Cultivation of selected halophytes in saline indoor farming and modulation of cultivation conditions to optimize metabolite profiles for human nutrition

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Publication-based dissertation

Submitted for the degree
"doctor rerum naturalium"
(Dr. rer. nat.)
in Food Chemistry

to the Faculty of Science
Institute of Nutritional Science
Department Food Chemistry
University of Potsdam

and
non-academic institute
Leibniz Institute of Vegetable and Ornamental Crops (IGZ)

Potsdam, July 2023

Place and Date of Disputation: Nuthetal, 5th February 2024

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Published online on the
Publication Server of the University of Potsdam:
https://doi.org/10.25932/publishup- 62697
https://nbn-resolving.org/urn:nbn:de:kobv:517-opus4- 626974

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Abbreviations

ABA	Abscisic acid
ABTS	2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid
ADI	Acceptable daily intake
CKD	Chronic kidney disease
CVD	Cardiovascular disease
DGE	German Nutrition Society (Deutsche Gesellschaft für Ernährung)
DPPH	2,2-diphenyl-1-picrylhydrazyl
DWC	Deep water culture
EPS	Epithiospecifier protein
EUE	Energy use efficiency
FAO	Food and Agriculture Organization of the United Nations
GABA	γ-Aminobutyric acid
GLS	Glucosinolate
GOGAT	Glutamine synthase
GS	Glutamine synthase
HPLC-TQ-MS	High performance liquid chromatography coupled to a triple quadrupole mass spectrometer
HVAC	Heating, Ventilation and Air Conditioning
HY5	Elongated hypocotyl 5
IC	Ion chromatography
IF	Indoor Farming
ITC	Isothiocyanate
JA	Jasmonic acid
LED	Light emitting diode
LHC	Light harvesting complex
LUE	Light use efficiency
MRM	Multi reaction monitoring
$NADP^+$/NADPH	Nicotinamide adenine dinucleotide phosphate
NCD	Non communicable diseases
NFT	Nutrient film technique
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells

NiR	Nitrite reductase
NPQ	Non-photochemical quenching
NR	Nitrate reductase
NUE	Nutrient use efficiency
OECD	Organization for Economic Cooperation and Development
PAR	Photosynthetic active radiation
PIF1	Phytochrome-interacting factor 1
PPFD	Photosynthetic photon flux density
PPNE	Photosynthetic photon number efficacy
PSI	Photosystem I
PSII	Photosystem II
PSM	Plant secondary metabolites
ROS	Reactive oxygen species
RuBisCO	Ribulose-1,5-bisphosphate carboxylase-oxygenase
RUE	Resource use efficiency
SA	Salicylic acid
SDG	Sustainable development goal
SOD	Superoxide dismutase
SUE	Land surface use efficiency
UHPLC-DAD-ToF-MS	Ultra-high performance liquid chromatography with diode array detection coupled to time-of-flight mass spectrometry
UVB	Ultraviolet B
UVR8	UV resistance locus 8
VF	Vertical farming
WHO	World Health Organization
WUE	Water use efficiency

Introduction

1. New agrifood systems and food sources

1.1 Global challenges in the transformation of agrifood systems

The world is facing several global crises such as war, the COVID-19 pandemic or climate change. This affects agrifood systems, which need to adapt to this changing world in order to ensure food security. These new agrifood systems will be shaped by socio-economic patterns and global trends that introduce new food sources.

Agrifood systems, like all other planetary systems, are subject to planetary boundaries. These planetary boundaries, such as global freshwater use, population growth, biodiversity loss, greenhouse gas (GHG) emissions, or cropland use, are being pushed by anthropogenic impacts (Springmann et al., 2018). According to the Food and Agriculture Organization (FAO), 2019, four billion people faced water scarcity at least one month a year, and 80% of countries will lack sufficient water for agriculture and food production (FAO, 2019). With 70% of the world's freshwater use, the agricultural sector is one of the greatest pressures on planetary boundaries (FAO, 2019). The UN predicts that the world's population will grow to 9.7 billion by 2050 (United Nations, 2022). The world is facing a huge loss of biodiversity, with nearly 25% of all species already threatened with extinction (IPBES, 2019). Changes in land mass, such as salinization, and natural disasters caused by climate change, such as wildfires and floods, reduce the available cropland. These pressures on planetary boundaries threaten to destabilize the ecosystem.

Beyond planetary boundaries, there are also other pressures on agrifood systems. For example, changes in land management, such as urbanization. According to the Organization for Economic Cooperation and Development (OECD), 2020, more than 76% of the world's population lived in urban areas, and the number is growing (OECD, 2020).

International trade disruptions in recent years have been shown to affect the resilience of agrifood systems (Fader et al., 2013, Kummu et al., 2020). Events such as the war in Ukraine or the COVID-19 pandemic have demonstrated the fragility of international trading systems and the far-reaching economic consequences of such outcomes (Glauben et al., 2022, Stojetz et al., 2022). This can lead to increased food insecurity, as shown for example in the “Life with corona”-survey (Stojetz et al., 2022). Whether or not it will be possible to guarantee food security with all these upcoming challenges in the future is a question that is gaining attention.

This question is the subject of the food4future project (German Federal Ministry of Education and Research [Grant number 031B0730 A]), which studies future agrifood systems from different perspectives, including food science, agricultural science, socio-economic science, or

anthropological science, among others. The aim of the project is not only to develop a new agrifood system with new food sources, but also to identify the demands and challenges that agrifood systems will have to face. For example, global macro- and micro-trends that could have an impact on the future of food production have been identified, including macro trends such as urbanization, globalization, and nutrition as a lifestyle or moral aspects of nutrition will shape future agrifood systems (Preiss et al., 2022). But also micro-trends such as individualized diets or the use of alternative (plant-based) food sources have to be taken into account (Preiss et al., 2022). To meet this, the vision of food4future is to develop an urban indoor farming system with alternative food sources that contribute to healthy, sustainable, and resilient diets in the future. This is in line with the literature, which states that new agrifood systems must be resilient and sustainable, but also provide healthy diets (Springmann et al., 2018, Willett et al., 2019, Gerten et al., 2020). Therefore, food4future is investigating new food sources, such as halophytes (salt-tolerant plants), algae, crickets, and medusa, to be produced in saline indoor farming as an alternative agrifood system and its implementation in urban areas. Urban food production creates a link again between where food is consumed and where it is produced. In order to achieve a more sustainable production, it is essential to reduce the water footprint (Hoff et al., 2013). Indoor farming can increase water use efficiency compared to other agricultural systems (Pennisi et al., 2019). Saline water is a largely untapped resource that can be used in saline agriculture to conserve freshwater resources. That is why food4future focuses on saline indoor farming.

1.2 New agrifood systems

When considering new agrifood systems, important factors such as the average income, resources or nutritional needs of a region must be taken into account (Vaidyanathan, 2021). The resources of a region mainly determine the possibility of implementing new agrifood systems. For example, there are differences in soil resources that limit the cropland. Infrastructure, water supply, economic resources, government funding and subsidies also influence farmers' decisions to adopt certain agricultural practices (Niles et al., 2015). A positive example of a local agrifood system can be found in urban agriculture. A study by Iida et al. (2023) found positive results on wellbeing and food security concerns of urban agriculture in walkable neighborhoods during the COVID-19-pandemic and stated the effectiveness of these urban agricultures. Another local, sustainable, and resilient agrifood system could be vertical farming, which is an emerging field. Vertical farming does not rely on soil resources or weather conditions, however the economic resources, water

supply and infrastructure of a region need to be taken into account (Paucek et al., 2023). Especially in Europe, were all these resources are given, vertical farming is an upcoming field.

