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Title

Morphological and physiological responses of halophytes: *Atriplex halimus, A. lentiformis, A. nummularia, Amaranthus caudatus* and *Chenopodium quinoa* Willd. to increasing levels of salinity

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Preface

This master's thesis has been conducted under The Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen (KU) and the International Center for Agricultural Research in Dry Areas (ICARDA) under the program Diversification and Sustainable Intensification of Production Systems (DSIPS) as the finalizing component in achieving my degree as Cand. Scient in Agricultural development. 3 months were spend in Amman, Jordan conducting field work as a mandatory element of a master's thesis in Agricultural Development. The work was partially funded by scholarships through University of Copenhagen and my supervisor Sven-Erik Jacobsen. The DSIPS program funded the entire field work conducted in Jordan. Images of leaves were processed by Sawsan Hassan at ICARDA to provide estimates of leaf area. The analyses of soil and water were performed by the NCARE laboratory. The remaining data collection and analysis is my original work.

Victoria K. S. Munkager 15.01.2015

Abstract

7 % of the world's agricultural lands are adversely affected by salinity, a state that makes them less capable of supporting agricultural systems. Assessing growth and physiological mechanisms associated with high tolerance in halophytes may provide useful tools in understanding and breeding for salinity tolerance as well as assisting in providing alternative underutilized candidates for reclamation and remediation of saline soils. This study evaluated the salinity tolerance of five halophytes: *Atriplex halimus*, *A. lentiformis*, *A. nummularia*, *Amaranthus caudatus* and *Chenopodium quinoa* Willd. variety *titicaca* exposed to moderate to severe levels of salinity. 25 days after sowing, seedlings were exposed to 5 different levels of salinity with electrical conductivity values of the saturated paste ranging from 8.7 dS/m to 105.8 dS/m. After approx. eight weeks of salinity exposure growth, plant water relations, water use efficiency, stomatal resistance, chlorophyll content and total leaf Na^+ and K^+ were measured. None of the five species survived more than 23 days of salinity exposure at the two highest salinity levels. Although significant growth reductions in response to increasing salinity were observed for all species there were clear between-species distinctions to be made pertaining to the degree of salinity stress experienced by the plants and which underlying mechanisms were mostly affected. Amaranth and quinoa were found to be Na^+ includers consequently altering the ratio between leaf K^+/Na^+ and inducing premature leaf senescence due to ionic stress. Contrastingly, all three atriplex species were found to be Na^+ excluders indicating that control of Na^+ uptake and transport plays an essential role in the salinity tolerance of atriplex. The stomatal resistance was adversely affected in nummularia, amaranth and quinoa and to some extent in halimus and lentiformis indicating that increasing salinity provokes a water conserving strategy in the plants which resulted in either unaltered or improved water status in all of the species. Reductions in chlorophyll content in response to salinity were observed in some but not all of the species and it is hypothesize that in quinoa, a C_3, and amaranth, a C_3-C_4, species chlorophyll content reductions lower the risk of photoinhibition under salinity-induced stomatal-limiting photosynthesis inhibition. Overall it can be concluded the significant differences in plant response, both morphological and physiological, to salinity exists, not only between species, but also within the same species over a salinity exposure gradient of EC: 8.7-26.8 dS/m.

Resumé

7 % af verdens landbrugsarealer kan karakteriseres som saltholdige. Høje koncentrationer af salte resulterer i en reduktion af jordens overordnede produktivitet grundet et ufavorabelt osmotisk potentiale og høje salt koncentrationer. Ved at undersøge hvordan vækst og underliggende fysiologiske mekanismer påvirkes af at vokse i saltholdige jorde kan man komme tættere på at forstå individuelle arters ydeevne samt hvilke mekanismer der er ansvarlige for salttolerance. Denne forståelse kan være essentiel viden for planteforædling af mere modstandsdygtige arter samt til at klarlægge om arter kan anvendes til at forbedre produktiviteten af saltholdige jorde der ellers ikke kan understøtte væksten af saltsensitive arter. I dette eksperiment blev fem forskellige saltelskende arter udsat for moderat til ekstremt saltholdige jorde. Arterne i forsøget var: *Atriplex halimus*, *A. lentiformis*, *A. nummularia*, *Amaranthus caudatus* og *Chenopodium quinoa* Willd. varietet *titicaca*. 25 dage efter såning blev plantespirerne udsat for fem forskellige grader a saltstress, hvoraf den laveste behandling havde en elektrisk ledningsevne på 8.7 dS/m og den højeste 105.8 dS/m. Efter 8 uger med saltstress blev plantevækst, vandindhold, vandudnyttelseseffektivitet, stomatal konduktans, klorofyl indhold og bladkoncentration af Na^+ og K^+ målt. Ingen af de fem arter overlevede længere end 23 dage i de to mest saltholdige behandlinger. Plantevæksten blev betydeligt reduceret som et resultat af øget saltstress for alle fem arter, men analyserne afslørede derudover at der er forskel mellem arter i grad af påvirkning samt hvilke fysiologiske parametre der påvirkes mest. Amarant og quinoa viste tydelige tegn på akkumulation af Na^+ i deres blade i takt med saltstress, hvilket resulterede i at forholdet mellem K^+/Na^+ blev reduceret og at individuelle blade viste tegn på tidlig ældning. Derimod var indholdet af Na^+ uændret af graden af saltstress hos de tre atriplex arter hvilket indikerer at disse arter har effektive mekanismer til at styre optag og bevægelse af Na^+ i planten. Stomatal konduktans var negativt påvirket af salt for nummularia, amarant, quinoa og i mindre grad halimus og lentiformis, hvilket signalerer at saltstress giver planterne incitament til at konservere vand ved at lukke stomata. Effektiviteten af denne mekanisme var tydelig at se på det uændrede eller forbedrede vandindhold i planterne. Indholdet af klorofyl i planternes blade blev kun reduceret i nogle af arter. En mulig hypotese for forholdet mellem kloropfyl og stomatal konduktans er, at quinoa og amarant, som ikke er C_4 arter, har en eller flere mekanismer, der regulerer klorofylindholdet i forhold til graden af stomatalbegrænsende fotosynteseinhibering. Overordnet set er det muligt a konkludere at der findes betydelige forskelle mellem de forskellige arters reaktion på saltstress, men at der også hersker forskelle indenfor de individuelle arter i takt med at graden af saltstress stiger fra 8.7 til 26.8 dS/m.

List of abbreviations

C_3	Photosynthetic pathway in which the first product of CO2 fixation is a 3-carbon intermediate
C_4	Photosynthetic pathway in which the first product of CO2 fixation is a 4-carbon intermediate
CCAFS	Climate Change, Agriculture and Food Security
CCI	Chlorophyll content index
CGIAR	Consultative Group on International Agricultural Research
DAS	Days after sowing
DAT	Days after treatment exposure
DSIPS	Diversification and Sustainable Intensification of Production Systems
DW	Dry weight
DVCT	Digital Vegetation Charting Technique
E	Transpiration
EC	Electrical conductivity
EC_e	Electrical conductivity of a saturated paste extract
ET	Evapotranspiration
FW	Fresh weight
ICARDA	International Center for Agricultural Research in the Dry Areas
NCARE	National Center for Agricultural Research and Extension
r_s	Stomatal resistance

Table of contents

1. Introduction

7 % of the world's agricultural lands, primarily in arid and semi-arid regions, are adversely affected by salinity, a state that makes them less capable of supporting agricultural systems (Geissler et al. 2009). Water scarcity is an increasing global phenomenon leading to expansion and exacerbation of salinity on agricultural lands due to accumulation of salts in the soil rhizosphere from poor water irrigation sources (Qadir and Oster 2004). One third of all irrigated agricultural land is reported to be significantly affected by salinity (Tester and Davenport 2003). IPCC (2013) predicts a global increase in temperature of 3.5°C by the turn of the century. Increased temperatures may intensify the degree of salinity by an increased upward movement of soluble salts due to higher rates of soil evaporation (Barret-Lennard 2002; Shabala and Munns 2012). To accommodate the future needs of a predicted increase in the global population (IPCC, 2007), within a world with scarcity of unexploited land suitable for commercial cash-crop agriculture (Qadir and Oster 2004), authors have suggested the use of underutilized species with high salinity tolerance for the reclamation and remediation of saline soils (Barret-Lennard 2002; Ravindran et al. 2007; Rabhi et al. 2010; Shabala 2013; Qadir et al. 2014).

1.1 Salinization, definitions and drivers

Salinization is the accumulation of water-soluble salts as Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-} and CO_3^{2-} in the soil solum or regolith (Barret-Lennard 2002; Qadir and Oster 2004; Munns and Shabala 2012). Salinity is distinguished in two forms, that which is naturally occurring (primary salinity) and that which is induced by human activity (secondary salinity). Primary salinity can be almost impossible to combat since its sources of salts and cause of upward movement are naturally occurring (Munns and Shabala 2012). The most dominant sources of salts are rainfall, aeolian deposits, weathering of the bedrock, and current as well geologically recent seawater intrusion (Rengasamy 2006; Tester and Davenport 2003). Secondary salinity is human induced due to land-use change or irrigation (Munns and Shabala 2012). Irrigation with limited drainage or leaching can lead to salinity as salts are continuously applied to the soil (Munns and Shabala 2012). Land-use change from deep-rooted native perennials to shallow-rooted annuals can raise the water table and move soluble salts to the rhizophere (Munns and Shabala et al. 2012). In arid and semi-arid areas, low precipitation and high evapotranspiration rates intensifies salinization for both primary and secondary salinity as the water budget is insufficient to leach excess salt from the soil (Rengasamy 2006).

The commonly used classification of soil salinity is defined by an electrical conductivity of the soil's saturated paste extract (EC_e) exceeding 4 dS/m, equivalent to an osmotic pressure of approximately 0.2 MPa (Richards 1954; Munns and Tester 2008). Above the threshold of 4 dS/m the growth of most crops is severely affected but the specific adverse impact of a given EC_e-value on plant growth depends on factors such as soil texture, soil moisture and distribution, and composition of salts in the soil (Richards et al. 1954). Saline soils which also have a high sodium exchangeable percentage are defined as having sodic properties

resulting in alkalinity and poor physical structure with crusting, reduced infiltration, increased soil strength and reduced aeration (Rengasamy 2006; Munns and Shabala 2012).

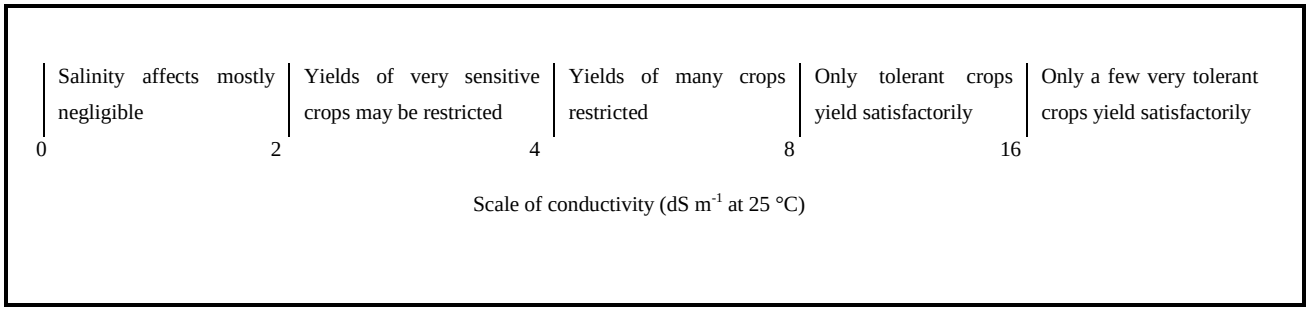


Table 1 Overview of salinity tolerance thresholds. Reproduced from Richard et al. (1954)

1.2 Plant response to salinity

The impact, not necessarily adverse, of salinity imposed on plants affects in two distinctive ways (Munns and Tester 2008). Due to elevated levels of salt ions, the external osmotic pressure increases in the soil medium which reduces the osmotic gradient between soil and plant, subsequently making the passive uptake of water less favorable (Tester and Davenport 2003). The osmotic effect is rapidly experienced by plants at the onset of salinity exposure (Munns and Tester 2008). Secondly, plants are affected by the uptake and accumulation of Na^+ and Cl^- after continuous salinity exposure i.e. the ionic effect (Munns and Tester 2008). The relative importance of tolerance conferring to either the osmotic or ionic stress imposed on plants has yet to be elucidated and may vary between species and as the degree of salinity intensifies (Roy et al. 2014).

Halophytes are salt tolerant plants that maintain the ability to complete their life cycle at EC-values higher than 4 dS/m (Richard et al. 1954). Some halophytes, due to inherent mechanisms of tolerance show optimal growth under moderate salinity compared to no salinity (Adolf et al. 2012; Belkheiri and Mulas 2013; Glenn et al. 2013), which illustrates the importance of assessing salinity over a range of salinities in comparison to using zero salinity as control. By testing the plants within a range of soil salinity levels it guides research in better understanding specific plant responses to salinity (Tester and Davenport 2003).

The following sections will describe how underlying physiological mechanisms are affected in halophytes from both ionic and osmotic impact and how they confer to alterations in plant growth. The sections described will be stomatal resistance, chlorophyll content, Na^+ and K^+ content in leaves, K^+/Na^+-ratio, water relations and water use efficiency (WUE).

1.2.1 Stomatal resistance

Uptake of CO_2 for photosynthesis and water vapor loss is regulated by small epidermal pores, the stomata, present on the abaxial and adaxial leaf surfaces (Lambers et al. 2008). Swelling of guard cells opens stomata and allows for CO_2 diffusion and loss of water vapor and shrinking closes stomata (Pandey et al. 2007).

Measurements of stomatal resistance (s/cm) provide insight to the aperture of stomata where increases in resistance indicate a decreased rate of CO_2 and water vapor due to an increased rate of stomatal closure. The degree of stomatal resistance is affected by environmental conditions including temperature, atmospheric CO_2 concentrations, as well as abiotic stresses such as drought and salinity (Munns and Tester 2008). As a response to the water deficient conditions caused by the increased osmotic potential in the saline soil, stomatal resistance increases rapidly after exposure initially from the altered water relations in the plant and subsequently by a local synthesis of Abscisic acid (ABA)(Munns and Tester 2008; Pérez-López et al. 2012). Stomatal resistance was significantly increased in quinoa plants exposed to 100 mM NaCl in comparison to a control (Razzaghi et al. 2011; Adolf et al. 2012). Pérez-López et al. (2012) found that salinity had an adverse impact on net photosynthetic rate, primarily due to increased stomatal resistance in barley (*Hordeum vulgare*). Contrastingly, Munns and Tester (2008) argued that the net photosynthetic rate measured per leaf area is unaffected by salinity and Cuin et al. (2010) failed to find a significant association between stomatal resistance and yield in 25 cultivars of wheat (*Triticum aestivum* L. and *Triticum turgidum* L. ssp. *durum*).

1.2.2 Chlorophyll content

Chlorophyll, is an integral part of energy harvesting from irradiance, energy which is used for photosynthesis (Alberts et al. 2004). By emitting waves with peak wavelength at 650 nm (absorbed by chlorophyll cells) and peak wavelength at 940 nm (no absorption by chlorophyll cells), measurements of chlorophyll content can be obtained nondestructively (Spectrum Technologies Inc. 2009). Several studies have found differences in the response to salinity between salt tolerant and salt sensitive plants. Reductions in chlorophyll content as salinity increases were found in glycophytes such as maize (*Zea mays*)(Cha-Um and Kirdmanee 2009) and rice (*Oriza sativa*)(Kanawapee et al. 2012). Kanawapee et al. (2012) furthermore found that more sensitive rice cultivars showed greater reduction in chlorophyll content than less sensitive, albeit still salt sensitive, cultivars. For salt tolerant plants, Adolf et al. (2012) found an increase in chlorophyll content for a salt tolerant variety, utusaya, of the halophyte quinoa grown at 100 mM NaCl compared with 0 mM NaCl, whereas the less tolerant halophytic variety titicaca had a decrease in chlorophyll content. Contrastingly, Shabala et al. (2012) found another quinoa variety, 3706, classified with titicaca to resemble in degree of salt tolerance, to be unaffected in chlorophyll content when comparing 0 mM NaCl and 400 mM NaCl salinity exposure. Seemingly there is no clear-cut conclusive results pertaining to response in chlorophyll content to salinity exposure and literature is limited at best in explaining the mechanisms involved in alterations of chlorophyll content due to salinity. Lutts et al. (1996) found that mature, non-senescing leaves of rice (*Oriza sativa*), had higher degrees of chlorophyll degradation than young leaves which may be associated with the higher Na^+ accumulation observed in older leaves and hence leaf senescence (Kanawapee et al. 2012). Adolf et al. (2012), argues that due to similar physico-chemical properties of Na^+ and K^+, Na^+ competes with K^+ for binding sites and distorts essential metabolic processes including chlorophyll biosynthesis. Reductions in

chlorophyll content may be explained either by a reduced chlorophyll biosynthesis or an increased chlorophyll degradation, or a combination of both.

1.2.3 Water use efficiency

WUE defined as the ratio of biomass per water use, provides insight to the amount of net biomass accumulation produced per unit of water. WUE is affected by water vapor pressure inside the leaf and temperature as well as stomatal aperture (Lambers et al. 2008). WUE and stomatal resistance are positively correlated because as stomata closes, the marginal reduction in transpiration decrease less than the marginal reduction in intercellular CO_2 (Lambers et al. 2008). Plants with the C_4 photosynthetic pathway are generally associated with higher WUE than C_3 plants because of their low inter-cellular CO_2 which allows for a higher stomatal resistance (Lambers et al. 2008; Glenn et al. 2012). In both halophytes and glycophytes, WUE has been found to improve from salinity exposure under water limiting conditions (Ayala and O'leary 1995; Glenn et al. 1998).

1.2.4 Na^+

The uptake, movement, accumulation and sequestering, and overall effect of Na^+ in plants have received extensive attention by researchers throughout the years (Blumwald et al. 2000; Tester and Davenport 2003; Flowers and Colmer 2008; Munns and Tester 2008; Cuin et al. 2009). Na^+ accumulation is primarily a phenomenon observed in the leaf blade, whereas roots have been found to maintain near constant concentrations regardless of salinity level of the soil medium through regulation of uptake, efflux and reallocation to the xylem (Munns 2002; Tester and Davenport 2003). Accumulation of Na^+ in leaves due to continuous loading from the xylem is a significantly slower process than the osmotic effect. The reduced cell volume, due to the water deficit experienced by the plant through high external osmotic potential, exacerbates the rate of Na^+ accumulation in the cytoplasm (Hasegawa et al. 2013). In the cytosol, toxic concentrations of Na^+ destabilizes membranes and proteins and impairs cellular and physiological processes including cell division and elongation and distorts mineral nutrient homeostasis (Greenway and Munns 1980; Hasegawa et al. 2000; White and Broadley 2001; Hasegawa et al. 2013). Consequently, individual leaf life span is reduced by accelerating senescence, reducing net shoot biomass production. If the rate of senescence is not counteracted by new leaf growth, which is primarily inhibited by osmotic stress, the photosynthetic capacity of the plant will be insufficient for plant survival (Munns and Tester 2008).

To avoid reaching toxic concentrations of Na^+ in the cytosol, two types of mechanisms have been defined in plants: Na^+ exclusion and tolerance (Munns and Tester 2008). No apparent correlation between total leaf Na^+ and salinity tolerance has been found for halophytic dicotyledonous species, suggesting that some species may predominantly adopt a Na^+ exclusion strategy whereas other species rely more heavily on Na^+ tolerance (Tester and Davenport 2003; Cuin et al. 2009;). Na^+ exclusion from the leaves involves reduced influx of Na^+ from soil to roots and increased efflux from roots back to the soil, which contributes to a lower rate loaded into the xylem and hence less Na^+ is translocated to the shoots. In halophytes, the net uptake of Na^+

by roots curtails as external salinity increases to high concentrations, which indicates that the role of Na^+ exclusion plays a more dominant role at high salinity (Glenn et al. 1998). Na^+ tolerance is not commonly associated with a higher tolerance of metabolic mechanisms in the cytosol and reallocation to roots via the phloem which is considered insignificant or non-existing. Na^+ tolerance is rather associated with a higher degree of compartmentalization in vacuoles and salt bladders or a prioritizing of maintaining lower concentrations in young, metabolically active leaf tissue by Na^+ loading in mature leaves (Tester and Davenport 2003; Shabala and Cuin 2007; Flowers and Colmer 2008; Shabala et al. 2014). In *Arabidopsis thaliana*, intracellular compartmentalization of Na^+ in the vacuole was induced by ABA and osmotic stress (Zhang and Shi 2013). The high osmotic potential of the vacuoles and salt bladders due to Na^+ accumulation causes an unfavorable osmotic gradient between the vacuole and the cytosol which can be counteracted by cytosolic K^+ and synthesis of organic compatible solutes including sucrose, proline and glycine betaine (Munns and Tester 2008; Shabala et al. 2014). Salt bladders are a common physiological trait of halophytic species within the family Chenopodiaceae. The salt bladders are classified as trichomes as they are modified epidermal hairs and are usually made up of two different cells, a stalk cell and a bladder (Atwell et al. 1999; Shabala 2013). Salt bladder selectively secretes Na^+ into external bladder cells which may rupture once the storage capacity has been reached subsequently releasing Na^+ onto the leaf surface (Smaoui et al. 2014). In mangroves, increased tolerance of Na^+ has been associated with the presence of bladder cells (Tester and Davenport 2003) but Ball et all (1988) found the rate of sequestration into bladder cells insufficient to the rate of Na^+ uptake.

