Thesis to obtain the degree of Master of Science

Effect of dynamic salinity on morphology, sodium, and potassium uptake and distribution of four contrasting sweet potato varieties under field conditions

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Abstract

Undernutrition persists as a global challenge, especially in low and middle-income countries. Addressing micronutrient deficiencies, notably vitamin A, is crucial for improving nutritional status and reducing child mortality. Sweet potato (*Ipomea batatas* L.), rich in protein, minerals, vitamins, and fiber, is a promising crop to fight malnutrition. However, its productivity is hindered by soil salinity, a growing concern globally. This thesis investigates four sweet potato cultivars (cv. Bie, Bita, Melinda, and Supermargarete) from the International Potato Center (CIP) under dynamic salinity induced by drip irrigation. Located near Maputo, Mozambique, the field trial assesses morphological and physiological characteristics. Following an establishment period, we conducted both destructive and non-destructive sampling every 10 days over eight cycles. Our objective was to comprehensively evaluate crop growth, development, and sodium/potassium dynamics in the aboveground biomass, aiming to explore the effects of salinity across diverse cultivars and identify potential salinity tolerance mechanisms. Throughout the trial, the soil salinity levels remained moderate, resulting in only subtle treatment differences. Assessing dry weight, we observed that cv. Bie, Bita, and Melinda exhibited tolerance to moderate salinity, while cv. Supermargarete displayed sensitivity. Morphological responses were diverse across cultivars concerning the allocation of resources in different plant parts as well as branching activity and leaf morphology and distribution. Interestingly, the salt treatment did not yield an increase in shoot sodium concentration indicating insufficient salinity induction. Notably, cv. Supermargarete stood out as the only cultivar showing a slight rise in sodium concentration and a decrease in aboveground biomass potassium concentration under saltwater irrigation. For future trials it is important that a higher soil electrical conductivity is reached earlier in the trial period.

Keywords: Salinity stress, soil salinity, sweet potato, morphology, physiology

List of Abbreviations

Abbreviation	Meaning
AGB	Aboveground biomass
ANOVA	Analysis of variance
BI	Bie
BT	Bita
Cv.	Cultivar
CEC	Cation Exchange Capacity
CIP	International Potato Center
Cl^-	Chloride
dS m^{-1}	Dezisiemens per meter
DAOT	Days after onset of treatment
DAP	Days after planting
DW	Dry weight
EC	Electrical conductivity
ECe	Electrical conductivity of the soil saturation extract
ESP	Exchangeable sodium percentage
FW	Fresh water
IIAM	Instituto de Investigação Agrária de Moçambique
K^+	Potassium
N	Nitrogen
Na^+	Sodium
ME	Melinda
P	Phosphorus
ppm	Parts per million
RGR	Relative growth rate
RLAR	Relative leaf area ratio
SLA	Specific leaf area
SM	Supermargarete
SW	Salt water
USDA	United States Department of Agriculture
VPD	Vapor pressure deficit

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1 Introduction

Undernutrition, which summarizes stunting, wasting, underweight and micronutrient deficiency is a pressing global problem. Most vulnerable is the population of low and middle-income countries. In these countries nearly half of all child deaths under the age of five are caused by undernutrition. In particular, the lack of iodine, iron and vitamin A is a threat for public health and development (World Health Organization, 2021).

Sweet potato (*Ipomea batatas* L.) is one of the most important staple foods in low-and middle-income countries. It has high potential for improving food security due to its drought tolerance, wide ecological adaptation, and short development period (Motsa et al., 2015). Compared to other important food crops such as cassava, wheat and rice, sweet potato produces more biomass and nutrients per hectare (International Potato Center, 2022). Its vitamin, mineral, fibre, and protein content exceed those of many other dietary staples. Orange- and yellow-fleshed cultivars additionally are valuable sources of the vitamin A precursor *β-carotene* (Motsa et al., 2015). Vitamin A deficiency is a severe public health problem in Africa, with serious effects particularly on pregnant women and young children. Consequences include a weakened immune system, blindness and a higher prevalence of child death (World Health Organization, 2009). Sweet potato also has been utilized for animal nutrition and industrial purposes due to its high content of starch and other secondary metabolites (Kim et al., 2013).

Sweet potato growth, productivity and quality can be seriously affected by salt stress, especially in arid and semi-arid environments (Ahanger et al., 2017; Rodríguez-Delfín et al., 2014). Salinisation, which is estimated to be increasing at a rate of 2 Mha per year, plays a crucial role in sweet potato distribution and adoption (Abbas et al., 2013; Arisha & Qiang, 2020). Soil salinity is defined as the accumulation of ions in the soil, e.g., Na^+, Cl^- or Ca^{2+} to a degree which disturbs plant growth and functioning. Salinization is commonly driven by capillary rise of saline groundwater, salt accumulation due to saline parent material or poor irrigation water quality and practices (Butcher et al., 2016). Salinity has a negative impact on crops by disturbing physiological processes such as photosynthesis, protein synthesis and signal transduction (Yang et al., 2020; Zörb et al., 2019). Although sweet potatoes are often grown on marginal land, salt stress is still a major factor limiting its productivity (Dasgupta et al., 2008).

To exploit the potential of sweet potato cultivation for food security in the Global South it is important to understand how salt tolerance is mediated in this crop. The proposed trial investigates the effect of increasing soil salinity on development, growth, and ion uptake of various sweet potato clones from the genetic panel provided by the International Potato Centre (CIP). Whereas salinity effects on sweet potato have been assessed in various studies under laboratory and greenhouse settings (Arisha & Qiang, 2020; Keso et al., 2017) the lack of field trials underlines the relevance of this research.

Objective of the Master´s thesis is to investigate differences in morphological and physiological traits in the selected cultivars of sweet potato in response to increasing soil salinity. Changes in morphological traits have been investigated based on both destructive and non-destructive data. In order to investigate how salinity affects plant growth, data has been collected on aboveground biomass, leaf area, branching activity, leaf number and shoot growth. The analysis of these traits provides information on whether and how soil salinity affects biomass accumulation in different parts of the plants.

The analysis of potassium (K^+) and sodium (Na^+) concentration of leaf blades, stems and petioles was performed to research how various cultivars take up and distribute those ions under salinity stress compared to no salinity stress. A plant´s ability to exclude Na^+ from the ABG or safely compartmentalize it has been shown to be crucial for salinity tolerance as well as the ability to maintain K^+ uptake under salinity (Munns & Tester, 2008). In connection with morphological data, this allows conclusions about the salinity tolerance of cultivars as well as possible tolerance mechanisms involved. Finally, results of phenotyping trials can be used to develop a field-based screening tool for salinity tolerance and improve models simulating sweet potato growth and yield under salinity.

2 State of the Art

2.1 Soil Salinity: Definition, global extent and causes

Saline soils have a an ECe of more than 4 dS m^{-1} at 25 °C according to the definition of Richards (1954). Saline soil furthermore has a pH in the saturation paste of less than 8.2. It is characterized by excessive soluble salts which have a negative impact on crop growth. Sodium chloride and sodium sulphate make up the greatest share of soluble salts, but also magnesium and calcium occur as sulphates and chlorides. Salinization is considered as being low with an ECe of up to 4 dS m^{-1}, medium up to 8 dS m^{-1}, high up to 16 dS m^{-1} and very high above 16 dS m^{-1}. A great share of crops is showing limited yields already at medium salinity levels (Abrol et al., 1988).

The global map of salt affected soils by FAO (2021a) shows the occurrence of salt-affected soils worldwide. Hereby, soils with ECe > 2 dS m^{-1} and/or an exchangeable sodium percentage (ESP) of > 15 % and/or a pH > 8.2 are classified as salt affected. The results based on data submitted by 118 countries suggest that about 4.4 % of the topsoil and 8.7 % of the total land area are salt affected, with about two thirds of affected soils located in areas with arid and semi-arid climates. Sodic and saline soils are a worldwide problem amounting to about 10 % of arable land in over 100 countries (Tanji, 2002). The share is even higher for the global irrigated land, where salt-affected soils were estimated to account for 20 % in 1995 and likely have been expanding ever since (Ghassemi et al., 1995; Qadir et al., 2014). Irrigated land makes up a share of about 15 % of the total cultivated area but accounts for about a third of the global food production, which underlines the severity of the salinity issue on irrigated land (Munns & Tester, 2008).

Salinization of soils by natural processes is called primary salinization. This includes for example salinization by soluble salts stemming from weathering of parent rock material and rainfall or streams transporting salts from depositions (Hassani et al., 2021; Zaman et al., 2018).

However, salinization often has anthropogenic causes. This is referred to as secondary salinization. Irrigation plays a major role in this process. Salinization by irrigation is based on an imbalance between water and salt input and output from the soil. This can be caused by the usage of low-quality irrigation water. Another important driver for secondary salinization is insufficient drainage in lower soil layers leading to saline water rising to the root zone. In semi-arid regions where irrigated agriculture is common, extensive irrigation systems provide great amounts of dissolved salts which accumulate in the soil due to

waterlogging or are transported into lower lying areas by seepage. In non-irrigated agriculture, salinization is often induced by clearing of deep-rooting vegetation to make land available for rain-fed cultivation. Thereby, the drainage capacity is reduced leading to rising water levels over a saline subsoil. Further causes for secondary salinization include misuse of fertilizers, soil amendments and sewage sludge as well as dumping of industrial wastewaters (Hassani et al., 2021; Pitman & Läuchli, 2002; Zaman et al., 2018). The proximity to the seaside is a risk factor as saline water can intrude into groundwater and rivers (Pitman & Läuchli, 2002). Climate change is also contributing to soil salinization due to rising sea levels and the expansion of drylands (FAO, 2021a).

2.2 General salinity effects on plants

The effect of salinity on plants depends on the salinity tolerance of the respective crop. Halophytes are plants able to complete their life cycles under lasting high salinity stress. Agricultural crops however are glycophytes which are not adapted to saline environments. Selective pressure by humans imposed with the domestication of crops has resulted in a decreased tolerance to several stresses and a decreased genetic variability, which complicates endeavours to increase salt stress tolerance (Cheeseman, 2015).

Only about 2 % of plants are halophytes, the remaining 98 % are glycophytes. Within the group of glycophytes, there is significant variation in terms of salinity tolerance, with some very sensitive and some fairly tolerant species (Radyukina et al., 2007). Rice is the most sensitive cereal, followed by durum wheat, bread wheat is moderately tolerant and barley is the most tolerant cereal. Dicotyledons vary even greater concerning their salinity tolerance with some extremely sensitive legume species, but also fairly tolerant species, e.g. Alfalfa, and even halophytes (Munns & Tester, 2008). It is currently assumed that both halophytes and glycophytes have similar protective mechanisms at their command, while mostly the way those mechanisms are controlled are vastly different (Radyukina et al., 2007).

The plant response to root zone salinity can be divided into two phases. The first effect of salinity is osmotic: Reduced shoot growth, leaf number and leaf expansion occur as an effect of increased external osmotic pressure. This can lead to an imbalanced uptake of nutrients as the increased concentration of Na^+ in the soil solution hinders the uptake of other cations like K^+ and Ca^{2+}. The second phase is characterized by ionic stress due to Na^+ accumulation in the shoot to a concentration that becomes toxic to the plant.

Generally, the osmotic effect has the greater effect on growth than the ionic effect, apart from very sensitive species or under high soil salinity. Tolerance traits can be classified into three categories: Osmotic stress tolerance, Na^+ exclusion from leaf blades and tissue tolerance to Na^+ and Cl^-. Osmotic tolerance is based e.g. on decreased stomatal closure or increased accumulation of organic solutes under osmotic stress. Exclusion mechanisms include for example increased sequestration of Na^+ into root vacuoles and altered transport mechanisms to reduce Na^+ transport to the shoot. Tissue tolerance is based on cellular and intracellular compartmentalization of toxic ions with the goal of preventing toxic concentrations in the cytoplasm. An example therefore is the inclusion of Na^+ into leaf vacuoles. A high share of salt tolerant crops, e.g. barley, display includer mechanisms. Including behaviour can lead to reduced K^+ requirement of crops under salt stress and could thus be an interesting aspect to introduce by breeding under the condition that mechanisms are in place to prevent Na^+ release from the vacuole (Munns & Tester, 2008; Zörb et al., 2019; Zörb et al., 2014).

The osmotic stress and ion toxicity caused by salinity do not only reflect in the physiology and morphology of crops but also, more critically, in their yield. About 90 % of the world´s nutrition is derived from only 30 crops, which all show a yield decline of 50 - 80 % under moderate salinity (ECe 4-8 dS m^{-1}). Salt tolerance can be assessed by calculating the crop specific salinity threshold at which yield declines in comparison to non-stressed conditions and the slope at which yield decreases with increasing salinity beyond this threshold. Particularly in the early growth stages, salt stress has a severe impact on growth and yield (Maas & Hoffman, 1977; Zörb et al., 2019).

2.3 Morphological response to salt stress in sweet potato

There is a general scarcity of literature on salinity effects on sweet potato. Especially limited are the studies investigating the morphological effects of salinity and salinity tolerance traits in sweet potato (Mondal et al., 2022). However, the general effects of salinity and specific effects on other crop species have been documented.

Salinity can affect plant growth at different extent. According to Acosta-Motos et al. (2017), plant growth under saline conditions initially experiences a decline due to a reduction in soil water potential, known as the osmotic phase. Subsequently, a distinctive impact manifests as salt injury in leaves. This occurs because of a swift escalation in salt concentration within the cell walls or cytoplasm, triggered by the inability of vacuoles to continue sequestering incoming salts, marking the onset of the ionic phase. A commonly used indicator of plant growth is the accumulation of biomass, that can be indicated as dry weight (DW) and measured in grams (g).

Mondal et al. (2022) exposed 12 contrasting genotypes of sweet potato to 0, 50, 100 and 150 mM of NaCl. They found out that increasing salinity affected the development of total dry weight and leaf dry weight differently across the studied genotypes. The threshold at which salinity started to impact biomass accumulation was 15 and 13 mM for sensitive varieties (CIP 189151.8 and CIP 440181 respectively) and 76 and 82 mM for tolerant varieties (CIP 194281.2 and CIP 440004 respectively). Both sweet potato cultivars Huambachero and Untacip showed a decrease in the dry weight of leaves in salt stressed plants compared to those under control conditions (Rodríguez-Delfín et al., 2012). The lower values of leaves dry weight were linked to a significant lower leaf area in the plants under salt water treatment. Two different varieties of sweet potato (CIP 188002.1, salt tolerant and CIP 189151.8, salt sensitive) both showed reduced dry weight under salt water treatment with different atmospheric moisture conditions (Mondal et al., unpublished). These findings indicate soil salinity negatively impact the biomass accumulation of sweet potato, but the extent of the negative effects depends on the genotype. Other crop species negatively affected by saline conditions in dry weight accumulation are soybeans, maize and tomatoes, among others (Çarpıcı, 2009; Tanveer et al., 2020). However, the effect of soil salinity on the dry weight accumulation of sweet potato plants varies with the level of salinity. Research by Afaf et. al. (2009) found that at 10% and 30% seawater levels, salinity promoted plant height, the number of leaves and side branches, and dry weight of shoots. However, these parameters were reduced at 50% seawater salinity level. This indicates that lower levels of salinity may have a positive

effect on dry weight accumulation, while higher levels can lead to a reduction. Therefore, the impact of salinity on dry weight accumulation in sweet potato plants is dependent on the specific salinity level.

The effect of salinity on leaf area in plants is well-documented. Munns & Tester (2008) explained that salinity stress can decrease leaf size, leading to stunted growth of plants. This is attributed to the depressive effects of salinity on leaf chlorophyll contents, which can reduce photosynthesis and lead to a reduction in leaf area. Additionally, research on maize genotypes and halophytes has shown that salinity stress significantly decreases leaf size, leaf area, and leaf expansion, ultimately impacting the growth and physiological attributes of the plants (Dikobe et al., 2021; Rozentsvet et al., 2022; Yu et al., 2019). Therefore, salinity stress generally leads to a reduction in leaf area in plants, which can have significant implications for their growth and productivity. However, this relationship is not always straightforward. The effect of salinity on leaf area is indirect, as the primary consequence of salinity is a significant reduction in the size of individual leaves or the quantity of branches, which is reflected on the leaf area (Munns & Tester, 2008). Moreover, a possible response mechanism to salt induced osmotic stress involves an immediate limitation of cell expansion in young leaves, leading to stomatal closure. A poor response to osmotic stress would lead to enhanced leaf growth and stomatal conductance. The subsequent increase in leaf area would be advantageous only for plants with an ample supply of soil water, such as irrigated crops, but it may be undesirable in environments with limited water availability (Munns & Tester, 2008). In sweet potato, leaf area has been proven to be affected by salinity as well. The varieties Japanese Yellow and Blackie both showed a reduction in the number of leaves and leaf area when irrigated with a 50 mM of NaCl solution, although the reduction in leaf area was different across the two cultivars, being smaller for cv. Blackie, classified as tolerant (Kitayama et al., 2020). Mondal et al. (2022) showed an increasingly larger decrease in leaf area, as well as leaf number and leaves dry weight, at increasing root zone salinity. Cultivars Huambachero and Untacip as well were significantly affected by salinity when looking at their leaf area (Rodríguez-Delfín et al., 2014). Additionally, they found that the tolerant cv. Untacip had higher values of specific leaf area (SLA). According to Negrão et al. (2017a), SLA (defined in their study as leaf area ratio, LAR), is an important parameter when studying plant's responses to salinity stress. Particularly, the relative leaf area ratio (RLAR) serves as an indicator of the impact of salinity on leaf thickness. A decrease in RLAR under salinity stress might be an adaptive response, considering the potential

increase in leaf thickness attributed to thicker cell walls or a possibly greater volume for the sequestration of salts. These findings concur on the negative effect of salinity on the leaf area and leaves number development in sweet potato.

Another plant growth parameter that is often studied for salinity related research is the length of the vine (plant height) and/or of the side branches. According to Munns & Tester (2008), a salt induced ionic stress can negatively impact the shoot growth. Additionally, it can suppress buds' development, thus affecting the formation of side branches. A negative salinity effect on shoot length was found by Chartzoulakis et al. (2002) in six olive cultivars. Shoot dry weight was negatively affected by salinity in two cultivars of peas at levels of NaCl comprised between 50 and 160 mM (Alarcón et al., 1999; Hernández et al., 2001). Two sweet potato cultivars, Japanese Yellow and Blackie, showed a decline in shoot height by 34.6% and 26.7% under a 50 mM NaCl treatment, respectively (Kitayama et al., 2020). Mondal et al. (2022) showed how both the main vine length and side branches length had smaller values across 12 sweet potato genotypes when exposed to a 150 mM salt treatment. In vitro apex cultivation of sweet potato showed a reduction of growth parameters under salt treatment. The number of shoot and shoot length were both negatively affected by salinity, as well as leaf number (Dasgupta et al., 2008). A reduction in shoot length of potato plants in saline environment was found in a field trial by Mahmud 2018, where plant height where plant height was reduced between 32.82% and 60.15%. The findings highlight the importance of shoot length and shoot development when investigating plants' response mechanisms to salinity.

2.4 Ionic response to salt stress in sweet potato

As in this study the Cl^- concentrations of the collected samples were not determined, the literature review will focus on Na^+ and K^+. In most species, Na^+ seems to cause toxic effects sooner than Cl^-. K^+ is relevant to analyse as its uptake is often negatively affected by the presence of excessive Na^+ in the soil. It is widely accepted that the restricted uptake of Na^+ under salt stress and the maintenance of a high tissue K^+ concentration and K^+ Na^+ ratio are beneficial under salt stress (Munns & Tester, 2008).

2.4.1 Na^+ and K^+ concentration in the aboveground biomass

Keso et al. (2017) exposed five sweet potato cultivars to 0, 200 and 600 mM NaCl respectively in a pot trial. The 200 mM NaCl treatment led on average to a 16 % increase of the Na^+ concentration in the aboveground biomass (AGB). The AGB K^+ concentration was significantly lower in the 200 mM salt treatment with an average reduction of 34.9 %. The extent of the reduction of the K^+ concentration caused by the 200 mM salt treatment varied greatly between varieties, with a maximum reduction of 83 % and a minimum reduction of 1.7 % compared to the control. Aboveground biomass and Na^+ concentration were significantly negatively correlated as well as K^+ and Na^+ concentration in the aerial biomass.

Yu et al. (2018) exposed the relatively salt sensitive sweet potato cultivar (cv.) Xushu 32 to salt stress of 150 mM NaCl for 15 days in a hydroponic trial. They found a significantly higher Na^+ concentration in the leaves, stems and roots (~ 22, 55 and 30 mg g^{-1} DW respectively) under salt stress in comparison to the control. K^+ concentration in leaves stems and roots (~ 60, 50 and 65 mg g^{-1} DW respectively) was significantly reduced compared to the control. The low capacity of cv. Xushu 32 to maintain K^+ concentrations under salinity is interpreted as a sensitivity trait.

Kitayama et al. (2020) conducted a pot experiment irrigating two different sweet potato cultivars with salt water with a concentration of 0, 25 and 50 mM NaCl respectively for 21 days. The salt sensitive cv. Japanese Yellow showed its peak Na^+ concentration in its stems and leaves at 50 mM NaCl with concentrations of 82.3 mg g^{-1} DW and 42.0 mg g^{-1} DW respectively, each more than 2-fold the concentration of the more tolerant cv. Blackie. However, also cv. Blackie showed a significant increase in the Na^+ concentration in stems and leaves. K^+ and Na^+ concentration were negatively correlated both in the stems and leaves. Cv. Blackie showed higher Na^+ concentration in the root which

indicates that this cultivar manages to exclude toxic Na^+ from the shoot to a certain extent by accumulating it in the root.

On the contrary, in a study by Fan et al. (2015), the transgenic sweet potato lines showed higher Na^+ concentration in the leaves ranging from 33 to 34 mg g^{-1} DW compared to the sensitive wild-type cultivar with 28 mg g^{-1} DW under 200 mM salt stress. The researchers included a gene coding for a vacuolar N^+/H^+ antiporter into cv. Xushu-22 and compared its performance to the wild-type sweet potato. Also, K^+ concentrations were significantly higher in the leaves of transgenic lines, with values ~ 27 mg g^{-1} DW versus ~15 mg g^{-1} DW in the wild-type sweet potato. The authors conclude that the transgenic lines´ ability to compartmentalize Na^+ into leaf vacuoles helps to avert toxic effects of Na^+.

Mondal et al. (unpublished) cultivated two different sweet potato cultivar (salt tolerant CIP 188002.1 and salt sensitive CIP 189151.8) in a nutrient solution with 0 and 50 mM respectively at two different vapour pressure deficits (VPD) (low: ~0.76 kPa; high: ~2.27 kPa). For both varieties higher Na^+, K^+ and Cl^- concentrations were found in the petioles than in leaf blades whereas the VPD did not have a significant effect on leaf blade concentrations. Under low VPD, the Na^+ concentration in the leaf blade of the tolerant cultivar was 20 mg g^{-1} DW versus 36 mg g^{-1} DW in the sensitive cultivar. The sensitive cultivar showed significantly higher leaf blade Na^+ concentration under both VPD. The tolerant cultivar managed to protect its leaf blades from Na^+ which reflects in a 4.5 times higher Na^+ concentration in the petioles than in the leaf blades. In comparison, the sensitive cultivar only showed a 1.9 times higher concentration of Na^+ in the leaf petioles than in the leaf blades (under low VPD). Both varieties were found to display a higher Na^+ and K^+ concentration in older leaves than younger leaves under the high VPD while under low VPD ion concentrations were more similar across the leaf positions. Regarding the K^+ concentration, the tolerant cultivar showed an about two times higher value in the petioles than the sensitive cultivar (47 versus 22 mg g^{-1} DW, low VPD). A pronounced accumulation of K^+ in the petioles and younger leaves is probably a tolerance trait.

This is in accordance with Tester and Davenport (2003) who claim that damage caused by Na^+ is determined by the extent of Na^+ accumulation in the leaves, the compartmentalization between different leaf parts (e.g., leaf blade and petiole), cells and leaf positions. Na^+ accumulation in the leaf tissue can lead to premature senescence and necrosis specifically in the older leaves. However, a high Na^+ concentration in the leaf blade alone does not suffice to classify a plant as sensitive to salt stress as plants vary in

tissue tolerance. Combining senescence data of the old leaves with Na^+ concentration is a better measure (Munns & Tester, 2008).

2.4.2 Na^+ and K^+ uptake

Mondal et al. (2022) exposed 12 sweet potato genotypes to four salt concentrations from 0 to 150 mM in a hydroponic trial for four weeks. They found that total K^+ content per plant showed a linear decrease with increasing salinity in all tested genotypes. Under 0 mM NaCl, K^+ content was between 250 and 350 mg per plant while under maximum salinity, K^+ contents per plant were only between 80 and 180 mg. Na^+ uptake and accumulation followed a quadratic function for all cultivars with maximum Na^+ content per plant at either 50 or 100 mM NaCl. Maximum Na^+ contents varied between 150 and 300 mg per plant. Higher Cl^- uptake was observed at any concentration while showing a very similar pattern to Na^+ uptake. Furthermore, differences between shoot K^+ content at 0 mM and at 75 mM NaCl were strongly negatively correlated with respective thresholds for dry matter accumulation under salt stress. This indicates that maintaining high K^+ shoot tissue content under salt stress is an important factor in salinity tolerance of sweet potato. A hydroponic trial by Mondal et al. (unpublished) also confirms that K^+ uptake is negatively correlated with the imposition of salt stress while Na^+ and Cl^- uptake are positively correlated.

Rodríguez-Delfín et al. (2014) planted two different varieties of sweet potato in wooden containers and irrigated with three levels of NaCl (0, 8 and 14 mM) for a total of 145 days. Phosphorus, magnesium, K^+ and Na^+ uptake (mg $plant^{-1}$) to the leaves were significantly affected by the imposed salt stress with all ions apart from Na^+ being taken up less. The cultivar Untacip showed significantly lower uptake of Na^+ compared to the cultivar Huambachero. While Untacip showed higher yield under low salt stress (8 mM NaCl), there was no yield difference at higher salt stress (14 mM NaCl) because Untacip showed a strong yield decline as a function of increasing salinity.

Begum et al. (2015) exposed 10 sweet potato genotypes to 5 levels of salt stress ranging from 1.8 dS m^{-1} to 20 dS m^{-1} in a hydroponic system for two weeks. The roots and shoots were then analyzed for Na^+ and K^+. Increasing salinity increased shoot Na^+ uptake in all genotypes while showing the highest tissue Na^+ content at 15 dS m^{-1}. K^+ uptake was inversely correlated with increasing salinity showing a reduction of 60 % in comparison with the control.