1.2.1 Indoor farming systems

In addition, indoor farming has several advantages over conventional agriculture, including increased productivity, reduced fertilizer and pesticide use, resilience to natural disasters, minimal food miles, better adaptation to consumer demands, and resource efficiency in energy, water, and light use (van Delden et al., 2021, Benke and Tomkins, 2017). The terms vertical farming and indoor farming are not clearly defined and are used for different systems. One definition for vertical farming that can be found by Banerjee and Adenaeuer (2014) is: "a system of commercial farming whereby plants, animals, fungi and other life forms are cultivated for food, fuel, fiber or other products or services by artificially stacking them vertically above each". Another definition can be found by van Delden et al. (2021): "multi-layer indoor crop production system". Indoor farming also has many definitions and vertical farming can be part of indoor farming and *vice versa*. For clarification, indoor farming is defined in this thesis as an indoor plant production system with controlled climate conditions and artificial lighting, without multi-layer production. Thus, indoor farming systems can be scaled up to vertical farming systems, so that criteria that affect vertical farms are also important to consider from the outset.
To approximate the profitability of a system the resource use efficiency (RUE) can be estimated. This includes water use efficiency (WUE), land surface use efficiency (SUE), nutrient use efficiency (NUE) and the light use efficiency (LUE).

1.2.1.1 Water-supply and fertilization

Considering the growing systems in indoor farming, there are also several ways of classification. The most common system used is hydroponics at 51%, aeroponics at 20%, aquaponics at 9%, and soil-based systems at 13%, and other systems by 6% (Wong et al., 2020). In hydroponics, plants are in direct contact with water, whereas in aeroponics, plants grow in air or mist. Hydroponic systems can be further divided into many subcategories that describe the nutrient and water supply. For example, in deep water culture (DWC), where the roots are fully submerged into an oxygenated nutrient solution (van Delden et al., 2021). In a nutrient film technique (NFT) system, the roots are exposed to a film material, which is soaked in nutrient solution (van Delden et al., 2021). The NFT systems are widely used, due to its high flexibility, the minimal use of water and nutrient solution

and the user-friendly handling. Advantages of an NFT system are the precise nutrient supply, the automatic system, the optimal plant density, and recirculation of nutrient rich water, among others (Gillani et al., 2023, Olympios and Choukr-Allah, 1999). On the other hand, a disadvantage of the NFT system are a low buffer capacity, which leads to the risk of disease and infection (Olympios and Choukr-Allah, 1999). In the NFT system, plants are supplied with nutrients through an applied nutrient solution.

1.2.1.2 Saline indoor farming

In a saline indoor farming system, the plants are supplied with saline water. In theory, any indoor farming system could be feasible, but in practice, some indoor farming systems have technical disadvantages. Saline water has the disadvantage that crystallized salts form a precipitate when the water evaporates. This can clog valves or hoses, making aeroponic systems less suitable. As mentioned above, suitable crops for saline agriculture are halophytes. They can be irrigated with saline water of varying salinity. A common parameter used to evaluate nutrient solutions is electrical conductivity (EC in dS m^{-1}), which indicates the concentration of salts in a solution. For context, the average salinity of seawater, depending on numerous factors, is about 3.5% salinity (equivalent to 25 dS m^{-1} or 600 mM salt) (Antonov et al., 2010), while the concentration in tap water, also depending on numerous factors, is close to zero (0.01% or 2 mM, in Großbeeren, Germany) (unpublished data). Depending on nutrient solution and plant species EC-values can differ in nutrient solutions, in average there EC level is between 1-2 dS m^{-1} (van Delden et al., 2020). Another way to monitor salt concentration and nutrient supply is to determine the concentration of anions (chloride, nitrate, phosphate, and sulfate) in the nutrient solution.

1.2.2 Cultivation conditions

A major advantage of indoor farming is the ability to fully modify environmental factors. This can be used to optimize the nutrient profile and yield of a crop, improving sustainability and economic outcomes. One of the most important factors is light, which is not only essential for photosynthesis and thus yield, but is also the most energy consuming factor in indoor farming (van Delden et al., 2021). The light use efficiency (LUE) is a variable used to evaluate the sustainability of a commercial product (Jin et al., 2022). It is expressed in marketable fresh weight per joule of electricity consumed by the lighting system (Jin et al., 2022). Other factors like temperature, CO_2 or humidity can also influence plant growth and nutritional profile. There is a need for further

research to optimize climate conditions in vertical and indoor farming systems (Benke and Tomkins, 2017).

1.2.2.1 Light in indoor farming systems

Due to the artificial lighting situation in indoor farming, the light automatically differs from greenhouse or field cultivation. Greenhouses include natural light, although there may be additional artificial lighting. Natural and artificial light can differ in various factors, like the diurnal changes (square/sinusoidal), the intensity, a homogeneous or heterogeneous spectra or the light direction. However, artificial lighting can imitate natural light in spectral quality and light quantity, there are still differences in the influence on plant metabolism, for example in sugar metabolism (Annunziata et al., 2017). Even for different artificial lighting sources, such as light emitting diodes (LEDs) lamps, fluorescent lamps or sodium-vapor lamps huge differences in the influence on plant metabolism can be found (Annunziata et al., 2017). One aspect of spectral quality is UV light. Being a part of the sunlight spectra, but missing in most artificial lighting, its influence on plant metabolism was underestimated in research, but in recent years, with the possibility of UV LED lamps emerging, this research field is growing (Schreiner et al., 2012, Yoon et al., 2022). UV light (100 to 380 nm), with UVA and UVB reaching the earth and thus being interesting when considering plants, can be applied as broad or narrow banded UV light using filters or specific LED lamps.

These light conditions can be actively used to modulate specific nutritional aspects, like enhancing health-promoting compounds. For example, the irradiance levels or spectral qualities (different colored LEDs) showed to influence pigment content (Naznin et al., 2019, Lefsrud et al., 2006, Frede et al., 2019). Further UVB light can be used to enhance contents of plant secondary metabolites (PSM) and thus promote health benefits (Badmus et al., 2022b, Heinze et al., 2018, Schreiner et al., 2012).

1.3 Halophytes as new food sources

New food sources are an essential part of healthier, more sustainable and resilient new agrifood systems of the future and could support a planet-friendly diet. Insects, seafood, and algae have been in the spotlight in recent years during the search for new sources of proteins and foods in general (Parodi et al., 2018, Weindl et al., 2020). One approach is to increase the proportion of plant-based foods in the diet, as they are more sustainable and healthier than animal-based foods, for example,

they have been shown to have lower GHG emissions (Xu et al., 2021). Refocusing attention on neglected plants, biodiversity can be increased, which simultaneously serves sustainability, health, and resilience (Baldermann et al., 2016). As "food production without freshwater" is defined as a micro-trend shaping new agrifood systems, halophytes are coming to the fore as new foods due to their high nutritional and pharmaceutical potential and their suitability for saline agriculture (Preiss et al., 2022).