1.2.5 K^+ and K^+/Na^+

Increased concentrations of Na^+ in the soil medium reduces the activity of K^+, along with other essential nutrients, lowering the availability to plants. Internally, Na^+ competes with K^+ for uptake sites at the plasma membrane due to a similarity between the hydrated ionic radii between the two cations (Cuin et al. 2009). Additionally K^+ efflux in roots is exacerbated through membrane depolarization caused by high concentrations of intracellular Na^+ (Shabala and Cuin 2007). K^+ is known to activate more than 50 enzymes, it is needed in high concentration for protein synthesis and disturbances to cytosolic K^+ homeostasis can lead to cell death (Tester and Davenport 2003; Demidchik et al. 2010). Maintaining a high ratio of K^+/Na^+ indicates the level of control the plant demonstrate over influx and efflux of Na^+ and K^+ and has been suggested as a parameter of salinity tolerance (Adolf et al. 2012). Cuin et al. (2009), found strong correlation between salinity tolerance in wheat cultivars and retention of K^+ in leaf sap.

1.3 Species

The five species used in this experiment is described in the five following section. *Table 2* provides a general overview of some of the immediate differences between the species.

1.3.1 *Atriplex halimus* L.

Halimus is a perennial dicotyledonous halophytic C_4 shrub (Walker et al. 2014), originating from the Mediterranean Area (Le Houérou 1992) but cultivated in Algeria, the Arabian Peninsula, Egypt, Iraq, Israel, Jordan, Libya, Morocco, Spain and Tunisia (Walker et al. 2014). The primary purpose of cultivating halimus is production of year round fodder in the form of fresh standing fodder, as well as silage suitable for sheep, goat, cattle and camels (Walker et al. 2014). Le Houérou (2010) reports that compared to other species of Atriplex it is less susceptible to overgrazing. Alternative uses include reduction of soil erosion and reclamation of marginalized soils in arid and semi-arid areas (Abbad et al. 2004). Several studies found that optimal growth of halimus is achieved at a salinity level above 0 mM NaCl and suggests that moderate concentrations of Na^+ stimulates plant growth (Bajji et al 1998; Hamid et al. 2011; Alla et al. 2012; Belkheiri and Mulas 2013). Various literature attributes the salinity tolerance of *A. halimus* partially due to the vesicular hairs on the leaf surface secreting accumulated salts (Walker et al. 2014; Gharaibeh et al. 2011; Mozafar and Goodin, 1970). Halimus characterized as a large sized plant within the *Atriplex* genus forming up to 3 m tall upright shrubs with a well developed root system extending down to 10 m deep and silver-green leaves (Le Houérou 1992; Walker et al. 2014). Natural reproduction is primarily through seed dispersal but vegetative propagation via shoot-borne roots is possible (Walker et al. 2014). For cultivation, precultivation of seeds or cuttings in nurseries is recommended over direct sowing due to low germination rate of seeds (Bajji et al. 2002). Halimus is a monoecious plant where the ratio between male and female flowers is affected by day length and has been found to fail setting reproductive flowers close to the equator (Le Houérou 1992; Talamali et al. 2003; Walker et al. 2014). Halimus grows well with high evapotranspiration and low mean annual precipitation of 100-400 mm and 400-600 mm for arid and semi-arid areas respectively (Walker et al. 2014). Optimal soil textures for growth include silty, loamy, and clayey soils whereas coarse sandy and heavy clay soils produce poor results and should be avoided completely (Le Houérou 2010). Halimus grows well in neutral to alkaline soils as well as saline soils, where it often forms mono-specific stands and outcompetes other species (Walker et al. 2014).

Figure 1 Image taken prior to harvesting of representative replicates of halimus at EC_e-levels: 8.7, 16.0 and 26.8 dS/m

1.3.2 *Atriplex lentiformis* L.

Originating from North America, lentiformis is a perennial, dicotyledonous halophytic shrub with a C_4 photosynthetic pathway (Le Houérou 2010; Glenn et al. 2013). Lentiformis is a dioecious shrub (Le Houérou 1992). Commonly, lentiformis is utilized as a forage shrub for browsing animals, but has also been

introduced to rehabilitate marginalized rangeland (Le Houérou 1992). Le Houérou (1992) reports that lentiformis is one of the more resilient species within the genus of *Atriplex* and can endure regular browsing. Regular browsing or trimming twice a year is required to maintain high palatable yields to avoid an overproduction of unpalatable woody tissue (Le Houérou 1992). Large sized Atriplex plant reach up to 3 m in height and a diameter of 5 m (Le Houérou 2010). Le Houérou (1992), suggests 4-6 m row space and inter-row space of 1-2 m for optimal growth. Self reseed (Le Houérou 2010). Lentiformis grows well under saline-sodic soils with heavy clay textures (Le Houérou 2010). Glen et al. (2013) reports that moderate salinity enhances growth in lentiformis due to osmotic adjustment by utilizing Na^+ as inorganic solutes. Sandy soils should never be utilized for cultivation of *A. lentiformis* (Le Houérou 1992). *A. lentiformis* is an erect medium sized Atriplex plant with silvery-green leaves and a well developed root system that can reach water tables down to 10 m depth (Le houérou 1992). Lentiformis has epidermal bladder cells capable of accumulating Na^+ (Shabala 2014).

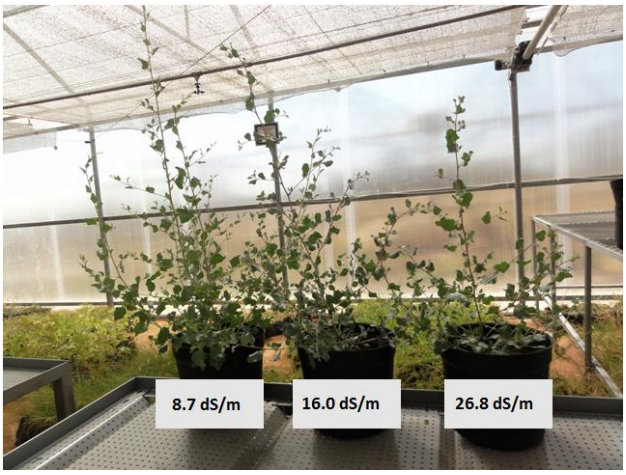


Figure 2 Image taken prior to harvesting of representative replicates of lentiformis at EC_e-levels: 8.7, 16.0 and 26.8 dS/m

1.3.3 *Atriplex nummularia* L.

Nummularia is a perennial dicotyledonous C_4 halophytic shrub originating from Australia (Le Houérou 2010). Plants of nummularia are generally dioecious but monoecious plants do occur (Souza et al. 2014). Nummularia is the species amongst introduced Atriplex species that cover the most land in Syria, Jordan, Egypt, Saudi Arabia, Libya, and Tunisia (Le houérou 2010). Nummularia is used as a forage crop in arid and semi-arid areas due to its high palatability compared to other Atriplex species, but is prone to over-browsing and may fail to re-grow. Browsing or trimming is nevertheless essential to maintain palatable yield and prolongs the lifespan of plants up to 40 years (Le Houérou 1992). Alternatively it is also used for rehabilitation of marginalized land and as an ornamental plant primarily at resorts (Le Houérou 1992). Plants grow to a height of 2-3 m, have one to few erect stems with silvery-green leaves and fruiting bracteoles (Jepson Flora Project 2013). Out of the three identified subspecies: spp. *nummularia*, spp. *omissa* Parr-Smith and spp. *spathulata* Parr-Smith, the subspecies *nummularia* is the most common (Le

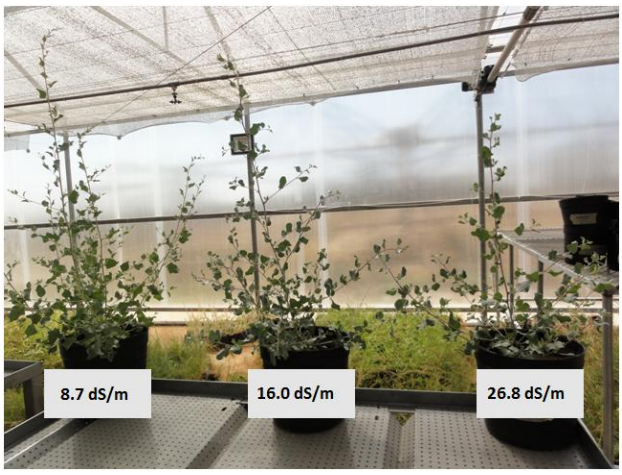


Figure 3 Image taken prior to harvesting of representative replicates of nummularia at EC_e-levels: 8.7, 16.0 and 26.8 dS/m

Houérou 2010). Studies have found moderate salinity to stimulate growth and survival, albeit with severely inhibited growth at salinity levels as high as 1000 mM NaCl (Belkheiri and Mulas 2013) and 1500 mM NaCl (Bazihizina et al. 2012). Salt excreting bladder cells have been suggested to confer, at least partly, to the high tolerance to salinity (Batanouny 1965; de Souza et al 2012; Hussin et al. 2013). Nummularia grows in arid and semi-arid areas with 150-400 mm mean annual precipitation and tolerates moderate drought. The roots of nummularia can go as deep as 10 m reaching a deep-lying water table (Le Houérou 1992; Le Houérou 2010). Medium textured soils are most suitable for cultivation and sandy soils should be avoided completely (Le Houérou 1992).

1.3.4 *Amaranthus caudatus* L.

Amaranth is an ancient dicotyledonous annual pseudo-cereal originating from the Andean region in South America, where it has been cultivated and consumed for more than 5000 years (Stallknecht and Schulz-Schaeffer 1993; Lamothe et al. 2015). Amaranth is commonly characterized as encompassing the C_4 photosynthetic pathway, but a recent study proposed that *Amaranthus cruentus* cv Amaranteca more correctly should be classified as a C_3-C_4 plant (Joaquín-Ramos et al. 2014). The genus *Amaranthus* L. includes 60 species of amaranth, where some encompass the ability to produce grains. Leaves of amaranth, both grain and non-grain producing, are a nutritive source of human consumption and livestock feed (Kaufmann and Weber 1990; Stallknecht and Schulz-Schaeffer 1993; Kaur et al. 2010). *Amaranthus caudatus* is promoted as one of the three primary grain species along with *A. hypochondriacus* and *A. cruentus* (Stallknecht and Schulz-Schaeffer 1993). Besides being gluten-free, the grains contain 12-18 % protein which is high compared to cereals (Kaufmann and Weber 1990; Kaur et al. 2010).The protein has been reported to contain a high percentage of the amino-acids lysine, methionine, and cysteine much like quinoa (Lamothe et al. 2015). In the Andean region, amaranth is cultivated in a variety of systems including self-seeding or broadcast seeding under slash and burn (Early 1990).

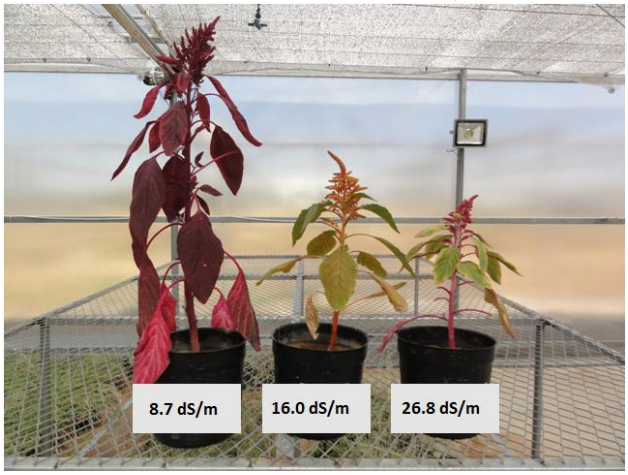


Figure 4 Image taken prior to harvesting of representative replicates of amaranth at EC_e-levels: 8.7, 16.0 and 26.8 dS/m

It is commonly found cultivated in systems with maize, as intercropping either between or within rows of maize or in crop rotation systems where maize is followed by amaranth cultivated in mono-culture (Early 1990). Plants of *Amaranthus caudatus* are obligate short day length plants (Cassab et al. 1999), have a main stem axis and show great variety in height ranging from 91-274 cm for cultivars grown in Montana (Stallknecht and Schulz-Schaeffer 1993). The stem terminates in a large apical branched unisexual inflorescence know for its many different colors of purple, orange, red or gold (Stallknecht and Schulz-

Schaeffer 1993). It has been reported to tolerate drought, heat, and pests (Kaur et al. 2010), and can grow under a wide range of irradiance and temperature (Aziz et al. 2011). Little is known about the salinity tolerance of amaranth but reportedly it tolerates salinity better than the cash-crops wheat, maize, sorghum, and cotton (Omamt et al. 2006; Joaquín-Ramos et al. 2014).

1.3.5 *Chenopodium quinoa* Willd. variety *titicaca*

Chenopodium quinoa Willd. is a annual, dicotyledonous, halophytic C_3 pseudocereal originating from the Peruvian and Bolivian Andes, where it has been cultivated since 5000 AD (Repo-Carrasco et al. 2003; Hariadi et al. 2011). Its small seeds, used for human consumption, are characterized by high quality protein due to the combination of amino acids, particularly lysine, methionine and cysteine (Repo-Carrasco et al. 2003; Lamothe et al. 2015). The seeds have been promoted as a gluten-free food high in iron, manganese, copper, zinc, and soluble dietary fibers (Jacobsen 2003; Lamothe et al. 2015). Quinoa has a 1-2 m high erect stem which terminates in a panicle inflorescence but inflorescence also rises from axils of lower leaves (Bertero et al. 1995). Quinoa plants are gynomonoecious and primarily self-pollinating (Bhargava et al. 2006; Zurita-Silva et al. 2014). The stem and inflorescence vary in color between shades of yellow, purple and red depending on variety (Bhargava 2013). Both leaves and stems have salt secreting bladder cells (Shabala et al. 2014). The highly branched root system of quinoa includes a tap root and can go as deep as 1.5 meter (Bhargava 2013). Great variability exist within the species which have adapted to a wide range of conditions (Adolf et al. 2012). Titicaca is an early maturing variety, selected and propagated under non-saline conditions in Denmark from a cross between lines originating in Southern Chile (Hariadi et al. 2011; Adolf et al. 2012). Cultivation of quinoa is possible between latitudes 20°N and 40°S and in altitudes ranging from sea level to almost 4000 meters above sea level (Jacobsen 2003). Pertaining to photoperiodicity, the titicaca is daylength neutral but other varieties have been found to be long or short day plants (Christiansen et al. 2010; Shabala et al. 2013). Quinoa is a drought tolerant plant that requires a minimum of 200 mm precipitation per growth cycle; with germination, bud formation, and flowering as the three phonological stages most susceptible to drought (Aguilar and Jacobsen 2003). Quinoa is a halophyte which reportedly has optimal growth at 100 mM NaCl and can tolerate salinity exposure as high as 500 mM NaCl (Hariadi et al. 2011; Eisa et al. 2012). Titicaca was classified by Shabala et al. (2013) as belonging to the group of lowest salinity tolerance out of 14 varieties. Adolf et al. (2012) found that titicaca was one of the most affected varieties out of 14 in terms of

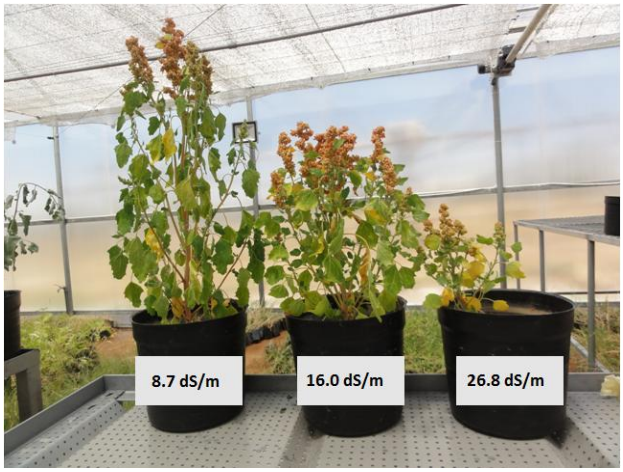


Figure 5 Image taken prior to harvesting of representative replicates of quinoa at EC_e-levels: 8.7, 16.0 and 26.8 dS/m

height reduction going from salinity of 0 to 100 mM NaCl but that biomass production was unaffected. In addition to salinity and drought tolerance, quinoa is tolerant to frost, wind and hail (Adolfet al. 2012). Optimal soil textures for cultivation of quinoa is sandy-loam to loamy-sand, as growth is susceptible to excess water in poorly drained soils (Bhargava 2013).

1.4 Objective

The objective of this study was to elucidate morphological changes and underlying physiological mechanisms affected by and conferring to salinity exposure within each species. Secondly, between-species differences in response to salinity exposure were assessed to identify whether the five halophytic species vary in their way of tolerating the increasing levels of salinity. It is hypothesized that at least the highest levels of salinity exposure will have severe detrimental impact on plant growth either due to osmotic stress, ionic stress or a combination thereof.

Family	Genus	Species	Alternative name	Origin	Photosynthetic pathway	Duration	Dicot/monocot	Salt bladders
Amaranthaceae[c] Subfamily: chenopods[j]	Atriplex L.[c]	*Atriplex halimus* L.	Saltbush[l]	Mediterranean region[b, g]	C_4[b]	Perennial[l]	Dicot[l]	Yes[k]
Amaranthaceae[c] Subfamily: chenopods[j]	Atriplex L.[c]	*Atriplex lentiformis*	Big saltbush[l] Quail bush[l]	SW USA[g]	C_4[m]	Perennial[l]	Dicot[l]	Yes[j]
Amaranthaceae[c] Subfamily: chenopods[j]	Atriplex L.[c]	*Atriplex nummularia* Lindl.	Bluegreen saltbush[l]	Australia[h]	C_4[h]	Perennial[l]	Dicot[l]	Yes[a]
Amaranthaceae[c] Subfamily: chenopods[j]	Chenopodium[c]	*Chenopodium quinoa* Willd. variety *titicaca*		Andes[f]	C_3[i]	Annual[l]	Dicot[l]	Yes[j]
Amaranthaceae[c]	Amaranthus L.[c]	*Amaranthus caudatus* L.	Love-Lies-Bleeding[b] Kiwicha[d]	Andes[f]	C_4 or C_3-C_4[e]	Annual[l]	Dicot[f]	No[*]

Table 2 Overview of some of the essential differences between halimus, lentiformis, nummularia, amaranth and quinoa. References: a. Batanouny 1965; b. FAO Ecocrop 2007?; c. ITIS 2014; d. Jimenez et al. 2013; e. Joaquín-Ramos et al. 2014; f. Lamothe et al. 2015; g. Le Houérou 1992; h. Le Houérou 2010; i. Shabala et al. 2012; j. Shabala et al. 2014; k. Smaoui et al. 2011; l. USDA 2012?; m. Zhu and Meinzer 1999. *To my knowledge salt excreting bladder cells have not been identified in amaranth.

2 Materials and method

2.1 Plant material and growth conditions

The species used for this experiment include: *Amaranthus caudatus*, *Chenopodium quinoa* Willd. variety *titicaca*, *Atriplex halimus*, *A. lentiformis*, *A. nummularia*. Seeds of halimus, lentiformis and nummularia were collected in 2013 from the ICARDA rangeland pastoretum in Aleppo, Syria and the seeds of quinoa and amaranth were donated from the University of Copenhagen. To enhance rapid germination, the seeds were rinsed in tap water every 3 hours for 24 hours, and kept in plastic containers with 2 l tap water in-between rinsing (Hussin et al. 2013; Piovan et al. 2014). The seeds were pre-cultivated for 25 days, i.e. initially in a greenhouse nursery between 14.05.14-04.06.14 and subsequently for an additional 4 days at the NCARE (National Center for Agricultural Research and Extension) research station "Mushaqqar" (31°46'26.13"N 35°48'9.07"E) (See map of location in Appendix A) where the remaining part of the experiment took place. For each species, the seeds were sowed in peat moss in polystyrene trays with approximately 10 seeds per compartment. On the 8th of June 2014, the seedlings were transplanted to 5.1 L pots (height: 18cm, diameter: 19cm) containing 4000±2 g of dry soil and thinned down to one seedling per pot. The first day (08.06.14), the plants were irrigated in small amounts over 4 hours to allow for slow percolation in the dry soil and to avoid leaching. 1 day after treatment exposure (DAT), the plants were watered to 80±4 % of the soil water holding capacity every 1-6 day. The water holding capacity was estimated by watering 3 pots for each of the 5 treatment levels, with small quantities of water every 10 minutes until leaching was observed. The pots were left overnight to reach the stage of saturation i.e. 100 % water holding capacity. See *Equation 1* calculations of the weight at 80 % water holding capacity. Irrigating up to 100 % water holding capacity was attempted but leaching occurred due to poor properties of the soil and it was reduced to 80 %. In general, the pots were susceptible to leaching through cracks in the soil. In order to avoid leaching, irrigation was done over 2-4 watering sessions per pot during a couple of hours. The water used for irrigation had an average EC_e-value of 0.80±0.25 dS/m. To limit the effect of the greenhouse, the pots were randomly placed and redistributed after every watering session. Within each treatment, each species had 6 individual pots.

$$WHC_{80} = 0.8 * \frac{(\sum_{i=1}^{n} SS_i - DS_i)}{n} + \frac{(\sum_{i=1}^{n} DS_i)}{n} \qquad \textbf{Equation 1}$$

For equation 1:WHC_{80}= Average weight of pots at 80 % water holding capacity; SS_i= weight of pot "i" at saturation; DS_i= Weight of pot "i" with dry soil. n= number of pots, i.e. 3 per treatment.

The day and night temperatures were set to 28±2°C and 18±2°C respectively. Ambient relative humidity ranged between 60-99 % with an average daily value of 76 %. From May to August, average daily photoperiod was 14 h, and average monthly irradiance was 319±14.5 Wh/m^2 based on 2010 data (NCARE IMIS, 2010). Due to a high rate of evapotranspiration, the greenhouse shading system was activated (10.07.14), blocking approx. 46 % of total irradiance at plant level. The estimate is based on photosynthetic

photon flux density measurements with and without shading at 11.00 and 12.30, (23.07.14), made with a LI-1600 steady state porometer (LI-COR, Inc., Lincoln, NE, USA).