2.4.3 K^+ Na^+ ratio

Salt stress impacts ion ratios in plants due to Na^+ influx via pathways of K^+. Cultivars that can discriminate better between Na^+ and K^+ regarding their transport to the shoot have a lower K^+ Na^+ ratio in the shoot which translates also into a lower ratio in the cytosol of shoot cells. A physiological cytosolic K^+ concentration in turn is a prerequisite for protein synthesis (Blumwald, 2000; Flowers & Hajibagheri, 2001). Maintaining a high K^+ Na^+ ratio in the aboveground tissue under salt stress has been proven to be a tolerance indicator linked to yield and biomass development for a wide range of crops, e.g. in wheat (Khan et al., 2009), barley (Flowers & Hajibagheri, 2001), rice (Asch et al., 2022) and maize (Akram et al., 2007).

Begum et al. (2015) exposed 10 genotypes of the Bangladesh Agricultural Research Institute (BARI) for two weeks to five levels of salinity up to 20 dS m^{-1} in a hydroponic trial. They defined the value 1.0 as a threshold for the K^+ Na^+ ratio (of root and shoot) under which plant growth is seriously impaired. The salinity level at the threshold was found to be 8 d Sm^{-1} in this hydroponic trial. Based on this, the authors classified sweet potato as a moderately salt tolerant crop.

In a pot trial exposing five sweet potato genotypes to a soil salinity of 200 mM NaCl, the K^+ Na^+ ratio of the AGB was found to rank between 2.35 and 1.48 depending on the cultivar. The two genotypes with the highest K^+ Na^+ ratio, RAB 45 and KAV 11, were classified as the most salt tolerant by the authors and also showed less reduction of the K^+ concentration under salt stress compared to the other tested genotypes (Keso et al., 2017).

In a pot trial, Fan et al. (2015) exposed a wild type line and three genetically modified sweet potato lines including the gene for a vacuolar Na^+/H^+ antiporter to 200 mM salt stress. Significantly higher K^+ Na^+ ratios were found for the genetically modified (between 1 and 1.5) than the wild type line (~ 0.5). While both lines showed higher Na^+ and lower K^+ concentration under salt stress, the genetically modified lines reduced their K^+ concentration to a way lesser extent. This explains how they maintained a higher K^+ Na^+ ratio under salt stress.

In a study by Mondal et al. (2022) the genotypic threshold for DW reduction and the respective K^+ Na^+ ratio in the aboveground tissue at this threshold were negatively correlated, while a positive correlation was expected. The same thresholds showed no correlation with the K^+ Na^+ ratio at a theoretical salt concentration of 75 mM. The authors

theorize that salinity tolerance in the tested cultivars was not based on the relation of K^+ to any other analysed element but rather on the ability to maintain high tissue K^+ concentrations under salt stress.

3 Materials and Methods

3.1 Trial site

The trial location was about 40 km South-West of Maputo close to the city Boane at the Umbeluzi river, at 26°01'31'' South and 32°17'54'' East at an elevation of 26 to 30 meters above sea level. The river provided the irrigation water. The total area of the trial was 6,237 m^2.

According to the Köppen-Geiger climate classification, Maputo has a tropical savanna climate with dry winters (Kottek et al., 2006). In the wet season from October to March, Nwalate research station encounters a unimodal rainfall pattern, from April to September there is an extended dry period (Ramírez et al., 2021). The annual average temperature in Maputo is 22.9 °C and annual rainfall is 713 mm. During the trial period from April to October, the climate data shows that there is on average less than 60 mm of monthly rainfall. June, July, and August are the driest months of the year with less than 20 mm of monthly rainfall. July is the coldest month of the year with an average temperature of 19.3 °C (see climate diagram in Appendix A) (Climate-Data.org, n. d.).

For this trial it was not possible to set up a weather station. However, weather data from the nearby Umbeluzi research station from the year 2019 is available from another publication (see Table 1) (Ramírez et al., 2021).

Table 1 Maximum, minimum and average temperature as well as rainfall from April to October 2019 at the Umbeluzi Research Station. Note: Data in the table below is based on Ramírez et al. (2021).

Month	Tmax (°C)	Tmin (°C)	Ta (°C)	RH (%)	Rain (mm)
Apr	30.3 ± 0.55	19.5 ± 0.23	23.9 ± 0.23	38.7 ± 0.99	35.43
May	30.0 ± 0.45	14.7 ± 0.47	21.3 ± 0.32	40.2 ± 1.17	0.00
Jun	28.2 ± 0.44	11.7 ± 0.39	19.2 ± 0.26	44.1 ± 1.41	48.23
Jul	28.9 ± 0.54	10.6 ± 0.44	19.2 ± 1.29	46.0 ± 1.27	19.77
Aug	29.0 ± 0.57	14.5 ± 0.45	21.0 ± 0.32	48.4 ± 1.15	75.76
Sep	29.2 ± 0.87	15.0 ± 0.58	21.1 ± 0.46	48.8 ± 1.26	13.78
Oct	30.5 ± 0.87	16.8 ± 1.17	22.4 ± 0.50	52.8 ± 1.94	11.1

*Tmax – maximum temperature; Tmin – minimum temperature; Ta – average temperature; RH – relative humidity.

Soil samples for a general soil analysis were taken before treatment onset from three soil depths (0-16 cm, 17-32 cm and 33-48 cm) and analyzed in the soil laboratory of the Instituto de Investigação Agrária de Moçambique (IIAM). The soil was classified as a clay soil according to the soil texture classification system of the United States Department of Agriculture (USDA) with approximately 60 % clay, and about 20 % of each sand and silt in all three analyzed soil layers (USDA, 1987).

Table 2 Results of the general analysis of the soil at the Nwalate Research Station

Depth (cm)	pH	ESP (%)	Effective CEC (meq/100 g)	Organic matter (%)	Olsen P (ppm)	Total N (%)	C/N	%		
								Sand	Silt	Clay
0-16	7.2	15.7	79	3.39	0.87	0.14	14.34	25	20	55
17-32	7.0	16.8	80	4.01	0.84	0.14	16.60	20	21	59
33-48	7.1	18.7	82	3.16	0.63	0.13	14.54	16	21	63

*ESP = Exchangeable sodium percentage; CEC = Cation exchange capacity; P = Phosphorus; N = Nitrogen; C/N= Carbon Nitrogen ratio.

The effective cation exchange capacity (CEC) in all soil layers was approximately 80 meq/100g while soil pH was approximately 7. The exchangeable sodium percentage (ESP) was 18.7 %, 16.8 % and 15.7 % in the upper, middle and lower soil layer respectively. The ESP is a measure of soil sodicity and describes the share of Na^+ in the total exchangeable cations. If it exceeds 6 %, a soil is classified as sodic, which applies to the soil at the trial site as the ESP was ranging between 15.7 % and 18.7 % (Wiesman, 2009, pp. 105–106). An Olsen phosphorus (P) value of at least 10 mg P kg^1 is widely thought to be beneficial for plant growth (Pierzynski, 2000). Olsen P in the analyzed soil samples was found to be < 1 mg P kg^1 indicating a P deficiency. The percentage of total Nitrogen (N) observed was 0.14 % for the upper and middle soil layer and 0.13 % for the lower soil layer (see Table 2).

3.2 Trial design

The trial entailed two sub-trials, the screening and physiology trial. This thesis is based on data obtained from the physiology trial. This trial covered an area of 2,178 m^2, with a width of 27 m and a length of 81 m. Its goal was to describe the detailed effects of salinity on six different sweet potato cultivars as well as potential mechanisms of salt stress tolerance.

The physiology trial was planned to consist of three treatments: a freshwater treatment (FW), a saltwater treatment with early onset (SW1) and a saltwater treatment with later onset (SW2). As planting and the beginning of sampling after planting were delayed due to various technical issues, SW2 was dropped rendering this block as reserve sampling plots for the SW1 (see Figure 1).

FW plots were located in freshwater irrigation section which was supplied with freshwater. The SW1 plots were situated in the saltwater irrigation section which was supplied with saltwater by a separate irrigation system. Irrigation treatments could not be randomized as the trial area is located on a slope. To avoid leaching of salt water into freshwater treatment plots, SW1 and SW2 plots were allocated further down on the slope than FW plots.

Within a treatment there were 3 replicates per variety, adding up to a total of 18 plots per variety and treatment and 54 plots for the whole physiology trial. Each plot contained a total of 135 plants in 9 rows with 15 plants each. Rows were spaced one meter apart from each other and each row was provided with an irrigation line. A plot consisted of 15 sampling units of 3 x 3 = 9 plants. Plants in the middle of a sample unit were surrounded with border plants and could thus be used for destructive sampling. Accordingly, 15 plants per plot were available for destructive sampling (see Figure 1).

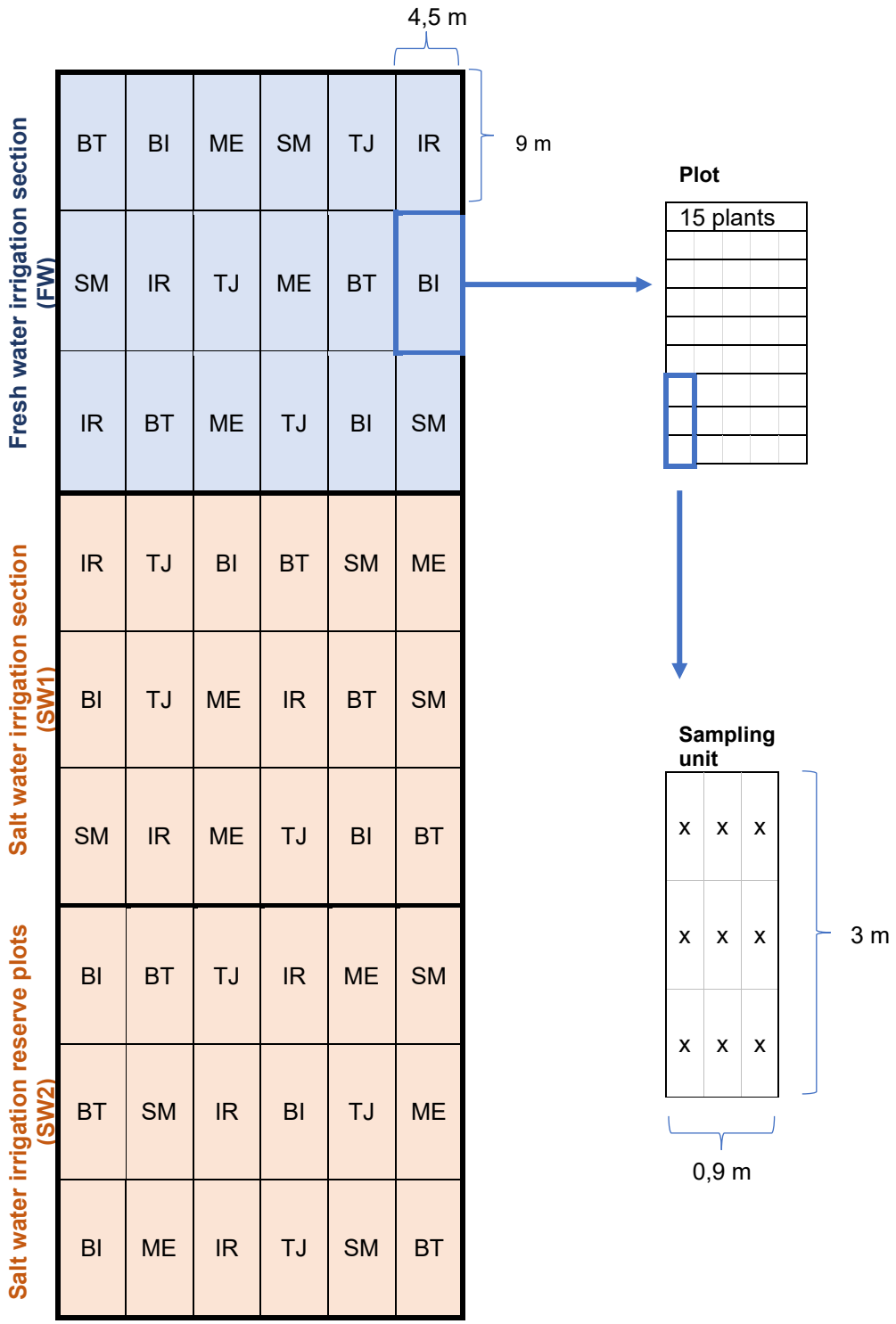

Figure 1 Trial design with irrigation sections, plots, and sampling units (BI= Bie, BT= Bita, ME= Melinda, SM=Supermargarete, TJ= Tio Joe). Note: Figure was created by the authors.

3.3 Planting material and planting

The planting material was kindly provided by the International Potato Center (CIP) in Maputo. The selection of cultivars depended on the amount of planting material available locally. Furthermore, as there is no scientific literature on the salinity tolerance of the available Mozambican sweet potato varieties, they were selected to cover a wide range of different characteristics concerning e.g., leaf shape, flesh colour or growth form (see Table 3).

Table 3 Properties of the CIP sweet potato cultivars used in the physiology trial. Note: Table was compiled from information from International Potato Center (2019).

Cultivar name	CIP code	Country of origin	Drought tolerant	Leaf shape	Growth	Flesh colour
Bie	CIP 112291.1	Mozambique	n.a.	Triangular	Semi-erect	Cream; secondary colour: purple
Bita	CIP 112290.1	Mozambique	n.a.	Moderate lobes	Semi-erect	White; Secondary colour: purple
Irene	CIP 106764.1	Mozambique	yes	Almost divided, very deep lobes	Erect	Intermediate orange
Melinda	CIP 106763.1	Mozambique	yes	Moderate lobes	Spreading	Pale orange
Super-margarete	unreleased	Mozambique	n.a.	Almost divided, very deep lobes	n.a.	Purple
Tio Joe	CIP 106769.1	Mozambique	yes	Triangular	Spreading	Dark orange

Cuttings were made the day before planting both from the multiplication site at the Maputo Office of CIP as well as at the Nwalate research station, where the field trial was located. Healthy vines were cut after every three nodes to produce cuttings. Leaf blades and petioles were fully removed apart from the petiole and leaf blade growing out of the top node. Cuttings were stored overnight in water buckets. Planting took place on 21-22/06/2022. During planting, two nodes were buried in the soil, whereas one node remained above the soil. The planting distance was 30 cm according to the distance of emitters on the irrigation lines. Prior to planting, the field was prepared by ploughing and establishing ridges of one meter distance and a height of approximately 30 cm.

3.4 Agronomic management of the trial

No fertilizer was applied to the field before or during the trial. Weeding was performed manually, based on necessity. Due to visible pest damage, an insecticide (Imidacloprid) was applied on 23/07/2022. The dosage was based on the recommended use by the producer.

3.5 Irrigation treatments

The irrigation amount was determined using reference values for crop evapotranspiration (ETc) from a study on crop water requirement of sweet potato in Nigeria (Opafola et al., 2018). Months similar in climate characteristics were used to estimate the reference evapotranspiration (ETo) which was calculated per plant and day based on the area of soil occupied by one sweet potato plant. Monthly crop coefficients (Kc) reflecting the developmental stage of the plant were taken to calculate the ETc. per plant and day (ETc = ETo * Kc). For the calculations, effective rainfall was assumed to be zero.

A monthly irrigation schedule was developed per irrigation section. For this, the saltwater section was divided into two irrigation sections for more uniform water distribution. For every of the three irrigation sections, ETc per plant and day was multiplied by the number of plants and calculated per week. The resulting water amount was then split into two irrigation events per week and divided by the drip rate measured at 1 bar (15 PSI) at the outlet to determine the duration of one irrigation event. On DAP 94, the number of irrigation events was increased to three per week due to first visible symptoms of water deficiency. Saltwater irrigation for the respective sections was started after an implementation phase of 57 days. The electrical conductivity of the saltwater treatment was 3 dS m^{-1} (2,000 ppm NaCl). After lack of visible treatment effects, the salt load was increased at DAP 94 to reach a planned electrical conductivity of 4.5 dS m^{-1} (3,000 ppm NaCl). Both EC values correspond to the category of moderately saline irrigation water (Rhoades et al., 1992).

The freshwater section and saltwater section were supplied with freshwater and saltwater respectively from two different irrigation systems (see Figure 2). Freshwater was pumped from the river into a freshwater reservoir (reservoir 1) with a capacity of 30,000 L using pump A. From there, pump B pumped the freshwater in underground tubes up into the freshwater section, where irrigation lines were fed from the underground tube system.

For the saltwater irrigation, the water was pumped from the freshwater reservoir into two connected reservoirs (reservoir 2 and 3) with a capacity of each 10,000 L. In reservoir 3,

concentrated salt solution was added to create the saline irrigation water. The concentrated salt solution was created by dissolving ordinary uniodized NaCl from the supermarket in a 5 L container filled with river water. Manual shaking helped to dissolve the salt. The other reservoir (reservoir 2) is filled with fresh water and can also be connected to pump C to flush the saltwater irrigation system with some fresh water after every sampling event to prevent clogging of the system. During the establishment phase, both reservoirs 2 and 3 were filled with freshwater.

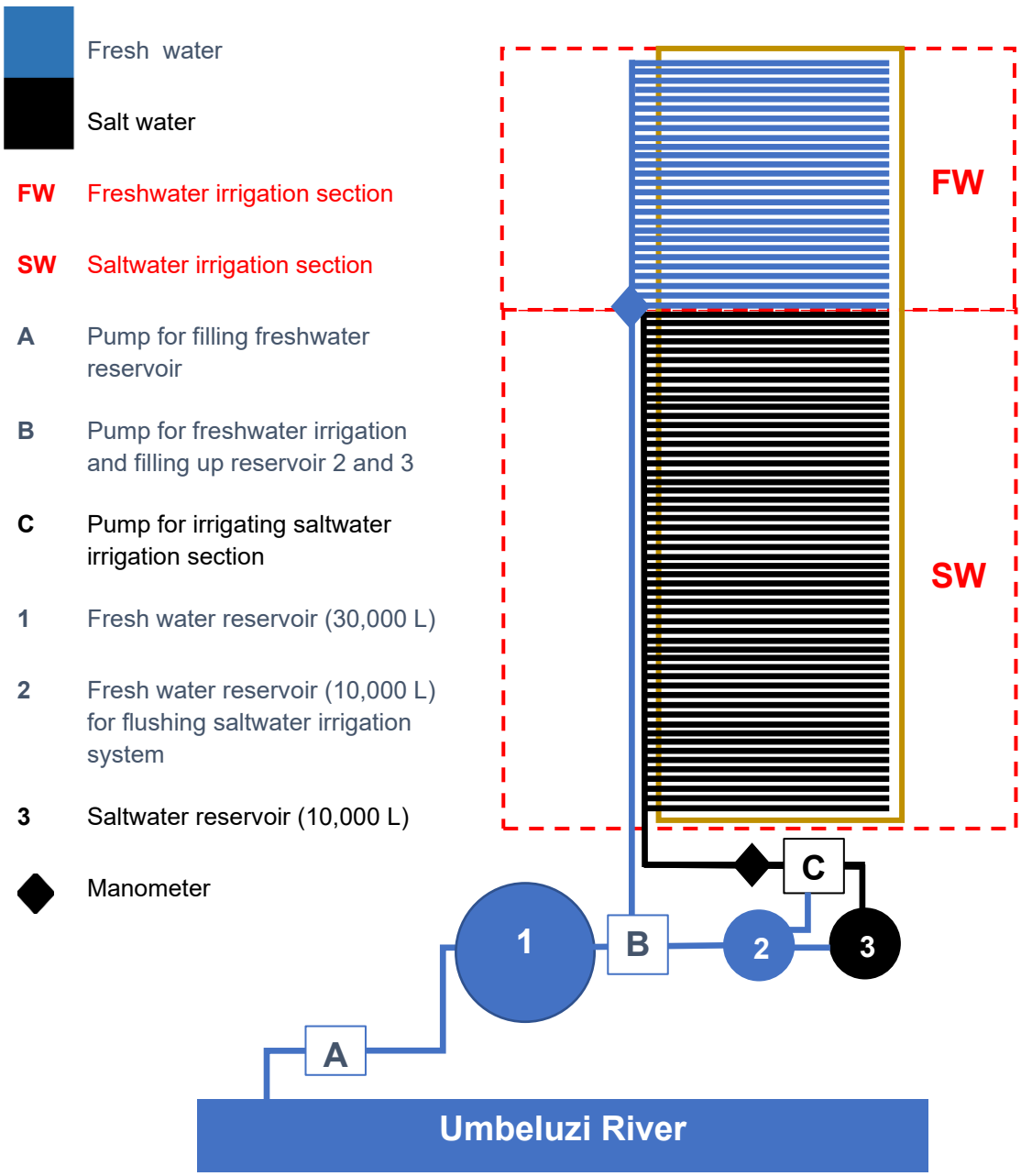

Figure 2 Set up of the fresh and saltwater irrigation system including reservoirs, pumps, manometers and irrigation lines. Note: Figure was created by the authors.

3.6 Sampling methodology

Sampling events were conducted approximately every 10 days for a total of 8 times. Sampling involved destructive and non-destructive plant sampling as well as soil sampling.

3.6.1 Non-destructive plant sampling

The growth of the plant was tracked using coloured cords to mark each vine. This vine marking process was performed on the middle plants of every sampling unit on every sampling event (15 plants per plot). The vine emerging from the top node was defined as vine 1 whereas for all the other vines, data was summed up within sections and noted down as vine 2. Every 10 days, a cord was attached around each vine above the last fully developed leaf. Only vines were marked whereas side branches and secondary side branches were not considered. Those markings were used to distinguish different sections of each vine, which were named “O” (old), “M” (middle) and “N” (new) (see Figure 3). Thereby, the new section contains the vine growth since the last sampling event. The old section is the part of the vine that grew in the establishment phase after planting but before treatment onset. Accordingly, vines that appeared later do not have an old section. Every new section, one sampling event later becomes part of the middle section of the vine, which is thus continuously growing over sampling events. For the non-destructive measurements, one plant per plot was randomly chosen and the same plant was measured at every sampling event throughout the whole trial. The parameters measured include following measurements per vine (1,2) and section (O, M, N):

- Vine length (cm)
- Side branch length (cm)
- No of side branches
- No of side branches on side branches
- No. of leaves
- No. of leaves attached directly to the vine (excluding side branch leaves)
- No. of senescent leaves (senescent leaves were defined as leaves of which at least 50 % of the area seemed photosynthetically inactive based on the colour)
- No. of senescent leaves attached directly to the vine.
- No. of flowers (flower buds as well as unfolded flowers)

Two people worked together on one plant using a ruler as equipment. The data was recorded in the non-destructive data sheet (see Appendix B).

With this sampling method, new growth in the form of side branches in the old and middle section was treated as part of the old and middle section and not noted as new growth. Tracking side branch growth with cords would have been not feasible due to time restraints and would have also increased the number of samples immensely.

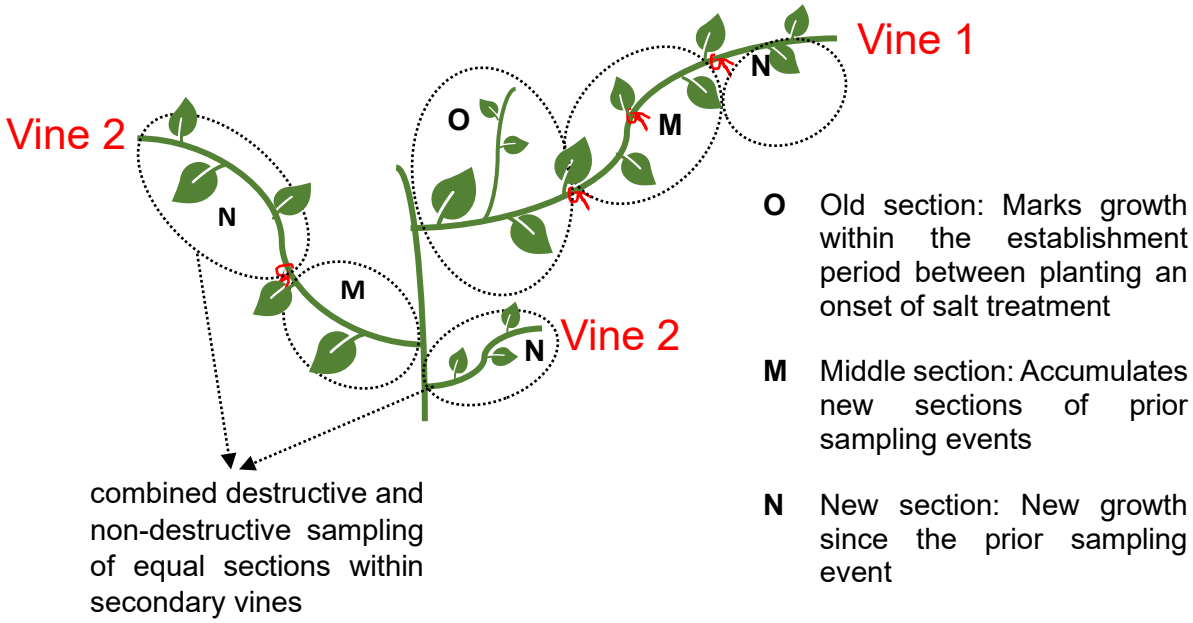

Figure 3 Schematic graphic of vine marking procedure dividing vines in old, middle and new sections for destructive and non-destructive sampling. Note: Figure was created by the authors.

3.6.2 Destructive plant sampling

One plant per plot, 36 plants per sampling event, was sampled destructively every 10 days. The sampling units from which the plants were taken were randomized. The complete plant including tuberous roots was removed carefully with a shovel. In a shed next to the field, the plant was divided into parts based on the vine marks. Per vine and section, leaf blades, stems, petioles, flowers, flower petioles, senescent leaves and senescent petioles were packed separately in paper bags with labels to uniquely identify each sample. Plant parts from secondary vines were aggregated within sections (see Figure 3) for the destructive sampling as well.

All leaf blades were photographed for later leaf area analysis before packing as described in section 3.11. and fresh weight of tuberous roots was noted.

3.6.3 Soil sampling

Every sampling event also included soil sampling. One soil sample per plot and sampling event was taken next to the plant that had been removed for destructive sampling at the respective sampling event. Soil samples were taken manually with a soil sampler. Each soil sample was divided to create three separate samples for the soil layers 0-16 cm, 17-32 cm, and 33-48 cm respectively.