1.3.1 Characterization of halophytes and their potential as alternative vegetables

Halophytes are a diverse group of extremophiles that grow in various saline habitats, such as coastal areas or salt marshes. About 2500 known species of halophytes are found in different plant families, with the *Amaranthaceae* representing the largest plant family of halophytes (Flowers and Al-Azzawi, 2023). There are several ways to classify halophytes, according to their habitats, their salt tolerance mechanisms, or the chemical composition of their shoots (Flowers and Colmer, 2008, Bergmeier, 2016). One of the most popular definitions was made by Flowers et al. (1986), which can be found in many publications, describing halophytes as "plants that can survive and reproduce at 200 mM NaCl". This is a handy definition in writing, but when defining a plant as a halophyte in an experimental setup, it is an inadequate definition that does not take into account the complexity of an environmental setup, as shown, for example, by the different salt tolerance levels of *A. hortensis* in 1.3.2.3 Influencing factors on salt tolerance. Halophytes are traditional vegetables that have been consumed for centuries. One of the most prominent crops derived from a halophyte is the sugar beet (*Beta vulgaris*) with its halophyte counterpart *Beta vulgaris* ssp. *maritima* (Aronson, 1985). Examples of traditional use of wild halophytes as vegetables are *Chenopodium album* (*Amaranthaceae*), which is consumed as a salad or cooked vegetable in Indian regions; sea spinach (*Tetragonia* ssp.), which was introduced as a spinach version in the 18th century; or sea kale (*Crambe maritima*), which is used as a salad or cooked vegetable (Panta et al., 2014, Dagar, 2005). In the last decade, there has been growing interest in the cultivation of halophytes (Centofanti and Bañuelos, 2019). Halophytes offer the possibility of being crops adapted to challenging environments, such as saline soils, and can diversify our diet. However, halophytes cannot compete with domesticated crops in terms of profitability to date, so they are mainly sold as gourmet vegetables (Boscaiu Neagu and Vicente Meana, 2013). The most cultivated and consumed halophytes are *Salicornia* and *Sacrocorina* ssp. (Centofanti and Bañuelos, 2019). Traditionally used in salads or as vegetables, they were grown for home consumption or sold in local markets. Nowadays, for example, *S. europaea* or *Salicornia bigelovii* are mainly sold to

Europe or the USA and produced in Mexico (Ventura and Sagi, 2013, Ventura, 2011). Other gourmet vegetables are the sea fennel (*Crithmum maritimum*) or “agretti” (*Salsola soda*) (Centofanti and Bañuelos, 2019). Quinoa (*Chenopodium quinoa*) is a halophyte that has conquered the international market, however it is mostly used as a "pseudocereal" (Centofanti and Bañuelos, 2019). Halophytes are also grown for oilseed and biodiesel production, such as *S. bigelovii*, and for fodder, *Salicornia*, *Suaeda*, or *Atriplex* ssp. (Centofanti and Bañuelos, 2019).

1.3.2 Salt stress and salt tolerance

Considering saline indoor farming, salt stress response and the salt tolerance mechanism of halophytes (salt-plants) are essential to understand and thus identify the optimal salt levels for cultivation.

1.3.2.1 Salt stress response

Salt stress occurs when plants are exposed to substantial concentrations of sodium (Na^+) and chloride (Cl^-) ions and can be divided into three phases (Schubert, 2017, Parihar et al., 2015). Phase 0, in which root nutrient uptake is initially reduced and root growth is restricted, occurs immediately after salt exposure. This leads to nutrient deficiency. For example, Ca-deficiency occurs through a salt-induced slow phloem movement of Ca. There is also a shift in the Na/K ratio, which is essential for maintaining the cell potential. Phase I, the osmotic stress phase, and Phase II, the ionic stress phase, occur when plants are exposed to prolonged salt stress. During the osmotic stress phase, the plant responds to the stress conditions in a variety of ways, including the accumulation of osmolytes such as proline or glycine betaine. Reduced water availability leads to a decrease in osmotic potential and therefore turgor. This leads to a reduction in distance growth and leaf size, which also counteracts water loss through transpiration. An essential signaling molecule in the salt stress response is abscisic acid (ABA). Application of ABA has been shown to improve salt stress response in *Vicia faba* (Sagervanshi et al., 2020, Zhu, 2002). During salinity stress, ABA has been shown to regulate, among other things, stomatal opening (Golldack et al., 2014). In the ionic stress phase, the plant must deal with the toxicity of accumulated Na^+/Cl^-, with chloride toxicity being an underestimated problem. These salt stress phases can occur in glycophytes (non-halophytic plants) as well as halophytes depending on the salinity level. However, halophytes have different coping mechanisms for salinity stress.

1.3.2.2 Salt tolerance mechanisms

The salt tolerance mechanisms for salt stress can be categorized into three main mechanisms: The salt-excluding (SEL), salt-excreting (SER), and salt-accumulating (SAL) mechanisms. In the salt-exclusion mechanism, salt exclusion occurs at the root surface (Fig. 1A), where initial Na^+/Cl^- uptake by the root cortex is stopped by increased formation of the endodermis and Casparian strip (Hasanuzzaman et al., 2014, Chen et al., 2018, Matsushita and Matoh, 1991). There is also translocation of Na^+/Cl^- through the xylem (Fig. 1B) and compartmentalization of Na^+/Cl^- in the vacuoles of the xylem parenchyma. By recycling Na^+/Cl^- back into the root through the phloem (Fig. 1C), ions are accumulated in basal plant organs and young tissue is protected. The mechanism of salt excretion is mainly based on the excretion of salt through Na^+/Cl^- bladders or glands (Fig. 1D) (Hasanuzzaman et al., 2014, Zhao et al., 2020). Salt bladders, consisting of a bladder cell that sits on the leaf surface and stores ions in vacuoles of the apically located basal cell, the epidermal bladder cell (EBC), which explode and die, and then the ions are washed out (Yuan et al., 2016). While structurally different, salt glands are consistent with a two- or more multicellular structure of epidermal cells (Yuan et al., 2016, Hasanuzzaman et al., 2014). The mechanism of salt accumulation is based on the uptake of Na^+/Cl^- and water by the root and their accumulation in the cell vacuoles (Fig. 1E) (Hasanuzzaman et al., 2014). This leads to a dilution of the cell sap and to stem or leaf succulence.

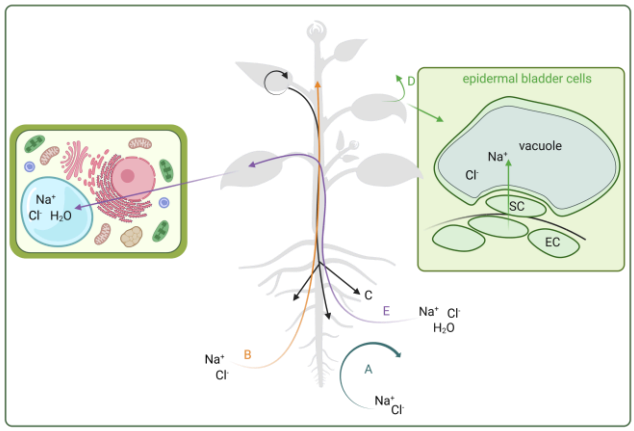

Figure 1 Salt tolerance mechanisms of halophytes. (A), Exclusion of ions at the root surface; (B), translocation of ions through the xylem; (C), recirculation into the root; (D), excretion by salt bladders/glands, epidermal bladder cell according to Zhao et al. (2020); (E), accumulation of salt and water in the cell vacuole. SC, stem cells; EC, epidermal cells. This figure was created using BioRender.com.