Salinity treatment was initiated 25 DAS. The plants were exposed to 5 different levels of naturally saline soil with EC_e-values of 8.7 dS/m, 16.0 dS/m, 26.8 dS/m, 92.0 dS/m and 105.8 dS/m. Subsamples of soil were collected at the NCARE research station "Al-Karama" south of the Jordan Valley (31° 55' 50.05" N 35° 34' 11.89" E) (See map in Appendix A) from 8 different locations with varying salinity. Based on EC_e and mM NaCl-values, 5 subsamples, illustrating the broadest range of salinity, were selected for the experiment. *Table 2* provides an overview of the soil specific characteristics of the 5 final samples. The soil was sieved through a 2mm mesh and thoroughly mixed. A tissue was placed between the soil and the bottom of the pot to cover the drainage holes. The plants were harvested on the 03.08.14, equivalent to 81 days after sowing (DAS) and 56 DAT. All three atriplex species were still in their vegetative stage whereas amaranth and quinoa, the two annuals, were in the seed filling stage, classified as developmental stage 15 for quinoa (Jacobsen and Stølen 1993).

EC_e (dS/m)	NaCl (mM)	pH
105.8	1551	7.8
92.0	1183	7.8
26.8	260	7.9
16.0	303	7.8
8.7	104	7.9

Table 3 EC_e, mM NaCl and pH

2.2 Sampling and measurements

2.2.1 Plant growth

Plant height was measured 56 days after commencement of treatment. As described by Belkheiri and Mulas (2013), leaf succulence was assessed from the ratio between fresh weight and dry weight of three randomly selected fully developed but non-senescing leaves from each plant. The dry weight was determined after oven drying at 60°C to constant weight. On 57 DAT, plants were cut at soil level to collect total aboveground biomass. Plant material was separated into leaves and stems which included inflorescence for quinoa and amaranth. Fresh weight was measured and dry weight was determined after oven drying at 60°C to constant weight. The weight of leaves used for determining succulence was added to the weight of total fresh weight and dry weight for leaves. Leaf area was measured using the Digital Vegetation Charting Technique (DVCT) in software VegMeasure. Images of individual leaves beneath a transparent 1 cm^2-grid with a known, fixed area were processed by VegMeasure providing an estimate of percentage leaf of total image area through color differentiation as described by Louhaichi et al. (2010).

2.2.2 Plant transpiration and water use efficiency

The loss in total weight between watering, i.e. the estimate of evapotranspiration (ET) was used as a proxy for transpiration (E), neglecting the weight of plants as described by Razzaghi et al. (2011). Water use efficiency, also called integrated water use efficiency, was defined as total dry matter production per unit total evapotranspiration (Martinez et al. 2003).

2.2.3 Stomatal resistance and chlorophyll content index (CCI)

In the period 51-55 DAT (76-80 DAS) stomatal resistance was measured in accordance with the product manual (LI-COR Inc. 1989) using a LI-1600 steady state porometer (LI-COR, Inc., Lincoln, NE, USA) on 3 fully developed, non-senescing leaves for each replicate between 8.00-15.00. **Fejl! Henvisningskilde ikke fundet.***Figure 6* illustrates the location of measurements on the individual leaf. For some plants, the leaves were so small that the measured area overlapped in the three measurements. For amaranth, data points are missing for two plants. One replicate had died in treatment 26.8 dS/m. For one plant in treatment 16.0 stable measurements were not possible and are therefore missing. CCI was measured on the same leaves and at the same time as stomatal resistance using a chlorophyll meter SPAD-502 Chlorophyll Meter (Minolta Inc., Osaka, Japan) in accordance with the product manual (Spectrum Technologies, Inc. 2009). The product manual suggests avoiding large veins which was sometimes impossible for amaranth.

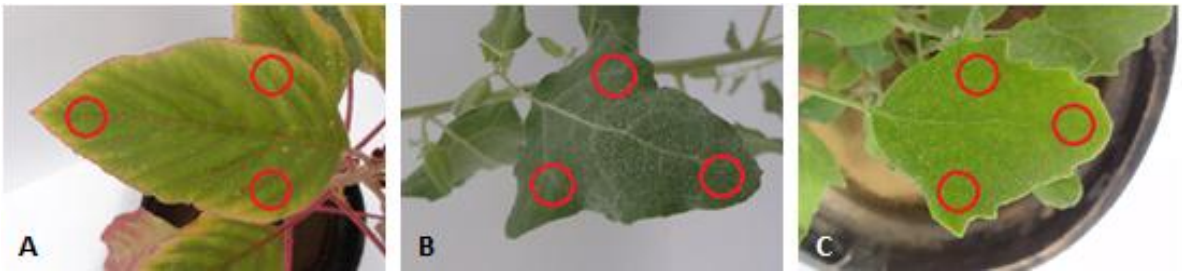


Figure 6 The red circle indicates where the stomatal resistance and CCI measurements were taken on A) Amaranth, B) Halimus, lentiformis and nummularia, C) Quinoa.

2.2.4 Total Na^+ and K^+ content

Measurements of total Na^+ and K^+ content were undertaken in Denmark at the Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen (KU). The leaves were dried again at 60°C to constant weight before grinding, were pulverized using a grinder, and dried for 24 h at 60°C. Three out of 6 replicates were randomly selected for the analysis. 100 mg of dried material was mixed with 15 % H_2O_2 in a 3.5 % HNO_3 solution and shaken thoroughly. Serving as a reference 3 samples with the NIST certified reference material of apple leaves were prepared similarly to the leaf material along with 12 blanks. The samples were digested in an Ultrawave microwave for approximately 20 minutes. The digested samples were transferred to Falcon tubes and diluted to 50 ml in 3.5 % HNO_3. Seven standard solutions were prepared for both Na^+ and K^+ by diluting 1000 ppm Na^+ and 1000 ppm K^+ stock solutions in a 3.5 % HNO_3 solution. The seven concentrations of standard solutions for Na^+ and K^+ were 0, 1, 5, 10, 15, 30, 50 ppm. An atomic absorption spectrometer (PerkinElmer, model 3300) was used to analyze the Na^+ and K^+ content. A preliminary run of the digested samples showed that the samples had to be diluted 10-fold to fit within the standard curve of the standard solutions. After dilution the full analysis was run. Controls between measurements of the 1 and 10 ppm standard solutions showed that the accuracy of the flame photometer had

drifted more than 5 % between control 3 and 4 for the Na^+ measurements as can be seen in *Table 4*. Consequently two standard curves for Na^+ were estimated. The initial standard curve (SC1) was used for measurements taken before Control 4 and the final standard curve (SC2) was used for measurements taken after control 4. As it can be seen in *Table 5* the measurements had not drifted more than 5 % for K^+ and only one standard curve was estimated. The three standard curves are illustrate in *Figure 7*.

ppm [Na^+]	Initial standard curve (SC1)	Control 1	Control 2	Control 3	Control 4	Final standard curve (SC2)
0	0.000					0.001
1	0.106	0.105 (-0.9 %)	0.104 (-1.9 %)	0.102 (-3.8 %)	0.101 (-4.7 %)	**0.100 (-5.7 %)**
5	0.341					**0.317 (-7.0 %)**
10	0.488	0.480 (-1.6 %)	0.473 (-3.1 %)	0.467 (-5.3 %)	**0.462 (-5.3 %)**	**0.458 (-6.1 %)**
15	0.592					**0.555 (-6.2 %)**
20	0.681					**0.638 (-6.3 %)**
30	0.814					**0.767 (-5.8 %)**
50	1.024					**0.966 (-7.7 %)**

Table 4 The absorbance results for Na^+ in brackets is given the percentage deviation of the absorbance compared to the first standard curve (SC1 Figure 8). Control 1, 2, 3, 4 were taken during measurements. Highlighted in bold are the results that have drifted more than 5 % from initial standard curve (SC1).

ppm [K^+]	Initial standard curve (SC1)	Control 1	Control 2	Control 3	Control 4	Final standard curve (SC2)
0	0.000					0.000
1	0.043	0.043 (0.0 %)	0.043 (0.0 %)	0.044 (2.3 %)	0.044 (2.3 %)	0.044 (2.3 %
5	0.226					0.228 (0.9 %)
10	0.415	0.416 (0.2 %)	0.416 (0.2 %)	0.416 (0.2 %)	0.417 (0.5 %)	0.417 (0.5 %)
15	0.574					0.576 (0.3 %)
20	0.698					0.702 (0.6 %)
30	0.907					0.915 (0.9 %)
50	1.241					1.246 (0.4 %)

Table 5 The absorbance results for K^+ in brackets is given the percentage deviation of the absorbance compared to the first standard curve (SC1 Figure 8). Control 1, 2, 3, 4 were taken during measurements. None of the controls had drifted more than 5 % from initial standard curve (SC1).

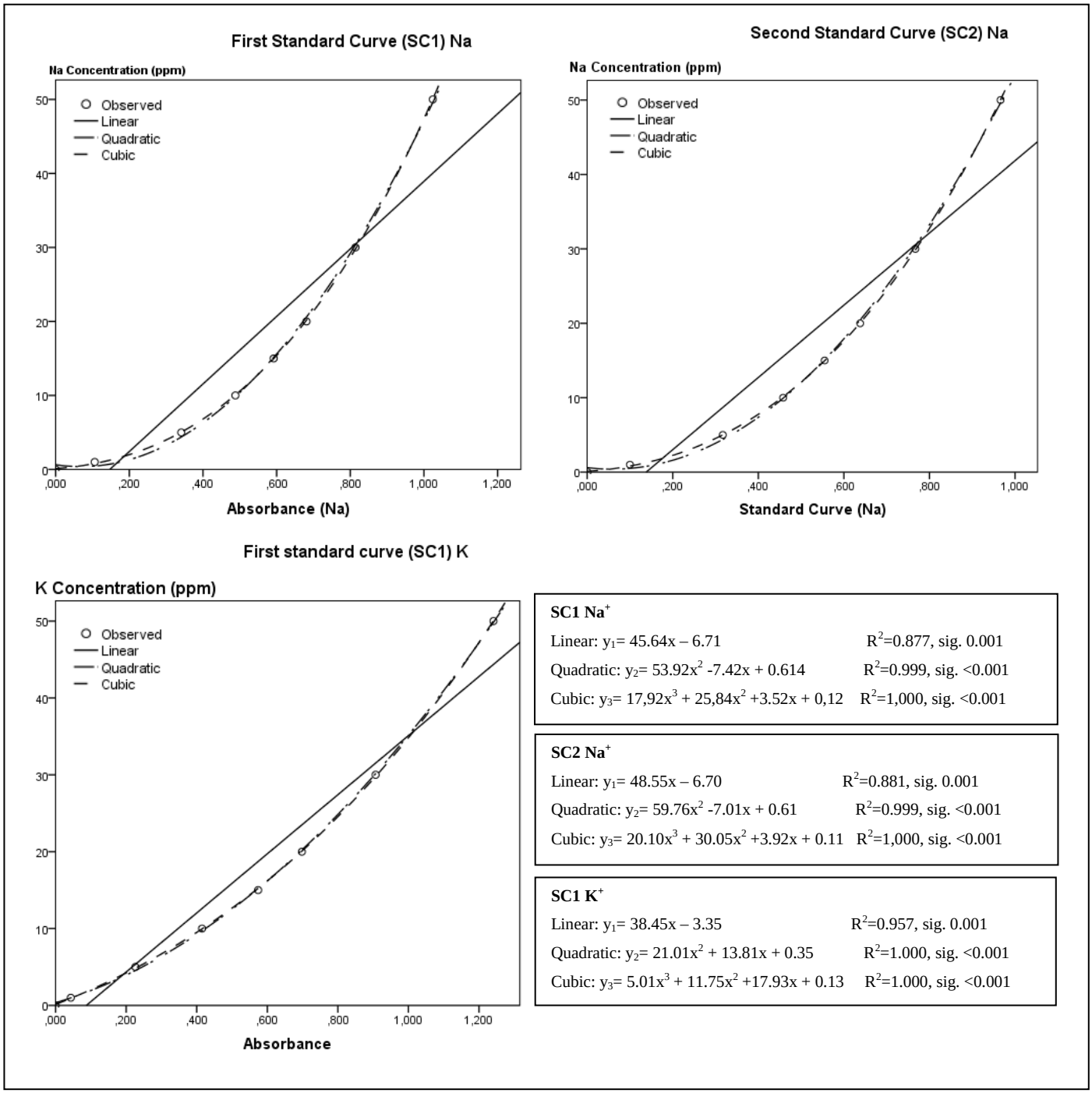


Figure 7 Standard curves 1 and 2 (SC1, SC2) for Na^+ and standard curve for K^+. Boxes on the lowest right corner present the best fit equations for the linear, quadratic and cubic equations.

2.3 Method and materials for analyses done by the NCARE laboratory

The following section describes the methodology and materials used for soil and water analysis which were analyzed in Amman by the local NCARE laboratory staff.

2.3.1 Soil analysis

To estimate pH, 50 g of soil sample, sieved through a 2 mm mesh, was mixed with 50 ml distilled water to a 1:1 soil water solution, thoroughly mixed and allowed to stand for an hour with stirring every 10 minutes.

After 1 hour the pH of the samples was estimated with a pH meter that was calibrated against two buffer solutions of pH 4.0 and 7.0. Electrical conductivity of a saturated paste (EC_e), was estimated with a calibrated conductivity bridge on a filtrated water saturated soil water sample. To estimate Na^+ content, a filtrated, thoroughly mixed 1:5 soil and ammonium acetate (1.0 Eq/l) sample was analysed for Na^+ content with a flame photometer at 589 nm against a standard curve of standard solutions with concentrations consisting of 25 ppm LiCl, 1.0 Eq/l NH_4OAc and 8 different concentrations of Na^+: 0, 20, 40, 60, 80, 100, 150, 200 ppm. The results were converted from ppm to mM by the following equation:

$$mM\ Na = ppm * \frac{Wt}{V*A} \quad \textbf{Equation 2}$$

In Equation 2 Wt= Weight of air dry soil (g); V= Volume of soil extract (ml); A= atomic weight of Na.

Soluble Cl^- content was estimated from the same sample as EC_e by adding 5-10 ml soil saturation extract in a wide-mouth Erlenmeyer flask (150 ml), adding 4 drops of 5 % K_2CrO_4 and then titrate against 0.01 N $AgNO_3$ until reddish-brown color appears. The concentration of Cl^- was determined from Equation X and X.

2.3.2 Irrigation water analysis

Samples of the water used for irrigation were collected the 6 times that the water tank was refilled. The NCARE laboratory performed the analysis of EC-value, Na^+ and Cl^- concentration in accordance with the official laboratory manual (ICARDA 2013). For estimating electrical conductivity, a calibrated conductivity meter was used. As reference material a 0.01 mM KCl solution with an EC-value of 1.413 dS/m (25°C). Measurements were done at 25°C. The water samples were filtered through Whatman filter paper no. 42 and analyzed for Na^+ content by a flame photometer at 589 nm wavelength against a standard curve made from 8 standard solutions with 25 ppm LiCl and 0, 20, 40 60, 80, 100, 150, 200 ppm Na^+. The Na^+ concentrations given in ppm are converted to mM by division with 23 i.e. the molar weight of Na^+. Cl^- was determined by the Mohrs's titration method. 10 ml of the water sample was transferred to a 250 ml Erlenmeyer flask and 4 drops of K_2CrO_4 (5%) is added. The solution was titrated against $AgNO_3$ (1.0 mM) until a permanent reddish-brown colour appears i.e. the point where there is no more Cl^- to react with the silver and the silver starts to react with chromate. The concentration of Cl^- was calculated from Equation 3 and 4.

$$N_{AgNO_3} = \frac{10*N_{NaCl}}{V_{AgNO_3}} \quad \textbf{Equation 3}$$

For equation 3: N_{AgNO3}=Eq/l of $AgNO_3$ solution; V_{AgNO3}= Volume of $AgNO_3$ solution (ml); N_{NaCl}= Eq/l of NaCl solution.

$$Cl\left(\frac{mEq}{L}\right) = \frac{(V_1 - B)*N*1000}{V} \quad \textbf{Equation 4}$$

For equation 4: V_1= Volume of 0.01 Eq/l $AgNO_3$, titrated for the sample (ml); B= blank titration (only used for soil analysis); N= Eq/l of $AgNO_3$ solution; V= Volume of the sample (ml).

2.4 Statistical analyses

One-way ANOVA (analysis of variance) followed by the Tukey's range test was used to analyse differences between treatments within each individual species. All one-way ANOVA analyses were evaluated on a 5 % significance level. A preliminary analysis was always undertaken prior to running an ANOVA to test for normal distribution of observations with the Shapiro-Wilk test and homoscedasticity with the Levene's test (based on median) at significance levels 1 % and 5 % respectively. Whenever the criteria of homoscedasticity was not met and the one-way ANOVA showed a statistically significant difference between treatments, the one-way ANOVA analysis was replaced with a Welch's ANOVA analysis followed by the Games-Howell post-hoc test. Both analyses were evaluated on a 5 % significance level. A two-way ANOVA was done to determine any interaction between species and treatment (species*treatment). The Levene's test was used to test for heteroscedasticity with an α-value of 0.050. Whenever the criteria of homoscedasticity was not met the α-values for the two-way ANOVA and the LSD post-hoc test were changed from 0.050 to 0.010 to reduce the risk of type I error. In the case of statistically significant interaction a two-by-two factorial ANOVA test was run with similar α-value as for the two-way ANOVA.

The best fit regressions for the Na^+ and K^+ standard curves were tested with linear and non-linear quadratic and cubic regressions. The cubic regression was selected as the best fit for both Na^+ and K^+ as it yielded the highest R^2, lowest p-value and most accurate Na^+ and K^+-concentrations for the certified reference material. IBM SPSS statistics 22.0 was used for all statistical analyses.

3. Results

In the following section, results will be presented for the following parameters: survival rate, dry weight, fresh weight/dry weight-ratio, leaf area, leaf succulence, water use efficiency, stomatal resistance, CCI, leaf Na^+, leaf K^+ and leaf K^+/Na^+-ratio. Statistically significant differences between treatments based on one-way ANOVA (or Welch's in case of heteroscedasticity) are always reported when significant at a 5 % level. Appendix F reports the specific p-value as a supplement for the curious reader. Pertaining to number of observations, n=6 for halimus, lentiformis, nummularia and quinoa and n=5 for amaranth unless otherwise stated.

3.1 Survival rate

For all five species, no plants survived the entire 56 days of exposure to salinity for the two highest EC_e-values: 105.8 and 92.0 dS/m as is illustrated in *Figure 9*. After 12 DAT all replicates were dead at 105.8 dS/m for lentiformis, nummularia and amaranth. After 17 DAT the last replicate remaining in treatment 105.8 dS/m was dead for halimus and quinoa. For treatment 92.0 dS/m the last replicates died between 12 and 23 DAT. Amaranth and quinoa showed the smallest difference in survival rate between treatment 105.8 and 92.0 dS/m where quinoa had a small delay in response between the highest and second highest salinity exposure and amaranth showed no difference. The three Atriplex species showed greater difference between

the two treatments. The difference in DAT where the last replicate died between 105.8 and 92.0 dS/m for halimus, lentiformis and nummularia is 6, 9 and 5 DAT respectively. For treatments 8.7, 16.0 and 26.8 dS/m all 6 replicates survived all 56 DAT for halimus, lentiformis, nummularia and quinoa. One replicate died in the 26.8 dS/m treatment for amaranth 23 DAT. Amaranth had all 6 replicates alive for the lowest treatment. For halimus, lentiformis, nummularia and quinoa, the data suggests that the threshold for survival lies somewhere between 26.8 and 92.0 dS/m since all replicates died at 92.0 dS/m and above and all replicates survived at 26.8 dS/m and below. The one amaranth replicate that died at 26.8 dS/m may indicate that the threshold for survival lies close to 26.8 dS/m. Since only one replicate died it may be uncorrelated to the salinity stress and caused by another unknown factor.

3.2 Height

Height decreased as a response to increasing EC_e-values for halimus, amaranth and quinoa. As EC_e increased from 8.7 dS/m to 16.0 dS/m, plant height was statistically reduced for halimus (p-value: 0.002) and amaranth (p-value< 0.001). Between treatment 16.0 dS/m and 26.8 dS/m height was not reduced further. The statistically significant reduction in height for quinoa occurred as EC_e increased from 16.0 dS/m to 26.8 dS/m (p-value: 0.002). For lentiformis and nummularia no statistically significant effect on height by the salinity treatments was identified.

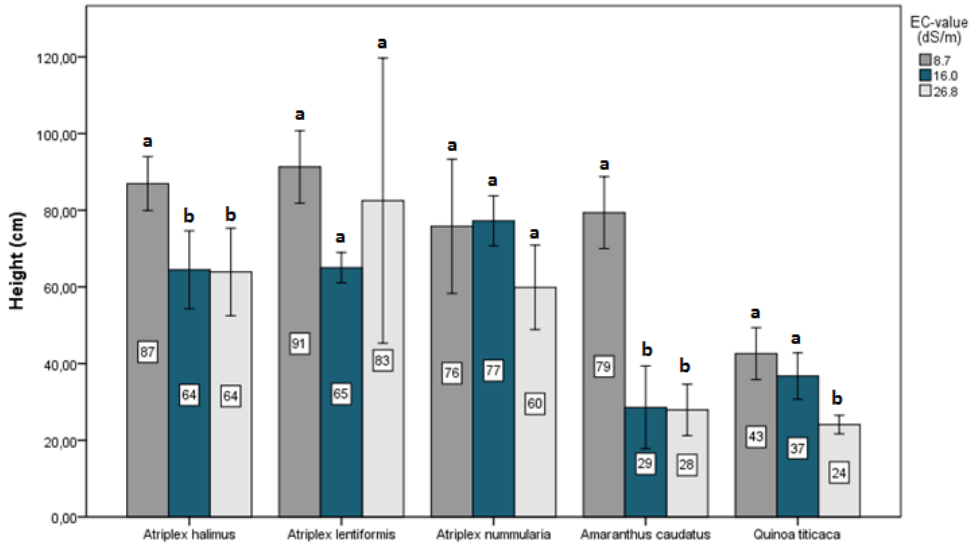


Figure 8 Height (cm) for species halimus, lentiformis, nummularia, amaranth and quinoa. Difference in letters above columns indicates a statistically significant difference in mean value between treatments within one species. n=6 Except for amaranth: n=5.