Soil samples were supposed to be analysed for EC during the trial to monitor the salt accumulation in the soil. Due to time constraints however, the salt accumulation could not be monitored, and samples were analysed only after the trial in the soil laboratory of the IIAM. Therefore, the 1:5 weight to volume method was used as described in a standard operating procedure by FAO (2021b) on three lab repetitions of each sample. The soil was air-dried and sieved through a 2 mm sieve. The EC Meter (WTW pH/Cond 3320 SET 2, Xylem Analytics Germany GmbH, Weilheim, Germany) was calibrated using standard solutions. 10 g of soil were mixed with 50 ml of distilled water and shaken for 60 minutes. After letting the samples rest for 30 min the EC was measured in the supernatant.

3.7 Salt accumulation in the soil

The EC data is currently available only for sampling event 1 (DAP 57) and sampling event 7 (DAP 122). As the soil samples on DAP 57 were taken before the onset of the SW treatment, the table below shows the average EC and ECe values over all plots, irrespective of the irrigation section. To estimate ECe values from the EC 1:5 values the factor 6.1384 was used which was derived from a publication of Kargas et al. (2022). With ECe values of 3.2, 2.6 and 2.3 dS m^{-1} in the upper, middle and lower soil layer respectively, the soil at the trial site can be classified as slightly saline even before the onset of the SW treatment (Abrol et al., 1988). To evaluate the EC data of sampling event 7 (DAP 122), the average was taken separately for freshwater and saltwater irrigation section plots. The ECe values in the FW section at DAP 122 were very similar to the salinity levels recorded at treatment onset. In the SW irrigation section, ECe values were elevated with a maximum of 6.3 dS m^{-1} in the first layer of the soil indicating that the salt water irrigation system was effective in inducing moderate salinity (see Table 4).

Table 4 Soil salinity in three different soil depths at treatment onset and 65 days after treatment onset

DAP	Treatment	Depth (cm)	EC 1:5 (dS m^{-1})	ECe (dS m^{-1})	Classification
57	n.a.	0-16	0.5	3.2	Slightly saline
57	n.a.	17-32	0.4	2.6	Slightly saline
57	n.a.	33-48	0.4	2.3	Slightly saline
122	FW	0-16	0.6	3.4	Slightly saline
122	FW	17-32	0.5	3.3	Slightly saline
122	FW	33-48	0.3	2.0	Non-saline
122	SW	0-16	1.0	6.3	Moderately saline
122	SW	17-32	0.8	5.0	Moderately saline
122	SW	33-48	0.6	3.9	Moderately saline

*DAP= Day after planting; EC= Electrical conductivity; ECe= Electrical conductivity of the soil saturation extract.

The map in Appendix C shows the EC 1:5 value of each plot´s soil sample at DAP 122 (sampling event 7) by sampling depth. The map indicates that there was a high variation of EC values between plots. However, there was no visible pattern to this variation.

3.8 Monitoring of soil humidity

Soil humidity measurement was performed every 10 days along with sampling. A FDR (Frequency domain reflectometry) sensor (PR2/4, Delta-T Devices Ltd, Cambridge, United Kingdom) was used to determine the volumetric water content of the soil. This sensor measures water content at four different depths from 10 cm to 40 cm in a 10 cm interval. The sensor was inserted into tubes that had been installed after planting and remained in the field over the whole trial period. For each measurement, three replicates were created by turning the sensor by 120 °. From the two available calibration settings for the soil type, organic and mineral, mineral was chosen.

The tubes for the FDR sensor were installed on the ridge in the middle between plants. In the first and last plot per row (Plot 1 and 6), each two tubes were installed. One in the upper part of the plot, and one in the lower part. In the middle plots of each row (Plot 3 and 4) only one tube was installed in the upper and lower part of the plot respectively. Accordingly, 18 tubes were installed in the fresh water section and 36 in the saltwater section. Soil humidity was measured to monitor the irrigation system as equal volumes of water were supposed to be supplied with each irrigation event between and within irrigation sections.

The FDR values recorded at four different depths at sampling event 1 (DAP 57) were mapped (see Appendix D). The map shows that there was some variation of volumetric water content over the field. However, the differences in soil moisture over the field seem to follow no pattern. Fresh water and salt water irrigation system had similar soil moisture. Particularly dry or moist spots are probably caused by dead plants or problems with the irrigation material (e.g., unwanted holes, closed emitters).

3.9 Dry weight determination

Dry weight determination of plant samples took place after drying samples for 48 h at 70 ° C in the drying oven (Universalschrank Um, Memmert GmbH + Co. KG, Schwabach, Germany) followed by 10 minutes in the desiccator. Weighing was done with a precision scale (QUINTIX224 – 1S, Sartorius Lab GmbH & Co. KG, Göttingen, Germany). Samples that had been dried before but stored at room temperature afterwards were dried again for at least another 12 h at 70 ° C before weighing.

3.10 Sodium and potassium analysis

The samples of sampling event 7, sampled at DAP 122 (corresponding to DAOT 60) were transported dried, in paper sampling bags to Germany. Leaf blades, stems and petioles of four selected cultivars, Bie, Bita, Melinda and Supermargarete, were analyzed for Na^+ and K^+ in the department laboratory of the Institute of Agricultural Sciences in the Tropics of the University of Hohenheim. Before grinding, the samples were redried in a drying oven (Universalschrank Um, Memmert GmbH + Co. KG, Schwabach, Germany) at 70 ° C for at least 12 hours. The extraction method was hot water extraction, which has been validated by Asch et al. (2022). Samples greater than 0.1 g were milled with a ball mill by adding one big, three medium and six small metal milling balls to each sample. Stems and petioles underwent prior grinding with a cutting mill (IKA A10, IKA®-Werke GmbH & CO. KG, Staufen, Germany).

After milling, milling balls were removed with a magnet and 0.1 – 0.15 g of each sample were weighed into 15 ml centrifuge tubes using a precision scale (QUINTIX224 – 1S, Sartorius Lab GmbH & Co. KG, Göttingen, Germany). From samples greater than 1 g, three subsamples of each 0.1-0.15 g were taken for separate analysis to ensure that a representative amount of each sample was analysed. Subsamples were taken from the complete milled sample. If there was less than 0.1 g of the sample available after grinding, either 0.05 g or 0.25 g were weighed in. 10 ml of denoised water were added to each sample, followed by short mixing on a shaker (MS2 Minishaker, IKA®-Werke GmbH &

CO. KG, Staufen, Germany). Then samples were autoclaved for 60 minutes at 120 ° C (SANOclav M-MCS, Wolf-Maschinenbau – SANOclav, Geislingen, Germany). After the extraction, samples were filtered into 100 ml flasks using a filter paper (Qualitative filter paper 413, VWR International bvba, Leuven, Belgium). Deionized water was added to the filtrate to reach a volume of 100 ml. Samples with a weigh-in of 0.05 g or 0.025 g were filled up to 50 ml or 25 ml respectively. The diluted filtrate was measured with a flame photometer (Jenway PFP 7, Cole-Parmer, Vernon Hills, United States). The flame photometer was calibrated using K^+ and Na^+ standards with the concentrations 12.5 ppm, 25 ppm, 50 ppm and 100 ppm respectively. The standards were created from commercial 1000 ppm K^+ and Na^+ standards (Jenway Flame Photometry Standard, Cole-Parmer, Vernon Hills, United States) by dilution with deionized water. The sample volume (25 ml, 50 ml, or 100 ml) was considered when calculating tissue concentrations of K^+ and Na^+ from flame photometer measurements.

Samples with a dry weight below 0.1 g were filled completely into microtubes (Micro tube 2 ml with cap, Sarstedt AG & Co. KG, Nürnbrecht, Germany) and the weigh-in was noted. Then about 16 Zirconium-Silicate spheres (Lysing Matrix D, MP Biomedicals, Solon, USA), 10 small beads and 6 medium beads, were added and grinding was performed in the fast prep (Fast-prep 24, MP Biomedicals, Solon, USA) with a speed of 5 m/s for 20 seconds. Afterwards, deionized water was added to fill up to a volume of 25 ml, while the rest of the procedure was the same as explained before.

3.11 Leaf area analysis

Before packing leaf blades into sampling bags during destructive sampling, photos of the leaf samples were taken for later leaf area analysis. Leaf blades were spread out flat on a white background. Mobile phones were used to take photos. To avoid distortion by uneven surfaces a spirit level was used to adjust the surface on which leaves were spread out as well as the construction holding the phone. The distance between the phone camera and the white surface was about 30 cm. The photos were analyzed using the software ImageJ to determine the leaf area per sample in cm^2.

3.12 Specific calculations

The relative leaf area ratio (RLAR) was calculated according to Negrão et al. (2017b), as follows:

$$RLAR = \frac{LAR_{salt}}{LAR_{control}}$$

where LAR is equal to: $$LAR = \frac{Leaf\ area}{Dry\ weight_{leaves}}$$

3.13 Data evaluation

Data organization and calculations were performed in Excel (Microsoft, Excel 2021). All graphs were produced using SigmaPlot (Sigmpaplot 12.5., Systat Software Inc.).

Direct comparisons of means between FW and SW treatment could not be performed as treatments were not randomized over the field. However, for the ion data, the effect of the SW treatment was calculated for each repetition as the difference between each value under SW treatment and the mean of the FW repetitions within the same cultivar. This resulted in three values that could be compared between cultivars to determine if the SW treatment had a significantly different effect on cultivars. For the comparison of means, a one-way analysis of variance (ANOVA) was performed in RStudio (version 4.3.2, R Foundation) with the cultivars as independent variables and effects of SW treatment on ion concentration, content and partitioning as dependent variables followed by a post-hoc Tukey´s HSD test (see p-values in Appendix E). To ensure that the testing requirements for the one-way ANOVA were met, Shapiro-Wilk normality test and Levene´s test for homogeneity of variance were performed.

4 Results

4.1 Morphological traits

4.1.1 Dry weight development per plant part

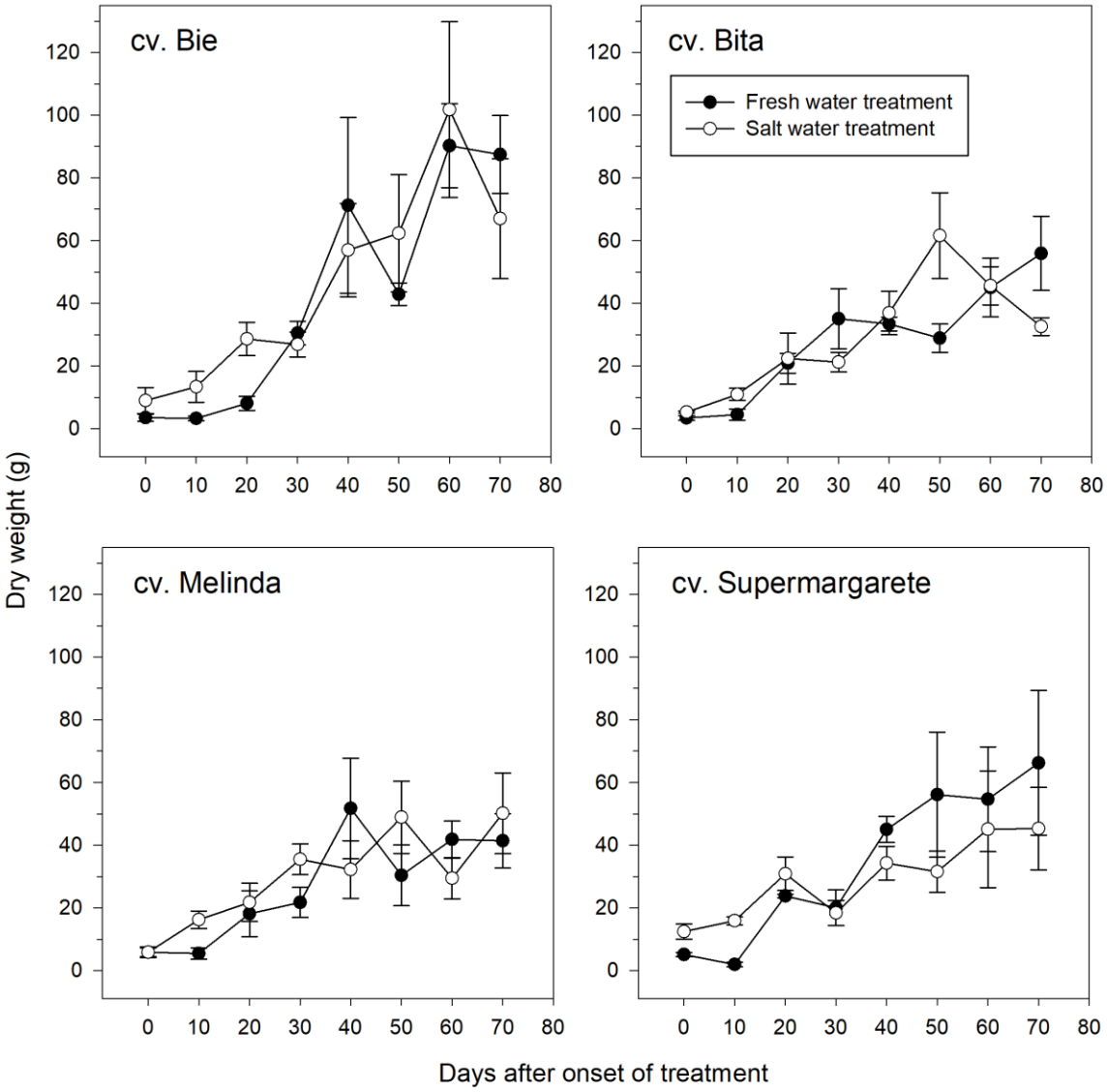

Figure 4 Average aboveground (leaf blades, stems and petioles) dry weight (g) development per plant under fresh and salt water treatment with standard error; n=3.

Cv. Bie shows the higher accumulation of dry biomass with 101.7 ± 28.0 g under SW treatment 60 days after onset of treatment (DAOT). Overall, cv. Bie exhibits a higher accumulation of biomass through the whole sample period, compared to the other cultivars. The SW treatment has a growth stimulating effect on all cultivars during the early stages of plant development: in cv. Bie, cv. Bita and cv. Supermargarete plants under SW treatment appear to accumulate more biomass during the first three sample events, while cv. Melinda until the fourth sample event. Between 20 and 30 DAOT, the plants under SW treatment start accumulating biomass at a lower rate than the plants

under FW treatment. This is clearly visible especially in cv. Supermargarete, which shows a higher accumulation of biomass under FW treatment.

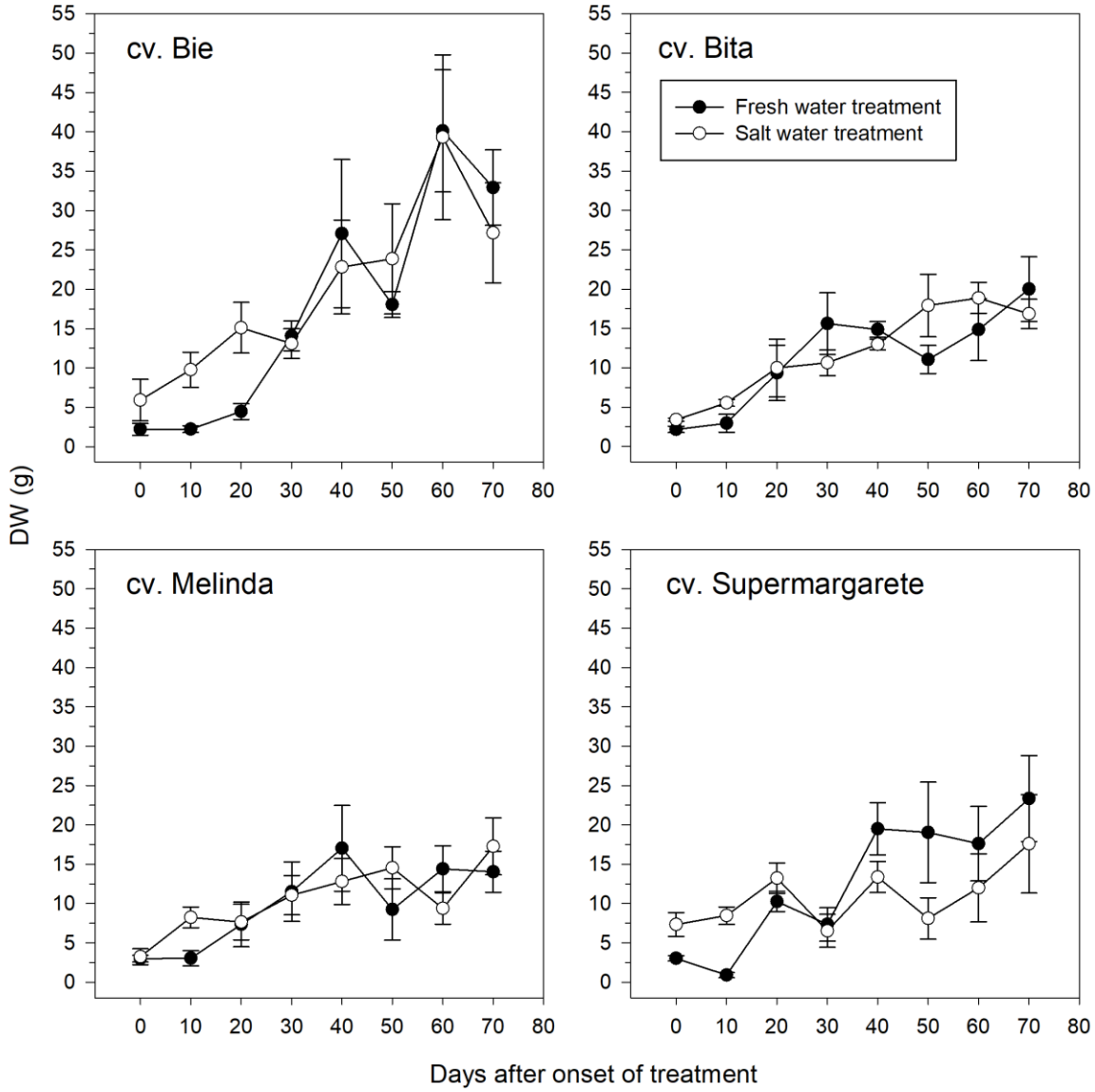

Figure 5 Average dry weight (g) of leaf blades development per plant under fresh and salt water treatment with standard error; n=3.

The accumulation of biomass in leaf blades follows the same pattern of total dry weight accumulation seen in Figure 4. Highest levels are reached in cv. Bie at 60 DAOT with 40.1 ± 7.7 g of leaf blades per plant under FW treatment and 39.3 ± 10.4 g of leaves per plant under SW treatment respectively. Cv. Supermargarete shows a clear difference between the two treatments from 40 DAOT, as plants under FW treatment have more leaves biomass than the salt stressed ones. Both cv. Bie and cv. Supermargarete display higher values of leaves DW under SW treatment in the first 20 days of treatment. Cv. Bita shows the same early stages development, but only in the first 10 days of treatment. After that, the two treatments have a synchronized and alternating development. This is visible

for the middle and late stage of development of all cultivars except Supermargarete. As seen in Table 5, leaves represent the highest share of the total dry aboveground biomass 10 DAOT in cv. Bie under SW treatment (67.5 ± 1.7%). Generally, the percentage share of the total aboveground biomass allocated to leaves is higher in the first 20 DAOT, in all cultivars and under both treatments. Cv. Supermargarete shows a clear difference between the two treatments from 40 DAOT, since plant under fresh water treatment have more leaves biomass than the salt stressed ones.

Table 5 Average percentage of leaves over the total aboveground biomass with standard error, per plant; BI = cv. Bie, BT = cv. Bita, ME = cv. Melinda, SM = cv. Supermargarete, FW = fresh water treatment, SW = salt water treatment; n=3.

	Days after onset of treatment							
	0	**10**	**20**	**30**	**40**	**50**	**60**	**70**
BI, FW	60.1±2.9	70.4±0.4	58.2±3.4	47.7±2.6	41.6±2.6	43.9±3.3	44.9±2.9	38.9±0.9
BT, FW	65.8±4.5	70±4.6	42±12.2	47.2±2.2	44.8±0.4	39.2±2	32.3±3.4	36.5±0.3
ME, FW	54.4±5.6	57.4±2	45.4±4.6	49.6±6.4	32.9±1	27.9±7.6	34.6±2.4	35.2±1.6
SM, FW	59.7±2	53±4.2	43.7±7.6	40.2±7.3	43.4±3.8	38.9±2.7	35.4±3.4	40.5±5
BI, SW	67.5±1.7	62.4±2.6	54.1±3.9	50.5±1.3	42.8±3.8	41.2±2.5	40.9±2.4	43.7±3.2
BT, SW	65±1.3	59.6±3.3	45.3±0.3	52±3.7	41.1±2.8	37.8±7.9	32.6±6.6	41.5±2.7
ME, SW	54.8±1.4	51.2±0.8	34.7±2.7	31.3±4.6	41.7±4.1	31±2.8	32.3±0.7	37.4±3.6
SM, SW	58.7±0.8	53.6±5.1	44.9±2.3	32.5±7.4	43.2±7.2	26.5±5.7	32.4±4.3	39.2±4.1

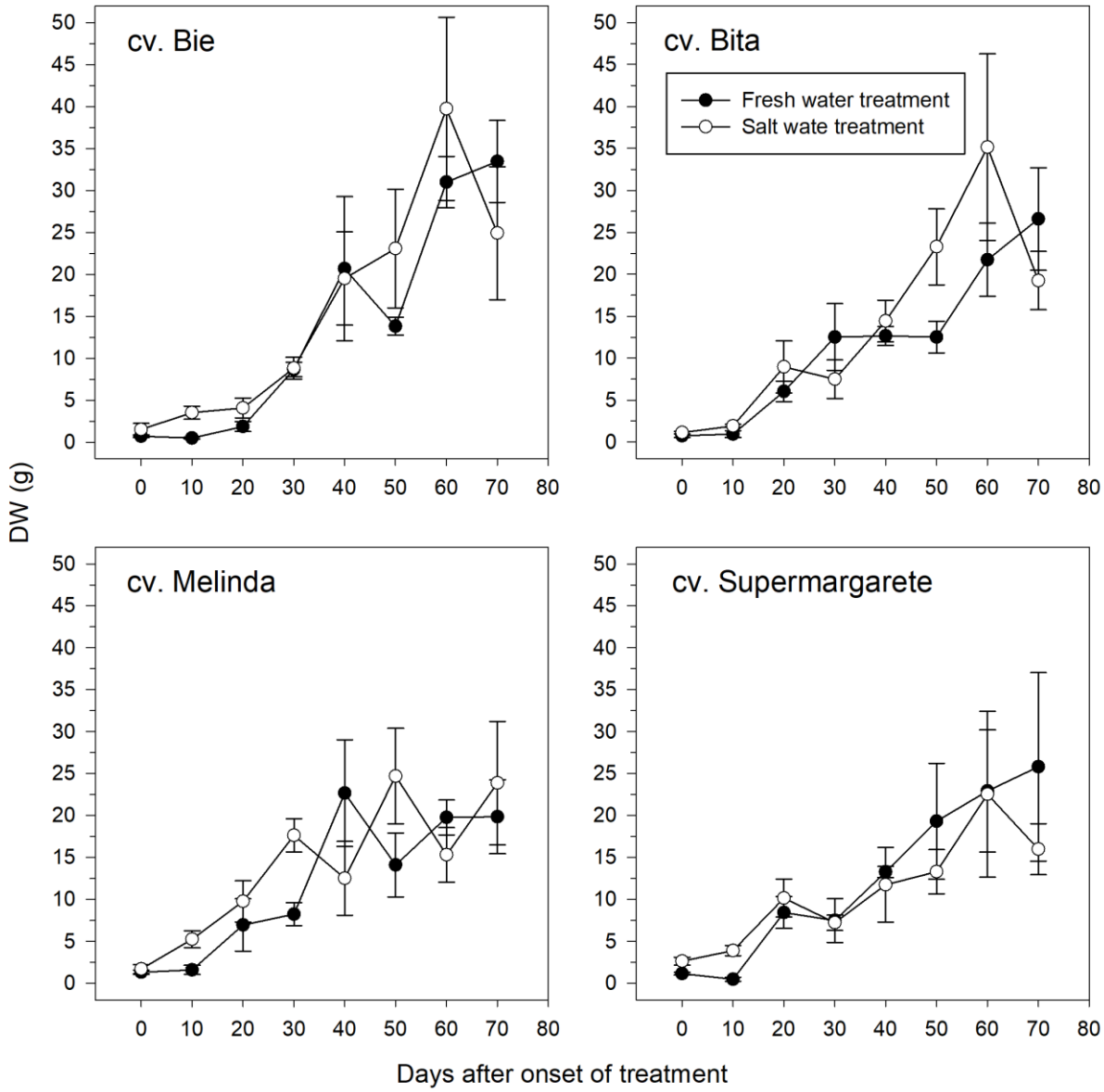

Figure 6 Average dry weight (g) of stems development per plant under fresh water and salt water treatment with standard error; n=3.

The development of stems dry weight has an upward trend, and it follows the same trajectory of the total dry weight (Figure 4). The highest value is registered in cv. Bie 60 DAOT under SW treatment (39.7 ± 10.9). The highest value under FW treatment (33.4 ± 4.8) is also in cv. Bie, but 70 DAOT. Cultivars Bie, Melinda and Supermargarete have higher values of stems dry weight under SW treatment in the very early stages of development, while this is not detectable in cv. Bita. At later stages of development, in both cv. Bie and cv. Melinda, the two treatments display an alternating development. However, in cv. Supermargarete plants under FW treatment have more stems biomass from 40 DAOT onwards. Cv. Bita, instead, has higher values under SW treatment between 40 and 60 DAOT. In cv. Bie and cv. Bita we can see a rapid growth in stems biomass in later stages of development, while cv. Melinda and cv. Supermargarete follow

a steadier curve in both treatments. Table 6 shows the percentage of the total aboveground biomass represented by stems. Higher values can be seen at the later stages of development in all cultivars and both treatments.

Table 6 Average percentage of stems over the total aboveground biomass, per plant; BI = cv. Bie, BT = cv. Bita, ME = cv. Melinda, SM = cv. Supermargarete, FW = fresh water treatment, SW = salt water treatment; n=3.