1.3.2.3 Influencing factors on salt tolerance

For the halophyte *A. hortensis* a salt tolerance between 100 mM and 514 mM is reported in the literature . Glenn et al. (2012) reported a salt tolerance of 514 mM, while Calone et al. (2021) reported a salt tolerance of 360 mM, Shekhawat et al. (2006) of only 100 mM and Kachout et al. (2009) of 250 mM. Since the plants in these studies are all greenhouse grown, the question arises as to why the salt tolerance levels are reported so differently. Environmental factors, such as light, temperature or humidity, influence the salt stress response of plants. In particular, salt stress affects photosynthesis, thus light regime is an important factor that may influence the salt tolerance of halophytes. Salinity affects photosynthesis in several ways: To counteract water loss during salinity stress, stomata opening is reduced, which limits CO_2 exchange and reduces photosynthetic carbon gain; imbalance in the Na/K ratio leads to a lower electrochemical gradient between the thylakoid interior and the stroma, resulting in less proton exchange and thus a lower stromal pH, which affects ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO), ATP synthase activity and NADPH reductase activity (Fig. 4) (Parihar et al., 2015, Hameed et al., 2021, Huang et al., 2019, Carillo, 2018). This leads not only to a reduced carbon gain, but also to an excess of electrons split by excited chlorophyll, and thus increased formation of reactive oxygen species (ROS). To scavenge ROS, the plant accumulates antioxidants, such as proline and glycine betaine, carotenoids, or flavonoids. Considering the light condition, a high light intensity, but also UVB light, can lead to the formation of ROS (details are shown in Fig. 7). The combination of high light intensity and therefore high photon flux, together with salinity, leads to over-excitation of the photosynthetic apparatus. Thus, more ROS are produced than the antioxidant system can scavenge, leading to necrosis and cell death.

However, halophytes are shown to have an adaptive mechanism to counteract photooxidation and inhibition during photosynthesis under salinity. For example, morphological adaptations can be found, such as a higher number of chloroplasts or relatively unstacked thylakoids (Bose et al., 2017, Lovelock and Ball, 2002). Stomatal aperture, photosynthetic rate, and water loss are also linked and shown to be regulated by ABA (Lovelock and Ball, 2002). Furthermore, a study by Carillo (2018) shows that there are differences between proline and GABA accumulation in low and high light under salinity. Their hypothesis is that the accumulation of GABA leads to the activation of glutamine synthase (GOGAT), which converts glutamine to glutamate, which causes an increased activity of ferredoxin, an enzyme in the photosynthetic process that transfers electrons split off from chlorophyll to $NADP^+$. This would ultimately lead to reduced ROS accumulation and thus protect plants from damage.

In addition to light intensity, spectral quality can also affect photosynthetic activity. For example, RuBisCo activity also depends on the light spectra and has its maxima under fully homogeneous spectra (Geiger et al., 1991). Furthermore, the absorption of green light is increased at higher salt concentrations due to thicker leaves that have a higher concentration of chlorophyll per leaf area.
Coming back to the different salinity tolerance levels reported for *A. hortensis*, the light conditions are not mentioned in all experimental setups, so it can only be speculated that different light conditions could have led to the different salinity tolerances, but also influencing factors like temperature, humidity, salt composition, watering, soil composition, etc. could affect salinity tolerance and thus salinity tolerance levels are individual for a certain experimental setup. Salt tolerance is therefore not a fixed level, but rather a range. It is also important to consider the halophyte species and the mechanism of salt tolerance, since halophytes have different photosynthetic adaptations.

1.3.3 Halophyte selection

The halophytes focused on in this thesis (*Brassica oleracea* var. *palmifolia*; *Cochlearia officinalis*, *Atriplex hortensis*, *Chenopodium quinoa* and *Salicornia europaea*) were selected according to different criteria. Certain technical criteria were obtained in a preliminary experiment, such as seed germination quality, plant performance and carotenoid content, which were taken into account. For example, *Crambe maritima*, was eliminated due to poor germination. Nutritional properties such as carotenoid content have been considered, as well as the high nutritional value of *C. quinoa* and *S. europaea* (Patel, 2016, Hulkko et al., 2022, Pathan and Siddiqui, 2022). Furthermore, regionality is important when considering consumer acceptance and introducing plants into a natural ecosystem. *A. hortensis*, *S. europaea* and *C. officinalis* are naturally occurring in the German flora (Müller, 2011). The ability to utilize the seeds and leaves of the plant increases the yield per acre and accounts for *A. hortensis* and *C. quinoa* (O'Leary, 1985, Chaudhary et al., 2023). The different levels of salt tolerance and the different salt tolerance mechanism of the selected species allow to investigate the influence of salt tolerance on a nutritional profile and offer research opportunities linking salt tolerance and indoor farming.
New food sources hold the potential for food safety issues, which is why the EU created the Novel Food Regulation (EU) 2015/2283 which states that “any food that has not been significantly consumed before May 1997 is considered a novel food” (European Parliament and European Council, 2015). This means that any newly introduced food must be implemented in the novel food regulation, which is a time and resource consuming process that can take years (EFSA Panel on

Dietetic Products et al., 2016). This limits the ability to actually introduce the food into the food market. Thus, plant species that are not subject to the Novel Food Regulation are advantageous. The selected halophytes are not considered to be novel foods, as they have been consumed in Europe for centuries, *B. oleracea* var. *palmifolia* (Thorness, 2009), used as a pharmaceutical plant, *C. officinalis* (Maat, 2004), or listed in the EU Novel Food Catalogue as food consumed before 1997 and therefore not subject to the Novel Food Regulation, *S. europaea*, *A. hortensis* and *C. quinoa* (Directorate-General for Health and Food Safety, 2023).

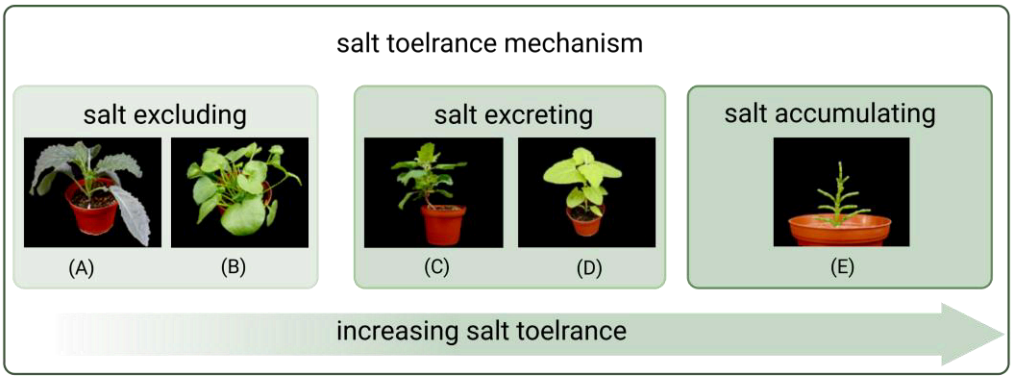

Figure 2 Selected halophytes and their salt tolerance mechanism. (A), *B. oleracea* var. *palmifolia* DC.; (B), *C. officinalis*; (C), *C. quinoa*; (D), *A. hortensis*; (E), *S. europaea*. This figure was created using BioRender.com.

1.3.3.1 *Brassica oleracea* var. *palmifolia* DC.

B. oleracea var. *palmifolia* (*Brassicaceae*), commonly known as palm kale, is grown primarily in Italy, in the northern Apennines, and is therefore also called Tuscan kale (Fig. 2A) (Thorness, 2009). In the last few years, it has received increasing attention in Italian gastronomy, but it has been little studied. Most of the available literature focuses on growth and yield (D'Antuono and Neri, 2003). Since it is a kale variety, it is not unexpected that the glucosinolates (GLS) glucobrassicin (I3M) and neoglucobrassicin (1MOI3M) are found, which are also present in common *B. oleracea* varieties such as kale, broccoli, or savoy cabbage (Fig. 4, Table S2) (Possenti et al., 2016). While neither the salt tolerance nor the mechanism of salt tolerance is known for *B. oleracea* var. *palmifolia*, salt tolerance up to 90 mM has been reported for *B. oleracea* (Flowers and Al-Azzawi, 2023). Thus, *B. oleracea* var. *palmifolia* is expected to be salt-tolerant at a low level and a salt-excluding plant as they show the lowest salt-tolerance.