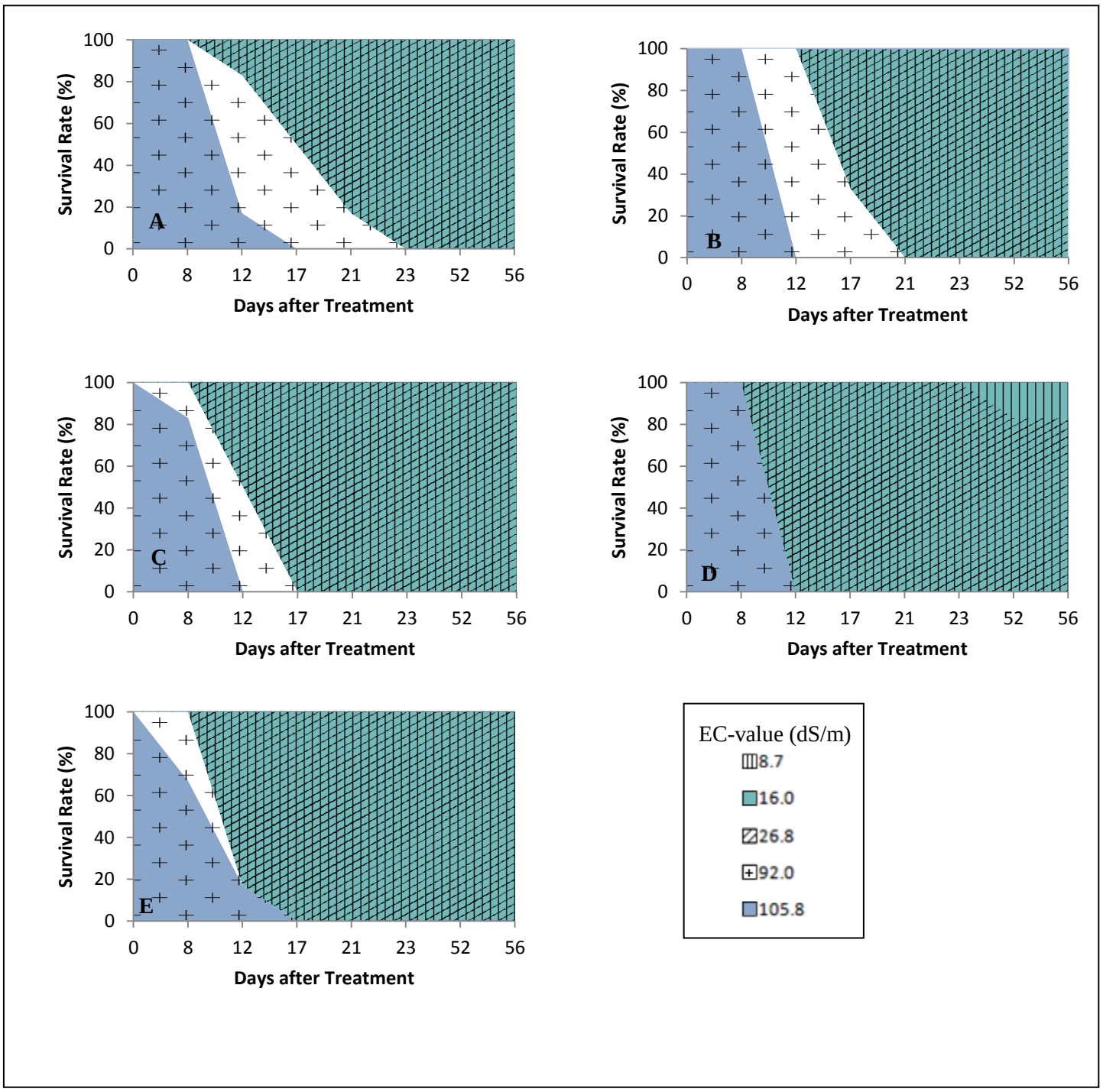


Figure 9 Survival rate over time of plants (given in percentage) for A. halimus, B. lentiformis, C. nummularia, D. amaranth, E. quinoa. 100 % = 6 alive replicates, 83 %= 5 alive replicates, 66 %= 4 alive replicates, 50 %= 3 alive replicates, 33 %= 2 alive replicates, 17 %= 1 alive replicate.

3.3 Dry weight

The combined dry weight of stems and leaves, i.e. the total dry weight, decreased as EC_e-values increased for all five species. The statistically significant decrease in total dry weight occurred between treatment 8.7 dS/m and 16.0 dS/m for halimus (p-value<0.001), lentiformis (p-value<0.001) and amaranth (p-value<0.001). There was no statistically significant difference between treatment 16.0 dS/m and 26.8 dS/m. Nummularia had no statistically significant difference between the two lowest treatments but as the EC_e-value increased to

26.8 dS/m total dry weight decreased statistically (p-value: 0.001). For quinoa, total dry weight decreased statistically as the EC_e-value increased to 16.0 dS/m (p-value: 0.014) and decreased further as salinity rose from 16.0 to 26.8 dS/m (p-value<0.001). The one-way ANOVA for dry weight of leaves and the dry weight of stems yielded similar statistical differences between treatments as total dry weight for halimus, nummularia, amaranth and quinoa. For lentiformis the results were similar for total dry weight and dry weight of stem. The dry weight of leaves for lentiformis was statistically different between all treatments and decreased from 8.7 dS/m to 16.0 dS/m (p-value: 0.004) and from 16.0 dS/m to 26.8 dS/m (p-value: 0.034). Figure A and B in Appendix C present bar charts of dry weight of leaves and dry weight of stems. The measurements of total aboveground growth expressed on a fresh weight basis yielded the same trends as DW.

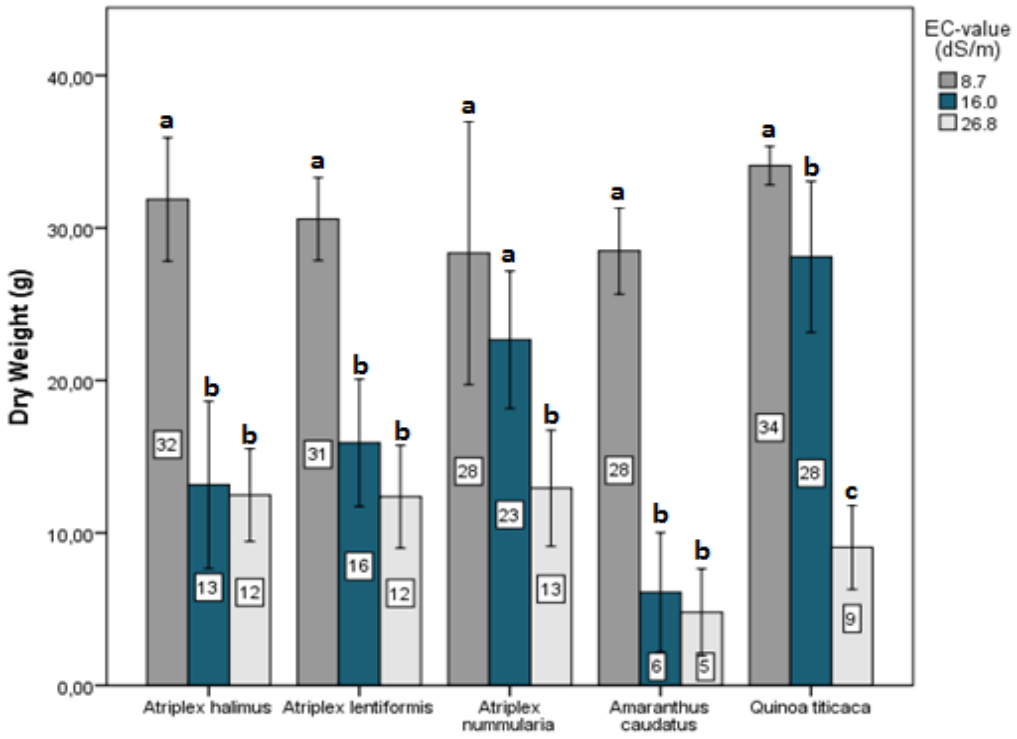


Figure 10 The total dry weight accumulation aboveground (shoot, leaves and inflorescence) measured in g. Difference in letters above columns indicates a statistically significant difference in mean value between treatments within one species.

3.4 Leaf area (cm^2)

Leaf area of lentiformis and nummularia was unaffected by treatment. Halimus had a statistically significant decrease in leaf area going from the lowest to the highest treatment (p-value: 0.024). Treatment 16.0 dS/m was not statistically different from either of the two other treatments. For amaranth leaf area was statistically higher than at 16.0 (p-value<0.001) and 26.8 dS/m (p-value<0.001). Between the two highest treatments there was no statistically significant difference. For quinoa, leaf area decreased as EC_e-values increased with a statistically significant difference between all three treatments. Between 8.7 and 16.0 dS/m the p-value was 0.034 and between 16.0 and 26.8 dS/m the p-value was <0.001.

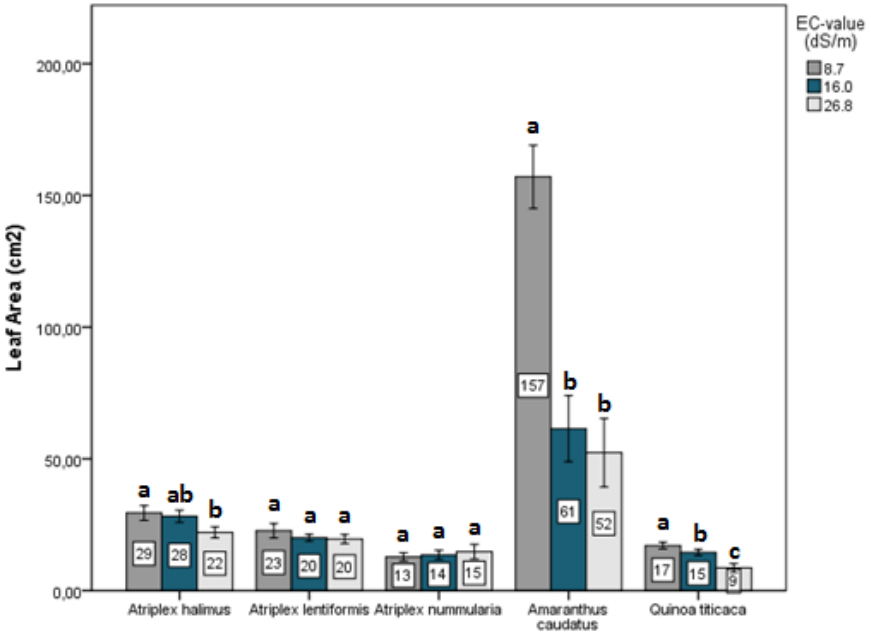
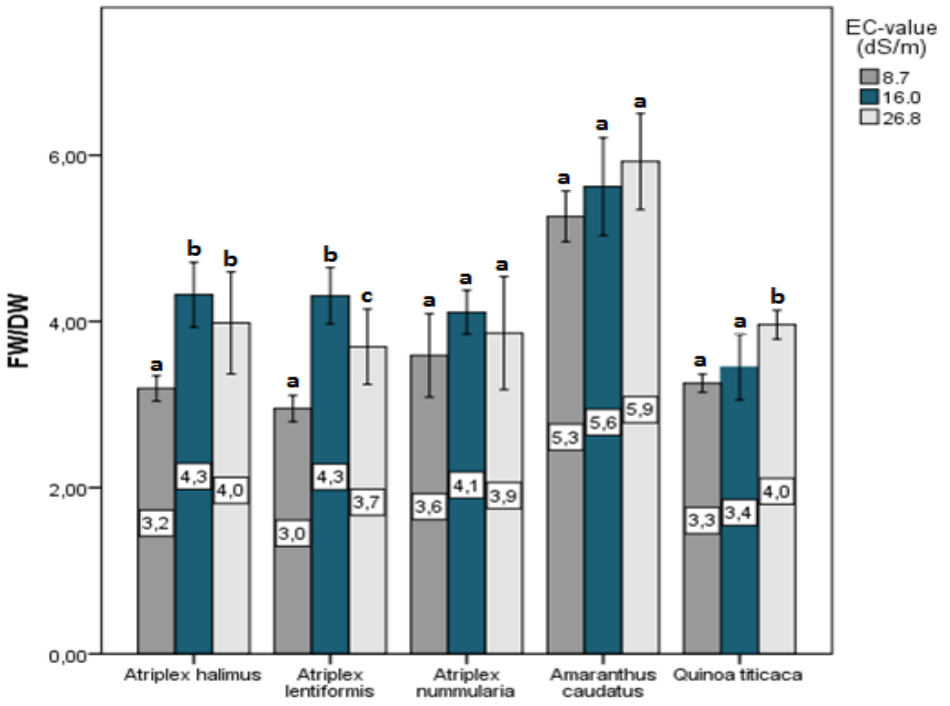


Figure 11 Leaf area (cm^2). n=18 except for amaranth at 26.8 dS/m n=15. Difference in letters above columns indicates a statistically significant difference in mean value between treatments within one species.

3.5 FW/DW

There was a statistically significant difference between all three treatments for lentiformis with an initial increase in FW/DW going from EC_e 8.7 dS/m to 16.0 dS/m (p-value<0.001) followed by a decrease in FW/DW going from 16.0 dS/m to 26.8 dS/m (p-value:0.050). The FW/DW ratio for 26.8 dS/m was significantly lower than the initial value at 8.7 dS/m(p-value: 0.016). The FW/DW at 8.7 dS/m was statistically lower than treatment 16.0 dS/m (p-value: 0.001) and 26.8 dS/m (p-value: 0.012) for halimus. Quinoa showed no statistically significant difference between treatment 8.7 and 26.8 dS/m but were both significantly lower than at 26.8 dS/m (p-values<0.001 and 0.007 respectively). The ratio between fresh weight and dry weight was not affected by treatment in nummularia and amaranth.



3.6 Leaf Succulence

Leaf succulence was unaffected for halimusand nummularia. The leaf succulence for lentiformis was significantly higher at 16.0 dS/m than at 8.7 dS/m (p-value <0.001) but between the highest and lowest salinity there was no statistically significant difference. For amaranth 16.0 dS/m was not difference from either of the two other treatments. The highest treatment had a significantly higher succulence than at the lowest treatment (p-value: 0.021). Treatment 26.8 dS/m yielded the highest succulence in treatment 26.8 dS/m which was statistically different from both 8.7 dS/m (p-value: 0.008) and 16.0 dS/m (p-value: 0.004).

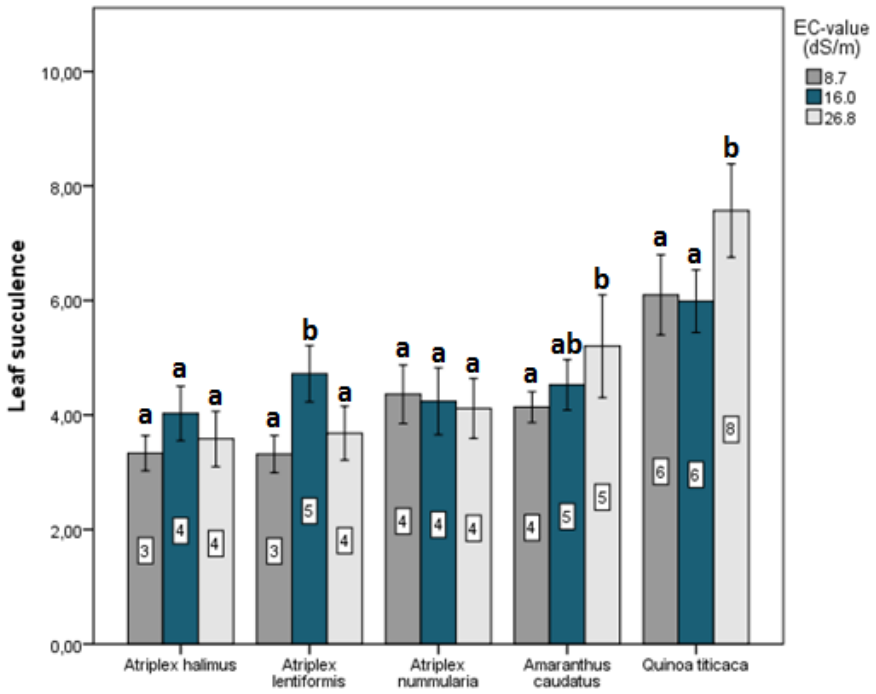


Figure 12 Leaf succulence measured as average FW/DW of 3 individual leaves. inDifference in letters above columns indicates a statistically significant difference in mean value between treatments within one species.

3.7 Water use efficiency (WUE)

For all five species, increasing salinity lead to a reduced WUE measured as dry matter (g) per kg unit of evapotranspiration. Halimus, lentiformis and amaranth all experienced a reduced WUE between treatment 8.7 and 16.0 dS/m with p-values <0.001 for halimus and amaranth and 0.003 for lentiformis. No further reduction in WUE was identified as salinity rose to 26.8 dS/m. For nummularia and quinoa the decline in WUE occurred between treatment 16.0 and 26.8 dS/m with p-values of 0.007 for nummularia and <0.001 for quinoa. The largest decline in WUE for a statistically significant difference was observed for amaranth. The WUE was reduced by 68 % compared to 51 % for quinoa, 45 % for halimus, 31 % for nummularia and lentiformis with the smallest reduction of 28 %. The cumulative water use is displayed in a graph in Appendix D.

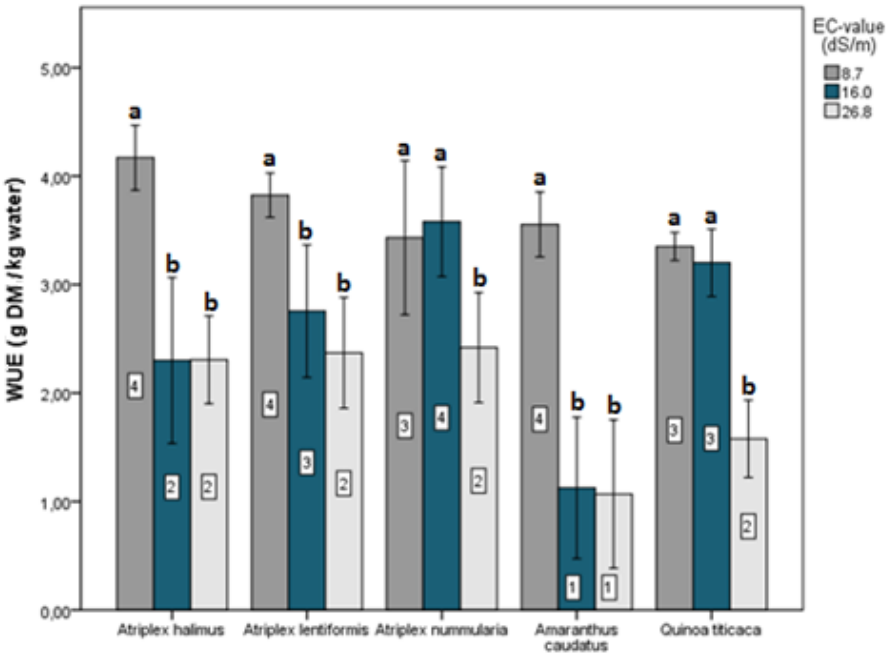


Figure 13 Water use efficiency measured as g dry matter/ kg water. Difference in letters above columns indicates a statistically significant difference in mean value between treatments within one species.

3.8 Stomatal resistance

For amaranth there was a positive correlation between EC_e-value and stomatal resistance with a statistically significant difference between all three treatment. The overall increase in stomatal resistance between treatment 8.7 dS/m to 26.8 dS/m was 7.89 s/cm equivalent to a 58 % increase. with salinity. For quinoa, the stomatal resistance of the highest EC_e-value was statistically different from the two lower treatments with an increase between the highest and lowest EC_e-value of 6.87 s/cm equivalent to a 57 % increase. Although the increase in stomatal resistance between treatment 8.7 dS/m and 16.0 dS/m was not statistical significant it indicates that stomatal resistance is positively correlated with salinity stress. For nummularia, the lowest EC_e-value yielded a stomatal resistance statistically smaller than for treatment 16.0 dS/m and 26.8 dS/m. The

rise in stomatal resistance was 3.35 s/cm (63 %) for treatment 16.0 dS/m and 3.17 s/cm (59 %) for treatment 26.8 dS/m. The trend for halimus and lentiformis is less unequivocal. For both species the lowest stomatal resistance was observed for the medium EC_e-value which was statistically lower than both 8.7 dS/m and 26.8 dS/m. The data therefore indicates that as salinity stress goes up from 8.7 to 16.0 dS/m stomatal resistance is decreased by 1.18 s/cm for halimus and 2.58 s/cm for lentiformis only to increase. As the EC_e went from 16.0 dS/m to 26.8 dS/m stomatal resistance for halimus and lentiformis increased with 1.81 s/cm and 3.5 s/cm respectively.