	Days after onset of treatment							
	0	**10**	**20**	**30**	**40**	**50**	**60**	**70**
BI, FW	22.2±2.5	15.9±0.2	23±1.4	29.8±1.2	29.7±2.8	33.8±2.7	36.1±2.4	39.4±0.4
BT, FW	20.5±4.3	17±4.7	30.2±7.1	35.9±2.8	38±1.7	44.3±2.2	51.7±5	47.8±0.9
ME, FW	23.4±1.2	27.7±1.8	36.3±3.3	39.1±2.3	48.1±4.6	51.7±3.5	49.5±2.5	48.1±1.2
SM, FW	22.5±0.6	23.3±8.7	35.2±8.7	34±6	31.2±4.5	38.2±1.1	41.4±2.7	36.8±5.5
BI, SW	15.9±0.9	22.6±1.9	16.3±6.6	34±0.6	34.8±0.9	38±0.7	40.2±1.1	36.6±2.1
BT, SW	21.4±1.5	20.6±1.6	42.6±3.4	35.1±7.5	44.2±3.8	48.1±7.5	52.6±0	46.1±2.6
ME, SW	27.1±3.7	32.1±0.9	47.1±2.3	51.5±4.2	37.3±3.8	51.3±3.3	53±1.5	45.1±5.4
SM, SW	21.4±0.8	25.2±4.8	32.9±2.4	44±7.3	31.8±13.4	47±0.4	50.5±5	40.9±7.2

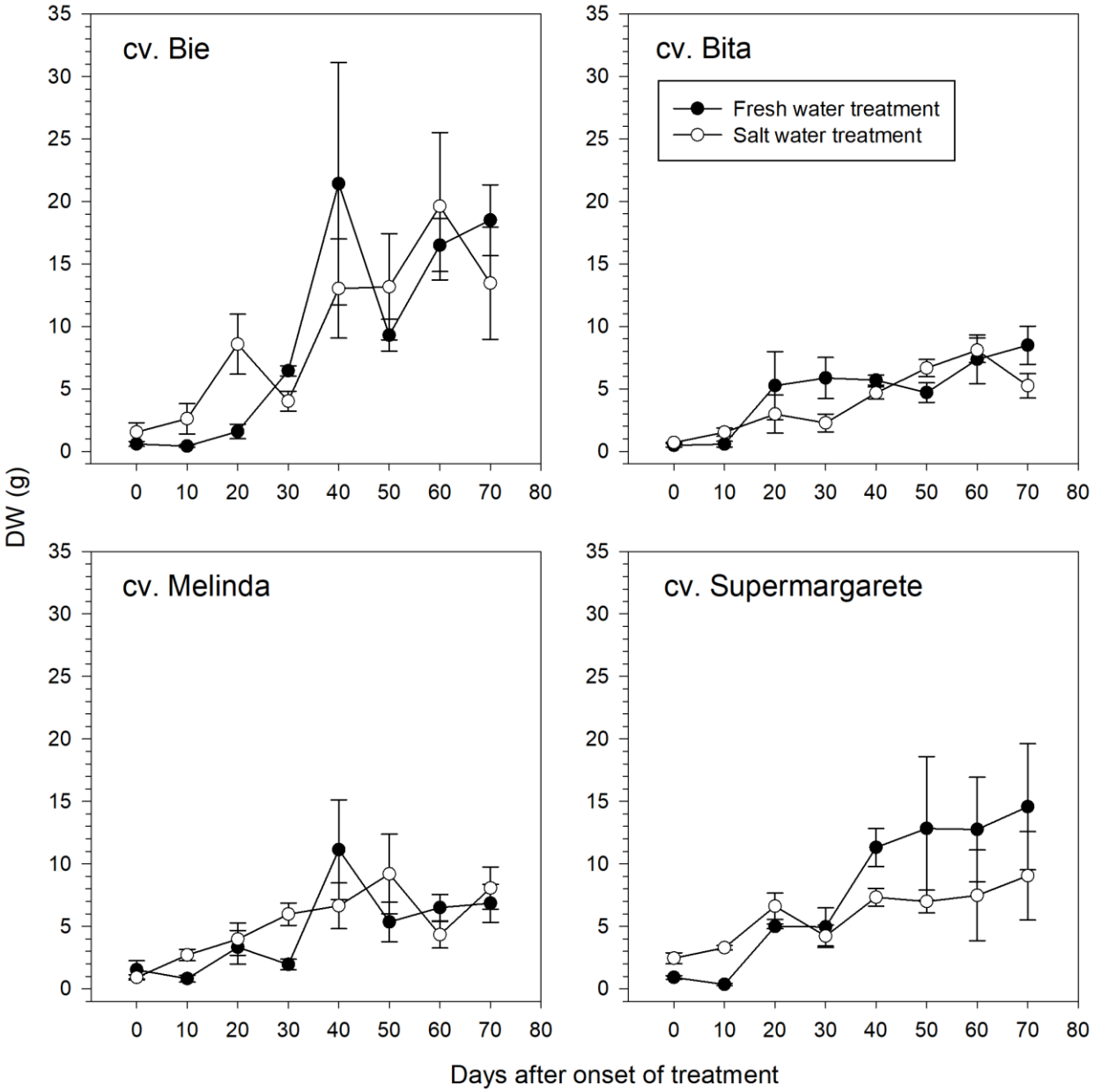

Figure 7 Average dry weight (g) of petioles development per plant under fresh and salt water treatment with standard error; n=3.

The development of petioles in the four cultivars follows the same trend of the total aboveground biomass, visible in Figure 4. Highest values are registered in cv. Bie, 40 DAOT with 21.4 ± 9.7 g under FW treatment, and 60 DAOT with 19.6 ± 5.8 g under SW treatment. Cv. Bie, cv. Bita and cv. Melinda display a synchronized and alternating development under the two treatments. Cv. Supermargarete has higher values under FW treatment from 40 DAOT onwards.

Table 7 Average percentage of petioles over the total aboveground biomass, per plant; BI = cv. Bie, BT = cv. Bita, ME = cv. Melinda, SM = cv. Supermargarete, FW = fresh water treatment, SW = salt water treatment; n=3.

	Days after onset of treatment							
	0	**10**	**20**	**30**	**40**	**50**	**60**	**70**
BI, FW	17.7±1.3	13.6±0.2	18.8±2.2	22.5±1.6	28.7±4	22.3±1.9	19±0.5	21.7±0.5
BT, FW	13.7±1.4	13.1±0.1	11.9±6.5	16.9±1.4	17.2±1.4	16.6±0.3	16±1.6	15.7±0.7
ME, FW	15.5±1.1	14.9±0.3	18.3±1.8	11.3±4.3	19±4	20.3±4.7	15.9±1.5	16.6±0.4
SM, FW	17.8±1.6	23.7±4.6	21.1±1.2	25.7±3.2	25.4±1.4	22.9±2.6	23.2±1	22.8±1.2
BI, SW	16.6±0.9	15±4.5	29.6±3.3	15.5±1.9	22.4±2.9	20.7±1.8	18.9±1.6	19.7±1.3
BT, SW	13.6±0.6	19.7±5	12.2±3.2	12.9±5.3	14.7±1.3	14.1±0.7	14.8±4.8	12.5±0.6
ME, SW	18.1±3.6	16.7±0.2	18.2±1.4	17.1±0.7	20.9±0.6	17.6±2.6	14.7±0.9	17.5±1.8
SM, SW	20±0.5	21.2±0.9	22.2±0.6	23.5±0.2	18.7±2.5	21±0.2	17.1±1.9	19.8±3.1

4.1.2 Length of the main vine

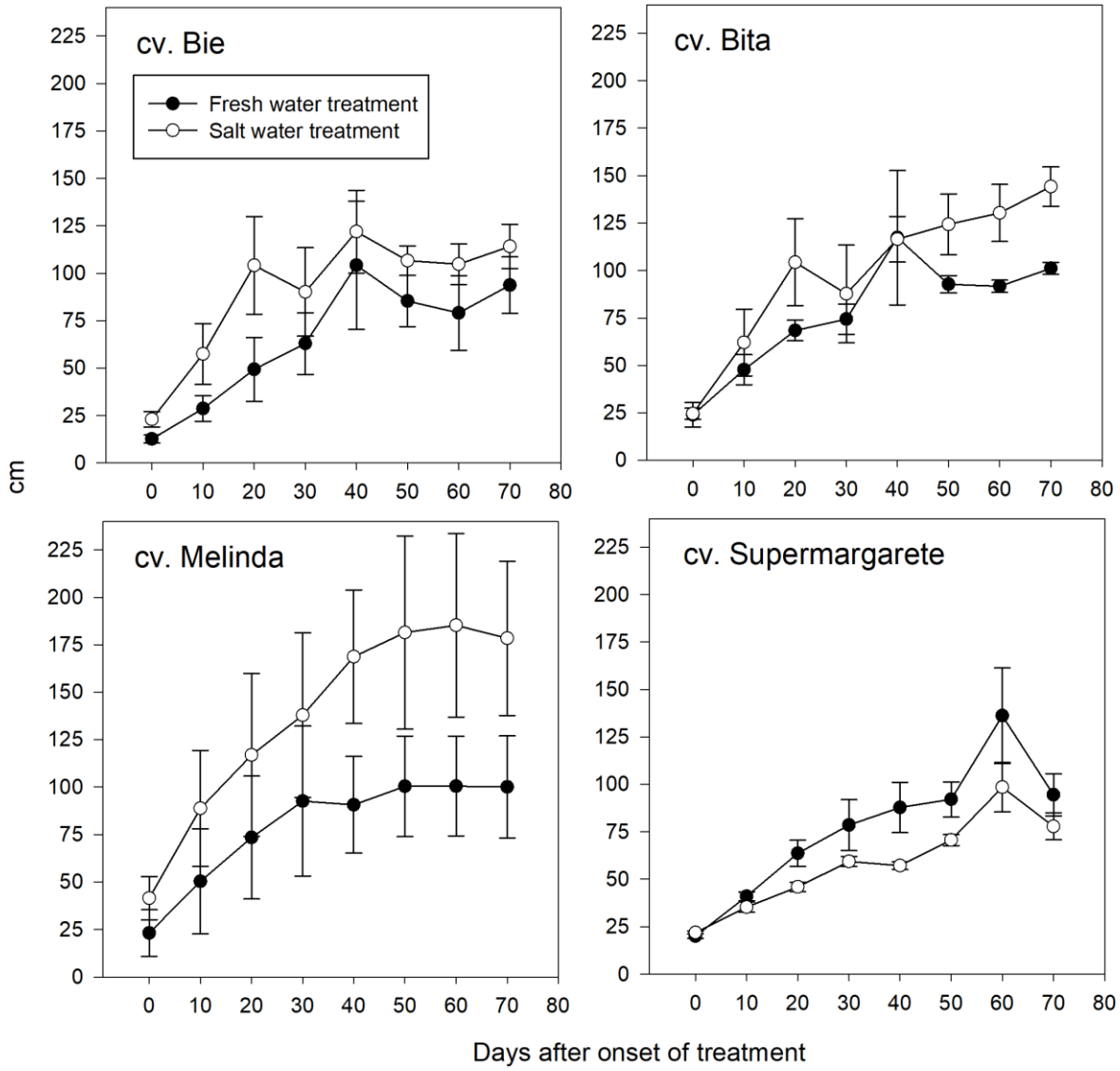

Figure 8 Average length (cm) development of the main vine per plant under fresh and salt water treatment with standard errors; n=3.

Vine length follows a growing curve in all cultivars and in both treatments. Longest vines are in cv. Melinda under SW treatment (185.2 ± 48.3 cm, 60 DAOT) and in cv. Supermargarete under FW treatment (136.1 ± 25.1 cm). Cv. Bie, cv. Bita and cv. Melinda clearly display how plants under SW treatment developed longer vines throughout the whole sampling period. The highest percentage difference is seen 20 DAOT in cv. Bie, where the salt stressed plants had 111.0 % longer vines than those under FW treatment. At later development stages (from 40 DAOT onwards), cv. Melinda showed larger differences between the two treatments, suggesting a higher sensibility to increasing soil salinity. On the other hand, cv. Supermargarete is the only cultivar that grew longer vines under FW treatment than the salt stressed plants.

4.1.3 Number of leaves and leaves distribution

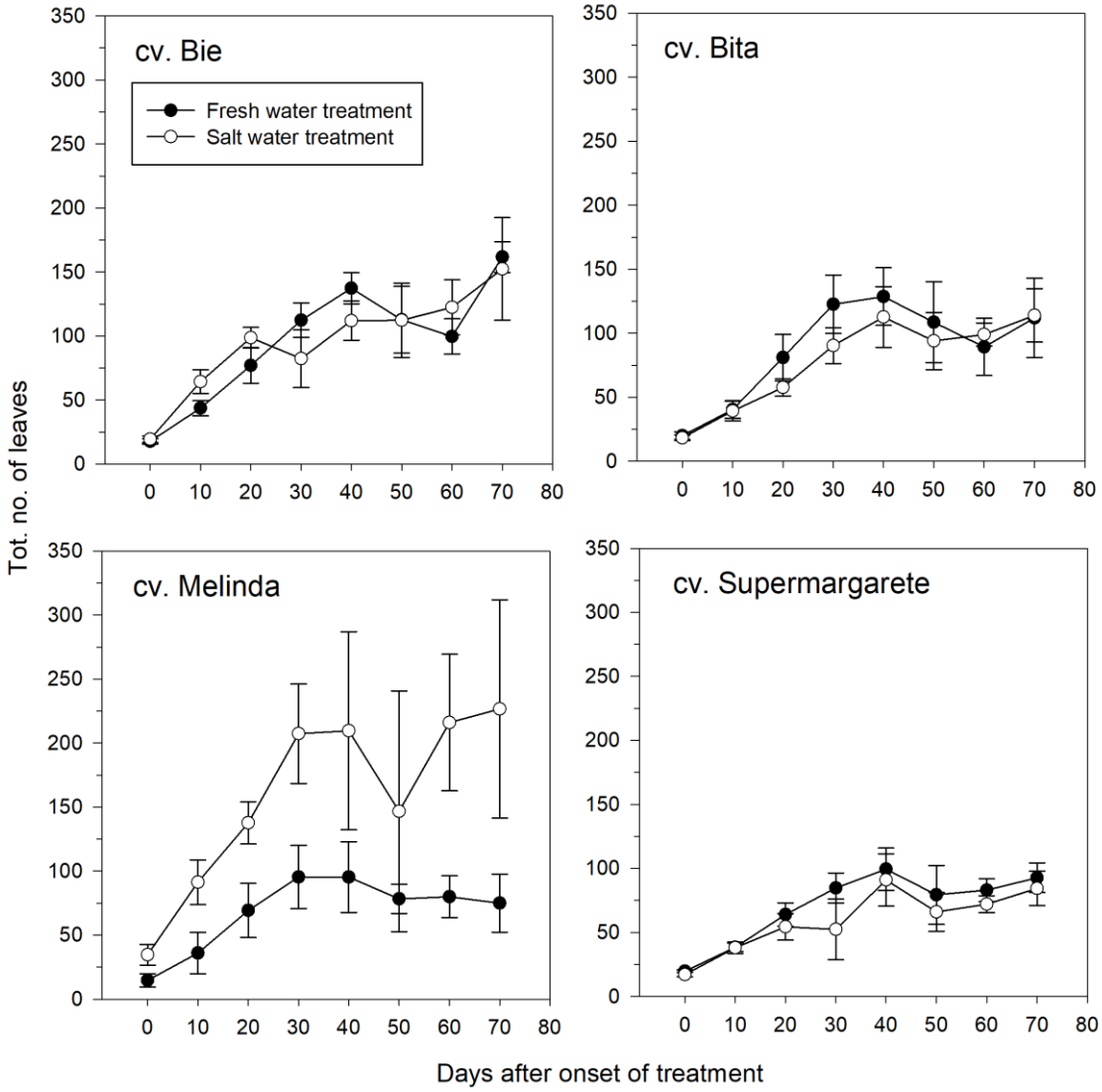

Figure 9 Average total number of leaves development per plant under fresh and salt water treatment, with standard errors; n=3.

The development of the total number of leaves appears quite different in each cultivar. Highest values are reached in cv. Melinda under SW treatment, with a peak at 70 DAOT with 226.6 ± 85.2 leaves. The highest percentage difference between treatments (see Table 8) can be seen at the same time point again in cv. Melinda (SW treatment is 202.2% larger in values than FW treatment). This is due to steady increment of the number of leaves in salt stressed plants, while there is a slight decrease in leaves number in the FW treatment at later stages of development. However, a discrete decrement, common to all cultivars under fresh water treatment, is detectable between 40 and 60 DAOT. Salt stressed plants of cv. Bita and cv. Supermargarete display the same trend at the same development stage. In both cv. Bita and cv. Supermargarete, the plants under FW treatment have higher values throughout the whole sampling period, except for cv. Bita

which, on 60 and 70 DAOT, has slightly higher values under SW treatment (10.8 % and 1.8 % higher than the fresh water treatment respectively, as seen in Table 8). Cv. Bie has an alternating development of leaves number under the treatments, with a peak of percentage difference at 10 DAOT (salt stressed plants have 47.3 % more leaves than those under fresh water treatment, Table 8). Overall, cv. Melinda shows the highest percentage differences between treatments throughout the whole observed period.

Table 8 Total number of leaves percentage difference between salt water and fresh water treatment. DAOT = Days after onset of treatment.

DAOT	*Bie*	*Bita*	*Melinda*	*Supermargarete*
0	9.4	-8.3	136.4	-13.6
10	47.3	-2.5	153.7	-0.9
20	28.1	-28.8	98.6	-15.1
30	-26.7	-26.4	117.5	-38.2
40	-18.4	-12.4	119.9	-8.4
50	-0.3	-13.5	87.2	-16.8
60	22.7	10.8	170.0	-13.3
70	-5.8	1.8	202.2	-9.0

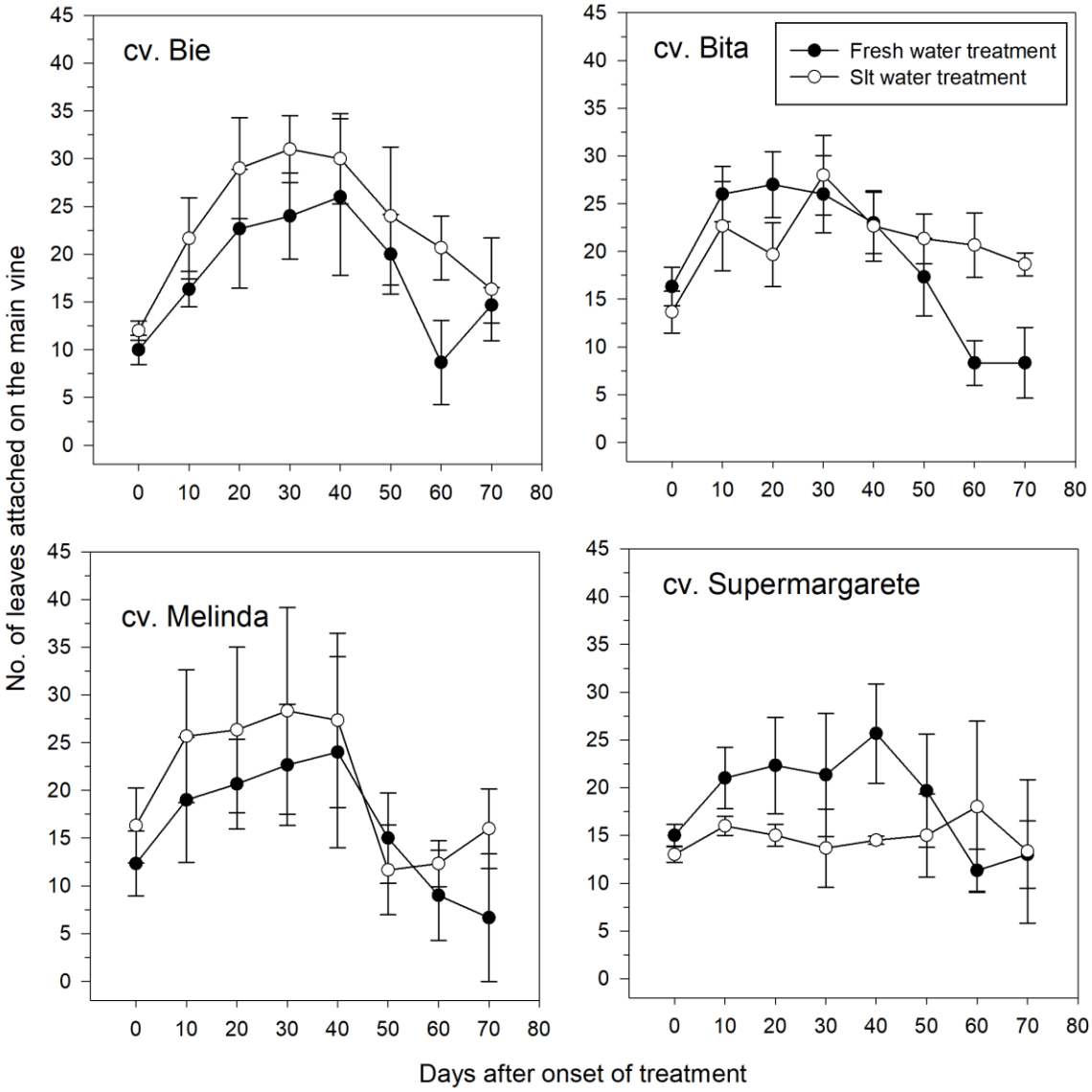

Figure 10 Average number of leaves attached to the main vine development per cv. under fresh and salt water treatment, with standard errors; n=3.

In all four cultivars, the total number of leaves attached to the main vine follows a generally similar pattern: a steady increase in values in the first 40 days of observations, followed by decreasing number of leaves from 50 DAOT onwards. This trend is detected in both FW and SW treatment, except for cv. Supermargarete, which displays less variation in the number of leaves from one sample event to the other under SW treatment. Both cv. Bie and cv. Melinda show higher values under SW treatment throughout the whole observation period. The highest number of leaves on the main vine (31 ± 3.5) is observed in cv. Bie under SW treatment 30 DAOT. Cv. Bita has an initial higher development of leaves on the main vine under FW treatment, but at later stages and increasing salinity, the plants under SW treatment display larger values, with decreasing values for the plants under fresh water treatment. Cv. Supermargarete as well has more leaves on the main

vine under FW treatment, especially in earlier development stages, since later it follows the same declining pattern described above. At higher level of soil salinity, Supermargarete plants under SW treatment have more leaves attached to the main vine compared to the non-stressed ones (60 and 70 DAOT). However, this is not due to an increasing number of leaves under salt water treatment, rather to a gradual decline in the values under FW treatment.

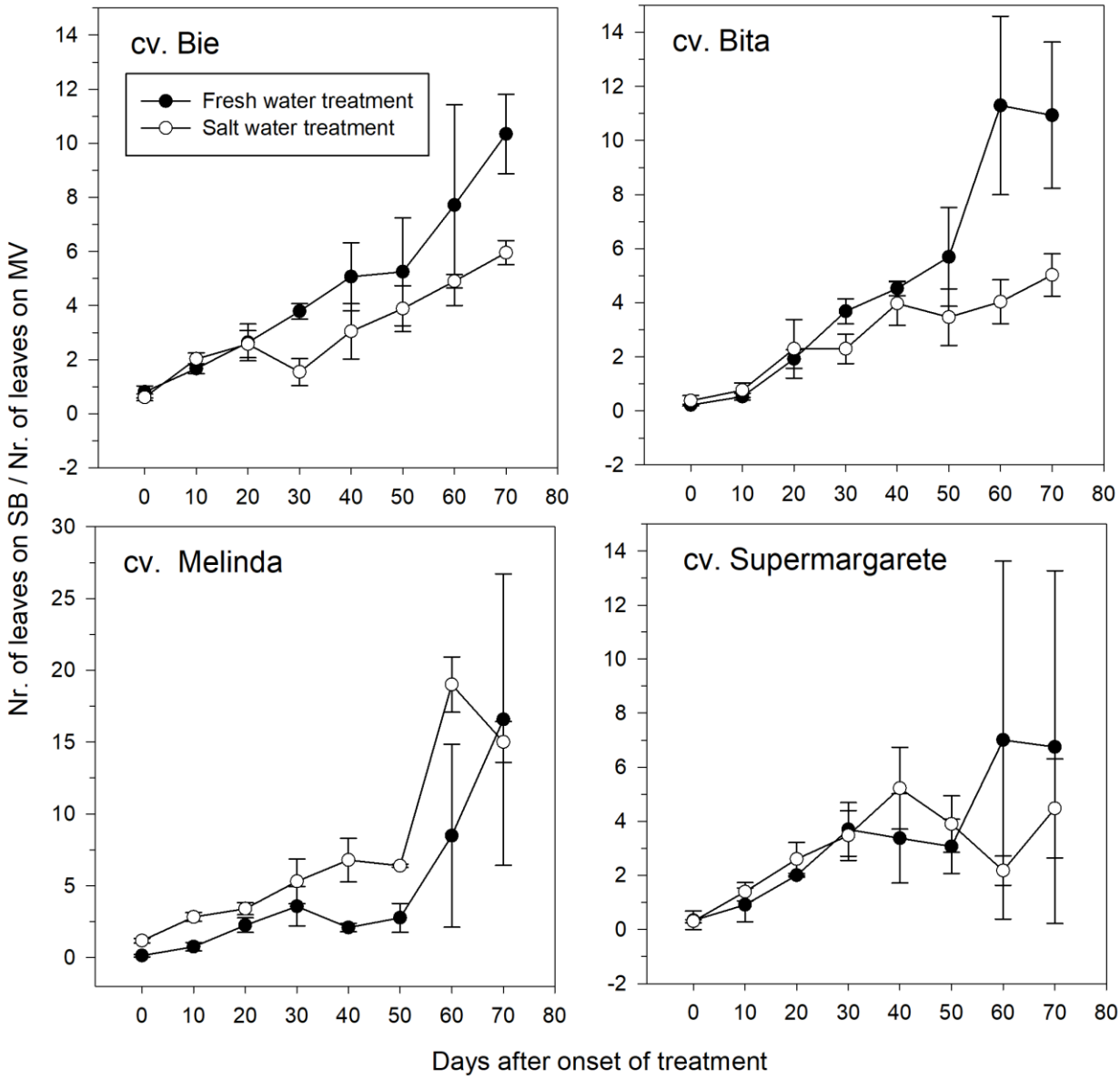

Figure 11 Average ratio between the number of leaves attached on the side branches and the number of leaves attached on the main vine development per plant under fresh and salt water treatment, with standard errors; n=3.