1.3.3.2 *Cochlearia officinalis* L.

C. officinalis is commonly known as scurvy grass because it was used by sailors to treat scurvy (Fig. 2B) (Maat, 2004). It is a perennial plant of the *Brassicaceae* family (Flowers and Al-Azzawi, 2023). It is reported to be salt tolerant up to 400 mM by de Vos (2013), who conducted the first cultivation study with *C. officinalis* to evaluate its potential as a food. Furthermore, there are no reports of salt bladders/glands or salt accumulation (de Vos, 2013), suggesting that this is a salt-excluding plant. Considering PSMs, the GLSs glucopurtanjivin (iPr) and glucococchlerian (sBu) are present in *C. officinalis* (Fig. 4) (Griffiths et al., 2001).

1.3.3.3 *Chenopodium quinoa* L.

C. quinoa, commonly known as quinoa, is native to the Andes and also belongs to the *Amaranthaceae* family (Fig. 2C) (Fuentes et al., 2009). Its salt tolerance is cultivar dependent, with Adolf (2013) describing salt tolerance of up to 500 mM for the *Hualhuas* and *Titicaca* varieties, and up to 110 mM for the Andean hybrid variety. Optimum growth is reported to be between 100 mM and 200 mM for *C. quinoa* Willd. (Hariadi et al., 2011). *C. quinoa* also has salt bladders and can therefore be considered a salt excreting plant (Fig. 1D) (Adolf, 2013). Quinoa has been cultivated in the Andes for over 7000 years, not only for its climate-resistant cultivation, but also for the exceptional nutritional value of its seeds, which contain essential amino acids, vitamins A, B2 and E, minerals calcium, magnesium, iron, copper, zinc and lithium, and are a good source of carbohydrates and essential fatty acids (Abugoch J., 2009). While most studies focus on the seed, the leaves are also edible and have been shown to have nutritional value, such as a richness in PSMs (Pathan and Siddiqui, 2022).

1.3.3.4 *Atriplex hortensis* L.

Commonly known as garden orache, *A. hortensis* is an annual plant in the *Amaranthaceae* family (Fig. 2D) (Flowers and Al-Azzawi, 2023). It is also a drought-tolerant plant, making it a climate-resilient crop (Flowers and Al-Azzawi, 2023). *A. hortensis*, like most *Atriplex* species, has been shown to have salt bladders and can therefore be considered a salt-excreting plant (Fig. 1D) (Schirmer and Breckle, 1982, Breckle, 2002, Kachout et al., 2009, Yuan et al., 2016). Their optimum growth is reported to be between no salt and 150 mM and their salt tolerance is between 100 mM and 514 mM (Wilson et al., 2000, Kachout et al., 2009, Calone et al., 2021, Glenn et al., 2012, Shekhawat et al., 2006). The seeds have comparable nutritional values to *C. quinoa* seeds,

such as essential amino acids (Wright et al., 2002). Although the leaves are edible, their nutritional properties are not well studied.

1.3.3.5 *Salicornia europaea* L.

S. europaea is inhabiting European coastal areas and is also a member of the plant family *Amaranthaceae* (Fig. 1E) (Flowers and Al-Azzawi, 2023). It is commonly known as glasswort and has a reported salt tolerance of up to 1000 mM (Lv et al., 2012, Flowers and Al-Azzawi, 2023). Its optimal salt levels are reported to be in the range of 200 mM to 400 mM (Lv et al., 2012, Glenn and O'Leary, 1984). *S. europaea* shows a stem succulence and is reported to be a salt accumulating plant (Araus et al., 2021, Song and Wang, 2014). Because of its high salt tolerance, the genus *Salicornia* is the subject of many halophyte cultivation studies (Ventura, 2011, Ventura and Sagi, 2013, Singh et al., 2014, Gunning, 2016, Khalilzadeh et al., 2021a, Khalilzadeh et al., 2021b).
Also, *S. europaea*, has shown to be rich in phytonutrients such as PSM, polyphenols, carotenoids, flavonoids, and/or fatty acids (Patel, 2016, Hulkko et al., 2022).

2. Nutritional properties of healthy diets in human nutrition

A global increase in adult obesity from 8.7% in 2000 to 13.1% in 2022 is a concerning phenomenon, as it is a risk factor for chronic kidney disease (CKD) and other non-communicable diseases (NCDs) (FAO, 2022, Jiang et al., 2023). Cancer, diabetes and cardiovascular disease (CVD) are the leading NCDs, which account for 74% of all deaths worldwide (WHO, 2022). One of the most important drivers of NCDs is an unhealthy diet (Wagner and Brath, 2012), however food insecurity can also be a driver of obesity and NCDs (Nettle et al., 2017, Nkambule et al., 2021). In addition to obesity and NCDs, malnutrition and hunger remain global health threats. The prevalence of undernourishment had increased again by 1.8% from 2019 to 2021, which leads to a further distance to reach the Sustainable Development Goal (SDG) 2: “Zero hunger” (FAO, 2022). Even though these are global phenomena, obesity, NCDs and mental health issues in particular are linked to Western diets. Higher intakes of fruits and vegetables and lower intakes of meat, solid fats, and added sugars would improve health and reduce the risk of NCDs in Western diets (Martin et al., 2013). Historically unhealthy dietary patterns in Western diets are related to the introduction of Neolithic and Industrial Age foods (Cordain et al., 2005).
The World Health Organization (WHO) defines health as “a state of complete physical, mental and social well-being and not merely the absence of disease” (WHO, 1946). The EAT-*Lancet*

Commission reported on a reference diet that takes into account human health and environmental sustainability (Willett et al., 2019). Healthy diets focus on meeting nutritional needs and reducing the incidence of NCDs and all-cause mortality. They suggest a range of 200-600 g of vegetables per day and distinguish between dark green, red and orange vegetables and other vegetables. They do not suggest a plant-based diet, but the main source of protein is based on legumes and nuts. However, this diet cannot meet all needs because there are several aspects that affect nutritional needs based on the diversity of people, such as age, gender, medical conditions, and physical activity levels (Willett et al., 2019).

2.1 Nutritional properties of plant-based diets

Plant-based foods are not only more sustainable and therefore healthier for the planet, but also healthier for people (Willett et al., 2019, Xu et al., 2021). This is due to the high levels of bioactive compounds such as fiber, PSM, vitamins, minerals and trace elements in fruits and vegetables. Since the importance of vitamins, minerals and trace elements for human health is well known, plant secondary metabolites (PSMs) have been the focus of research in recent decades. PSMs are increasingly known to have incredible protective properties against several NCDs. These protective properties are due to their ability to scavenge ROS. Several factors such as an unhealthy diet, stress or air pollution can lead to increased production of ROS in the body. This can cause oxidative damage to biomolecules such as proteins, DNA or lipids, which is further linked to the increase in NCDs such as cancer, CVD or respiratory diseases (Liu et al., 2018). Antioxidants, such as PSMs, have the chemical properties to convert these highly reactive radicals into stable, inert molecules that no longer interact with biomolecules.

The group of PSMs is very diverse in their metabolic pathways, chemical structure, and function in plants, so not all PSMs have positive properties. Some, such as alkaloids or saponins, have antinutritive or even toxic properties. However, they have one thing in common: they are formed in the secondary metabolism of plants. Although there are many PSMs, such as polyphenols, flavonoids, alkaloids, only carotenoids and GLS are subject to this thesis. Additionally, carotenoids not only have health-promoting effects in the human diet, but are also essential photosynthetic pigments, like chlorophylls. As described above, salt has a huge impact on photosynthesis and thus on carotenoids and chlorophylls. Hence, they can serve as indicators of nutritional quality, but also as clues to plant physiological processes. The content and profile of PSMs in plants varies from one plant species to another (Mageney et al., 2016, Wiesner et al., 2013, Wu et al., 2021).