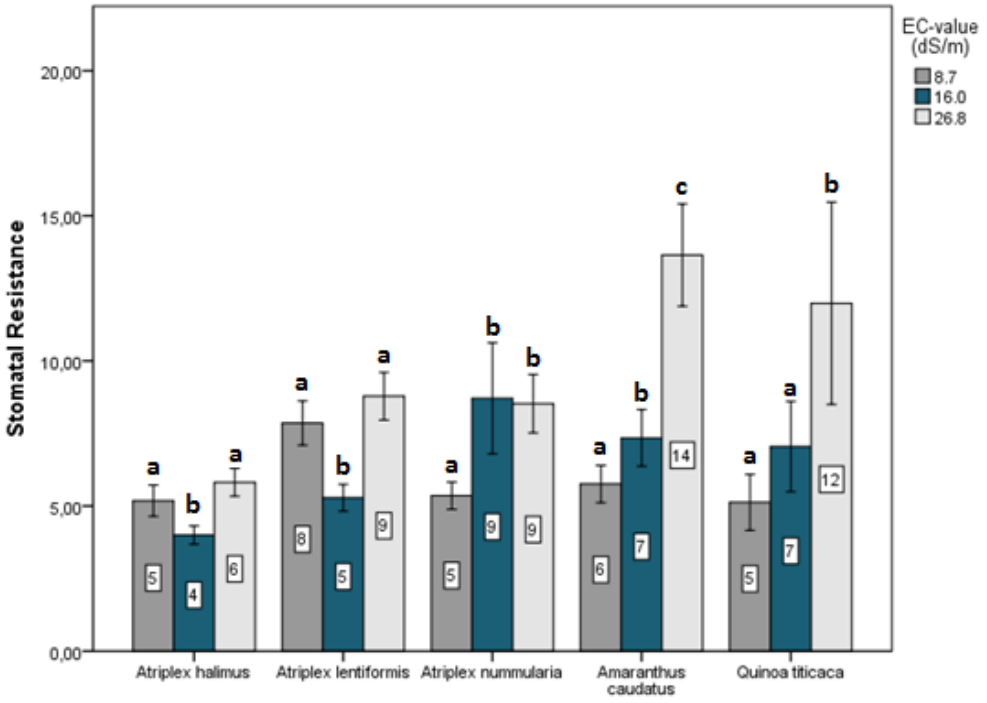


Figure 14 Stomatal resistance (s cm^{-1}) for species halimus, lentiformis, nummularia, amaranth and quinoa. Difference in letters above columns indicates a statistically significant difference in mean value between treatments within one species. n=54 for halimus, lentiformis, nummularia and quinoa. n=45 for amaranth at 26.8 dS/m and n= 51 for quinoa at 26.8 dS/m.

3.9 Chlorophyll content index (CCI)

For halimus and nummularia the range in salinity between the three treatments had no statistically significant effect on CCI. For lentiformis, the CCI was significantly lower at 16.0 dS/m compared with both 8.7 and 26.8 dS/m (both p-value<0.001). For amaranth, the CCI was 28.2 (±6.67) at an EC_e-value of 8.7 dS/m, with a small, statistically insignificant, increase at treatment 16.0 dS/m. The plants exposed to the highest treatment resulted in CCI statistically lower than the 8.7 (p-value< 0.001) and 16.0 dS/m (p-value< 0.001) treatments. The response in CCI to salinity for quinoa was similar to that of amaranth and decreased slightly between the lowest and medium treatment and was statistically reduced at the highest treatment (both p-values<0.001).

The data suggest that the salinity threshold where CCI is affected in amaranth and quinoa lies between an EC_e-value of 16.0 and 26.8 dS/m. Pertaining to the effect salinity has on CCI the data has not elucidated an overall trend applicable to all five species which appear to engage in different strategies as salinity is exacerbated. Overall it appears that the three Atriplex species have a higher amount of chlorophyll content than amaranth and quinoa. CCI was the only parameter where halimus and lentiformis proved to have a statistically significant difference between their response to salinity which also is apparent in *Figure 15*.

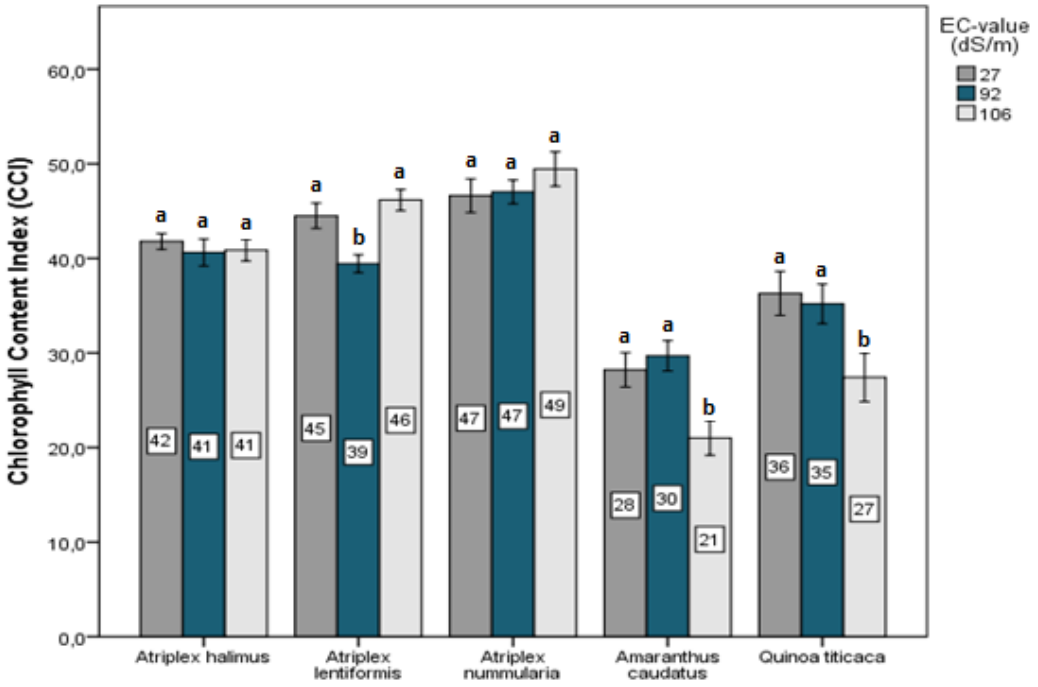


Figure 15 CCI (arbitrary units) for species halimus, lentiformis, nummularia, amaranth and quinoa. Difference in letters above columns indicates a statistically significant difference in mean value between treatments within one species. n=54 for halimus, lentiformis, nummularia and quinoa. n=45 for amaranth at 26.8 dS/m and n= 51 for quinoa at 26.8 dS/m.

3.10 Ion accumulation

Mean (±SD) for Na^+, K^+ and K^+/Na^+-ratio can be found in *Table 6*. As a supplement the bar charts using transformed data can be found in Figure F,G and H in Appendix C. For halimus and nummularia no statistically significant difference in total Na^+ content in leaves was found between treatments. For lentiformis there was only a statistically significant difference between treatment 8.7 and 16.0 dS/m resulting in a smaller Na^+ content at the lowest salinity level (p-value: 0.004). For amaranth Na^+ content was statistically reduced in treatment 26.8 dS/m when compared with 8.7 dS/m (p-value: 0.0013). The Na^+ content at 16.0 dS/m, with a high standard deviation, is also higher than at 8.7 dS/m albeit not statistically significant (p-value: 0.074). Results for quinoa indicate that Na^+ is increasing with salinity. Similarly to amaranth standard deviations were high at 16.0 and 26.8 dS/m and the one-way ANOVA failed to find any

statistically significant differences. Total leaf K^+ was unaffected by salinity exposure in nummularia, amaranth and quinoa. In halimus, the K^+ content was statistically higher at 16.0 dS/m compared to 8.7 dS/m (p-value: 0.034). For lentiformis treatment 16.0 dS/m K^+ content was significantly higher compared to 8.7 dS/m (p-value: 0.005) and 26.8 dS/m (p-value: 0.035). The K^+/Na^+ was unaffected in halimus, lentiformis, nummularia and quinoa. Amaranth maintained a higher K^+/Na^+ ratio at 8.7 dS/m compared to the highest salinity (p-value: 0.024) and medium salinity albeit this difference was not statistically significant (p-value: 0.053).

Species	**EC_e** dS/m	**Na^+** (µg/mg FM) Mean (±SD)	**K^+** (µg/mg FM) Mean (±SD)	**K^+/Na^+** (unitless) Mean (±SD)
Halimus	8.7	146.6±23.33	13.7±2.42	0.094±0.010
	16.0	257.2±41.76	23.0±1.36	0.091±0.013
	26.8	212.0±86.03	19.2±5.30	0.093±0.012
Lentiformis	8.7	109.2±9.34	12.6±2.59	0.114±0.014
	16.0	209.4±23.55	25.5±1.83	0.123±0.019
	26.8	156.8±64.63	16.2±3.38	0.109±0.025
Nummularia	8.7	223.7±67.17	13.1±7.30	0.059±0.033
	16.0	279.3±25.83	22.8±3.43	0.083±0.019
	26.8	269.2±102.03	21.8±5.37	0.084±0.014
Amaranth	8.7	1.87±0.22	15.8±2.40	8.43±0.34
	16.0	29.74±19.05	24.1±5.70	1.28±1.13
	26.8	11.09±3.83	24.0±0.25	2.32±0.72
Quinoa	8.7	12.85±0.56	28.91±1.57	2.25±0.06
	16.0	23.89±13.66	32.94±5.64	1.57±0.53
	26.8	53.08±25.99	38.19±3.64	1.58±0.70

Table 6 Overview of mean values (±SD) for Na^+, K^+ and K^+/Na^+. n=3.

4. Discussion

4.1 Salinity threshold for plant survival

Halophytes are plants that tolerate high levels of salinity where most crops, the glycophytes, fail to survive and reproduce (Richards 1954). Based on the commonly used threshold, 4 dS/m, for distinguishing between halophytes and glycophytes all five species can be classified as halophytes since they survived salinity exposure significantly higher than EC_e=4 dS/m. In the two highest treatments all six replicates of all five species died indicating that the threshold for survival lies somewhere between 26.8 and 92.0 dS/m. Amaranth only had 5 surviving replicates which showed signs of leaf senescence (*Figure 4*) in all three treatments and had an overall reduction in FW between 8.7 and 26.8 dS/m of 81 %. Quinoa similarly showed great reductions in FW (68 %) and leaf senescence at 26.8 dS/m (*Figure 5*). The three *Atriplex* species showed no signs of leaf senescence and plants appeared vigorous at 26.8 dS/m. Although it cannot be said with certainty where exactly in the range of 26.8-92.0 dS/m the threshold for survival lies for the individual species, it appears that for the three Atriplex species it is not close to 26.8 dS/m. Several previous studies support that the threshold for survival lies beyond 26.8 dS/m for halimus (Hamid et al. 2011; Alla et al. 2012; Belkheiri and Mulas 2013), lentiformis (Zhu and Meinzer 1999; Glenn et al. 2012) and nummularia (de Araújo et al. 2006; Bazihizina et al. 2012; Belkheiri and Mulas 2013). Studies have found survival thresholds above 92.0 dS/m for both halimus and nummularia. Belkheiri and Mulas (2013) observed 100 % survival at salinity exposure of 1000 mM NaCl (≈ EC_e of 100 dS/m) for halimus and nummularia and nummularia survived 1500 mM NaCl (≈ EC_e of 150 dS/m) in a study by Bazihizina et al. (2012). The discrepancies in where the threshold lies exactly may arise from varietal differences (Belkheiri and Mulas 2013) or due differences in seedling age at the onset of salinity exposure. The newly germinated seedlings of this study were exposed to salinity 25 days after sowing whereas the propagated cuttings in the studies by Belkheiri and Mulas (2013) and Bazihizina et al. (2012) were 50 and 84 days old respectively. It has previously been established that the seedling stage, along with germination, has been defined as a salt susceptible phenological stage of Atriplex species (Bajji et al. 2002). Variations in salinity tolerance has similarly been associated with varietal differences and found early establishment to be a salt sensitive stage for quinoa (Ruiz-Carrasco et al. 2011, Adolf et al. 2013). It may be concluded that the salinity threshold for survival depends on variety and phenological stage at onset of exposure.

Mere survival is not the only relevant criteria for success to consider when assessing the salinity tolerance threshold for a species. It is important to consider yield and reproductive capacity as well. Plants unable to reproduce, especially annuals, make farmers dependent on buying seeds in order to continue cultivation. The quality and quantity of grains were not analyzed for amaranth or quinoa but the reductions on size of amaranth and quinoa inflorescence, which were clearly visible (*Figure 4* and *Figure 5* respectively), indicate that seed quantity was reduced at 26.8 dS/m. Further analysis is needed to establish whether or not amaranth or quinoa are able to reproduce at high salinities.

4.2 Increasing salinity inhibits growth and reduces WUE

Adverse impact from increases in salinity was observed for all five species in DW and FW due to reductions in both leaves and stems. Height decreased significantly in response to salinity for halimus, lentiformis, amaranth and quinoa. Albeit statistically not significant, reductions in height appear to occur in nummularia as well. Although all plants were adversely affected the degree varied between some of the species. Halimus and lentiformis did not differ significantly in response to increasing salinity in either height, DW and FW indicating that salinity inhibits these two species in a similar manner. Amaranth and quinoa proved to most frequently adopt a different pattern of response than the other species. It seems rational that the three Atriplex species would resemble each other more and that amaranth and quinoa would differentiate themselves to a higher degree. For quinoa the differences were primarily caused by a higher overall reduction meaning that quinoa was significantly more inhibited in growth at the highest treatment compared to the lowest. Amaranth on the other hand differed from the other species primarily due to the higher degree of adverse impact induced at 16.0 dS/m compared with 8.7 dS/m. In general it was observed that for amaranth it was the shift from 8.7 to 16.0 dS/m that produced differences in growth whereas the difference between treatment 16.0 and 26.8 failed to show any significant effect. Even halophytes may experience growth reductions as a result of high intensity salinity exposure. Growth reductions due to increasing salinity have similarly been found in other studies for halimus (Bajji et al. 1998), lentiformis (Glenn et al. 2012) and amaranth (Omamt et al. 2010; Ormami and Hammes 2013). Contrasting findings have been found for both nummularia and quinoa. Bazihizina et al. (2012) found DW to decrease as salinity increased for nummularia whereas de Araújo et al. (2006) found that salinity initially had a stimulating effect on DW (0-300 mM NaCl) and that DW was only adversely affected after 300 mM NaCl. For quinoa salinity was found to reduce growth in two independent studies (Orsini et al. 2011; Eisa et al. 2012). Hariadi et al. (2011) found that in titicaca, height was more adversely affected by salinity than shoot FW which was barely statistically significant contradicting the findings of this study where FW was reduced 68 % but height was only reduced 43 %. Pertaining to nummularia the discrepancies between studies may be caused by varietal differences but this cannot be the explanation for quinoa since Hariadi et al. (2011) used the same variety as this study. Differences in water availability in the two experimental designs may be the explanation since symptoms of water deficiency causes reductions in cell expansion, stomatal conductance and CO_2 assimilation as well as loss of turgor (Razzaghi 2011). The differences in the relative impact on FW and height illustrates that there are factors besides the degree of salinity, such as water availability that may interact with the effect of salinity. Overall it can be concluded that the increasing levels of salinity severely inhibits growth due to alterations in both biomass production and height but the degree of inhibition varies between species.

The WUE decreased significantly with increasing salinity for all five species indicating the efficiency of utilizing water for carbon assimilation is adversely affected by salinity. The fact that the three atriplex species were the least affected is in accordance with the assumption that C_4 species have higher WUE

efficiency (Lambers et al. 2008; Glenn et al. 2012). Nevertheless it appears conspicuous that WUE declines for all five species because it contradicts the common assumption of increased WUE as salinity and drought intensifies (Ayala and O'leary 1995; Glenn et al. 1998; Lambers et al. 2008) as well as the findings of other studies on lentiformis (Glenn et al. 2012), nummularia (Bazihizina et al. 2012; Hussin et al. 2013), amaranth (Omamt et al. 2010) and quinoa (Eisa et al. 2012). Due to the inherent relationship between net photosynthesis, stomatal resistance and transpiration (Lambers et al. 2008), as explained in the introduction, it seems unlikely from a theoretical point of view that the decrease in WUE is caused by stomatal limiting photosynthetic inhibition. That means that an inhibition of the photosynthetic capacity due to non-stomatal limitation should explain the decrease in WUE. Whether this is the case is uncertain as only CCI was estimated and there is no clear correlation between CCI and WUE which could explain the reduced WUE for all five species. An alternative explanation is related to the methodology behind measuring evapotranspiration as well as the assumption the evapotranspiration works as a proxy for transpiration. This will be discussed further in Section

4.3 Osmotic effect of increasing salinity

Osmotic stress due to salinity induces rapid reductions in cell elongation and affected morphological parameters are reductions in emergence of new leaf material and smaller leaf area (Munns and Tester 2008). As salinity increases the soil osmotic potential becomes more negative and induces drought-like symptoms, which can be observed by increases in stomatal resistance (Munns and Tester 2008). Consequently it is possible to distinguish the osmotic effect from the ionic effect by observing stomatal resistance and leaf area.

Stomatal resistance increased in response to salinity for nummularia, amaranth and quinoa indicating that all three species were adversely affected by the osmotic component of the increasing salinity. In amaranth and quinoa, the significant reduction in leaf area provides additional evidence of the osmotic effect. Height, which was affected in all three species, may also indicate reductions in the rate of cell elongation. It therefore seems likely for osmotic stress to play a significant, albeit not necessarily exclusive, role in the growth inhibition observed for nummularia, amaranth and quinoa. Similar findings pertaining to increased stomatal resistance have been reported for nummularia (Bazihizina et al. 2012; Hussin et al. 2012), amaranth (Omamt et al. 2010; Ormami and Hammes 2013) and quinoa (Orsini et al. 2011; Razzaghi et al. 2011; Adolf et al. 2012). The down regulation of stomatal aperture under salinity has been suggested as an adaptive water conserving mechanism to cope with the unfavorable gradient in osmotic potential between soil and plant (Koyro et al. 2006). The increased stomatal resistance comes at a cost and is associated with a reduced rate of net photosynthetic assimilation, i.e. the stomatal limitation of photosynthesis (Martinez et al. 2003; Geissler et al. 2010; Omamt et al. 2010; Pérez-López et al. 2012). Although reductions in stomatal aperture almost certainly reduces plant photosynthesis the exact causality between growth inhibition and photosynthesis has not been clearly established and reductions in individual leaf area due to the osmotic effect cannot solely be explained by a down-regulated photosynthetic assimilation (Munns and Tester 2008).

A different response in stomatal resistance was observed for both halimus and lentiformis. Stomatal resistance was significantly lower at 16.0 dS/m compared to both 8.7 and 26.8 dS/m. It is conspicuous that stomatal resistance is lowest at 16.0 because it seems to indicate that either the degree of drought experienced by the plant is lowest at 16.0 dS/m or that the plants adopt a strategy under 16.0 dS/m salinity exposure to counteract the increasing drought stress by utilizing the available salts for osmotic adjustments but that this mechanisms is insufficient at the highest salinity level. Osmotic adjustment to favor water uptake by utilizing either inorganic solutes such as Na^+ and K^+ or organic compatible solutes has been suggested as a way for plants to adapt to water-deficient saline conditions but a study on halimus found that despite observed up-regulation of osmolytes such as proline, methionine and sucrose the increase was evaluated as too low to regulate the osmotic potential of the cytosol (Alla et al. 2012). Similar findings for halimus were reported by Bajji et al. (1998). In halimus a higher degree of Na^+ accumulation cannot explain a potential osmotic adjustment as it was unaltered but it appears that K^+ tended to be a little higher at 16.0 dS/m than at the other treatments. For lentiformis, K^+ was accumulated to a higher degree at 16.0 dS/m with a similar trend for Na^+. Nevertheless, it is uncertain to determine conclusively whether the improved stomatal resistance in fact is due to an osmotic adjustment since leaves were not tested for organic osmolytes and because estimates of total leaf Na^+ and K^+ does not elucidate whether it is stored in vacuoles, bladder cells of the cytosol. There is a slight tendency, albeit not statistically significant, for the FW/DW-ratio in leaves to be highest at 16.0 dS/m for both halimus and lentiformis, which could be an indication of improved leaf water status (Adolf et al. 2012). A study on halimus similarly found that relative water content of leaves was higher at the moderate salinity treatments (50 and 200 mM NaCl) compared to 0 and 500 mM NaCl (Hamid et al. 2011). Further analysis is needed to establish whether osmotic adjustment can account for the improved stomatal resistance at 16.0 dS/m but is insufficient at the highest salinity treatment with more progressive water stress.

For amaranth and quinoa FW/DW of leaves was significantly improved at the highest salinity treatment. The higher degree of Na^+ accumulation at 26.8 dS/m suggests that at Na^+ may act as an important osmolyte for improving leaf water status. The findings of Hariadi et al. (2011) concluded that between 80 and 100 % of the osmotic adjustment in quinoa leaves were due to inorganic osmolytes which supports the idea that for quinoa accumulation of leaf Na^+ is an essential adaptive mechanism to cope with the osmotic effect of salinity.

4.4 Species display variations in dealing with ionic stress.

Plants have two primary options to deal with the ionic effect toxic concentrations of Na^+ can cause in leaf cell cytosol. Firstly by salt exclusion through restricted Na^+ uptake by roots, minimized Na^+ loading into the leaf tissue and/or retranslocation of Na^+ from shoots to compartments. The second option involves tissue tolerance referring to accumulation of Na^+ in the leaves but compartmentalized into vacuoles or salt bladders (Munns and Tester 2008; Shabala et al. 2014). The response in the total Na^+ accumulation in leaves, which

includes cytosolic, vacuolar and salt bladder Na^+, measured over a varying range of salinities elucidates whether plants adopt a salt excluding strategy thereby limiting the loading of Na^+ from the xylem into the leaves.