Figure 11 shows the development of the ratio between the total number of leaves attached on the side branches and the total number of leaves attached to the main vine. All the studied cultivars have values above 1 already at early development stages, implying that

a larger number of leaves are attached to the side branches under both treatments. Cv. Bie shows that plants under FW have a larger proportion of their leaves attached to the side branches compared to the SW treatment from 30 DAOT onwards. Cv. Bita follows the same trend, but the proportion difference is more noticeable at later development stages. Contrarily, cv. Melinda shows higher values in the plants under SW treatment throughout the whole observed period. Cv. Supermargarete has similar values of the ratio until 50 DAOT, after which FW treatment has higher values than SW. However, the large standard error makes the results of cv. Supermargarete unreliable.

4.1.4 Leaf area

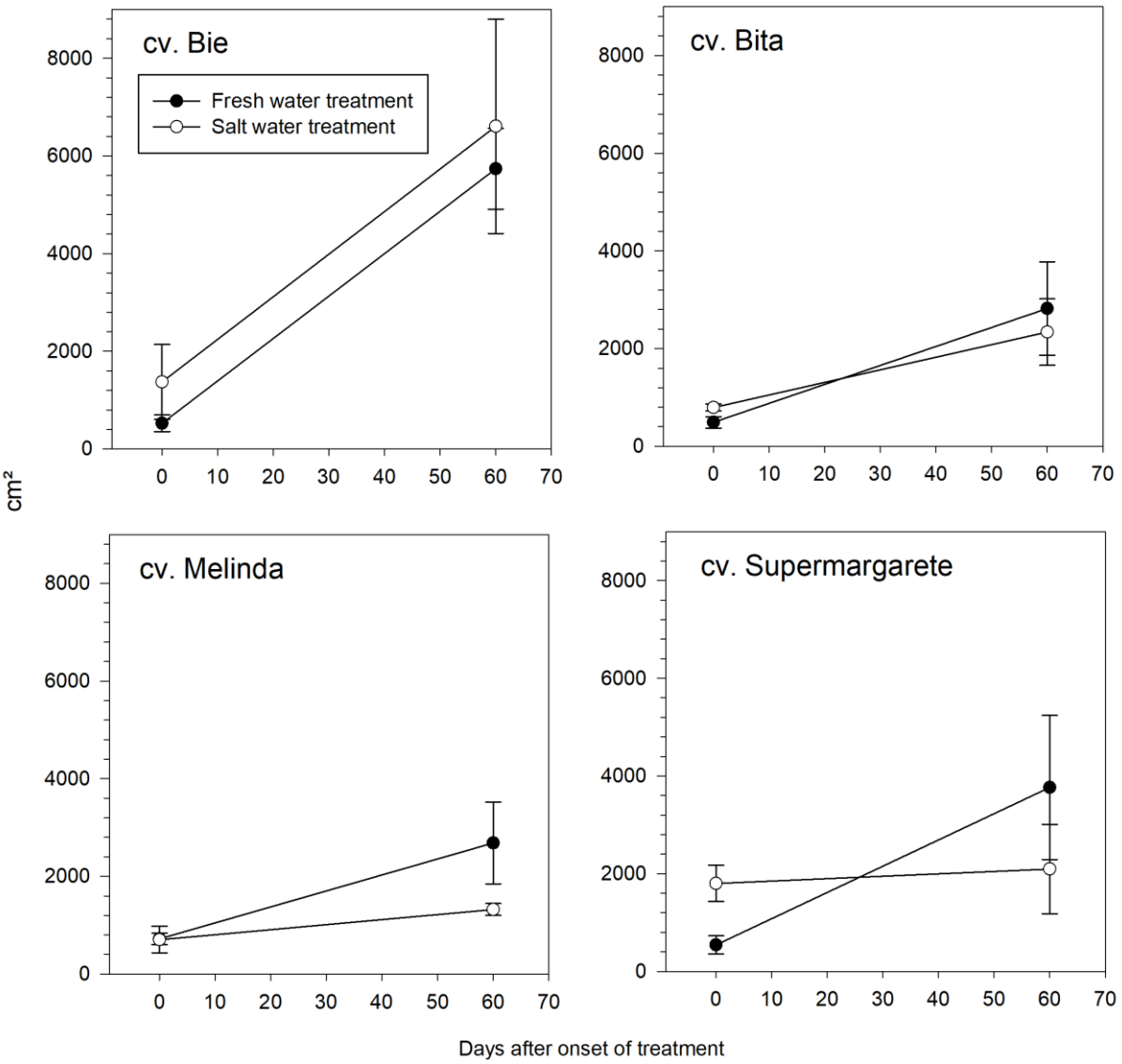

Figure 12 Average total Leaf Area (cm2) development per plant under fresh and salt water treatment, with standard errors; n=3.

Leaf area (cm^2) was measured for leaf samples from the beginning of the onset of treatment and 60 days after. The highest values are seen in cv. Bie 60 DAOT under both FW and SW treatment respectively with 5736.6 ± 829.3 cm^2 and 6605.5 ± 2194.6 cm^2. Cv. Bie, under both treatments, shows a steeply ascending curve, with a rapid increase in leaf area values. The growth rates of LA under FW and SW treatment are very similar, as seen in Table 9. Cv. Bita has similar but alternating values of LA across the two treatments. Cv. Melinda has higher values of LA at 60 DAOT under FW, starting from similar values to SW at 0 DAOT. Cv. Supermargarete shows a steep increase in LA under

FW, as well as the highest difference in the growth rate between FW and SW (see table below).

Table 9 Average absolute growth rate (cm^2d^{-1}) of leaf area under fresh water (FW) and salt water (SW) treatment; n=3.

Cultivar	*Bie*	*Bita*	*Melinda*	*Supermargarete*
FW	86.8	38.8	32.7	53.7
SW	87.2	25.7	10.3	4.9

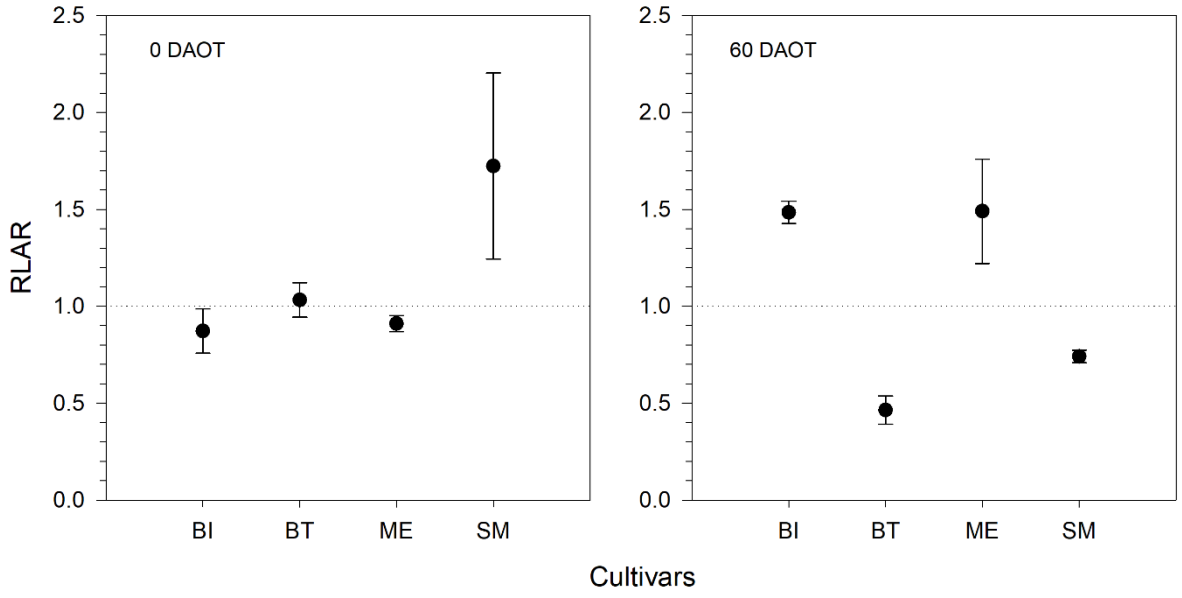

Figure 13 Average relative leaf area ratio (RLAR) per plant under fresh and salt water treatment, with standard errors; n=3.

The relative leaf area ratio RLAR has different values for each cultivar. The highest value (1.72 ± 0.47) is reached by cv. Supermargarete at 0 DAOT. The lowest value (0.46 ± 0.07) is reached by cv. Bita at 60 DAOT. At 60 DAOT, the RLAR of cv. Bie is equal to 1.47 ± 0.05, cv. Melinda is equal to 1.49 ± 0.26 and cv. Supermargarete is equal to 0.74 ± 0.03.

4.1.6 Branching activity

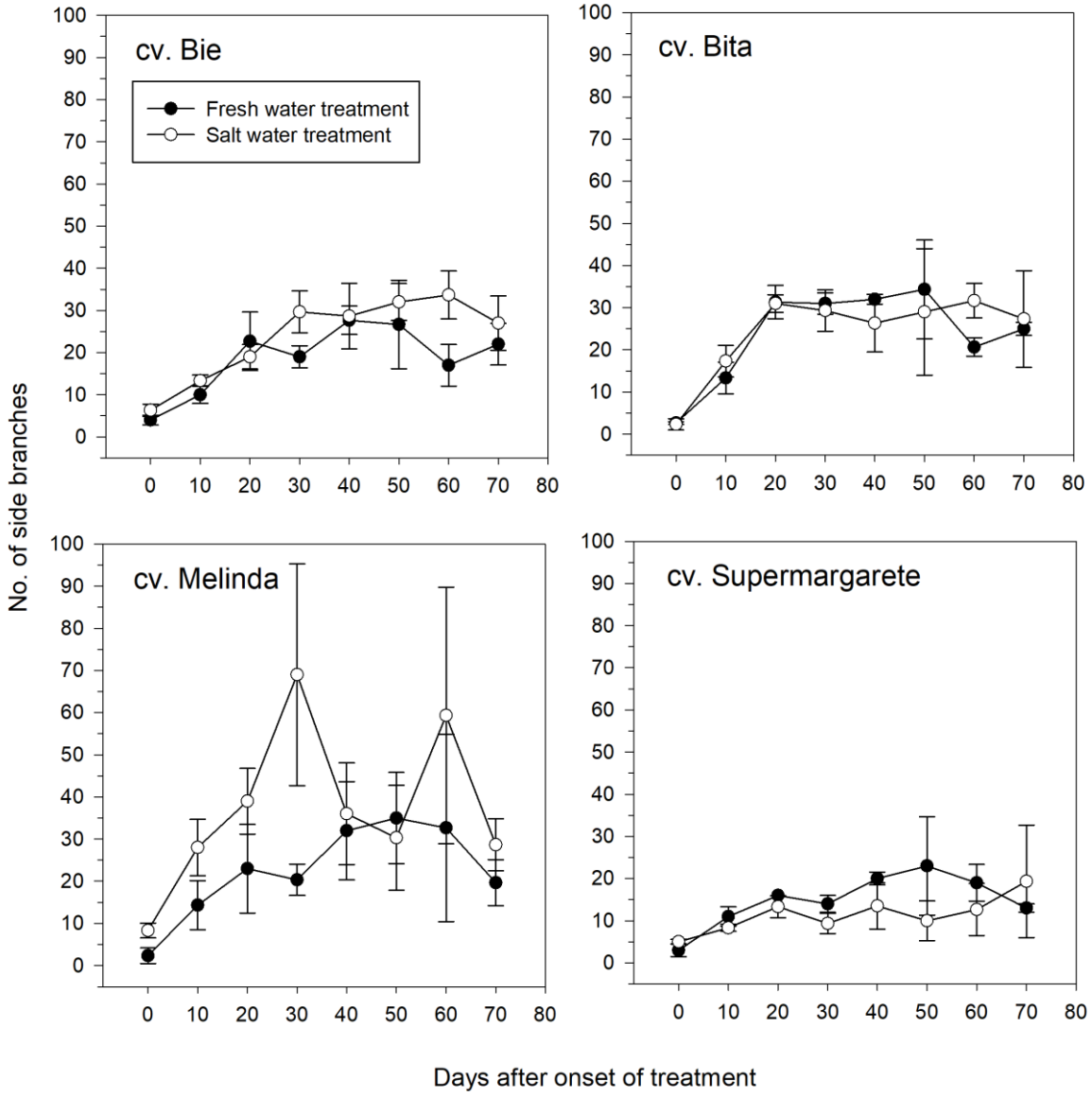

Figure 14 Average total number of side branches development per plant under fresh and salt water treatment, with standard errors; n=3.

Cv. Melinda clearly displays a tendency to develop a larger number of side branches. This tendency is pronounced in the plants under SW treatment, starting from the early stages of development. Indeed, the highest number of side branches is measured in cv. Melinda under SW treatment at 30 DAOT (69.0 ± 26.2). Cv. Bita seems to invest equal resources in its branching activity under both treatments. Cv. Bie, on the other hand, displays larger number of side branches at increasing salinity. Cv. Supermargarete can be generally assessed as the least active in terms of number of side branches, compared to the other cultivars.

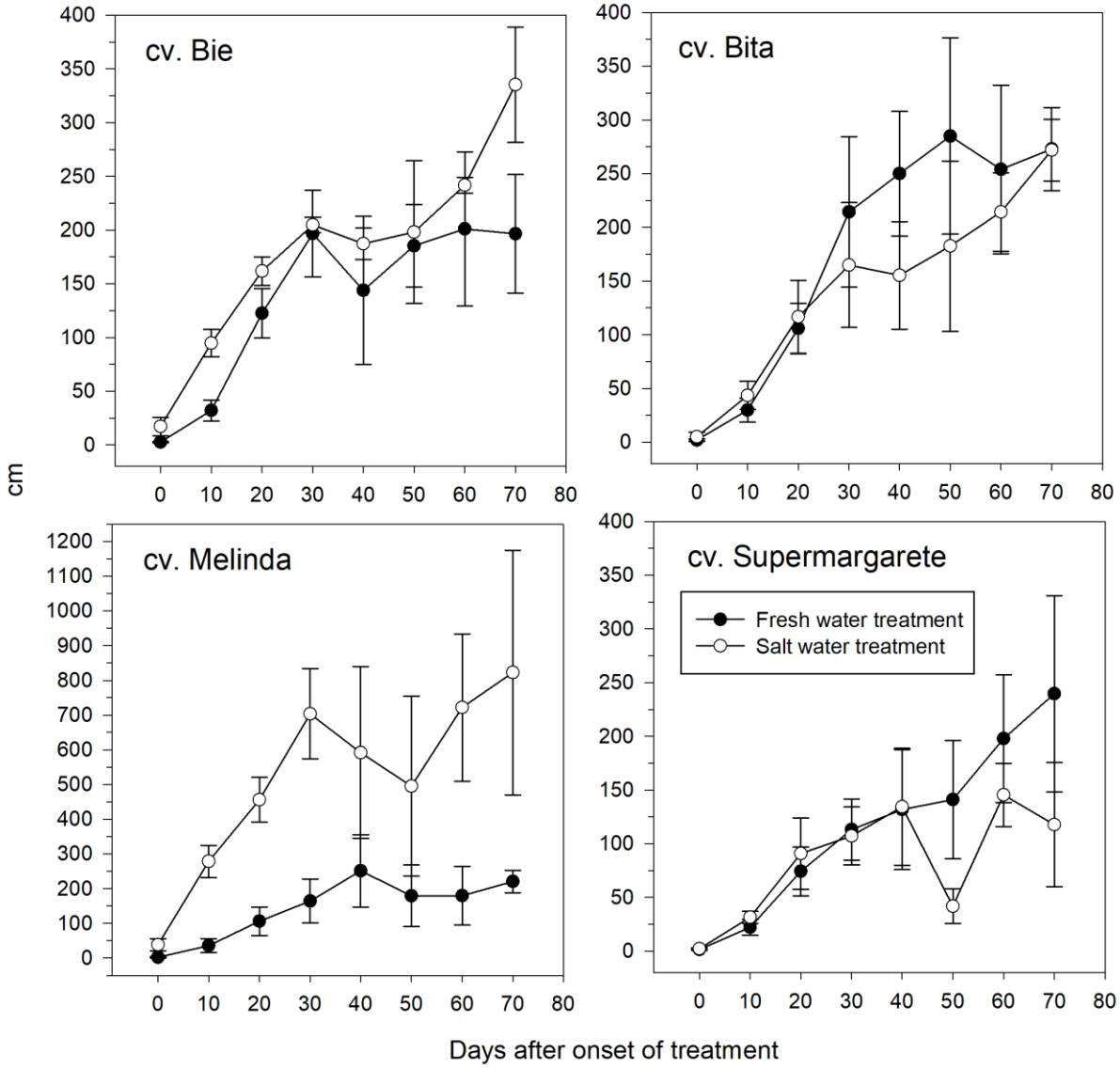

Figure 15 Average total length (cm) of side branches development per plant under fresh and salt water treatment, with standard errors; y-axis scale differs for improved readability; n=3.

Cv. Melinda shows higher side branch length compared to other cultivars. Plants of cv. Melinda under SW treatment also show considerably longer side branches compared to those under FW treatment, whereas plants of cv. Bita and cv. Supermargarete show the opposite. All four cultivars have a higher value of side branch length under SW treatment in the first 30 DAOT, even though in cv. Bita and cv. Supermargarete this is hardly visible. In cv. Bita, from 30 DAOT onwards, plants under FW treatment have longer side branches compared to the SW treatment. A similar pattern can be observed in cv. Supermargarete, where the plants under FW treatment start to have longer side branches from 40 DAOT. At the later stages of development of cv. Bita, plants under SW treatment have longer side branches, from 40 DAOT onwards. A clear effect of the salt treatment is visible in cv. Melinda, where plants under SW treatment have clearly longer side branches compared to the FW treatment.

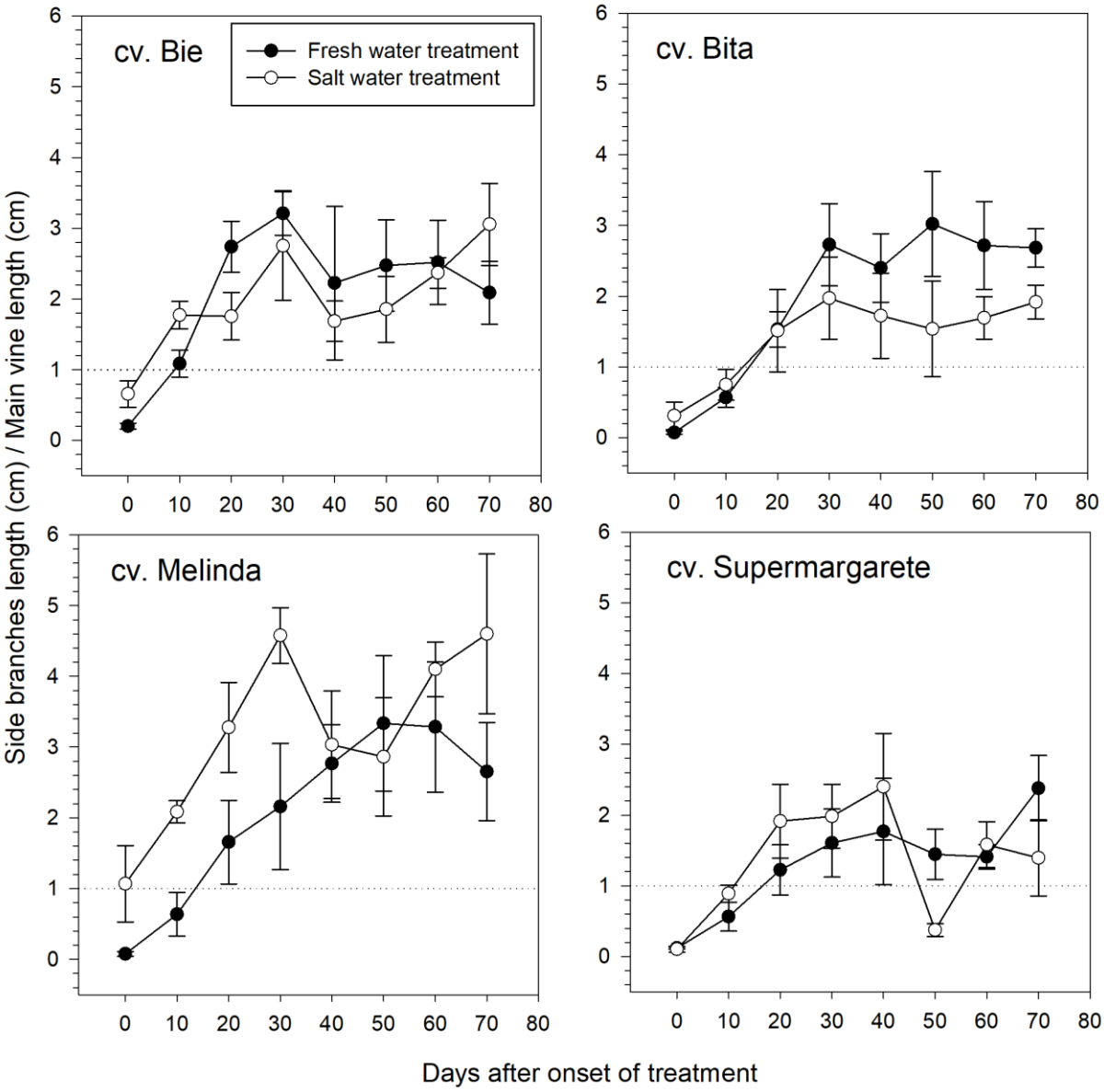

Figure 16 Average ratio between the length (cm) of side branches and the lenght (cm) main vine development per plant under fresh and salt water treatment, with standard errrors; n=3.

Figure 16 shows the development of the ratio between the total length (cm) of the side branches and the total length (cm) of the main vine. Values below 1 imply that the main vine is longer than the total length of the side branches. This situation is detectable only at early stages of development. At 20 DAOT all cultivars have values higher than 1 under both treatments. Cv. Bie shows higher values under FW treatment from 20 to 60 DAOT, while cv. Bita has an initial similar development of the ratio under FW and SW treatment, followed by constantly higher values under FW from 30 DAOT onwards. Cv. Melinda has two distinct curves, with the one under SW having higher values except between 40 and 50 DAOT. Cv. Supermargarete follows a similar trend.

4.2 Na^+ and K^+ content, partitioning and concentration

4.2.1 Na^+ and K^+ content

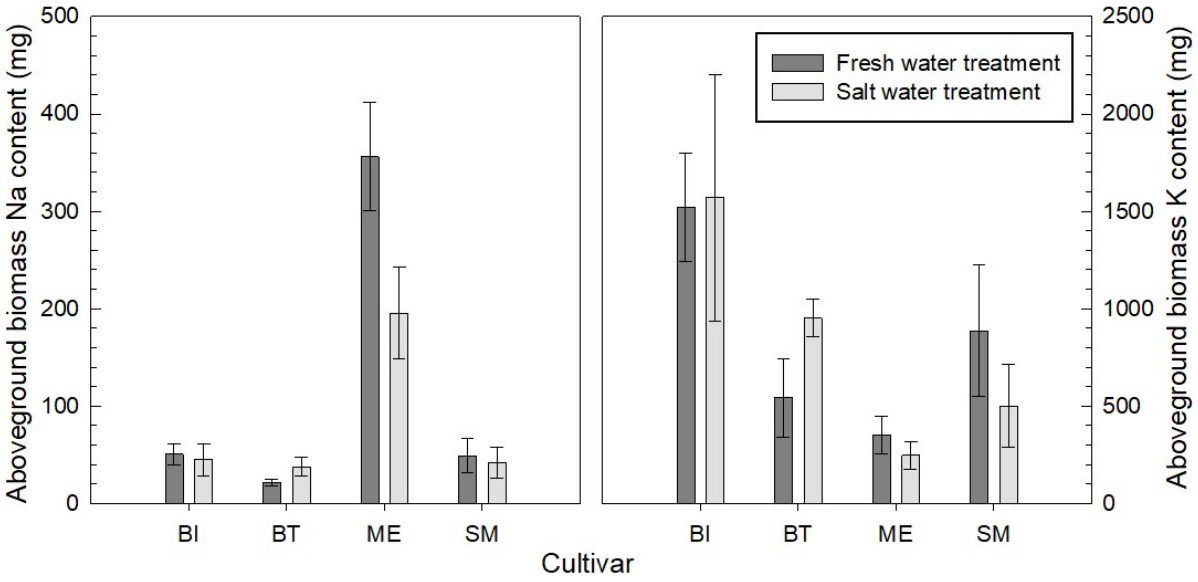

Figure 17 Average aboveground biomass (leaf blades, stems and petioles) Na^+ and K^+ content (mg) per plant under fresh water and salt water treatment with standard error (BI=Bie, BT= Bita, ME=Melinda, SM=Supermargarete); n= 3; 60 days after treatment onset; y-axis scale differs for improved readability.

Na^+ content of the AGB varies stronger between cultivars than treatments. Cv. Melinda has the by far highest aboveground biomass Na^+ content in both treatments while all other cultivars have Na^+ contents of up to only 50 mg per plant. However, cv. Melinda´s Na^+ content is about 45 % lower under SW compared to FW treatment. The Na^+ content of cv. Bita is increased under SW treatment by about 42 % compared to the FW treatment. The effect of the SW treatment on plant Na^+ content was significantly greater in cv. Melinda compared to any of the other three cultivars.

The K^+ content of the AGB is more variable between cultivars and treatments than the Na^+ content. Cv. Bie has the highest K^+ content under both treatments and cv. Melinda the lowest. Under FW treatment, cv. Supermargarete has the second highest K^+ content followed by cv. Bita. Cv. Bita has increased K^+ content by 43 % under SW treatment, while cv. Supermargarete´s K^+ content is reduced by about 44 % compared to the FW treatment. The effect of the SW treatment on the K^+ content of the AGB was not significantly different between any of the cultivars (see Appendix E).