2.2 Carotenoids and chlorophylls

Carotenoids and chlorophylls form the group of photosynthetically active pigments in plants. During photosynthesis carotenoids function primarily as accessory pigments. Carotenoids are important in their function of light harvesting in the light harvesting complex (LHC) and also scavenging ROS (Zakynthinos and Varzakas, 2016). Carotenoids exist in stereoisomers, described as *Z*- and *E*-isomers (Zakynthinos and Varzakas, 2016). Due to their multiple double bonds, a carotenoid can have several stereoisomers located at different double bonds. Carotenoids can be divided into carotenes and xanthophylls, both of which have phytoene as their biosynthetic precursor (Cazzonelli and Pogson, 2010). From phytoene, (all-*E*)-lycopene is formed in several steps. Here the carotenoid biosynthetic pathway splits in two. One pathway leads to (all-*E*)-lutein and the other pathway leads to (all-*E*)-β-carotene and other carotenoids according to Fig. 3. From the β-carotene branch, the plant hormone ABA is formed in several biosynthetic steps.

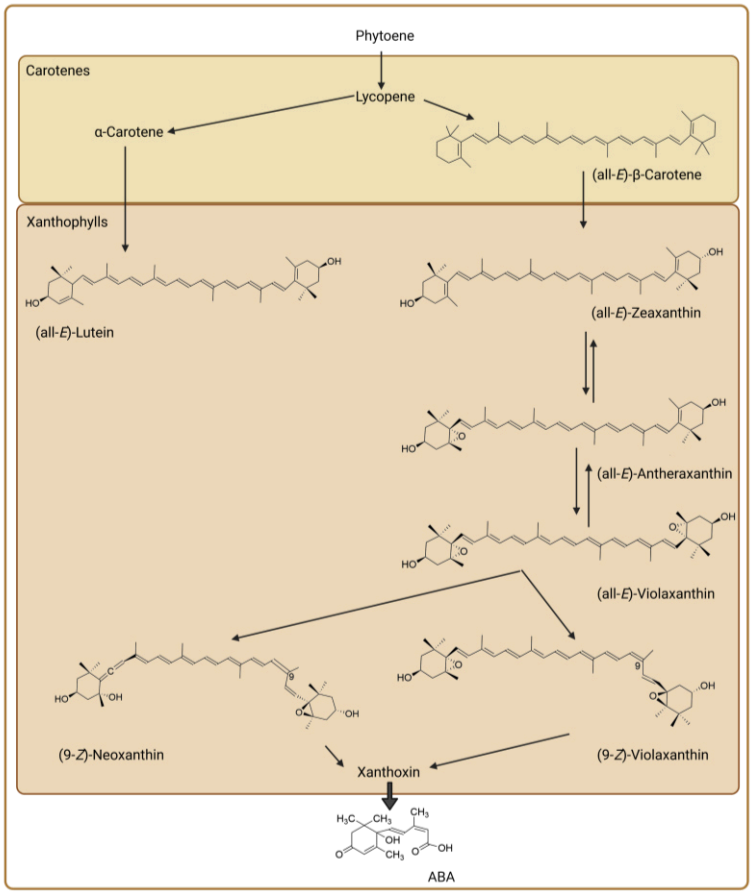

Figure 3 Chemical structure and biosynthetic pathway of selected carotenoids. ABA, abscisic acid. This figure was created using BioRender.com.

There are about 1100 different carotenoids known to date, which are colored red, orange or yellow, among others (Yabuzaki, 2017). The main sources of carotenoids are fruits and vegetables. Depending on the color, different carotenoids are present in higher amounts, for example, in tomatoes the carotenoid lycopene causes the red color, or in orange carrots it is β-carotene. In green leafy vegetables, the main carotenoids are β-carotene, lutein, violaxanthin, and neoxanthin (Britton and Khachik, 2009). In common green leafy vegetables, high levels of lutein and β-carotene can be found in kale or spinach with up to 5.9 mg 100 g^{-1} fresh mass and 8.3 mg 100 g^{-1} fresh mass, respectively (Table S1). Lower levels are found in Chinese cabbage and savoy cabbage (Table S1). This shows that the amount of carotenoids is highly dependent on the plant species, but there are also intraspecific variation, as shown for example, in *Brassica oleracea* var. *sabellica* (Mageney et al., 2016).

Considering the health-promoting effects of carotenoids, the activity of vitamin A, also known as provitamin A, is especially important for people on a vegan diet, since vitamin A can be found mainly in animal-based foods. Dietary carotenoids are converted in the intestinal mucosa to retinol (vitamin A1) and its esters and oxidation products (Cazzonelli, 2011). Carotenoids are one of the main sources of pro-vitamin A in the human diet (Zakynthinos and Varzakas, 2016, Grune et al., 2010). Only carotenoids that contain a β-ionone ring, such as retinol, are provitamin A carotenoids, such as β-carotene or β-cryptoxanthin. In human health retinol is important for the growth and development and essential for the visual process. The recommended intake of provitamin A is calculated using a retinol activity equivalent (DGE, 2020) and β-carotene has the highest retinol activity. Individual carotenoids have also been shown to have several other health-promoting properties (Table 1). Bioavailability and accessibility is also an important factor when considering health-promoting effects in foods, and can be influenced by differences in plant matrix, levels of carotenoid absorption inhibitors, levels of dietary fiber present, or the isomeric form of the carotenoid (Zakynthinos and Varzakas, 2016). For example, *cis*-lycopene preferentially accumulates in the human body, while the all-*E*-isomer predominates in food (Boileau et al., 2002). However, several other factors, such as how the food is processed or factors within the individual consuming the food, such as age, also have a major impact on the bioavailability and absorption of carotenoids in humans (Weber et al., 2020, Schmiedeskamp et al., 2022).

Table 1 Biological effects of major carotenoids in green leafy vegetables on human health.

Carotenoid	Health-promoting property	Reference
Lutein		
	Anti-apoptotic	(Park et al., 2020)
	Inhibits NF-κB-dependent inflammatory gene expression	(Kim et al., 2008b)
	Antimicrobial	(Mitra et al., 2021)
	Reduces the risk of CVD	(Mitra et al., 2021)
	Antioxidant	(Rodrigues et al., 2012)
Lutein + Zeaxanthin		
	Reduces the risk of age-related macular degeneration (AMD)	(Eisenhauer et al., 2017, Wang et al., 2007)
	Improve cognitive function	(Johnson, 2014)
β-Carotene		
	Anti-apoptotic	(Park et al., 2020)
	Pro Vitamin A activity	(Boon et al., 2010)
	Antioxidant	(Rodrigues et al., 2012)
	Anti-carcinogenic	(Milani et al., 2017, Fiedor and Burda, 2014)
Violaxanthin		
	Antioxidant, anti-inflammatory	(Milani et al., 2017)
	Anti-carcinogenic	(Gagez et al., 2012)
Neoxanthin		
	Anti-carcinogenic	(Gagez et al., 2012)
	Anti-bacterial	(Molnár et al., 2010)

Chlorophylls are the major photosynthetic pigments and act as the central light-harvesting molecules in photosystems. With magnesium as the central atom, they absorb light with maxima from 680 to 700 nm. In green plants, chlorophyll *a* and *b* are the forms of chlorophyll present. Considering the biosynthesis of chlorophylls, chlorophyll *a* is first synthesized from glutamate in 13 steps located in the chloroplasts (Chatterjee and Kundu, 2015). Secondly, chlorophyll *b* is formed from chlorophyll *a* in the chlorophyll cycle (Tanaka and Tanaka, 2011). However, in the chlorophyll cycle, chlorophyll *b* can also be converted to chlorophyll *a*. The chlorophyll cycle is the main regulator of chlorophyll levels in the photosynthetic apparatus.