In this study, total leaf Na^+ in halimus and nummularia were found to be unaffected by increasing salinity within the range of 8.7 and 26.8 dS/m. The results suggest that tolerance to salinity of halimus and nummularia lies in their ability to exclude Na^+ from the leaf tissue rather than their ability to sequester the Na^+ in vacuoles or salt bladders. Halimus and nummularia must have mechanisms to maintain constant leaf Na^+, which could include reduced net Na^+ uptake, reduced loading into the xylem or compartmentalization of Na^+ before reaching the leaves. No clear consensus has been established pertaining to the accumulation of Na^+ in leaves in response to salinity for halimus and nummularia. For nummularia, no difference in leaf Na^+ was found between treatments 100, 300, 600 and 800 mM NaCl (Belkheiri and Mulas 2013). Contrarily, two other studies found that within similar ranges of salinity leaf Na^+ to continuously accumulate as salinity exposure intensified (Ramos et al. 2004; Araújo et al. 2006). For halimus, the positive correlation between Na^+ accumulation in leaves and salinity exposure within the range of 50-500 mM NaCl found by Hamid et al. (2012) contradicts the findings of this study. Belkheiri and Mulas (2013) studied two clones of halimus and also found leaf Na^+ to increase with salinity in one of the varieties. The second clone had the highest Na^+ accumulation at 300 mM NaCl but between treatments 0,600 and 800 mM NaCl there was no difference. A study on halimus done by Alla et al. (2012) found lower leaf Na^+ accumulation at 50 mM NaCl but no difference between treatments 300 and 550 mM NaCl. It seems unlikely that the differences in findings between this and the aforementioned studies are caused by differences in experimental design since Belkeheiri and Mulas (2013) manage to find contradictions in response between two clones of halimus within the same experiment. The discrepancy between studies rather suggests a significant variation in mechanisms conferring to salinity tolerance within the species of both halimus and nummularia. Varietal differences in salinity tolerance and the associated mechanisms are considered a common phenomenon within halophytic species (Flowers and Colmer 2008; Munns and Tester 2008; Cuin et al. 2010; Adolf et al. 2013). The K^+/Na^+ ratio in halimus and nummularia was also unaltered by increasing salinity. It can be concluded that the reduction in growth, observed for both species, as salinity increased, was not caused by leaf Na^+ toxicity since neither Na^+ nor K^+/Na^+ was affected by increasing the levels of salinity exposure. This implies that the osmotic effect is the main stress factor induced by salinity in halimus and nummularia. Ramos et al. (2004) reached a similar conclusion for nummularia by comparing plant response to NaCl and KCl treatments. He found that biomass production was less adversely affected in the NaCl treatments than in the KCl treatments indicating that Na^+ helps alleviate the osmotic stress induced by high concentrations of cations, either as Na^+ or K^+, and that the high EC_e values rather than Na^+ toxicity impaired plant growth.

The results for lentiformis did not yield any clear trend and at 26.8 dS/m there was a large standard deviation indicating variation within between the three replicates in their leaf Na^+ accumulation in response to salinity.

As salinity exposure intensified there appeared to be an initial increase followed by a decrease. Similarly, Glenn et al. (2012) found that Na^+ content in leaves increased as salinity rose from 0 to 86 mM NaCl but then continuously dropped as salinity rose to 171 and 342 mM NaCl followed by a rise at 513 mM NaCl where it reached the highest Na^+ accumulation. Both studies elucidate two important pieces of information pertaining to the ionic effect from increasing salinity. Firstly, lentiformis, unlike the two other *Atriplex* species, does not solely rely on Na^+ exclusion in response to the ionic stress, since the Na^+ accumulation is altered by differences in degree of salinity. Secondly, it is possible that the relative roles of Na^+ exclusion and Na^+ tolerance in tolerating the ionic stress shift as salinity increases. The reasoning behind the increased rate of Na^+ exclusion when salinity is higher is not perfectly clear. Similar results are found for Na^+ measured on a dry weight basis, which implies that the difference is not due to differences in leaf water content. Stomatal resistance was highest at 16.0 dS/m meaning transpiration would consequently be lowest for this treatment. In theory, a reduced rate of water flow through the xylem and leaves would presumably carry less Na^+ to the leaves, which seems to contradict the aforementioned trend of lower Na^+ at higher levels of salinity (Tester and Davenport 2003). A final note worth mentioning pertaining to lentiformis is that Glenn et al. (2012) used pooled data and standard deviations are therefore missing, this study found a high standard deviation for treatment 26.8 dS/m and Zhu and Meinzer (1999) found a continuous Na^+ accumulation between 5 treatments ranging from 50 to 600 mM NaCl. Significant within-species differences may be the cause of the contradicting trends. It appears that further studies regarding Na^+ uptake and transport preferably with more replicates than 3, are needed to elucidate the exact mechanisms involved in lentiformis conferring to this trend shifting trend in leaf Na^+ accumulation.

There appear to be a tendency for titicaca to be salt includer as Na^+ content increased in leaves at higher salinities. For titicaca similar conclusions have been found previously where the highest rate of increase is observed as salinity rises from zero to 100 mM NaCl. (Hariadi et al. 2011; Adolf et al. 2012; Shabala et al. 2013). Both salt bladder and vacuolar compartmentalization have been attributed as mechanisms conferring to Na^+ tolerance in quinoa and Bonales-Alatorre et al. (2012) concluded that the Na^+ sequestration was sufficient to maintain low cytosolic Na^+ concentrations without impairment of the photosynthetic capacity. Two independent studies with quinoa found Na^+ accumulation to be higher in old leaves compared to young leaves of variety *titicaca* (Hariadi et al. 2011) and variety *KVL 52* (Bonales-Alatorre et al. 2012). This may indicate that old leaves are utilized as sinks to exclude Na^+ from young and more metabolically active leaves (Munns and Tester 2008). It may also merely be that the longer lifespan of old leaves leads to a greater Na^+ accumulation but not a difference between young and old leaves in the rate of Na^+ loading (Munns and Tester 2008). Bonales-Alatorre et al. (2012) furthermore found that young leaves relied to a large extent on bladder cell compartmentalization whereas old leaves had few, primarily ruptured, bladders cells and therefore relied on vacuolar compartmentalization. Orsini et al. (2011) found similar trend between old and young bladder cells but no alterations in bladder cell density in neither old nor young leaves in response to

increasing salinities (0-600 mM NaCl) which suggest that bladder cells do assist in sequestering Na^+ away from the cytosol but to a limited extent and that vacuolar compartmentalization also play a role in Na^+ tolerance in quinoa. The increased accumulation of leaf Na^+ in quinoa assists in osmotic adjustment in order to assure water uptake. Hariadi et al. found that between 80-100 % of osmotic cell turgor in leaves was attributed to the presence of the inorganic osmolytes Na^+ and K^+ and concludes that titiaca appear to osmotically adjust solely by utilizing inorganic osmolytes harvested from the soil which was similarly suggested by Ruffino et al. (2010) and Orsini et al. (2011).

Amaranth had an increase in Na^+ content implying that much like titicaca amaranth is a Na^+ includer. Similar results were found for *Amaranthus cruentus* and *A. tricolor* in a study conducted by Ormami and Hammes (2013). Amaranth does not have any salt secreting bladder cells and relies solely on vacuoles to compartmentalize Na^+.

4.5 Changes in K^+/Na^+ are caused by alterations in Na^+ but not K^+ accumu-lation in leaves

The K^+/Na^+-ratio, which was unaffected in the three *Atriplex* species, appeared to be declining with increasing salinity for both amaranth and quinoa. This effect is due to the increased accumulation of Na^+ as K^+ was unaffected by salinity. Since K^+ is an essential nutrient to plants responsible for activating over 50 enzymes and that Na^+ competes for major binding sites in metabolic processes, it has been reported that the ability to maintain a high cytosolic K^+/Na^+-ratio is one of the more important factors contributing to Na^+ tolerance in plants (Adolf et al. 2012). It is difficult to say precisely how the accumulated Na^+ affects the leaves since measuring total leaf Na^+ does not elucidate whether the excess Na^+ has been compartmentalized or is causing damage in the cytosol by replacing K^+. Furthermore it was highlighted in a study by Cuin et al. (2010) that it is important to distinguish between total leaf K^+ and leaf sap K^+ because the latter does not include structural K^+, which may be utilized by salinity stressed plants in order to restore the K^+/Na^+-ratio. Signs of senescence in the form of yellowing were observed on some leaves while others eventually died off (unpublished data) in treatment 26.8 dS/m for quinoa (see picture in *Figure 5*) and in both 16.0 and 26.8 dS/m for amaranth (See picture in *Figure 4*). This indicates that both plants experienced ionic stress consequently causing leaf senescence since osmotic stress does induce reductions in leaf elongation and production of new leaf material but not senescence. Further analysis is needed elucidating where in the leaf tissue Na^+ and K^+ is located to conclusively assess whether the altered K^+/Na^+-ratio is in fact indicative of ionic damage but it does appear that the ionic stress adversely impacts amaranth at 16.0 and 26.8 dS/m and quinoa at 26.8 dS/m.

4.6 Chlorophyll content

The chlorophyll content of leaves can be used to assess the non-stomatal limitation of photosynthesis as chlorophyll plays a crucial role in harvesting energy from irradiance, which is used to drive photosynthesis (Maksimovic et al. 2013). Reductions in chlorophyll content arise from either increased degradation of chlorophyll or inhibitions in chlorophyll biosynthesis (Santos 2004). Three different categories of response

to increasing salinity were revealed by the two-way ANOVA of the interaction between species and treatment. The chlorophyll content of amaranth and quinoa was significantly reduced by salinity. Similar results have been reported in previous studies on *Amaranthus tricolor (*Gambarova and Gins 2008) and titicaca (Adolf et al. 2012). Lutts et al. (1996) attributed reductions in chlorophyll content to the toxic effect of increased accumulation of Na^+, which is in accordance with this study's conclusion that amaranth and quinoa appear to be salt includers. This suggests that the decrease in chlorophyll content may be caused by degradation of chlorophyll but reductions in biosynthesis may still play a role. Reductions in chlorophyll content has been suggested to relate to plants with higher sensitivity to salinity (Cha-Um and Kirdmanee 2009; Adolf et al. 2012; Kanawapee et al. 2012; Saleh et al. 2012), although Shabala et al. (2012) failed to reach this conclusion.

Halimus and nummularia showed no salinity induced alterations in chlorophyll content. A previous study by Ben Hassine and Lutts (2010) reached similar conclusions pertaining to halimus exposed to well-watered saline conditions whereas drought, in the form of PEG, adversely affected chlorophyll suggesting that chlorophyll content is triggered by the osmotic effect rather than the ionic effect. 5-Aminolevulinic acid, an integral part of chlorophyll biosynthesis, is activated by K^+ (Santos 2004: Adolf et al. 2012). K^+ was not reduced by salinity in neither halimus nor nummularia and the ability to retain K^+ in leaves may play a part in maintaining chlorophyll content under salinity exposure in some species.

For lentiformis medium salinity exposure plants were found to have the lowest chlorophyll content compared with both treatment 8.7 and 26.8 dS/m. The reduction of chlorophyll at 16.0 dS/m does not seem to correlate with leaf K^+, which was highest at this salinity level for lentiformis. Santos (2004) studied biosynthesis and degradation of chlorophyll content in sunflower (*Helianthus annuus* L.) and found that moderate, but not severe, salinity stress induced an increase in the enzyme chlorophyllase, which is considered as the initial step in chlorophyll degradation due to removal of phytol. At severe salinity exposure the activity of chlorophyllase was inhibited and the decrease in chlorophyll was rather associated with inhibition of chlorophyll biosynthesis due to a salinity induced up-regulation of 5-Aminolevulinic acid dehydrogenase. This observation of two different mechanisms resulting in chlorophyll reduction could explain why chlorophyll content initially decreased at 16.0 dS/m only to increase again at 26.8 dS/m if the relative degree of impact differs between chlorophyll degradation and biosynthesis. Estimates of chlorophyll content has been found to be affected by leaf water content as well as leaf thickness (Merenco et al. 2009) and the differences in chlorophyll content may solely arise from the differences in leaf FW/DW of lentiformis. Reductions in chlorophyll content may assist the plant by reducing the rate of flow of electrons through the photosystem and reduce the risk of photoinhibition. The reduction in CCI for amaranth and quinoa may be associated with the increase in stomatal resistance to reduce the risk of photoinhibition (Santos et al. 2004).

4.7 Comments on experimental design

4.7.1 Transient differences in drought intensity between treatments

All plants were watered up to 80 % field capacity at each watering session to assure plants had similar water availability. The calculations were done without taking into account the weight of the plants as was done by (Razzaghi et al. 2011). By accounting for the aboveground FW of plants at harvest it was found that by the end of the experiment plants were only watered up to approximately 70 % of water holding capacity. This estimate ranges between 68.6 and 75.3 % between species and treatments and is most likely even lower if belowground weight was included. This consequently has a two-fold impact on the stress induced in the plants. Firstly, it means that the relative role of drought, experienced by the plants, has been progressively exacerbated, as plants grew larger. Secondly, it means that, as plants progressively were watered further and further below the 100 % water holding capacity the less accurate became the EC_e-values used as proxies for the salinity exposure as they provide estimates of the electrical conductivity in a saturated soil paste. The actual EC-values of the soils, which the plants in this experiment have been exposed to, may be substantially higher (Rengasamy 2002; Shabala and Munns (in shabala 2012)). A counteracting effect may be seen from the Na^+ removal undertaken by the plants (Hariadi et al. 2011). On a slightly brighter side of things, this exacerbation of drought stress was occurring for all species and all treatments. As it has been concluded previously in this thesis the role of osmotic and ionic stress mechanisms may very well vary across a salinity gradient and it is therefore imperative to know if changes in EC-values and Na^+ availability in the soil is altered throughout the experiment. An improvement of the experimental design would have been to take assess the EC and concentrations of soil Na^+ during and by the end of the experiment. Additionally it would have been more optimal to water plants up to 100 % water holding capacity and on a daily basis. The first was not possible due to poor soil properties that resulted in leaching of water and soluble salts, which could have been avoided by using soil with better structure. The latter was unfortunately not possible due to transport restrictions.

4.7.2 Measurements of evapotranspiration

Although this method of using evapotranspiration as a proxy has been successful before (Razzaghi et al. 2011) it may be possible that due to the reduced growth in response to increasing salinity a higher degree of evapotranspiration may have been occurring overestimating the water use by the smaller more salinity stressed plants. Pots with similar soils as treatment 8.7, 16.0 and 26.8 dS/m but no plants, had an average daily water loss of 91±29 g of water (Appendix J.) which is equivalent to 10.5 % of the total water in the pots at 80 % water holding capacity. It is therefore likely that in the initial stages of the experiment where plants were small, evapotranspiration was high enough to affect the measurements of transpiration.

4.7.3 Obstacles in comparison of plant response to salinities between studies

Throughout my literature search, I have discovered two interesting issues that are important to consider when comparing different studies pertaining to plant response to salinity exposure. Firstly it is a matter of absence

in consensus regarding the proxies used for degree of salinity exposure. It is common practice in salinity experiments to induce salinity stress solely through addition of NaCl and use mM NaCl as the proxy for salinity (Cuin et al. 2009; Adolf et al. 2012; Bazihizina et al. 2012; Shabala et al. 2013) as opposed to using EC of the soil medium (Razzaghi et al. 2011; Kanawapee et al. 2012) or a combination of both (Ormant et al. 2010;). Bajji et al. (2002) reported salinity exposure in both osmotic potential and mM NaCl. EC-values are affected by the presence of Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, SO_4^{2-}, CO_3^{2-}, NH_4^+, $NO_3^-$and HCO_3^- and no exact relationship between EC and total dissolved salts has been established (Hardie and doyle in Shabala and Cuin 2012). It has been suggested that an EC_e-value of 4 dS/m is equivalent to an osmotic potential of 0.2 MPa (Munns and Tester 2008) and 40 mM NaCl in a soil where Na^+ and Cl^- are the predominant soluble salts (Hardie and Doyle in Shabala and Cuin 2012). This conversion in based on EC of a saturated paste and Shabala and Munns (in shabala 2012) conclude that the conversion from 4 dS/m could be equivalent to as high as 80-100 mM NaCl under non-saturated soil conditions. In the study by Ormant et al. (2010) which used the same soil for all treatments and but variations in amount of added NaCl, the conversion factor for converting EC_e-values to mM NaCl ranged between 6.1 and 8.3. In this study, which utilized naturally saline soil from different sites, the conversion factor ranged between 9.7 and 19. This illustrates that the relationship between EC_e and mM NaCl is not constant. Previous literature have highlighted the importance of distinguishing between the osmotic effect i.e. the EC or osmotic potential of soil and the ionic effect caused by Na^+ (Tester and Davenport 2003; Flowers and Colmer 2008; Munns and Tester 2008; Cuin et al. 2009; Hariadi et al. 2011;). It therefore seems imperative to provide EC_e-values alongside concentrations of NaCl of the soil medium especially since peer-reviewed research has a tradition for strengthening findings through comparison with similar studies.

Secondly, some studies use 0 mM NaCl as the control condition compared to only one treatment level of salinity when assessing halophytic plant response to salinity which is a twofold issue. In studies on halophytes which have been shown to achieve optimal growth at above-zero salinity levels due to an efficient utilization of Na^+ as an inorganic osmoticum e.g. quinoa (Hariadi et al. 2011), halimus (Alla et al. 2012), nummularia (Ramos et al. 2004), the moderate salinity level induces a positive effect whereas the zero salinity level appear to be the stressed treatment due to insufficient availability of Na^+. In combination with the absence of more than one salinity treatment the experimental design run the risk of overlooking important information because the plant mechanisms may be substantially different when comparing zero salinity to an optimal level of salinity i.e. the plant response is overall positive vs. comparing optimal salinity levels with varying levels of increasing salinity which impose actual stress on the plants i.e. the plant response to salinity is negative. To give an example, Belkheiri and Mulas found that between 0 and 100 mM NaCl leaf growth rate as well as leaf Na^+ increased for nummularia. Looking at the plant response between 300 and 800 mM NaCl leaf growth rate decreased and Na^+ remained unaltered. This variation in response has been found by several other studies (Hariadi et al. 2011; Alla et al. 2012; Eisa et al. 2012; Glenn et al.

2012; Hussin et al. 2013). The comment does not serve to discredit the findings of studies using two treatments whereby one has zero salinity it is merely aimed to highlight the relevance of looking at various levels of salinity to uncover potential differences in plant mechanisms in play under stimulating salinity exposure and inhibiting salinity exposure.

5. Conclusion

The effect of salinity on plant growth and physiological mechanisms is a multifaceted trait with numerous different responses dependent on species and level of salinity. Pertaining to survival and growth it was clear that all five species had a threshold below an EC_e of 92.0 dS/m when watered up to 80 % water holding capacity. Severe inhibition on growth, both in terms of dry weight and fresh weight, was observed in response to increasing salinity. Nevertheless all five species classifies as halophytes as they survived in the three lowest salinity treatments which were all significantly more saline than the halophytic threshold of 4.0 dS/m. In amaranth and quinoa, the growth reductions were associated with premature leaf senescence seen especially as yellowing of leaves. Despite an improvement in leaf water status, potentially due to adjustment of either stomata or the osmotic both species showed clear signs of premature leaf senescence, indicating that the Na^+ including strategy, seemingly in order to osmoregulate, comes at a high costs for amaranth and quinoa. Due to the potential relationship between increases in stomatal resistance and chlorophyll content reductions it may be hypothesized that amaranth and quinoa have mechanisms to down-regulate chlorophyll biosynthesis or up-regulate chlorophyll degradation in response to the salinity-induced increases in stomatal resistance as a defense mechanism against photoinhibition. The three atriplex species showed no tendency to accumulate neither more nor less Na^+ as salinity exposure intensified suggesting they have efficient mechanisms regulating Na^+ uptake and Na^+ loading into leaf tissue. Both halimus and lentiformis managed to improve their plant water status at 16.0 dS/m compared to both 8.7 and 26.8 dS/m. This could be caused be an efficient regulation of stomatal resistance which was also most optimal at 16.0 dS/m for both halimus and lentiformis. Nummularia had unaltered leaf water relations but an increase in stomatal resistance. It was generally observed that the plant response did not appear linear across the salinity gradient. A good example is the bell-curved trend of both water status and stomatal resistance in halimus and lentiformis which may indicate that different the relative role of the osmotic and ionic stress depends on the degree of salinity. It also highlights the great importance of assessing plants over varying levels of salinity in order to fully understand how the mechanisms conferring to salinity tolerance work.