4.2.2 Na^+ and K^+ content partitioning between plant tissues

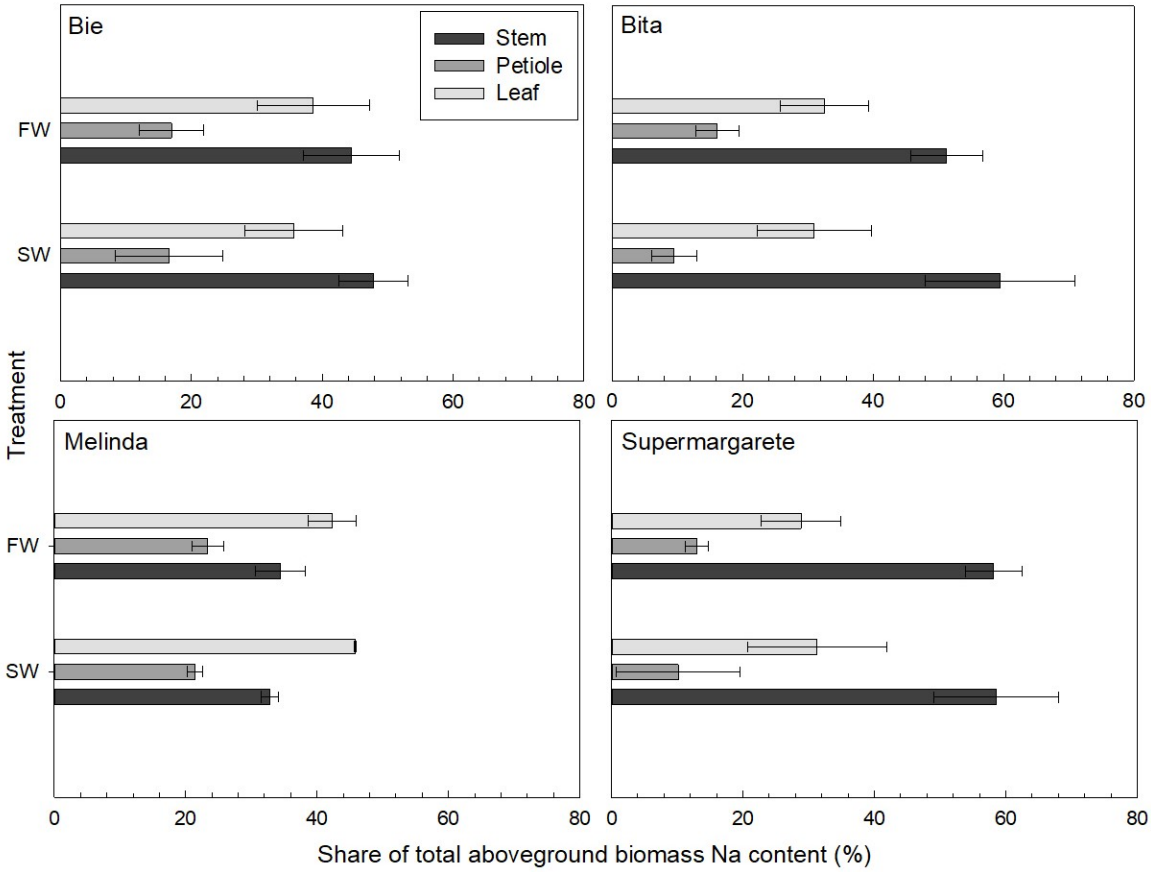

Figure 18 Average share (%) of leaf blade, stem and petiole Na^+ content in the total aboveground biomass Na^+ content with standard error (FW= fresh water treatment, SW = salt water treatment) n= 3; 60 days after treatment onset.

The Na^+ distribution follows a similar pattern between all cultivars and treatments. Cv. Bie, Bita and Supermargarete store the greatest share of the total aboveground Na^+ content in the stems, followed by leaf blades and petioles under both treatments. Thereby, cv. Supermargarete shows the most pronounced accumulation of Na^+ in the stems of all tested cultivars with 58.1±4.3 % in the FW treatment and 58.5±9.5 % in the SW treatment. On the other hand, the share of the Na^+ content stored in the leaf blades is the lowest in cv. Supermargarete with 28.9±6.1 % (FW) and 31.3±10.6 % (SW) respectively. Interestingly, leaf blades of cv. Melinda hold an even greater share of the total Na^+ content than stems under both treatments with 42.2±3.7 % (FW) and 45.8±0.2 % (SW). Treatment differences on Na^+ partitioning between plant parts are minor. In cv. Bita, the share of Na^+ stored in the petioles is reduced under SW treatment with 9.5 ±3.4 % versus 16.2±3.3 % under FW treatment. In turn, the share of Na^+ stored in the stems is slightly increased under SW treatment. The SW treatment effects on the Na^+ partitioning into the three plant parts are not significantly different between any of the cultivars (see Appendix E).

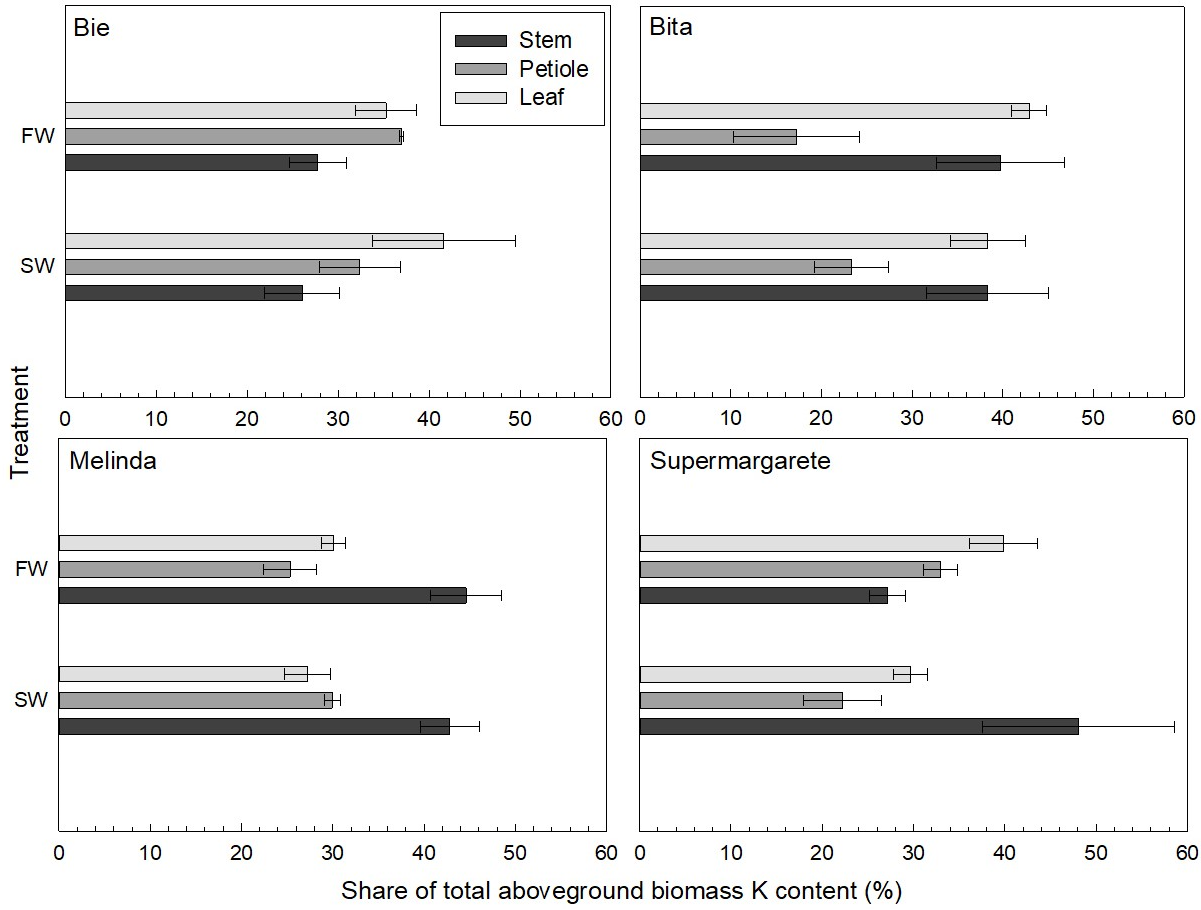

Figure 19 Average share (%) of leaf blade, stem and petiole K^+ content in the total aboveground biomass K^+ content with standard error (FW= fresh water treatment, SW = salt water treatment) n= 3; 60 days after treatment onset.

The K^+ partitioning between plant parts is more variable between cultivars than the Na^+ partitioning. In cv. Bie, K^+ partitioning under FW treatment over the three plant organs is close to even with the lowest share of K^+ stored in the stems. This pattern also occurs in cv. Supermargarete under FW treatment. Cv. Melinda stores the majority of K^+ in the stems with more than 40 %, the remaining K^+ is distributed evenly between leaf blades and petioles irrespective of the treatment. Cv. Bita stores equal shares of K^+ in leaf blades and stems (around 40 % each) and a lower share in petioles under both treatments.

Cv. Supermargarete is the only cultivar showing a different pattern of K^+ partitioning under SW treatment. Under FW irrigation, stems have the lowest share of K^+ with 27.2±2.0 % while under SW treatment, stems contain the largest share of K^+ with 48.1±10.5 %. Therefore, shares of K^+ in leaf blades and petioles are reduced equally under SW treatment. The effect of the SW treatment on the K^+ partitioning into any of the three plant parts is not significantly different between cultivars (see Appendix E).

4.2.3 Na^+ and K^+ content partitioning between old, middle and new plant sections

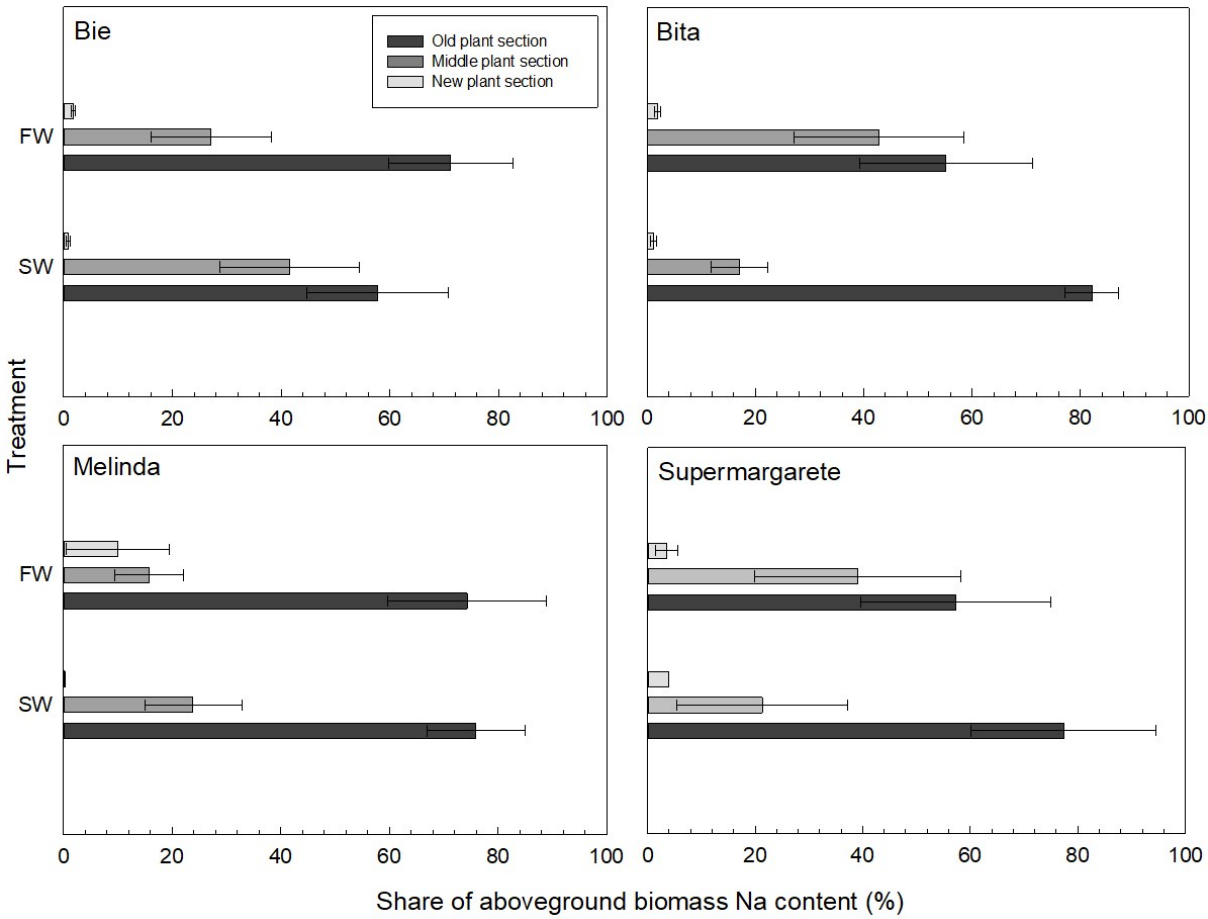

Figure *20 Average share (%) of old, middle and new plant section Na^+ content in the total aboveground biomass Na^+ content with standard error (FW= fresh water treatment, SW = salt water treatment), n= 3; 60 days after treatment onset.*

In all cultivars and both treatments, the greatest share of the total aboveground biomass Na^+ content is contained in the old plant section followed by the middle and lastly the new section. Na^+ stored in the middle section under SW compared to FW irrigation is increased in cv. Bie and Melinda by 14.4 % and 8.2 % respectively. A lower share of Na^+ stored in the middle section and in turn a higher share stored in the old section under SW treatment occur in cv. Bita and Supermargarete. The share of Na^+ stored in the middle section in cv. Bita is reduced from 42.8±15.6 % to 17.1±5.2 % and from 39.1±19.1 % to 21.3±15.1 % in Supermargarete.

Effects of the SW treatment on the Na^+ partitioning into old and middle section were not significantly different between any of the cultivars. The SW effect of Na^+ partitioning into the new section was significantly different between cv. Melinda and Bie (see Appendix E), while the other cultivars could not be tested due to missing repetitions.

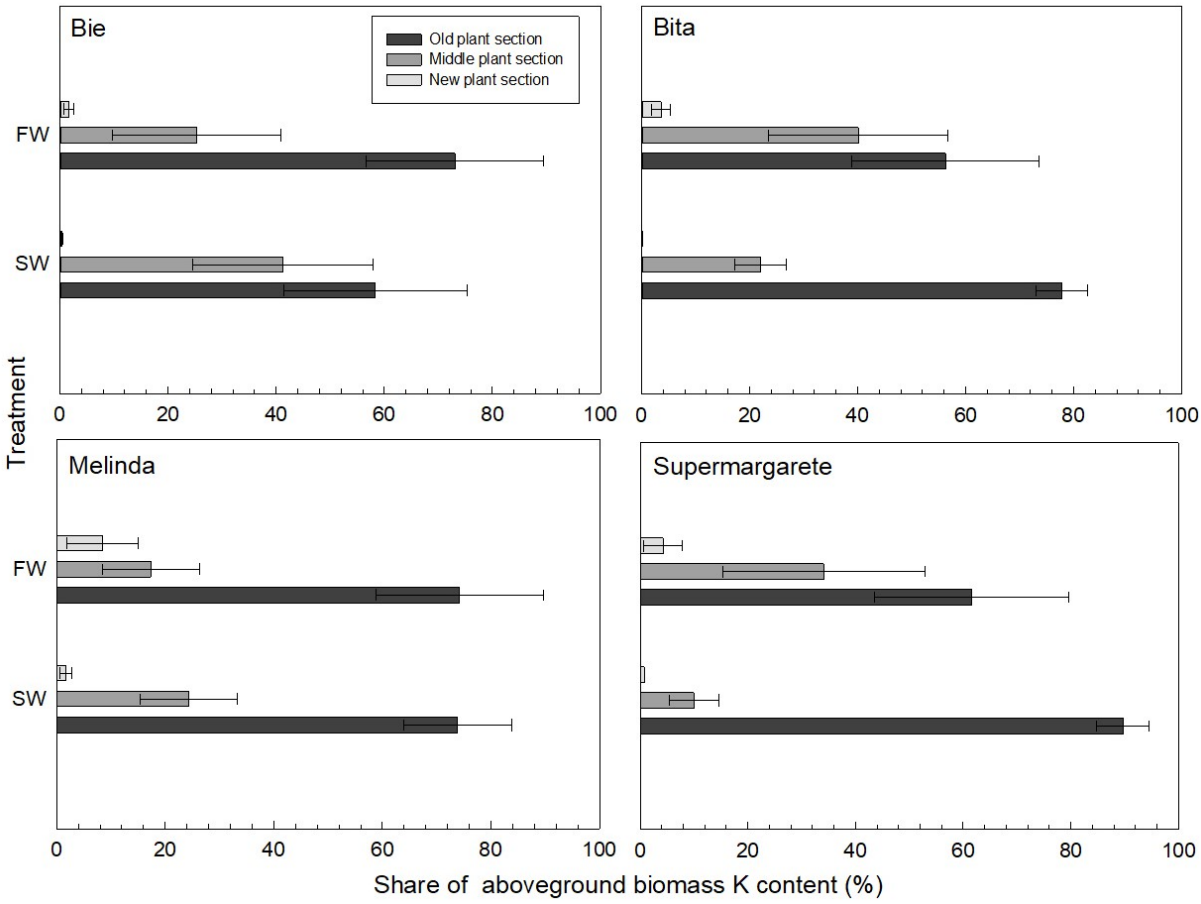

Figure 21 Average share (%) of old, middle and new plant section K^+ content in the total aboveground biomass K^+ content with standard error (FW= fresh water treatment, SW = salt water treatment); n= 3; 60 days after treatment onset.

As for Na^+, the highest share of K^+ is also stored in the old section, followed by the middle and new section in all cultivars and treatments. Treatments effects on K^+ partitioning follow the same pattern as observed for Na^+ partitioning.

Accordingly, cv. Bie and Melinda both have a higher share of K^+ stored in the middle section under SW treatment than FW treatment. Cv. Bie has a share of 25.3 % versus 41.3 % of K^+ content in the middle section and cv. Melinda of 17.4±9.0 % versus 24.4±8.9 % under FW versus SW irrigation. Cv. Bita and Supermargarete store a smaller share of K^+ in the middle section under SW treatment and a higher share in the old section. Cv. Bita stores 40.1±16.6 % of K^+ in the middle section under FW treatment and only 22.1±4.8 % under SW treatment. Cv. Supermargarete stores 34.1±18.7 % under FW treatment and 10.0±4.6 % of total K^+ under SW treatment in the middle section. The effect of the SW treatment on partitioning into the new plant section was found to be significantly different between cv. Melinda and Bie, while the other cultivars could not be tested due to missing repetitions (see Appendix E).

4.2.4 K^+ Na^+ ratio of the aboveground biomass

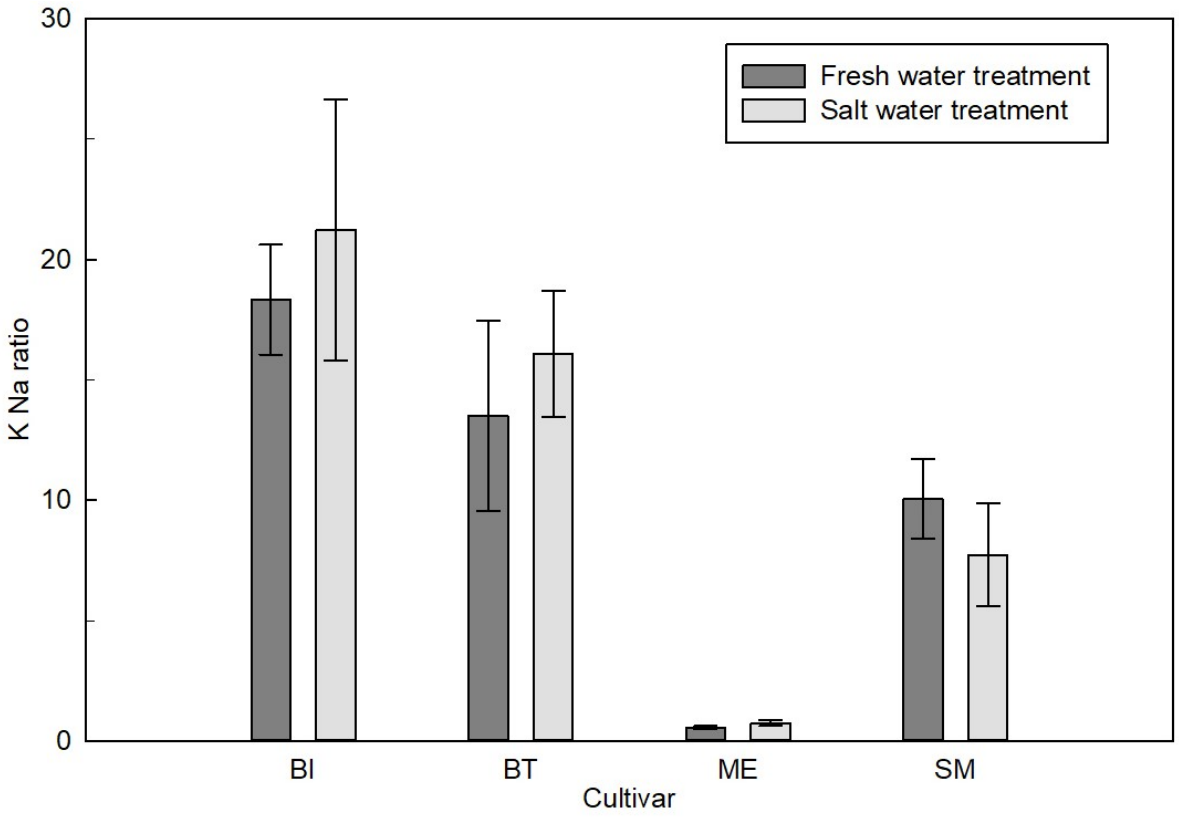

Figure 22 Average K^+ Na^+ ratio of the aboveground biomass (leaf blades, stems, and petioles) per cultivar with standard error (BI=Bie, BT= Bita, ME=Melinda, SM=Supermargarete); n= 3; 60 days after treatment onset.

The Ka^+ Na^+ ratio of the aboveground biomass is based on the ratio of K^+ and Na^+ content in mol of leaf blades, petioles and stems combined.

Under both treatments the K^+ Na^+ ratio declines in the order Bie > Bita > Supermargarete > Melinda. The K^+ Na^+ ratios of cv. Bie, Bita and Melinda are increased under SW compared to FW irrigation by 2.9, 2.6 and 0.2. respectively. Cv. Supermargarete is the only cultivar to show reduced K^+ Na^+ ratio under SW treatment with 7.7±2.2 as opposed to 10.1±1.7 under FW treatment.

The effect of the SW treatment on the K^+ Na^+ ratio of the AGB was not significantly different between any of the cultivars (see Appendix E).

4.2.5 K^+ Na^+ ratio of different plant tissues

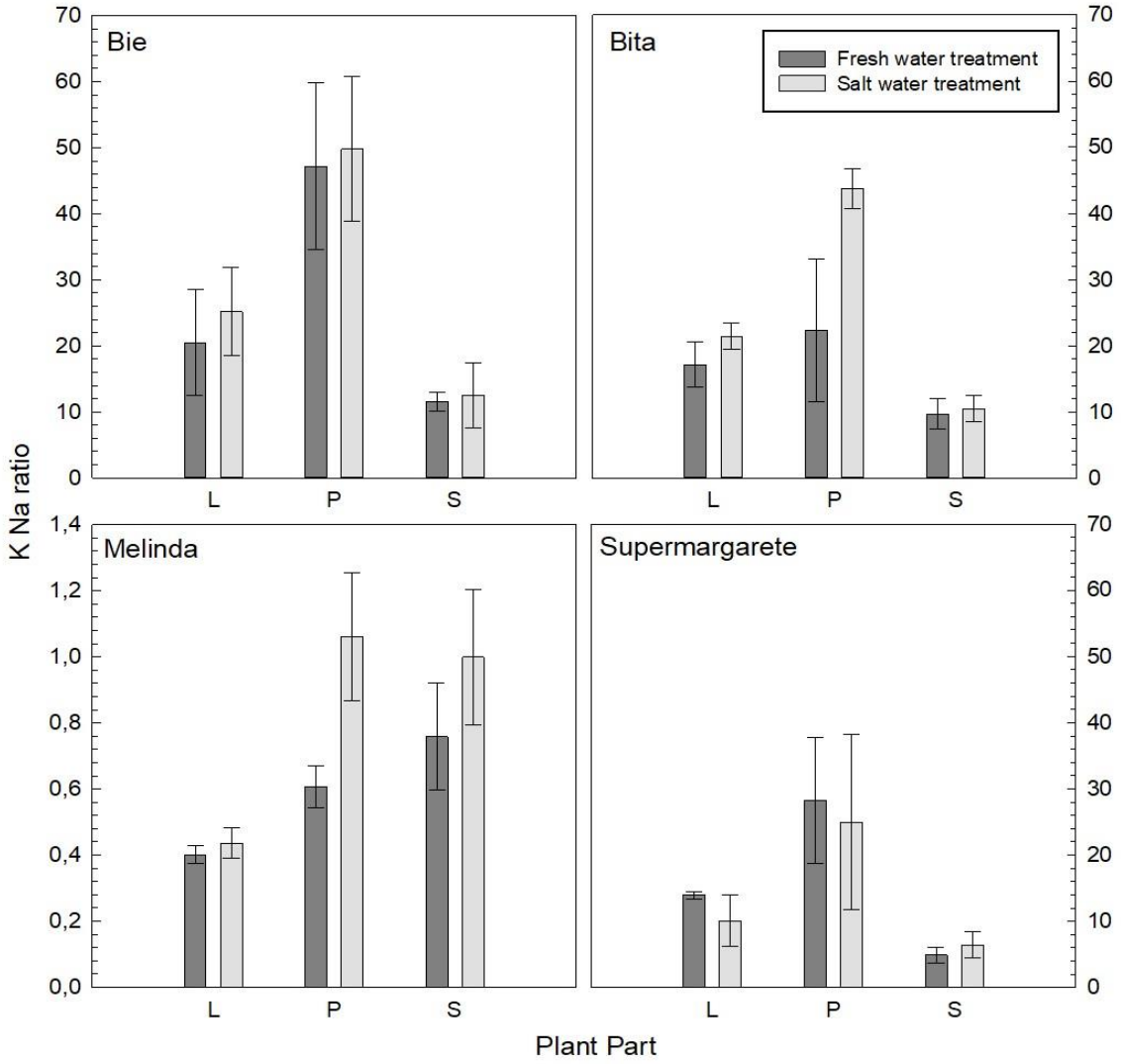

Figure 23 Average K^+ Na^+ ratio of leaf blades, stems, and petioles per cultivar with standard error (L= leaf blades, P= petioles, S= stems); n= 3; 60 days after treatment onset; y-axis scale differs for improved readability.