Chlorophylls have also been shown to have health-promoting effects, such as antioxidant and chemoprotective properties based on their ability to scavenge ROS (Harttig and Bailey, 1998, Chernomorsky et al., 1999). In vegetables, chlorophylls are mainly found in green leafy vegetables,

high levels are found in spinach or Pak choi, and low levels are found in white cabbage, for example, which is reflected in the depth of green (Limantara et al., 2015).

2.3 Glucosinolates

GLSs are PSMs found primarily in *Brassica* species. Depending on their biosynthetic precursor amino acids they can be categorized in indole, aliphatic and aromatic GLSs (Fig. 4). The GLS degradation products are responsible for the typical taste of kale, radish or mustard, but are also associated with health benefits. The GLS degradation products are formed by thermal or enzymatic (mechanic) degradation. Thermal degradation occurs during cooking and is influenced by several factors, such as the plant matrix in the prepared food (Renz et al., 2023, Baenas et al., 2019). Enzymatic degradation occurs with ß-myrosinase (thioglucosidase, E.C. 3.2.1.147) during a freezing, thawing or chopping (Possenti et al., 2016). In the plant, myrosinase is activated by the cell disruption that follows an insect attack. Myrosinase hydrolyzes to form thioglucose, sulfate, and an unstable aglycone, which spontaneously rearranges into several GLS degradation products. Depending on the chemical structure of the GLS nitriles, isothionitriles (ITCs), epithionitriles and vinyl oxazolidinethiones are formed. GLS degradation products play an important role in plant-insect/herbivore interactions (Possenti et al., 2016). The GLS breakdown products, when consumed in the diet, can have health-promoting effects against CVDs, neurodegeneration, diabetes, and various inflammatory disorders (Possenti et al., 2016). In particular, ITCs show anti-carcinogenic activity that affects multiple stages of cancer development, including induction of detoxification enzymes and inhibition of activation enzymes (Possenti et al., 2016). Nitriles, on the other hand, show no bioactivity or even adverse effects (Basten et al., 2002, Kupke et al., 2016). Food processing, but also pre-harvest factors, as well as the plant species, have an influence on GLS content and thus the formation of GLS breakdown products (Ilahy et al., 2020, Wu et al., 2021, Sikorska-Zimny and Beneduce, 2021).

Glucosinolates are mainly consumed in Western diets with varieties of *Brassica oleracea*, such as Brussels sprouts, kale, savoy cabbage, white cabbage, or red cabbage. Common GLSs in these vegetables are, for example, glucoraphanin, sinigrin or glucobrassicin, in total about 50 GLSs occur in common *Brassica* vegetables (Wu et al., 2021). However, the profile and amount of GLSs varies widely among these vegetables (Wu et al., 2021). For example, glucoraphanin values range from 1.09 to 58.94 mg 100 g^{-1} fresh mass. Amounts of total and selected GLSs of common *Brassica* vegetables are given in Table S2.

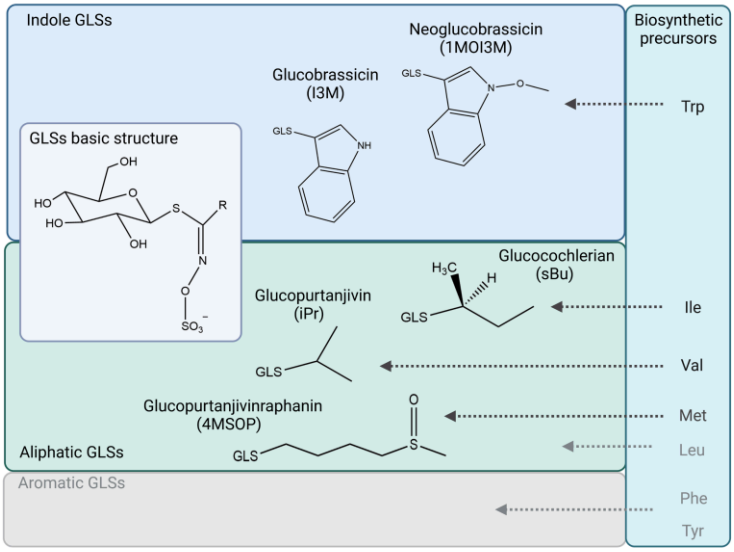

Figure 4 Chemical structure and biosynthetic precursors of selected glucosinolates. GLS, glucosinolate; I3M, indol-3-ylmethyl GLS; 1MOI3M, 1-methoxylindol-3-ylmethyl GLS; iPr, 2-propyl GLS; sBu, (1*S*)-1-methylpropyl GLS; 4MSOP, (*RS*)-4-(methylsulfinyl) butyl GLS; trp, tryptophan; ile, isoleucine; val, valine; met, methionine; leu, leucine; phe, phenylalanine. This figure was created using BioRender.com.

2.4 Minerals

Besides PSM, minerals can also be valuable compounds in food. However, they can also be a risk factor if their content is above the recommended levels. In halophytes a higher content of sodium and chloride can occur, compared to glycophytes. Sodium and chloride have important functions in the human body but can also be harmful if consumed in excess. Sodium is essential for maintaining cell potential and is involved in the active transport of several substances (Strohm et al., 2018). Chloride is part of the production of gastric acid, a component of digestive juices and is involved in the non-specific defense against pathogens (Strohm et al., 2018). On the other hand, sodium and chloride are associated with increased blood pressure, which can lead to hypertension, and increased risk of CVDs (Strohm et al., 2018). Therefore, the WHO recommends consumption of up to 5 g day^{-1} of sodium chloride, 3 g day^{-1} of chloride, and 2 g day^{-1} of sodium (WHO, 2012). The average sodium consumption in 2010 was 3.95 g day^{-1}, which would be equivalent to 9.88 g day^{-1} of sodium chloride, and the highest intakes were found in East and Central Asia and Eastern Europe (Powles et al., 2013). In Germany, the average intake of sodium chloride is at 6 g day^{-1} (Strohm et al., 2018). Thus, a reduction of salt uptake in the diets is recommended, which needs to be taken into account considering halophytes as new food sources.

Besides salt, sodium and chloride, nitrate is also of relevance as a risk factor in human nutrition. Nitrate does not affect human health directly, but through its metabolites, nitrite, and nitric oxide. Nitrite oxide is associated with wound healing and lowering blood pressure (Schäffer et al., 1996, Kapil et al., 2010). Nitrite and nitric oxide are toxicologically relevant because they are associated with the formation of methemoglobin and gastric cancer (EFSA, 2008). Therefore, the WHO recommends an acceptable daily intake (ADI) of 3.7 mg kg^{-1} body weight. This would lead to a limit intake of 323 mg day^{-1} for an average man in Germany (87 kg body weight; 40 to 45 years old), 254 mg day^{-1} for an average woman (69 kg body weight; 40 to 45 years old), and 69 mg day^{-1} for an average child (19 kg body weight; 4 to 5 years old) (Destatis, 2018). Unlike sodium chloride, which is mainly ingested with processed foods, nitrate is often ingested with vegetables (Weitzberg and Lundberg, 2011, EFSA, 2008). Among vegetables, green leafy vegetables such as spinach or lettuce have higher levels than root vegetables or fruit vegetables (Luo et al., 2022, Brkić et al., 2017). Luo et al. (2022) showed that the daily intake of nitrate from leafy vegetable consumption exceeded the recommended levels for adults in Shanghai, even though the fresh vegetables were within the safe level of 1 mg kg^{-1} nitrate. Additionally, to the plant species, the nitrate content is influenced by many factors such as the development stage of the plant, the harvest season, the plant organ consumed or the light regime (Brkić et al., 2017, Luo et al., 2022, Bian et al., 2020).