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8. Appendices

Appendix a. Muchaqqar

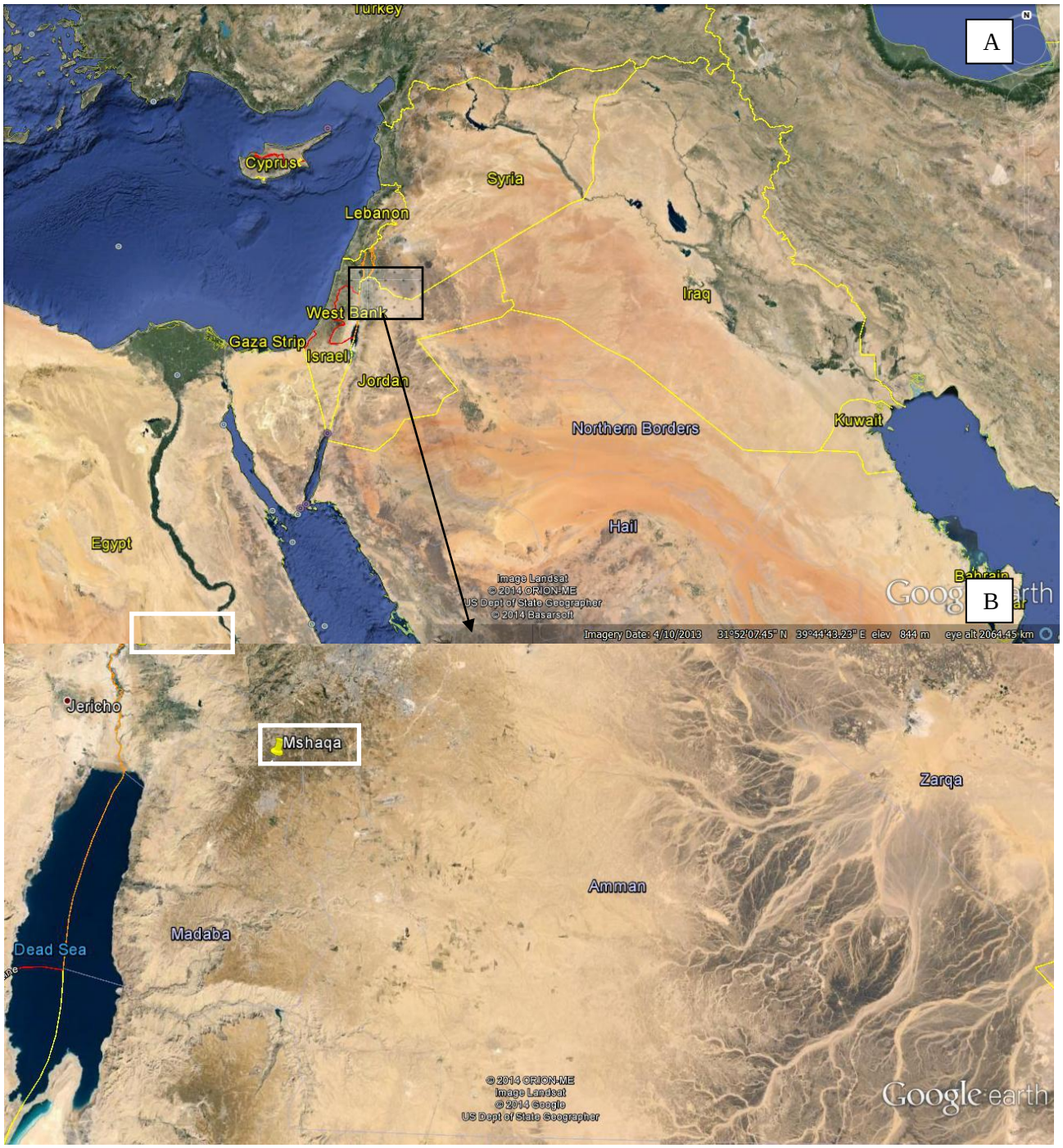
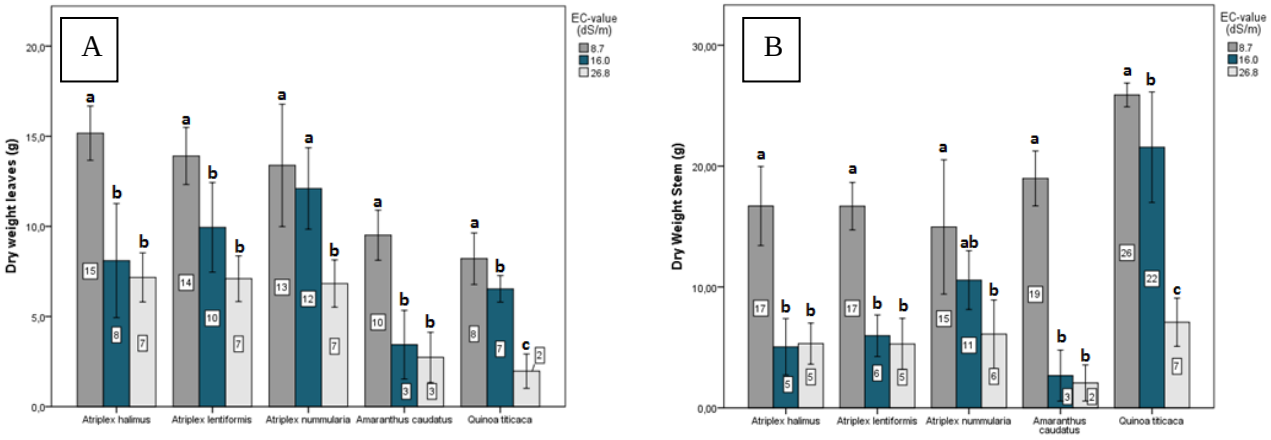


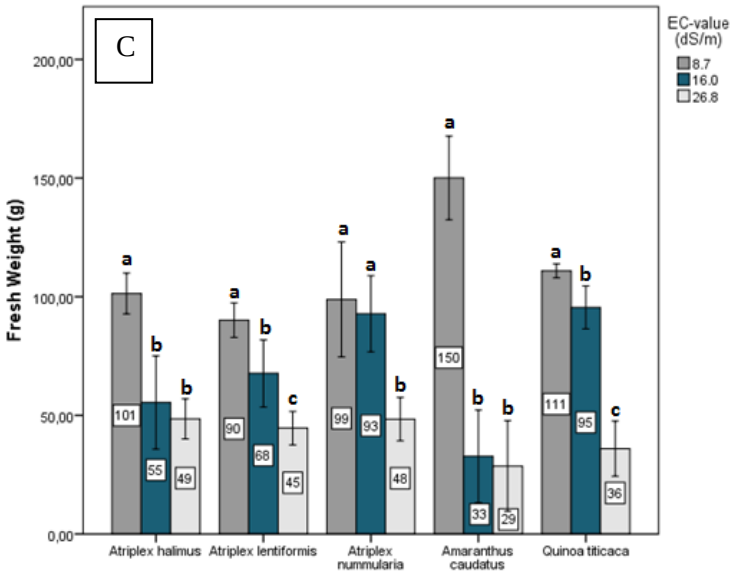
Figure xvi Picture A is a map over the location of the study areas in regards to Jordan. Picture B is an enlarged map of the section marked in a black square on map A. The yellow pins in white boxes shows the location of the study site of Al-karama and Mushaqqar (Mshaqa). The images have been produced in Google Earth.

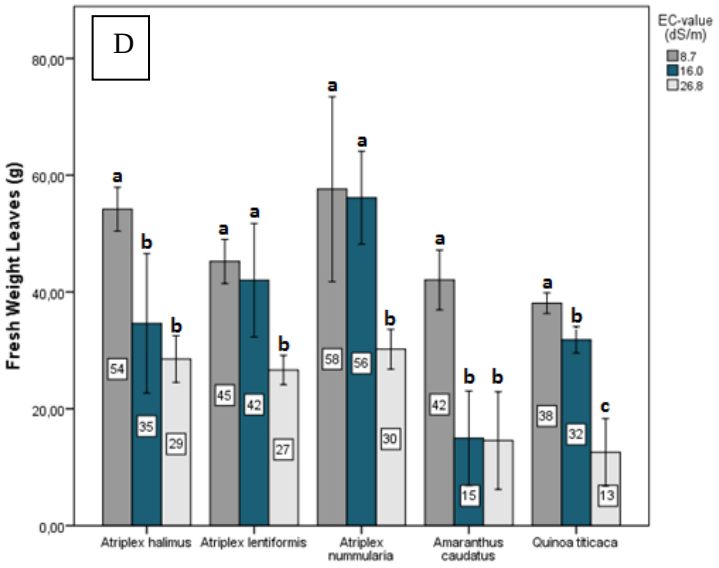
Appendix b. Survival rates

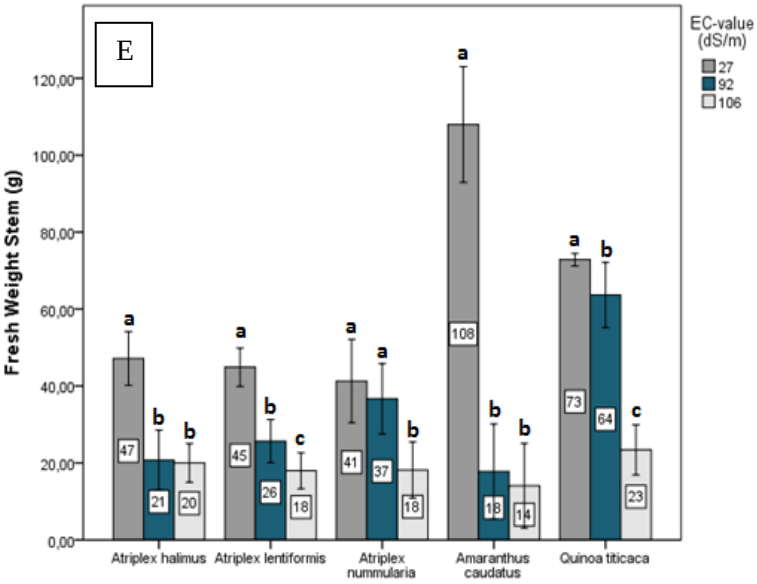
Species	EC (dS m^{-1})	0 DAT (08.06.14)	8 DAT (16.06.14)	12 DAT (20.06.14)	17 DAT (25.06.14)	21 DAT (29.06.14)	23 DAT (01.07.14)	52 DAT (30.07.14)	56 DAT (03.08.14)
Halimus	105.8	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**
	92.0	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**
	26.8	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**
	16.0	(6) **100 %**	(6) **100 %**	(5) 83 %	(3) 50 %	(1) 17 %	(0) **0 %**	(0) **0 %**	(0) **0 %**
	8.7	(6) **100 %**	(6) **100 %**	(1) 17 %	(0) **0 %**	(0) **0 %**	(0) **0 %**	(0) **0 %**	(0) **0 %**
Lentiformis	105.8	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**
	92.0	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**
	26.8	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**
	16.0	(6) **100 %**	(6) **100 %**	(6) **100 %**	(2) 33 %	(0) **0 %**	(0) **0 %**	(0) **0 %**	(0) **0 %**
	8.7	(6) **100 %**	(6) **100 %**	(0) **0 %**	(0) **0 %**	(0) **0 %**	(0) **0 %**	(0) **0 %**	(0) **0 %**
Nummularia	105.8	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**
	92.0	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**
	26.8	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**
	16.0	(6) **100 %**	(6) **100 %**	(3) 50 %	(0) **0 %**	(0) **0 %**	(0) **0 %**	(0) **0 %**	(0) **0 %**
	8.7	(6) **100 %**	(5) 83 %	(0) **0 %**	(0) **0 %**	(0) **0 %**	(0) **0 %**	(0) **0 %**	(0) **0 %**
Amaranth	105.8	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**
	92.0	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**
	26.8	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(5) 83,3%	(5) 83 %
	16.0	(6) **100 %**	(6) **100 %**	(0) **0 %**	(0) **0 %**	(0) **0 %**	(0) **0 %**	(0) **0 %**	(0) **0 %**
	8.7	(6) **100 %**	(6) **100 %**	(0) **0 %**	(0) **0 %**	(0) **0 %**	(0) **0 %**	(0) **0 %**	(0) **0 %**
Quinoa	105.8	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**
	92.0	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**
	26.8	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**	(6) **100 %**
	16.0	(6) **100 %**	(6) **100 %**	(1) 17 %	(0) **0 %**	(0) **0 %**	(0) **0 %**	(0) **0 %**	(0) **0 %**
	8.7	(6) **100 %**	(4) 67 %	(1) 17 %	(0) **0 %**	(0) **0 %**	(0) **0 %**	(0) **0 %**	(0) **0 %**

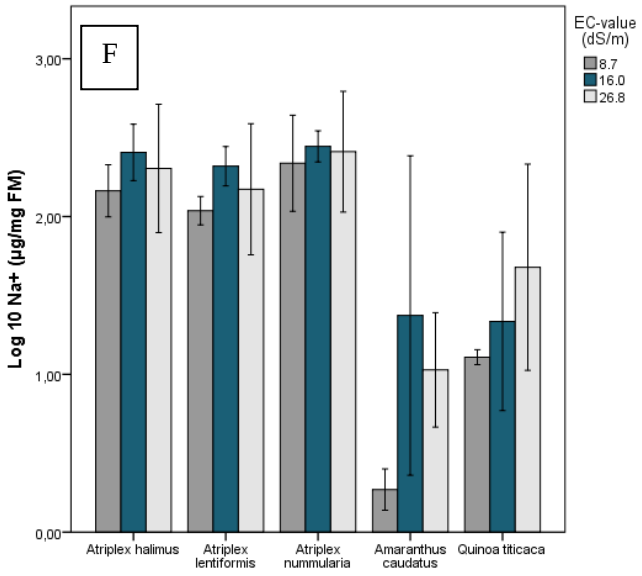
Table 7 Survival rate of replicates for halimus, lentiformis, nummularia, amaranth and quinoa exposed to salinity treatments. Given is alive plants in percentage of 6 replicates and in brackets the total count of replicates alive.

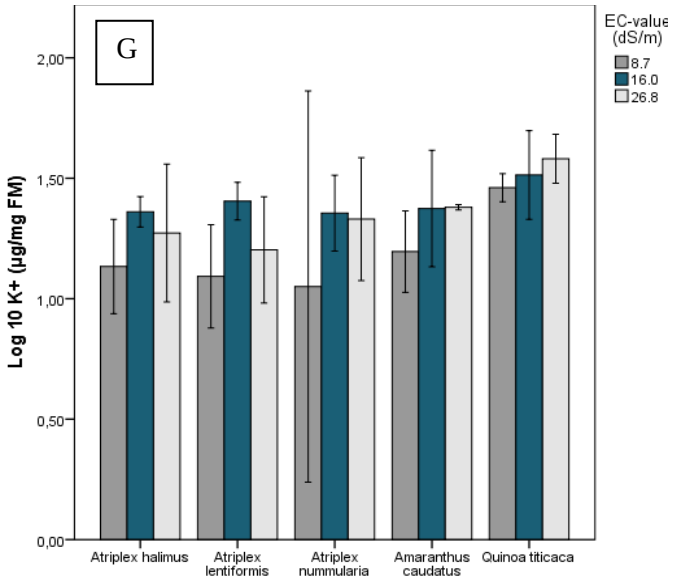


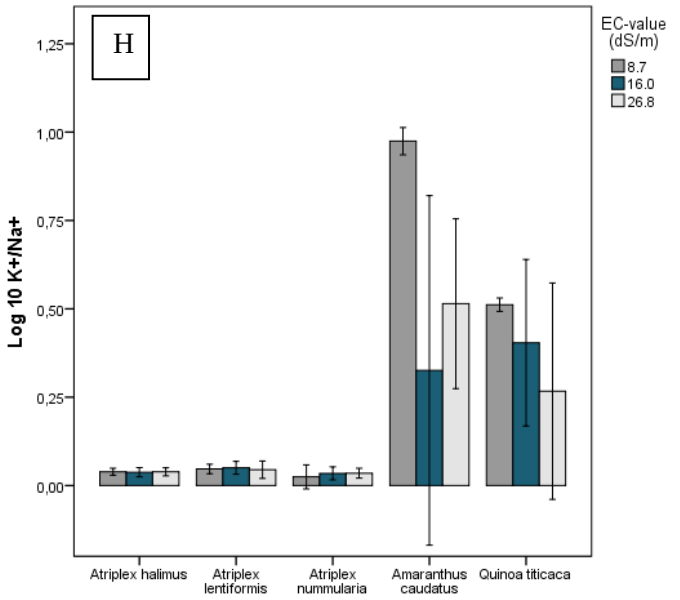

C
Fresh Weight (g)
200,00
150,00
100,00
50,00
0,00
EC-value (dS/m)
8.7
16.0
26.8
Atriplex halimus
Atriplex lentiformis
Atriplex nummularia
Amaranthus caudatus
Quinoa titicaca

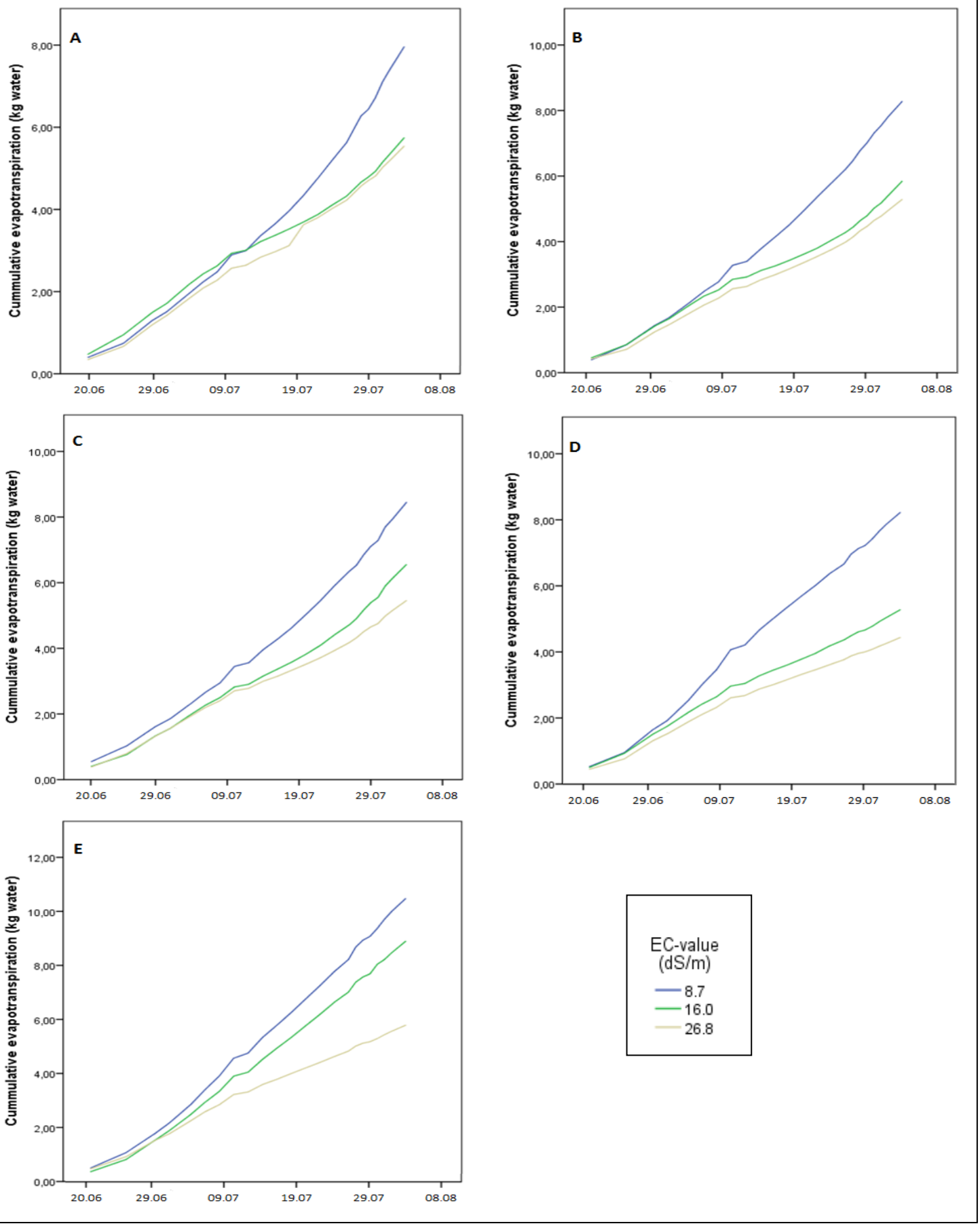

D
Fresh Weight Leaves (g)
80,00
60,00
40,00
20,00
0,00
EC-value (dS/m)
8.7
16.0
26.8
Atriplex halimus
Atriplex lentiformis
Atriplex nummularia
Amaranthus caudatus
Quinoa titicaca


E
Fresh Weight Stem (g)
120,00
100,00
80,00
60,00
40,00
20,00
0,00
EC-value (dS/m)
27
92
106
Atriplex halimus
Atriplex lentiformis
Atriplex nummularia
Amaranthus caudatus
Quinoa titicaca


F
Log 10 Na+ (µg/mg FM)
3,00
2,00
1,00
0,00
EC-value (dS/m)
8.7
16.0
26.8
Atriplex halimus
Atriplex lentiformis
Atriplex nummularia
Amaranthus caudatus
Quinoa titicaca


G
Log 10 K+ (µg/mg FM)
2,00
1,50
1,00
0,50
0,00
EC-value (dS/m)
8.7
16.0
26.8
Atriplex halimus
Atriplex lentiformis
Atriplex nummularia
Amaranthus caudatus
Quinoa titicaca


H
Log 10 K+/Na+
1,25
1,00
0,75
0,50
0,25
0,00
EC-value (dS/m)
8.7
16.0
26.8
Atriplex halimus
Atriplex lentiformis
Atriplex nummularia
Amaranthus caudatus
Quinoa titicaca


A
B
C
D
E
Cummulative evapotranspiration (kg water)
0,00
2,00
4,00
6,00
8,00
10,00
12,00
20.06
29.06
09.07
19.07
29.07
08.08
EC-value (dS/m)
8.7
16.0
26.8