Cv. Bie, Bita and Supermargarete have the highest K^+ Na^+ ratio in petioles, followed by leaf blades and stems under both treatments. Overall, the highest ratios are observed in cv. Bie´s petioles under both treatments. Only cv. Melinda has similar or even higher K^+ Na^+ ratios in stems compared to petioles.

Cv. Bita has an increased K^+ Na^+ ratio of leaf blades by 4.3 and petioles by 21.5 under SW treatment. In cv. Melinda, the K^+ Na^+ ratios of petioles and stems are increased by 0.5 and 0.2 respectively under SW treatment. Only cv. Supermargarete has reduced K^+ Na^+ ratios under SW treatment occurring in leaf blades and petioles which are lowered by 3.2 and 3.9 respectively compared to the FW treatment. The effect of the SW treatment on petiole K^+ Na^+ ratio was significantly different between cv. Melinda and Bita. The other cultivars´ SW effects on petiole K^+ Na^+ ratio could not be compared due to missing data or failed normality testing (see Appendix E).

4.2.6 Na^+ and K^+ concentration of the complete aboveground biomass

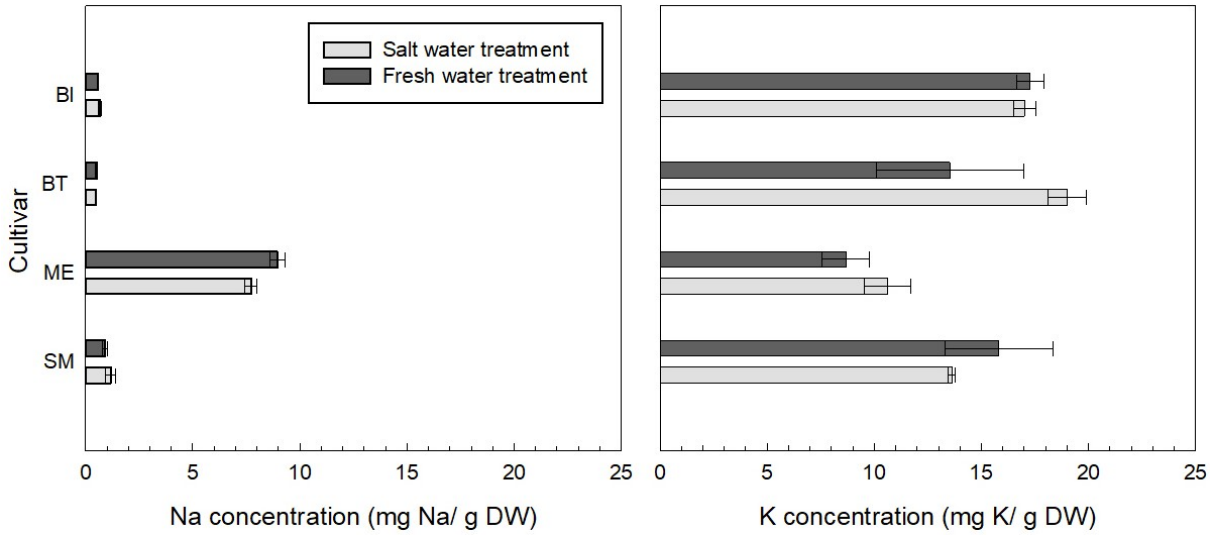

Figure 24 Average Na^+ and K^+ concentration of the aboveground biomass (leaf blades, petioles and stems) per cultivar with standard error (BI= Bie, BT= Bita, ME= Melinda, SM= Supermargarete); n= 3; 60 days after treatment onset.

Cv. Melinda has the highest AGB Na^+ concentration under both treatments with 8.9±0.3 (FW) and 7.7±0.3 mg Na^+ g^{-1} DW (SW). The other cultivars all have Na^+ concentrations on average below 1.5 mg Na^+ g^{-1} DW. Cv. Supermargarete has the second highest Na^+ concentration after cv. Melinda in both treatments. With a difference of 1.2 mg Na^+ g^{-1} DW between treatments, cv. Melinda has decreased Na^+ concentration under SW treatment. Cv. Supermargarete showed a slight increase in Na^+ concentration by 0.2 mg Na^+ g^{-1} DW under SW irrigation. SW treatment effects on the AGB Na^+ concentration were significantly different between cv. Melinda and Bita as well as cv. Melinda and Supermargarete.

K^+ concentrations are generally much higher than Na^+ concentrations in cv. Bie, Bita and Supermargarete. Under FW treatment, cv. Bie has the highest K^+ concentration with 17.3±0.6 mg K^+ g^{-1} DW, followed by Supermargarete, Bita and lastly Melinda. Under SW treatment cv. Bita has the highest K^+ concentration with 19.0±0.9 mg K^+ g^{-1} DW, followed by Bie, Supermargarete and Melinda. Cv. Melinda has the highest Na^+ concentration but it shows the lowest K^+ concentration under both treatments. While cv. Melinda and Bita have increased K^+ concentration under SW treatment compared to FW treatment, cv. Supermargarete is the only cultivar to show reduced K^+ concentration under SW treatment by 2.2 mg K^+ g^{-1} DW. The SW effect on the K^+ concentration was significantly different in cv. Bita compared to all other cultivars as well as between cv. Melinda and Supermargarete (see Appendix E).

4.2.7 Na^+ and K^+ concentration of different plant tissues

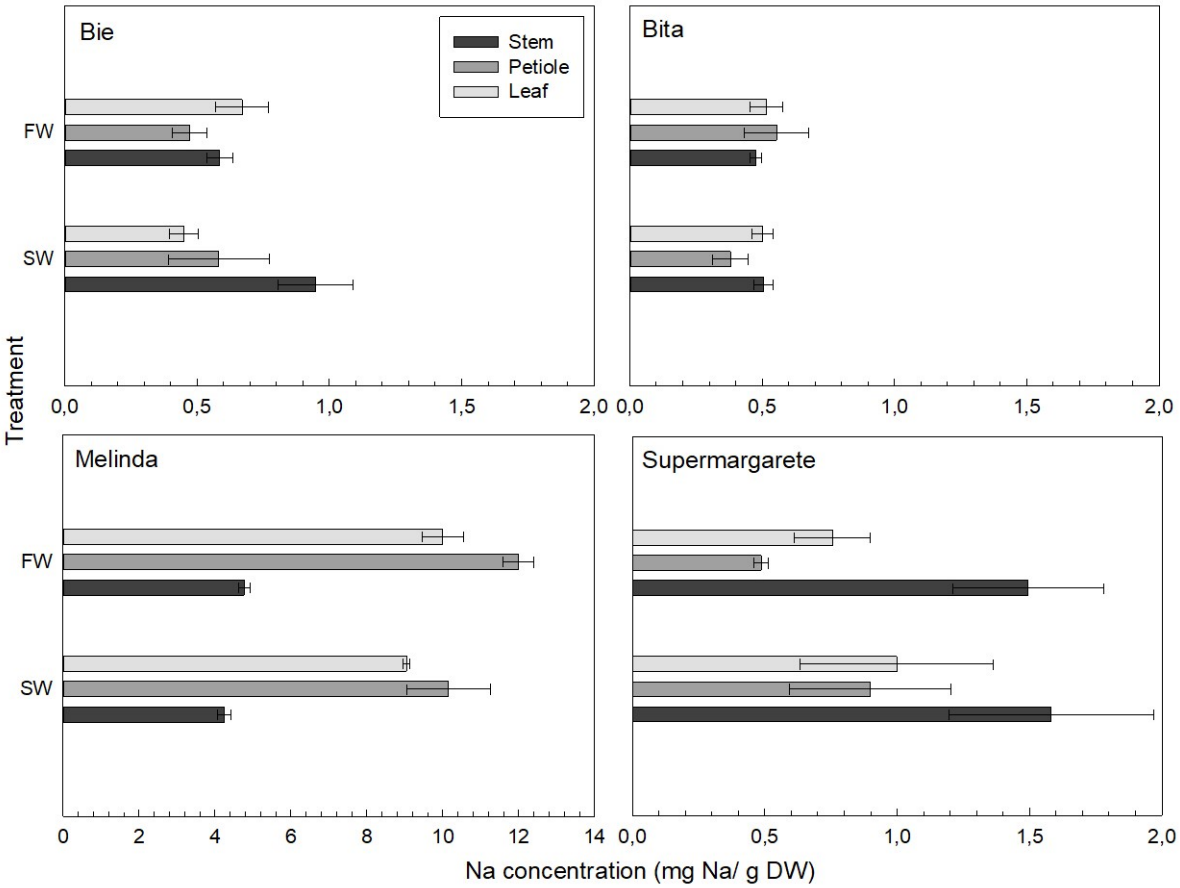

Figure 25 Average Na^+ concentration of leaf blades, stems and petioles per cultivar with standard error (FW= freshwater treatment, SW= saltwater treatment); n= 3; 60 days after treatment onset; x-axis scale differs for improved readability.

The Na^+ concentration of leaf blades, petioles and stems follows distinct, cultivar-dependent patterns. In cv. Bie and Bita, there are no pronounced differences in Na^+ concentrations under FW treatment between plant tissues and all values range below 0.7 mg Na^+ g^{-1} DW. Cv. Melinda shows pronounced differences between Na^+ concentrations of plant tissues with highest concentration found in petioles, followed by leaf blades and stems. In cv. Supermargarete, stems have the highest Na^+ concentration, followed by leaf blades and lastly petioles.

Increases of the Na^+ concentration under SW treatment occur in cv. Bie´s stems with an increase of 0.3 mg Na^+ g^{-1} DW and in leaf blades and petioles of Supermargarete by 0.2 and 0.4 mg Na^+ g^{-1} DW respectively. A decrease of the Na^+ concentration under SW treatment occurs in cv. Melinda´s leaf blades, petioles and stems by 1.0, 1.8 and 0.5 mg Na^+ g^{-1} DW respectively compared to the FW treatment. Significant effects of the SW treatment on the Na^+ concentration only occurred in leaf blades between cv. Melinda compared to cv. Supermargarete and Bita (see Appendix E).

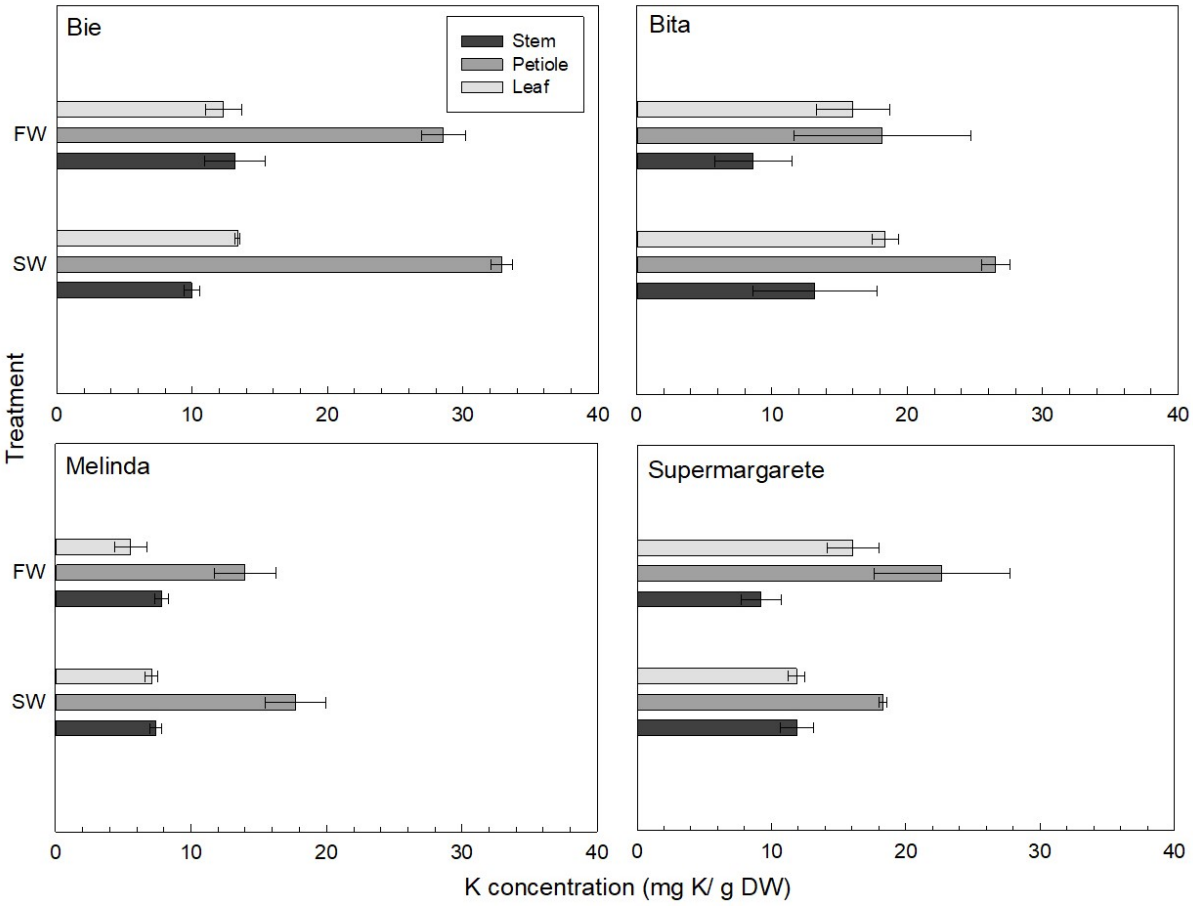

Figure 26 Average K^+ concentration of leaf blades, stems and petioles per cultivar with standard error (FW= fresh water treatment, SW= salt water treatment); n= 3; 60 days after treatment onset.

All cultivars have the highest K^+ concentration in the petioles. Depending on cultivar and treatment either stems or leaf blades have the second highest K^+ concentration. Petioles of cv. Bie have the overall highest K^+ concentration with 28.6±1.6 and 32.9±0.8 mg K^+ g^{-1} DW in FW and SW treatment respectively. Cv. Melinda has the lowest K^+ concentration under both treatments in all plant organs compared to the other cultivars.

While cv. Bie, Bita and Melinda have increased K^+ concentration in petioles under SW treatment, cv. Supermargarete is the only cultivar to show decreased petiole concentration by on average 4.4. mg K^+ g DW compared to the FW treatment. The leaf blade concentration of Supermargarete is also reduced from 16.1±2.0 mg K^+ g DW to 11.9±0.6 mg K^+ g DW under SW treatment. No significant differences in the effect of the SW treatment on K^+ concentration were found between any cultivars for any plant tissue (see Appendix E).

4.2.8 Na^+ and K^+ concentration of old and middle plant sections

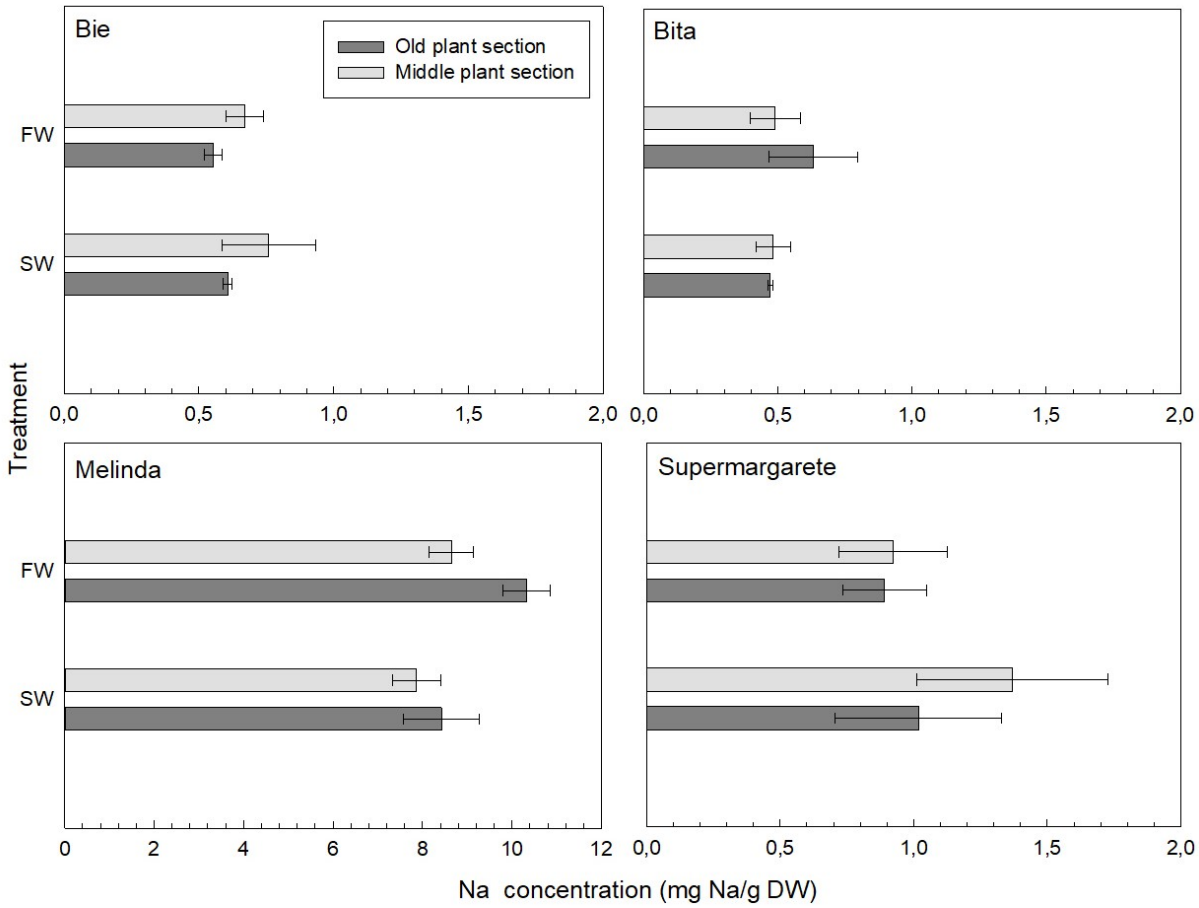

Figure 27 Average Na^+ concentration in the old and middle plant section per cultivar with standard error (FW = fresh water treatment, SW = salt water treatment); n= 3; 60 days after treatment onset; x-axis scale differs for improved readability.

New section concentrations could not be included in the graph due to insufficient data after eliminating datapoints of samples with a DW smaller than 0.1 g that were analysed using the fast prep (see section 3), which had unusually high concentrations. Cv. Melinda again has the highest Na^+ concentrations compared to the other cultivars reaching up to 10.3±0.5 mg Na^+ g^{-1} DW in the old section under FW treatment. Cv. Supermargarete has the second highest Na^+ concentrations after cv. Melinda with a maximum of 1.4±0.4 mg Na^+ g^{-1} DW in the middle section under SW treatment. Concentration differences between old and middle sections are minimal in all cultivars. In cv. Bie, the old section Na^+ concentration is slightly lower than the middle section in both treatments, while in cv. Melinda, the old section concentration is higher than the middle section in both treatments. Cv. Supermargarete has an increased middle section Na^+ concentration under SW treatment by 0.5 mg Na^+ g^{-1} DW compared to FW treatment. There were no significant differences between cultivars concerning the effect of the SW treatment on old and middle section Na^+ concentration (see Appendix E).

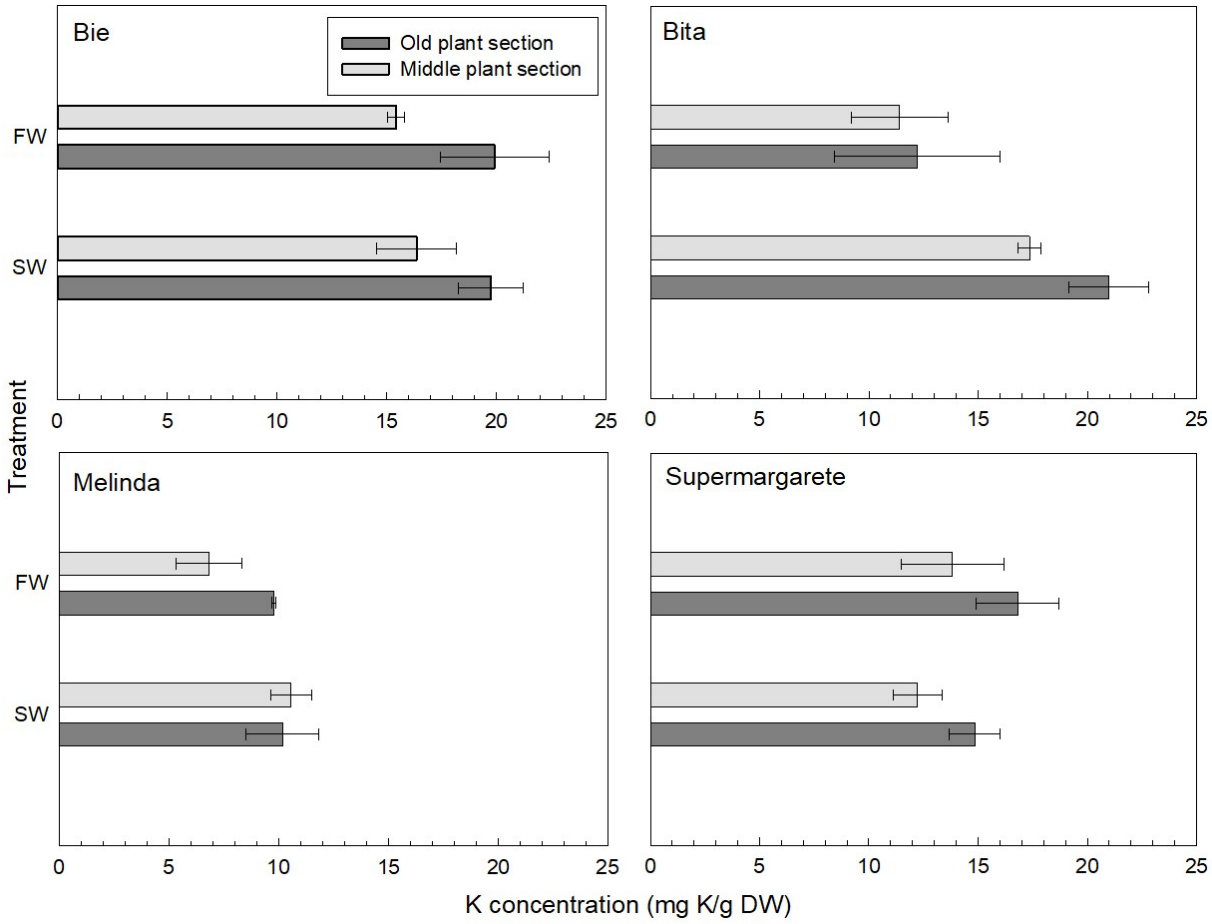

Figure 28 Average K^+ concentration in the old and middle plant section per cultivar with standard error (FW= freshwater treatment, SW = salt water treatment); n= 3; 60 days after treatment onset.

There is a tendency for higher K^+ concentration in the old than the middle plant section except for cv. Melinda under SW treatment and cv. Bita under FW treatment. Cv. Bie has the highest K^+ concentrations of all cultivars in both sections irrespective of the treatment and Melinda the lowest. Increased K^+ concentration under SW irrigation occurs in the middle section of cv. Melinda as well as the old and middle section of cv. Bita. Cv. Bita´s old and middle section K^+ concentration under SW treatment is increased by 8.8 and 6.0 mg K^+ g^{-1} DW respectively. Cv. Melinda´s middle section has a concentration of 6.8±1.5 mg K^+ g^{-1} DW under FW treatment versus 10.6±0.9 mg K^+ g^{-1} DW under SW treatment. A reduction of the K^+ concentration under SW treatment occurs only in cv. Supermargarete with an average reduction of 2.0 and 1.6 mg K^+ g^{-1} DW in the old and middle section respectively.

The SW effect on old section K^+ concentration is significantly different between cv. Bita and any other cultivar. The SW effect on middle section K^+ concentration is significantly different between cv. Bie and Bita, cv. Bie and Melinda, cv. Supermargarete and Bita as well as cv. Supermargarete and Melinda (see Appendix E).

5 Discussion

5.1 Cultivar dependent morphological changes in response to salinity

From a cross-cultivar overview, it is evident that that genetic differences play a role in the treatment effects on the branching activity of the plants, both in the number of side branches (see Figure 14) and in their length (see Figure 15). For example, treatment effect on side branches number of cv. Melinda indicates the presence of a potential genetic trait contributing to salinity response. The variation in main vine length responses represents another possible growth strategy among cultivars. Cv. Bie's and cv. Melinda's consistently longer main vine under SW suggests a unique adaptation strategy compared to other cultivars. From the study, it also emerges that different responses in RLAR (see Figure 13) among cultivars highlight diverse leaf thickness adaptation strategies.

However, when looking at the aboveground biomass accumulation as a growth trait and possible tolerance indicator, the response to the salinity treatment was not as diversified as the one seen for other growth traits. As seen in Figure 4, none of the studied cultivars display strong differences in terms of treatment effect. The salt water treatment has an initial growth promoting effect, as a N^+ uptake can be beneficial by taking over unspecific functions of K^+, such as providing osmolytes and facilitating water uptake (Rodríguez-Navarro & Rubio, 2006; Wakeel, 2013). This is common across all the studied cultivars and visible from 0 to 20 DAOT. Following this initial period, the curves for FW and SW treatments share similar alternating trends, suggesting that the soil salinity did not affect the plant's growth. Only cv. Supermargarete shows a later treatment effect: while in the early stages of development the salt stress has a stimulating growth effect, at later development stages it seems to reach a threshold above which DW accumulation is impaired. This compromises the growth of the stressed plants, while the ones under FW could thrive. From our results, it appears that this threshold was either not reached for the other cultivars or that Supermargarete was the only sensitive one among our genetic cluster.

Table 4 indicates a pre-existent condition of slight salinity in the soil. When considering that the plants grew for 57 days in these conditions before the SW treatment started, we can pose the hypothesis that they could have adapted to the existent soil salinity already at early stages of development. This consideration, joint with the results of the soil analysis at 122 DAP that show a moderate soil salinity, could explain how cv. Bie, cv. Bita and cv. Melinda did not show a treatment effect on the accumulation of aboveground

biomass. Thus, the synchronized and alternating curves of the total DW (g) shown in Figure 4, capture a situation where either the plants already found a way to cope with salt stress in the early stages of development (before 57 DAP) or the salt accumulated in the soil never reached a level high enough to actually stress the plants.