	EC (dS/m)	Height (cm) (n=6)	Leaf area (cm^2) (n=18)	DW (plant) (g) (n=6)	DW (leaves) (g) (n=6)	DW (stems) (g) (n=6)	FW (plant) (g) (n=6)	FW (leaves) (g) (n=6)	FW (stems) (g) (n=6)	WUE (DM/ET) (g_{DM}/kg_{water}) (n=6)
	8.7	87.0±6.70	29.5±5.64	31.9±3.86	15.2±1.44	16.7±3.11	101.3±8.20	54.2±3.60	47.2±6.64	4.17±0.28
Halimus	16.0	64.5±9.70	28.2±4.65	13.2±5.23	8.1±3.01	5.05±2.22	55.4±18.71	34.6±11.38	20.8±7.39	2.30±0.73
	26.8	63.9±10.87	22.1±4.22	12.5±2.90	7.2±1.30	5.3±1.62	48.5±8.06	28.5±3.80	20.0±4.80	2.31±0.39
	8.7	91.3±9.01	22.7±5.48	30.6±2.60	13.9±1.51	16.7±1.87	90.1±6.95	45.2±3.59	44.9±4.75	3.82±0.20
Lentiformis	16.0	65.0±3.76	20.1±2.68	15.9±3.98	9.9±2.36	6.0±1.64	67.7±13.48	42.0±9.25	25.7±5.34	2.76±0.58
	26.8	82.5±35.43	19.6±3.49	12.4±3.21	7.1±1.21	5.3±2.02	44.6±6.70	26.6±2.38	18.0±4.44	2.37±0.49
	8.7	75.8±16.68	12.8±3.13	28.4±8.23	13.4±3.23	15.0±5.29	98.9±23.08	57.6±15.08	41.3±10.35	3.43±0.68
Nummularia	16.0	77.2±6.21	13.5±3.47	22.7±4.29	12.10±2.15	10.6±2.31	92.8±15.30	56.2±7.57	36.7±8.74	3.58±0.48
	26.8	59.9±10.50	14.8±5.65	12.9±3.63	6.8±1.25	6.1±2.69	48.4±8.70	30.2±3.26	18.2±6.94	2.42±0.48
	8.7	79.4±8.93	157.1±24.18	28.5±2.70	9.5±1.33	19.0±2.17	150.1±16.80	42.1±4.88	108.0±14.35	3.55±0.28
Amaranth	16.0	28.6±10.28	61.5±25.17	6.1±3.73	3.4±1.81	2.7±2.01	32.7±18.61	15.0±7.70	17.8±11.78	1.13±0.62
	26.8	27.9±5.39*	52.4±23.45^	4.8±2.30*	2.7±1.12*	2.1±1.20*	28.6±15.39*	14.6±6.74*	14.1±8.85*	1.07±0.55*
	8.7	42.6±6.46	17.1±2.57	34.1±1.21	8.2±1.36	25.9±0.93	110.9±2.84	38.1±1.68	72.8±1.55	3.35±0.12
Quinoa	16.0	36.7±5.77	14.5±2.32	28.1±4.72	6.5±0.70	21.6±4.36	95.5±8.63	31.8±2.16	63.7±8.06	3.20±0.30
	26.8	24.1±2.30	8.6±3.44	9.1±2.62	2.0±0.91	7.1±1.89	36.0±11.05	12.6±5.50	23.4±6.21	1.58±0.34

*n=5; ^n=15; ¤n=45; ¨n=51

	EC (dS/m)	FM-DM (plant) (g_{water}) (n=6)	FM-DM (leaves) (g_{water}) (n=6)	FM-DM (Stems) (g_{water}) (n=6)	Succulence (g_{water}) (n=6)	r_s s/cm (n=54)	CCI (unitless) (n=54)	Na^+ ($\mu g/mg_{FM}$) (n=3)	K^+ ($\mu g/mg_{FM}$) (n=3)	K^+/Na^+ (n=3)
	8.7	69.5±4.38	39.0±2.26	30.5±3.58	0.43±0.13	5.18±1.97	41.8±3.06	146.6±23.33	13.7±2.42	0.094±0.010
Halimus	16.0	42.2±13.49	26.5±8.38	15.7±5.18	0.40±0.14	4.00±1.18	40.6±5.23	257.2±41.76	23.0±1.36	0.091±0.013
	26.8	36.0±5.69	21.3±3.14	14.7±3.27	0.29±0.11	5.81±1.76	40.9±4.18	212.0±86.03	19.2±5.30	0.093±0.012
	8.7	59.5±5.01	31.3±2.76	28.2±3.08	0.48±0.15	7.86±2.79	44.5±4.91	109.2±9.34	12.6±2.59	0.114±0.014
Lentiformis	16.0	51.8±9.67	32.1±7.16	19.7±3.82	0.45±0.12	5.28±1.70	39.4±3.49	209.4±23.55	25.5±1.83	0.123±0.019
	26.8	32.2±3.55	19.5±1.26	12.7±2.47	0.30±0.10	8.78±3.01	46.2±4.16	156.8±64.63	16.2±3.38	0.109±0.025
	8.7	70.5±15.41	44.2±12.05	26.3±5.09	0.34±0.14	5.36±1.71	46.6±6.51	223.7±67.17	13.1±7.30	0.059±0.033
Nummularia	16.0	70.1±11.35	44.0±5.60	26.1±6.61	0.31±0.11	8.71±7.03	47.0±4.60	279.3±25.83	22.8±3.43	0.083±0.019
	26.8	35.5±6.06	23.39±3.09	12.1±4.41	0.29±0.08	8.53±3.69	49.5±6.67	269.2±102.03	21.8±5.37	0.084±0.014
	8.7	121.6±14.53	32.6±3.77	89.0±12.41	2.70±0.44	5.76±2.35	28.2±6.67	1.87±0.22	15.8±2.40	8.43±0.34
Amaranth	16.0	26.6±14.88	11.5±5.93	15.1±9.78	1.05±0.46	7.35±3.25	29.7±5.86	29.74±19.05	24.1±5.70	1.28±1.13
	26.8	23.8±13.12*	11.8±5.62*	12.07.67*	1.15±0.62*	13.65±5.85¤	21.0±6.08¤	11.09±3.83	24.0±0.25	2.32±0.72
	8.7	76.8±2.40	29.9±1.46	47.0±1.72	0.46±0.11	5.12±3.53	36.3±8.55	12.85±0.56	28.91±1.57	2.25±0.06
Quinoa	16.0	67.4±4.09	25.3±1.86	42.1±4.04	0.38±0.09	7.05±5.71	35.2±7.68	23.89±13.66	32.94±5.64	1.57±0.53
	26.8	26.9±8.45	10.6±4.60	16.3±4.32	0.27±0.11	11.99±12.39¨	27.4±9.13¨	53.08±25.99	38.19±3.64	1.58±0.70

*n=5; ^n=15; ¤n=45; ¨n=51

ANOVA	EC (dS/m)	EC (dS/m)	Height (n=6)	Leaf area (n=18)	DW (plant) (n=6)	DW (leaves) (n=6)	DW (stems) (n=6)
Halimus	8.7	16.0	0.002**	0.718	<0.001***	<0.001***	<0.001***
		26.8	0.002**	<0.001***	<0.001***	<0.001***	<0.001***
	16.0	8.7	0.002**	0.718	<0.001***	<0.001***	<0.001***
		26.8	0.993	0.001**	0.958	0.721	0.980
	26.8	8.7	0.002**	<0.001***	<0.001***	0.000***	<0.001***
		16.0	0.993	0.001**	0.958	0.721	0.980
Lentiformis	8.7	16.0	0.114	0.137	<0.001***	0.004**	<0.001***
		26.8	0.758	0.062•	<0.001***	<0.001***	<0.001***
	16.0	8.7	0.114	0.137	<0.001***	0.004**	<0.001***
		26.8	0.352	0.926	0.188	0.034*	0.800
	26.8	8.7	0.758	0.062•	<0.001***	<0.001***	<0.001***
		16.0	0.352	0.926	0.188	0.034*	0.800
Nummularia	8.7	16.0	0.978	0.853	0.223	0.623	0.130
		26.8	0.084•	0.350	0.001**	0.001**	0.002**
	16.0	8.7	0.978	0.853	0.233	0.623	0.130
		26.8	0.058•	0.668	0.026*	0.004**	0.123
	26.8	8.7	0.084•	0.350	0.001**	0.001**	0.002**
		16.0	0.058•	0.668	0.026*	0.004**	0.123
Amaranth	8.7	16.0	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
		26.8	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
	16.0	8.7	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
		26.8	0.992	0.536	0.757	0.714	0.857
	26.8	8.7	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
		16.0	0.992	0.536	0.757	0.714	0.857
Quinoa	8.7	16.0	0.154	0.034*	0.014*	0.033*	0.043*
		26.8	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
	16.0	8.7	0.154	0.034*	0.014*	0.033*	0.043*
		26.8	0.002**	<0.001***	<0.001***	<0.001***	<0.001***
	26.8	8.7	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
		16.0	0.002**	<0.001***	<0.001***	<0.001***	<0.001***

*n=5; ^n=15; ¤n=45; ¨n=51

ANOVA	EC (dS/m)	EC (dS/m)	FW (plant) (n=6)	FW (leaves) (n=6)	FW (stems) (n=6)	WUE (DM/ET) (n=6)	FM-DM (plant) (n=6)
Halimus	8.7	16.0	<0.001***	0.001**	<0.001***	<0.001***	<0.001***
		26.8	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
	16.0	8.7	<0.001***	0.001**	<0.001***	<0.001***	<0.001***
		26.8	0.624	0.335	0.976	1.000	0.459
	26.8	8.7	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
		16.0	0.624	0.335	0.976	1.000	0.459
Lentiformis	8.7	16.0	0.003**	0.621	<0.001***	W: 0.012*	0.139
		26.8	<0.001***	<0.001***	<0.001***	W: 0.001**	<0.001***
	16.0	8.7	0.003**	0.621	<0.001***	W: 0.012*	0.139
		26.8	0.002**	0.001**	0.038*	W: 0.459	<0.001***
	26.8	8.7	<0.001***	<0.001***	<0.001***	W: 0.001**	<0.001***
		16.0	0.002**	0.001**	0.038*	W: 0.459	<0.001***
Nummularia	8.7	16.0	0.807	0.965	0.641	0.892	0.998
		26.8	<0.001***	0.001**	0.001**	0.017*	<0.001***
	16.0	8.7	0.807	0.965	0.641	0.892	0.998
		26.8	0.001**	0.001**	0.006**	0.007**	<0.001***
	26.8	8.7	<0.001***	0.001**	0.001**	0.017*	<0.001***
		16.0	0.001**	0.001**	0.006**	0.007**	<0.001***
Amaranth	8.7	16.0	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
		26.8	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
	16.0	8.7	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
		26.8	0.917	0.994	0.869	0.981	0.944
	26.8	8.7	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
		16.0	0.917	0.994	0.869	0.981	0.944
Quinoa	8.7	16.0	0.014*	0.001**	0.043*	0.603	W: 0.003**
		26.8	<0.001***	<0.001***	<0.001***	<0.001***	W: <0.001***
	16.0	8.7	0.014*	0.001**	0.043*	0.603	W: 0.003**
		26.8	<0.001***	<0.001***	<0.001***	<0.001***	W: <0.001***
	26.8	8.7	<0.001***	<0.001***	<0.001***	<0.001***	W: <0.001***
		16.0	<0.001***	<0.001***	<0.001***	<0.001***	W: <0.001***

*n=5; ^n=15; ¤n=45; ¨n=51

ANOVA	**EC** (dS/m)	**EC** (dS/m)	**FM-DM (leaves)** (n=6)	**FM-DM (Stems)** (n=6)	**Succulence** (n=6)	**Log 10 r_s** (n=54)
Halimus	8.7	16.0	0.003**	<0.001***	0.747	W: 0.651
		26.8	<0.001***	<0.001***	0.007**	W: <0.001***
	16.0	8.7	0.003**	<0.001***	0.747	W:0.651
		26.8	0.243	0.901	0.045*	W: <0.001***
	26.8	8.7	<0.001***	<0.001***	0.007**	W: <0.001***
		16.0	0.243	0.901	0.045*	W: <0.001***
Lentiformis	8.7	16.0	W: 0.970	0.001**	0.762	<0.001***
		26.8	W: <0.001***	<0.001***	<0.001***	0.181
	16.0	8.7	W: 0.970	0.001**	0.762	<0.001***
		26.8	W: 0.017*	0.004**	0.004**	<0.001***
	26.8	8.7	W: <0.001***	<0.001***	<0.001***	0.181
		16.0	W: 0.017	0.004**	0.004**	<0.001***
Nummularia	8.7	16.0	W: 0.999	0.997	0.680	W:0.002**
		26.8	W:0.017*	0.001**	0.452	W: <0.001***
	16.0	8.7	W: 0.999	0.997	0.680	W: 0.002**
		26.8	W: <0.001***	0.001**	0.927	W:0.499
	26.8	8.7	W: 0.017*	0.001**	0.452	W: <0.001***
		16.0	W: <0.001***	0.001**	0.927	W: 0.499
Amaranth	8.7	16.0	<0.001***	<0.001***	<0.001***	0.015*
		26.8	<0.001***	<0.001***	<0.001***	<0.001***
	16.0	8.7	<0.001***	<0.001***	<0.001***	0.015*
		26.8	0.995	0.874	0.826	<0.001***
	26.8	8.7	<0.001***	<0.001***	<0.001***	<0.001***
		16.0	0.995	0.874	0.826	<0.001***
Quinoa	8.7	16.0	W: 0.002**	0.078•	0.049**	0.001**
		26.8	W: <0.001***	<0.001***	<0.001***	<0.001***
	16.0	8.7	W: 0.002**	0.078•	0.049**	0.001**
		26.8	W:0.001**	<0.001***	0.011**	<0.001***
	26.8	8.7	W: <0.001***	<0.001***	<0.001***	<0.001***
		16.0	W: 0.001**	<0.001***	0.011**	<0.001***

*n=5; ^n=15; ¤n=45; ¨n=51

ANOVA	EC (dS/m)	EC (dS/m)	CCI (n=54)	Log 10 Na^+ (n=3)	Log 10 K^+ (n=3)	Log 10 K^+/Na^+ (n=3)
Halimus	8.7	16.0	0.325	0.078•	0.034*	0.939
		26.8	0.494	0.324	0.174	0.999
	16.0	8.7	0.325	0.078•	0.034*	0.939
		26.8	0.951	0.532	0.437	0.955
	26.8	8.7	0.494	0.324	0.174	0.999
		16.0	0.951	0.532	0.437	0.955
Lentiformis	8.7	16.0	<0.001***	W: 0.004**	0.005**	0.862
		26.8	0.103	W: 0.482	0.242	0.943
	16.0	8.7	<0.001***	W: 0.004**	0.005**	0.862
		26.8	<0.001***	W: 0.446	0.035*	0.686
	26.8	8.7	0.103	W: 0.482	0.242	0.862
		16.0	<0.001***	W: 0.446	0.035*	0.686
Nummularia	8.7	16.0	W: 0.923	0.531	W: 0.412	0.467
		26.8	W: 0.071	0.731	W: 0.459	0.437
	16.0	8.7	W: 0.923	0.531	W: 0.412	0.467
		26.8	W: 0.078	0.933	W: 0.934	0.998
	26.8	8.7	W: 0.071	0.731	W: 0.459	0.437
		16.0	W: 0.078	0.933	W: 0.934	0.998
Amaranth	8.7	16.0	0,430	W: 0.074•	W: 0.134	W: 0.053•
		26.8	<0.001***	W: 0.013*	W: 0.076•	W: 0.024*
	16.0	8.7	0,430	W: 0.074•	W: 0.134	W: 0.053•
		26.8	<0.001***	W: 0.468	W: 0.995	W: 0.418
	26.8	8.7	<0.001***	W: 0.013*	W: 0.076•	W: 0.024*
		16.0	<0.001***	W: 0.468	W: 0.995	W: 0.418
Quinoa	8.7	16.0	0.777	W: 0.379	0.457	W: 0.320
		26.8	<0.001***	W: 0.114	0.062•	W: 0.133
	16.0	8.7	0.777	W: 0.379	0.457	W: 0.320
		26.8	<0.001***	W: 0.310	0.308	W: 0.377
	26.8	8.7	<0.001***	W: 0.114	0.062•	W: 0.133
		16.0	<0.001***	W: 0.310	0.308	W: 0.377

*n=5; ^n=15; ¤n=45; ¨n=51

	EC (dS/m)	Height (cm) (n=6)	Leaf area (cm^2) (n=18)	DW (plant) (g) (n=6)	DW (leaves) (g) (n=6)	DW (stems) (g) (n=6)
Halimus	8.7	0.639	0.814	0.024	0.810	0.524
	16.0	0.794	0.093	0.980	0.985	0.935
	26.8	0.564	0.185	0.107	0.066	0.216
	Homo-geneity	0.268	0.430	0.603	0.257	0.491
Lentiformis	8.7	0.917	0.608	0.226	0.457	0.840
	16.0	0.572	0.644	0.659	0.742	0.563
	26.8	0.013	0.856	0.134	0.226	0.082
	Homo-geneity	0.200	0.108	0.244	0.065	0.518
Nummularia	8.7	0.667	0.059	0.019	0.043	0.095
	16.0	0.185	0.302	0.437	0.495	0.829
	26.8	0.563	0.250	0.119	0.045	0.060
	Homo-geneity	0.149	0.064	0.832	0.703	0.651
Amaranth	8.7	0.121	0.280	0.303	0.703	0.561
	16.0	0.375	0.002	0.280	0.070	0.653
	26.8	0.203*	0.307^	0.846*	0.775*	0.717*
	Homo-geneity	0.197~	0.319¨	0.354~	0.518~	0.163~
Quinoa	8.7	0.004	0.450	0.528	0.021	0.225
	16.0	0.880	0.350	0.034	0.204	0.010
	26.8	0.591	0.009	0.364	0.457	0.176
	Homo-geneity	0.594	0.509	0.393	0.783	0.374

*n=5; ^n=15; ~n=17; °n=45; ¨n=51

	EC (dS/m)	FW (plant) (g) (n=6)	FW (leaves) (g) (n=6)	FW (stems) (g) (n=6)	WUE (DM/ET) (g_{DM}/kg_{water}) (n=6)	FM/DM (plant) (n=6)	FM/DM (leaves) (n=6)
Halimus	8.7	0.042	0.147	0.771	0.804	0.105	0.316
	16.0	0.994	0.998	0.933	0.977	0.996	0.998
	26.8	0.765	0.299	0.377	0.364	0.742	0.410
	Homo-geneity	0.218	0.079	0.697	0.307	0.113	0.058
Lentiformis	8.7	0.291	0.760	0.493	0.036	0.531	0.600
	16.0	0.745	0.320	0.443	0.145	0.559	0.285
	26.8	0.107	0.623	0.107	0.386	0.190	0.367
	Homo-geneity	0.190	0.182	0.792	0.011	0.169	0.002
Nummularia	8.7	0.023	0.676	0.115	0.023	0.170	0.879
	16.0	0.649	0.191	0.647	0.888	0.410	0.063
	26.8	0.676	0.131	0.170	0.255	0.875	0.524
	Homo-geneity	0.504	0.132	0.920	0.953	0.200	0.038
Amaranth	8.7	0.519	0.042	0.070	0.920	0.454	0.028
	16.0	0.247	0.183	0.595	0.161	0.253	0.202
	26.8	0.706*	0.898*	0.359*	0.853*	0.650*	0.900
	Homo-geneity	0.643~	0.656~	0.124~	0.277~	0.728	0.417
Quinoa	8.7	0.434	0.560	0.449	0.448	0.071	0.646
	16.0	0.240	0.797	0.109	0.303	0.694	0.956
	26.8	0.467	0.388	0.178	0.464	0.510	0.364
	Homo-geneity	0.234	0.038	0.363	0.463	0.047	0.031

*n=5; ^n=15; ~n=17; ¤n=45; ¨n=51

	EC (dS/m)	FM/DM (Stems) (n=6)	Succulence (n=6)	r_s s/cm (n=54)	Log 10 r_s s/cm (n=54)	CCI (unitless) (n=54)	Na^+ ($\mu g/mg_{FM}$) (n=3)
Halimus	8.7	0.976	0.246	0.002	0.242	0.079	0.12
	16.0	0.942	0.499	0.042	0.502	0.088	0.835
	26.8	0.566	0.069	0.000	0.000	0.872	0.099
	Homo-geneity	0.539	0.293	0.014	0.001	0.001	0.073
Lentiformis	8.7	0.281	0.184	0.021	0.448	0.139	0.076
	16.0	0.570	0.969	0.000	0.173	0.263	0.571
	26.8	0.402	0.204	0.012	0.795	0.803	0.201
	Homo-geneity	0.739	0.503	0.005	0.621	0.258	0.026
Nummularia	8.7	0.117	0.320	0.054	0.284	0.031	0.067
	16.0	0.569	0.211	0.000	0.000	0.043	0.609
	26.8	0.406	0.077	0.000	0.442	0.073	0.203
	Homo-geneity	0.578	0.022	0.003	0.005	0.004	0.073
Amaranth	8.7	0.046	0.735	0.000	0.069	0.198	0.306
	16.0	0.579	0.202	0.000	0.017	0.000	0.298
	26.8	0.301	0.506*	0.028¤	0.384	0.346	0.538
	Homo-geneity	0.106	0.472	0.000	0.080	0.550	0.012
Quinoa	8.7	0.460	0.224	0.000	0.000	0.438	0.017
	16.0	0.758	0.109	0.000	0.000	0.545	0.017
	26.8	0.170	0.046	0.000	0.001	0.296	0.445
	Homo-geneity	0.173	0.724	0.002	0.249	0.305	0.033

*n=5; ^n=15; ˜n=17; ¤n=45; ¨n=51

	EC (dS/m)	Log 10 Na^+ (µg/mg_{FM}) (n=3)	K^+ (µg/mg_{FM}) (n=3)	Log 10 K^+ (µg/mg_{FM}) (n=3)	K^+/Na^+ (n=3)	Log 10 K^+/Na^+ (n=3)
Halimus	8.7	0.148	0.919	0.889	0.635	0.643
	16.0	0.749	0.794	0.886	0.468	0.394
	26.8	0.140	0.966	0.689	0.008	0.010
	Homo-geneity	0.102	0.0092	0.145	0.754	0.753
Lentiformis	8.7	0.081	0.367	0.433	0.726	0.732
	16.0	0.524	0.752	0.715	0.738	0.729
	26.8	0.283	0.610	0.708	0.726	0.738
	Homo-geneity	0.032	0.505	0.270	0.608	0.607
Nummularia	8.7	0.086	0.005	0.003	0.943	0.960
	16.0	0.651	0.341	0.385	0.223	0.226
	26.8	0.278	0.389	0.472	0.531	0.536
	Homo-geneity	0.089	0.254	0.022	0.477	0.471
Amaranth	8.7	0.277	0.599	0.533	0.650	0.667
	16.0	0.149	0.025	0.031	0.126	0.191
	26.8	0.693	0.077	0.078	0.885	0.768
	Homo-geneity	0.024	0.018	0.034	0.141	0.033
Quinoa	8.7	0.017	0.919	0.889	0.635	0.643
	16.0	0.027	0.794	0.886	0.468	0.394
	26.8	0.281	0.644	0.689	0.008	0.010
	Homo-geneity	0.035	0.272	0.338	0.049	0.033