Cv. Bie has a similar trend under both treatments when looking at the development of the total number of leaves (see Figure 9). This is well reflected on the development of leaf area, as seen in Figure 12. Indeed, while in absolute terms the SW displays higher absolute values, the growth rate of LA does not differ between the two treatments (86.8 $cm^2\ d^{-1}$ under FW and 87.2 $cm^2\ d^{-1}$ under SW). According to Munns and Tester (2008), high levels of LA in salt stressed plants correspond to a reduced response to osmotic stress. This is especially favoured by plants that can benefit from sufficient water availability, such as the case of our irrigated field trial, and explains the higher values in LA for the plants under SW treatment. Moreover, photosynthetic rates per unit of LA do not decrease in salt stressed plants, because of adaptation mechanisms in the anatomy and architecture of the leaves (Munns & Tester, 2008). This can explain why there are no substantial differences in the aboveground biomass accumulation of cv. Bie across the two treatments. However, when looking at the relative leaf area ratio (RLAR), cv. Bie has higher values at later stages of development, suggesting an increase in SLA values under SW treatment. Thus, the salt stressed plants have thinner leaves, exposing them to higher transpiration rates and resulting to larger losses in water (Munns & Tester, 2008). As suggested by Negrão et al. (2017a) , a decrease in RLAR values can be related to salinity tolerance. Cv. Bie can be classified a sensitive cultivar in this regard. But with the plants being constantly irrigated, the higher transpiration rate did not result in water stress. Distinct leaves distribution patterns emerge in cv. Bie across the two treatments. Less leaves are attached to the side branches than the main vine at intermediate and late development stages under SW treatment compared to the FW treatment, where the ratio shows higher values (see Figure 11). This trend can be linked to the total number of leaves attached to the main vine, which is higher in the SW treatment. It appears then that when cv. Bie is salt stressed, it invests more energies in distributing more leaves on the main vine, indicating either an adaptive response or the lack of a tolerance mechanism to cope with the salt stress. There is little difference in the branching activity of cv. Bie, given that only at later development stages we can see higher values in the SW treatment, both in the number of side branches (see Figure 14) and in the length of them (see Figure 15). These observations suggest that while the overall leaf area

development is similar between FW and SW, there are notable differences in leaf thickness and distribution in response to salt stress. The higher RLAR and the altered leaf distribution patterns under SW, joint with a constant water availability, indicate specific adjustments and adaptive responses to an osmotic stress in the plants (Munns & Tester, 2008; Negrão et al., 2017a).

Cv. Melinda presents a behavior very different from what has been seen in our field trial and in the scientific literature. Plants under SW treatment display a pronounced branching activity, longer main vines and a larger total number of leaves. Notwithstanding, this is not reflected on the aboveground biomass accumulation, as there is not a strong treatment effect on the dry weight. There are slight differences in the accumulation of stems DW (see Figure 6) and percentage of stems on the total WD (see Table 6) at intermediate stages of development, which can be linked to the higher branching activity under SW treatment. Internode length was not measured in our experiment, but it could have been a useful trait to better understand the complex dynamic of resource allocation in cv. Melinda. LA is affected by salinity. According to Munns and Tester (2008), a decreasing effect of salinity on LA is generally due to either smaller size of the single leaves or to a decrease in the stem and branch growth. Looking at the total number of leaves under SW treatment (see Figure 9), it emerges that the plants are investing in a higher number of leaves, but these appear to be smaller in size. Moreover, when looking at the RLAR, there is an increase in values from 0 to 60 DAOT (see Figure 13). This is resulting from a higher SLA under SW treatment at later development stages and thus thinner leaves. If we compare these results to the DW of leaves under SW (see Figure 5), which appears unchanged compared to the FW, we can speculate that the higher number of leaves is balanced by their smaller and thinner size. The spatial distribution of the leaves is affected by the salt treatment as well. Figure 10 shows that under SW, cv. Melinda has a larger number of leaves on the main vine, which is linked both to the larger total number of leaves and the longer main vine. However, from 50 DAOT, the number of leaves attached to the main vine under SW decreases drastically, possibly due to salt induced senescence. At the same time, the ratio between the number of leaves on the side branches and the number of leaves on the main vine (see Figure 11), shows an increase under SW, suggesting that the plants are investing in having more leaves on the side branches in response to the saline condition. We can therefore say that cv. Melinda's response to saline conditions consists in a variety of morphological adjustments, such as a marked branching activity in combination with the creation of smaller, thinner leaves,

which are also larger in number compared to the plants under FW treatment. It is however inexplicable how the pronounced branching activity (with larger values under SW compared to the other cultivars as well), is not reflected in the total aboveground biomass accumulation. We could speculate that not only the leaves, but the stems as well are thinner and therefore anatomically affected by the salinity, but we would need the side branches to be sampled separately to support this hypothesis.

Cv. Bita, like cv. Melinda and cv. Bie, does not show a strong treatment effect on the development of the total dry weight (see Figure 4). The SW treatment has a late effect on the petioles dry weight (see Figure 7) and a slight effect on the development of the percentage of stems of the total DW (see Table 6). When looking at the LA of cv. Bita, however, the effect of salinity is visible. Plants under FW have a 66% more rapid increase in LA compared to SW, as confirmed in Table 9. The effect of soil salinity on LA is linked to the development of the total number of leaves of cv. Bita. As seen in Figure 9, plants under SW treatment have less leaves from 20 to 50 DAOT, resulting in the overall less available photosynthetic surface. The reduction of leaves under salinity is in line with what was found by (Mondal et al., 2022). However, the value of RLAR decreases from 0 to 60 DAOT. At 50 and 60 DAOT, the dry weight of leaf blades is higher under SW treatment. The decrease of RLAR implies ticker leaves under SW treatment and therefore a larger volume of tissue where salt can be stored, likely contributing to salinity tolerance (Negrão et al., 2017a). In terms of distribution, the higher number of leaves attached to the main vine at late development stages under SW is explained by the longer vines from 50 to 70 DAOT in the stressed plants. The branching activity of cv. Bie does not appear to be affected by salinity, except for the length of side branches, which at later stages of development are slightly longer under SW treatment.

Cv. Supermargarete is the only among our genetic pool to show a treatment effect on the total dry weight development, as seen in Figure 4. As described previously in this chapter, plants under SW treatment have smaller values of DW from 40 DAOT onwards, making cv. Supermargarete a sensitive cultivar to salinity. The negative effect of soil salinity on the aboveground biomass accumulation is well described in the scientific literature (Acosta-Motos et al., 2017). The salinity effect is seen as well in the development of the percentage of leaves of the total DW (see Table 5), suggesting a change in the resource allocation pattern of the plants in response of salinity. Indeed, the hypothesis of cv. Supermargarete being a sensitive cultivar is further supported by the development of LA, which has a growth rate of 53.7 cm^2 d^{-1} and 4.9 cm^2 d^{-1} under FW and SW treatment

respectively, pointing towards an inhibition of leaf area development by salinity. The total values of LA (see Figure 12) indicates that the plants under FW are able to build a larger available surface for photosynthetic activity, thus contributing to the overall higher accumulation of above ground biomass under FW treatment. Decreasing values in RLAR (see Figure 13) signal an adaptive strategy of plants under SW treatment, suggesting a shift towards thicker leaves as a response to salinity stress. The faster development of leaf area under FW, coupled with decreasing values of RLAR, suggests that cv. Supermargarete excels in freshwater conditions, with a propensity for rapid leaf expansion and relatively thinner leaves. On the other hand, plants under SW treatment have smaller and slightly thicker leaves. This growth trajectory aligns with a strategy optimized for efficient light capture and photosynthesis in the absence of salinity stress. The longer main vine and longer side branches under FW (see Figure 8 and Figure 15) indicate an overall trend of greater vegetative growth and structural development. However, in contrast to cv. Melinda, cv. Supermargarete is not a branching cultivar. There is also a tendency to have a larger number of side branches under FW treatment. However, it must be noticed that, under field conditions, cv. Supermargarete was very sensitive to breaking by wind and sampling operations, which makes this data relatively unreliable. Total side branches length seemingly is shrinking towards the end of trial, which is another symptom of the breaking of the side branches. Overall, the results on DW and LA, joint to the elevated vegetative activity under FW, suggest that cv. Supermargarete is negatively affected by the soil salinity reached in this trial.

In conclusion, the level of soil salinity reached in our experiment stimulated a wide range of morphological changes in the studied cultivars, suggesting a complex set of adaptive mechanisms. The poor or absent treatment effect on the DW suggests that a higher level of salinity had to be reached to further stress the plants and further comprehend their development strategies. However, other morphological traits such as the LA, leaves distribution and branching activity, suggest an intricate dynamic difference in the growth patterns of cultivars of sweet potato in response to soil salinity. For future experiments, it is suggested to start the SW treatment at earlier development stages in order to better capture the onset of possible tolerance mechanisms and their dynamic. Moreover, it is suggested to increase the amount of salt provided with the irrigation water, to reach higher levels of soil salinity and thus better stress the plants.

5.2 Cultivar and treatment dependent differences in Na^+ and K^+ concentration, uptake, and partitioning

Using DW as a tolerance indicator, only cv. Supermargarete showed a clear tendency for reduced total DW under SW treatment from DAOT 40 on (see Figure 4). Cv. Supermargarete is thus classified as a salt sensitive cultivar while the others are salt tolerant at least to moderate soil salinity reached in this field trial.

Cv. Bie had a high AGB K^+ concentration with about 17 mg K^+ g^{-1} DW in both treatments (see Figure 24). The total K^+ content per plant was also greater compared to the other cultivars which was related to the K^+ concentration and high DW of cv. Bie plants (see Figure 17). Cv. Bie had the highest petiole K^+ concentration of all cultivars under both treatments (see Figure 26). A high concentration of K^+ in the petioles is physiological because of the important function of K^+ in the process of phloem loading for the transport of fixed carbon from the leaf blades to the sink (Ho et al., 2020). Considering that only 19.0 % (FW) and 18.9 % (SW) of the total DW were allocated to the petioles (at DAOT 60), a great share of total K^+ was accumulated in the petioles with 37.0 % and 32.4 % under FW and SW treatment respectively. Similarly, in Mondal et al. (unpublished), the salinity tolerant cultivar CIP188002.1 strongly accumulated K^+ into petioles even under no salinity stress while under salt stress of 50 mM both K^+ and Na^+ were compartmentalized into petioles to an extent that petioles stored an even larger share of the total plant Na^+ and K^+ content than their respective leaf blades. Na^+ and K^+ are hard to distinguish due to their similar ionic radii (Schachtman & Liu, 1999). Thus, an accumulation of Na^+ in the petioles under salinity could be a positive by-effect of a strong K^+ compartmentalization into petioles. There is a need for more research, exposing cv. Bie to stronger salinity stress to assess where excessive Na^+ will be stored or if its uptake will be avoided. In our research, soil salinity was not sufficient to increase Na^+ concentration of the AGB, which was 0.6 and 0.7 mg Na g^{-1} in FW and SW treatment respectively (see Figure 24). The SW treatment did also not affect the Na^+ content partitioning between plant organs of cv. Bie (see Figure 18).

Cv. Bita had the lowest Na^+ content per plant of all cultivars as well as a very low AGB Na^+ concentration of 0.5 mg Na^+ g^{-1} DW in both treatments in our research (see Figure 17 and Figure 24). Cv. Bita has been shown to avoid Na^+ uptake into leaf blades and stems under salinity stress in a study by Meierhöfer and Fleidl (2023). They found that cv. Bita had Na^+ concentrations of only 2 mg and 10 mg g^{-1} DW in leaf blades and stems combined in the FW and SW treatment respectively. Possibly, cv. Bita is able to avoid Na^+

transport into the shoot by, for example, storage of Na^+ in the roots as reported for cv. Blackie by Kitayama et al. (2020). Data from this trial for root ion contents is not available yet. Not only avoiding transport to the shoot but also re-transport from the shoot to the root by loading of Na^+ into the phloem is a mechanism observed under salt stress helping plants to protect the shoot from toxic effects of Na^+ (Pardo, 2010; Wakeel, 2013). Another explanation for the exclusion of Na^+ from stems and leaf blades is the compartmentalization of Na^+ into petioles which were not analysed by Meierhöfer and Fleidl (2023). However, this seems unlikely based on the decreased Na^+ concentration of the petioles and reduced Na^+ partitioning into petioles under SW treatment in our trial (see Figure 25 and Figure 18). While Na^+ content and concentration per plant were unaffected by the treatment, cv. Bita had higher total K^+ content per plant under SW treatment which was based not on DW differences (equal between treatments at DAOT 60), but higher K^+ concentration of the AGB under SW treatment (see Figure 24). This increase of the AGB K^+ concentration under SW treatment reflected in the significance testing which showed that the effect of the SW irrigation on the K^+ concentration was significantly different in cv. Bita compared to any other cultivar (see Appendix E). The K^+ concentration was equally increased in the old and middle section of the plant as well as over leaf blades, petioles and stems (see Figure 28 and Figure 26). The AGB K^+ Na^+ ratio was thus higher under SW than under FW treatment. This also manifested in an increased petiole and leaf blade K^+ Na^+ ratio under SW treatment (see Figure 23). A possible explanation for the higher K^+ concentration and uptake under SW treatment that also occurred in cv. Melinda could be increased availability of K^+ in the soil. The major share of K^+ in the soil is bound in primary and secondary minerals. It has been reported that irrigation with water containing high concentrations of Na^+ can lead to exchangeable K^+ being detached from clay minerals and becoming available to plants. On the other hand, this desorption also makes K^+ more vulnerable to leaching (Bar-Tal et al., 1991; Wakeel, 2013). Possibly also reduced external K^+ availability due to leaching could have led to an activation of K^+ uptake via high-affinity K^+ transporters like HAK1 or AKT1, overcompensating for the lower K^+ availability. Furthermore, high-affinity Na^+ transporters (HKT) are versatile and might react to salinity by mediating Na^+-K^+-symport instead of transporting two Na^+ ions (Rodríguez-Navarro & Rubio, 2006).

Cv. Melinda is a strongly Na^+ including cultivar. It had the highest AGB Na^+ content per plant and AGB Na^+ concentration with 8.9 mg Na^+ g DW under FW versus 7.7 mg Na^+ g^{-1} DW under SW treatment (see Figure 17 and Figure 24). Lower plant total Na^+ content

under SW treatment was due to lower DW of selected sampling plants than in the FW treatment at DAOT 60 (see Figure 4) as well as slightly reduced Na^+ concentration of the AGB under SW treatment (see Figure 24). This response to the SW treatment was found to be significantly different to cv. Bita and Supermargarete, which showed almost no change in Na^+ concentration (see Appendix E). Furthermore, cv. Melinda was the only cultivar to accumulate a greater share of the total plant Na^+ in the leaf blades than in petioles and stems (see Figure 18). This was due to the about two-fold higher Na^+ concentration in cv. Melinda´s leaf blades and petioles compared to the stems (see Figure 25). High Na^+ uptake, especially under K^+-limiting conditions, can be beneficial as Na^+ takes over unspecific functions of K^+, like providing osmolytes and facilitating water uptake (Rodríguez-Navarro & Rubio, 2006; Wakeel, 2013). Yet, strong partitioning of Na^+ into leaf blades and high leaf blade Na^+ concentration could be sensitivity traits. High leaf blade Na^+ concentration under SW treatment has been associated with decreased photosynthetic rate and leaf area in sweet potato (Kitayama et al., 2020). If this trend continues with higher soil salinity, the Na^+ concentration in the leaf blades could become toxic. This depends on the tissue tolerance of cv. Melinda´s leaf blades. Compartmentalization of Na^+ into leaf vacuoles via sodium hydrogen exchangers (NHX) in the tonoplast can be a strategy to protect leaf tissue from the adverse effects of Na^+ (Pardo, 2010). It is necessary to expose cv. Melinda to stronger salinity, to evaluate if the cultivar has mechanisms to prevent toxic accumulation of Na in$^+$ the shoot e.g., by compartmentation or reduced uptake. As Na^+ is transported to the leaf blades via the transpiration stream (Pardo, 2010), reduced transpiration, by e.g., stomatal closure, proposed as an adaptation mechanism in Mondal et al. (unpublished), could prevent overaccumulation of Na^+ in the leaf blades under more severe salinity stress. K^+ content and concentration of the AGB on the other hand were the lowest in cv. Melinda under both treatments. Like cv. Bita, also cv. Melinda had increased K^+ concentration under SW treatment. This increased AGB K^+ concentration is based on higher petiole and leaf blade K^+ concentration under SW treatment (see Figure 26). Lower total K^+ content per plant is due to lower DW of plants under SW treatment at DAOT 60. High AGB Na^+ content and low K^+ content resulted in cv. Melinda having the by far lowest K^+ Na^+ ratio of all cultivars under both treatments with 0.6 and 0.8 under FW and SW treatment respectively (see Figure 22). Low K^+ Na^+ ratios are often interpreted as a sensitivity indicator (Begum et al., 2015; Fan et al., 2015; Keso et al., 2017). Mondal et al. (2022) found that genotypic threshold for DW reduction and the K^+ Na^+ ratio at this threshold did not show any significant correlation. According to the researchers, maintaining tissue K^+ concentration

under salinity stress is a more suitable tolerance indicator. Thus, low K^+ Na^+ ratios of cv. Melinda are not necessarily pointing to salinity sensitivity.

Cv. Supermargarete has been shown based on morphological data to be sensitive even to the moderate soil salinity achieved in this trial. The ion data supports this theory. Cv. Supermargarete had slightly increased Na^+ concentration of the AGB under SW treatment based on increased leaf blade and petiole Na^+ concentration (see Figure 25). The Na^+ content per plant was unaffected by the treatment because of lower DW of plants under SW treatment (see Figure 4). The K^+ concentration of the AGB was reduced under SW treatment as well as the total K^+ content per plant (see Figure 24 and Figure 17). Partitioning of K^+ into both leaf blades and petioles was reduced under SW irrigation and partitioning of K^+ into stems increased due to decreased petiole and leaf blade K^+ concentration (see Figure 19). Accordingly, AGB K^+ Na^+ ratio was reduced under SW treatment compared to FW treatment while all other cultivars showed similar or increased K^+ Na^+ ratio under SW treatment (see Figure 22). Concerning different plant tissues, the reduction of the K^+ Na^+ ratio occurred in leaf blades and petioles. Several studies in sweet potato have associated a decline of the K^+ Na^+ ratio with salinity stress (Begum et al., 2015; Fan et al., 2015; Keso et al., 2017). The ability to discriminate between Na^+ and K^+ and maintain K^+ uptake under higher soil Na^+ concentration is an important basis of salinity tolerance (Schachtman & Liu, 1999). Our results indicate that cv. Supermargarete might show lower K^+-Na^+-selectivity than the other cultivars. The reduced aboveground biomass K^+ concentration suggests that the increased soil Na^+ under SW treatment interfered with K^+ uptake in cv. Supermargarete. This points to different transporters being involved in K^+ transport in this cultivar, possibly with lower affinity for K^+ over Na^+. It is also possible that increased Na^+ uptake enhanced leakage of K^+. Na^+ uptake into cells can lead to a depolarization of the cell membrane and an activation of K^+ outward-rectifying channels resulting in a decrease of cytosolic K^+ concentration (Wakeel, 2013). Due to high standard errors in cv. Supermargarete´s ion concentration data, the cultivar should be tested again to confirm the theory that Na^+ is included at the expense of K^+ even under moderate salinity stress.

In summary, only cv. Supermargarete showed slightly higher Na^+ concentration under SW treatment compared to FW treatment. Low shoot Na^+ concentrations in all cultivars in comparison to the literature point to insufficient soil salinity in the SW irrigation section. The absence of significantly different SW effects between cultivars concerning most variables including Na^+ and K^+ content of the AGB, Na^+ and K^+ content partitioning between plant tissues and AGB K^+ Na^+ ratio (see Appendix E) also suggests insufficient salinity stress. It is thus necessary to research the performance of all cultivars under higher salinity. Another issue is the slight salinity also in the FW section that remained over the whole trial period (see Table 4).

Consequently, there was no real control with non-saline soil and treatments were more similar in terms of soil salinity than intended. This explains why cultivar effects on ion content, concentration and partitioning were generally more pronounced than treatment effects. We cannot fully understand possible tolerance mechanisms or sensitivity traits under insufficient salinity stress. More research is needed to understand ion uptake and partitioning under higher salinity stress in the cv. Bie, Bita, Melinda and Supermargarete. The observed cultivar dependent differences on Na^+ and K^+ uptake and partitioning are likely connected to different activity and expression of ion transporters and channels. This variability between cultivars is a great genetic resource for salinity tolerance.

To improve the trial, the establishment phase of the plants before treatment onset should be reduced. The long establishment phase of 57 days after planting allowed the plants to generate a DW of about 30 -100 g per plant before treatment onset. Plants are more salt sensitive in the early development periods (Negrão et al., 2017a). It has been shown in wheat that the later salt stress was initiated (10, 56 or 101 DAP respectively) the less salt sensitive were the plants (Maas & Poss, 1989). Furthermore, the sectioning method should be refined, either to track the growth of side branches or to leave them out of samples for ion analysis. New growth on the side branches was not recorded as new growth but as belonging to the section that the side branch was attached to. Possibly concentration differences by plant age were concealed by combining plant parts of different ages. This might be the explanation for why Na^+ and K^+ concentrations showed little difference between old and middle plant section irrespective of the treatment (see Figure 27 and Figure 28).

5.3 Interplay of physiological and morphological responses

Both the results of the ion analysis as well as the morphological data reflect the low level of salinity reached during the field trial. No clear treatment effect was observed in cv. Bie, Bita and Melinda on total DW, DW partitioning between plant tissues, as well as Na^+ concentration or partitioning. What was clear from our field trial, is that variety differences play a key role in the partitioning patterns of biomass and ions, thus making sweet potato an excellent crop species for possible breeding efforts to adapt to saline soils.

Cv. Supermargarete was the only cultivar to show a continuously lower DW in the SW treatment than FW treatment from 40 days after treatment onset onwards. This indicates a negative effect of salinity on aboveground biomass. The ion data also suggests, that cv. Supermargarete is possibly salinity sensitive being the only cultivar with increased Na^+ concentration in the aboveground biomass even under moderate salinity (see Figure 24). Lower DW allocation to leaf blades and petioles under salinity stress coincides with higher Na^+ concentration (see Figure 25) and lower K^+ concentration (see Figure 26) in leaf blades and petioles under salinity stress. Possible reasons for lower DW allocation to leaf blades and petioles could be increased senescence and reduced leaf expansion. Reduced LA under SW treatment and reduced RLAR support this theory, indicating smaller but thicker leaf blades under salinity stress as an adaptive response to dilute sodium in the leaf tissue (Negrão et al., 2017a).

On the contrary, Melinda leaves were smaller and thinner under SW treatment. While leaves DW does not differ much, the total number of leaves increases under SW treatment. Melinda has a very strong branching activity under SW, but interestingly DW partitioning into leaf blades petioles and stems is similar between treatments (see Table 5, Table 6 and Table 7). There are more, smaller, and thinner leaves under SW treatment, which are distributed along the long side branches (see Figure 11). Melinda is characterized by high Na^+ uptake and the strong branching activity could have the purpose of compartmentalizing Na^+ in the plant. This could be an adaptive strategy of cv. Melinda to cope with the salt stress, but in order to investigate on that, it would be necessary to sample and analyse the side branches differently from the main vine. By doing so, useful data could be obtained on a possible dilution effect.

Cv. Bie's similar development of LA under FW and SW treatment allowed the plants to maintain an adequate growth under saline conditions. When looking at the partitioning of dry matter, it appears that petioles had a large share of DW. Cv. Bie appears to have thicker and/or longer petioles compared to other cultivars, under both treatments. This is

well reflected on the concentration of K^+ in the petioles, which is the highest among all cultivars. We could speculate that Na^+ might be stored in petioles as well if higher levels of salinity were reached in the soil. This could be an adaptive strategy for plants to efficiently store Na^+ and prevent its accumulation in the leaf blades. Cv. Bita on the other hand seems to be a tolerant avoider, as confirmed by the results of the DW development and the lowest content of Na^+ among the studied cultivars.

While the data collected in this trial is helpful to provide insight into the effects of salinity on ion uptake and partitioning as well as growth and development of the aboveground biomass, yield is also a critical factor. Most studies researching salinity stress in sweet potatoes do not include a yield analysis. It is possible that aboveground biomass is negatively affected by salinity while the yield is maintained or increased. A preliminary yield analysis of cultivars in the screening trial showed that the great majority of cultivars had increased tuber yield under SW treatment, even those that had reduced aboveground biomass. This underlines the importance of combining tuberous root yield data with morphological and physiological data.

The unclear treatment effect in this trial was due to insufficient salinity stress. Therefore, it is important for further studies to monitor soil salinity continuously throughout the trial to be able to adapt the salinity of the irrigation water accordingly. High standard errors between repetitions additionally complicated identifying effects of the salinity treatment. Especially for the non-destructive data collection, it could have been useful to measure more than three plants per cultivar and treatment. Due to constant measuring and marking as well as strong winds, plants frequently lost side branches but there were no spares to replace damaged plants. This likely decreased the data quality and increased standard errors. Further possible explanations for high variability of the data are unevenness in soil moisture (see Appendix D) and soil salinity (see Appendix C) over the field as well as natural variability among individual plants.

6 Conclusion

In conclusion, this research aimed to investigate the impact of soil salinity on growth, morphological adaptations, ion uptake and partitioning of four sweet potato cultivars. The findings show different responses among cultivars, revealing valuable insights into possible salt tolerance mechanisms.

The cultivar Supermargarete emerged as likely to be salt-sensitive, exhibiting reduced total dry weight and reduced potassium uptake under saline water treatment. Conversely, other cultivars demonstrated tolerance to at least moderate soil salinity.

The overall limited salinity stress imposed in this trial, evidenced by low shoot Na^+ concentrations across all cultivars, underscores the necessity for research under higher salinity levels. The lack of a true non-saline control further complicates the interpretation of results, emphasizing the need for a more controlled experimental setting.

In summary, the observed cultivar-dependent variations in Na^+ and K^+ dynamics and morphology serve as a valuable genetic resource for understanding and enhancing salinity tolerance in sweet potato. Further research under more rigorous salinity conditions is imperative to understand the mechanisms underlying cultivar-specific responses, contributing to the development of resilient sweet potato varieties for regions facing increasing soil salinity challenges.

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