Objectives

Considering saline indoor farming as a new agrifood systems with cultivation of halophytes as potential alternative vegetables for future food production, an assessment of the systems, as well as nutritional quality is needed. Due to the lack of studies on saline indoor farming systems with halophytes, the system had to be built up from the ground and several system optimizations (fertilization, temperature, humidity, light regime, growing material, germination) were carried out. In a pre-experiment the halophyte cultivation was tested and further plant species and salt levels were selected for the development of the indoor farming systems. To facilitate the implementation of new plant species in the future, halophyte species with different salinity tolerances and salinity tolerance mechanisms were selected as mentioned in 1.3.3 Halophyte selection. To meet the need for sustainable production and a healthy product, a sustainable use of water and a modulation of environmental conditions should be implemented to enhance health-promoting PSMs. Therefore, this thesis evaluated the system feasibility, nutritional properties, alternative water use, influence of light regime and salinity and modulating of light conditions, e.g., UVB light, of selected halophytes grown in saline indoor farming. To assess the plant performance and gain insight into the underlying mechanism of halophyte's responses to light and salinity, morphological parameters and signaling molecules, e.g., plant hormones, amino acids and ROS, as well as photosynthetic active pigments were analyzed. To evaluate the nutritional properties PSM and minerals, e.g., chloride and nitrate, were analyzed. As a basis for these studies, methods had to be adapted and developed due to the differences in plant material from halophytes to glycophytes, such as higher salinity and, in the case of *S. europaea*, the leafless stem succulence. Therefore, HPLC-TQ-MS MRM methods for the determination of plant hormones (abscisic acid, salicylic acid and jasmonic acid) and amino acids were established and optimized for the halophytic plant material. For the analysis of ROS, enzymatic methods for H_2O_2 and SOD activity were adapted. Anion (chloride, nitrate, sulfate and phosphate) determination was optimized for soil and plant material measured by ion chromatography. Carotenoids, chlorophylls and glucosinolates were measured by UHPLC-DAD-(Q)-ToF-MS. To further study the influence of oxidative stress on carotenoid accumulation, a method was developed to apply an oxidative stress-inducing substance.

This thesis was divided into four plant studies:

(1) The focus of the study published in publication I was to investigate the suitability of halophytes as alternative vegetables for human consumption produced in a saline indoor farming system and

how salinity affects the nutritional profile. Therefore, five selected halophyte species were grown in a saline indoor farming system in a NFT system at different salinity levels. Plant performance and optimal salinity level were assessed by phenotype, yield, anions and stress markers (phytohormones and pigments). The influence of salinity on the nutritional profile was assessed by the content of PSMs (carotenoids, chlorophyll, glucosinolates) and minerals (chloride and nitrate).

(2) The objective of the study published in publication II was to determine if brine water is feasible as an alternative saline water source for indoor halophyte cultivation as a food crop. This study was performed with the halophyte *S. europaea*, as it has the highest salt tolerance. The plants were grown in indoor cultivation and exposed to two regional brine waters, an artificial seawater and an artificial sodium chloride solution. The saline water solutions were tested against a control, assessed as optimal growth condition in study (1). The plant growth and carotenoid and chlorophyll contents were analyzed to test the feasibility of the brine waters as water sources and evaluate their influence on nutritional properties.

(3) The subject of the study published in publication III was the interaction between salinity and light regime on the food quality of halophytes. Therefore, the selected halophytes were cultivated in the NFT system in indoor farming and in a greenhouse. The plant performance and salt stress response were assessed by morphological parameters, plant hormones, anion content and photosynthetic active pigments. Further the nutritional properties were evaluated by analyzing the content of selected PSM.

(4) The aim of the study published in publication IV was to determine the optimal UVB dose to increase carotenoid content and to elucidate the UVB response of halophytes. Therefore, in a first experiment, *C. officinalis*, *A. hortensis* and *S. europaea* were exposed to different doses of supplemental narrow-band UVB light and growth and pigment content were analyzed. Due to indifferent responses to UVB light, the further experiments were focused on *S. europaea*. Growth, plant hormones and photosynthetic active pigments were evaluated to be able to differentiate between eustress and distress response. Further, to evaluate the involvement of oxidative stress in the UVB response, ROS were assessed and a menadione treatment was performed as an additional experimental point of view. To gain some insight into the interaction of UVB light and salinity, an experimental setup with two different salt levels was also performed.

Manuscripts

Overview

Publication I

Title	Comprehensive characterization of selected phytochemicals and minerals of selected edible halophytes grown in saline indoor farming for future food production
Authors	M. Fitzner, M. Schreiner, S. Baldermann
Published in	*Journal of Food Composition and Analysis*, 122, 105435; IF: 4.3
DOI	10.1016/j.jfca.2023.105435
Own contribution	Organization and performance of the experimental study, adaptation and further development of the methodologies for the analysis of phytohormones and minerals, data curation, writing original draft, corresponding author

Publication II

Title	Utilization of regional natural brines for the indoor cultivation of *Salicornia europaea*
Authors	M. Fitzner, A. Fricke, M. Schreiner, S. Baldermann
Published in	*Sustainability*, 2021, 13, 12105; IF: 3.9
DOI	10.3390/su132112105
Own contribution	Organization and performance of the experimental study, data curation, writing original draft, corresponding author

Publication III

Title	The interaction of salinity and light regime modulates photosynthetic pigment content in edible halophytes in greenhouse and indoor farming
Authors	M. Fitzner, M. Schreiner, S. Baldermann
Published in	*Frontiers in Plant Science.* 2023;14 IF: 6.6
DOI	10.3389/fpls.2023.1105162
Own contribution	Organization and performance of the experimental study, adaptation and further development of the parts of methodologies

	for the analysis of phytohormones and minerals, data curation, writing original draft, corresponding author

Publication IV

Title	Between eustress and distress: UVB induced changes in carotenoid accumulation in halophytic *Salicornia europaea*
Authors	M. Fitzner, M. Schreiner, S. Baldermann
Published in	*Journal of Plant Physiology*; IF: 4.3
DOI	10.1016/j.jplph.2023.154124
Own contribution	Organization and performance of the experimental study, adaptation and further development of the parts of methodologies for the analysis of phytohormones, amino acids and ROS, data curation, writing original draft, corresponding author

Publication I

Comprehensive characterization of selected phytochemicals and minerals of selected edible halophytes grown in saline indoor farming for future food production.

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Published in: *Journal of Food Composition and Analysis*, 122, 105435
DOI: 10.1016/j.jfca.2023.105435

Journal of Food Composition and Analysis 122 (2023) 105435

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Comprehensive characterization of selected phytochemicals and minerals of selected edible halophytes grown in saline indoor farming for future food production

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ARTICLE INFO

Keywords:
Carotenoids
Glucosinolates
Future food
Edible Halophytes
Saline Agriculture
Salt

ABSTRACT

Sustainable and resilient agri-food systems are needed to ensure food security in the face of increasing urbanization, water scarcity and climate change. Saline indoor farming offers a flexible use of urban production areas with resource-saving water management. Salt-tolerant plants (halophytes) are crops that can be grown in saline environments, have excellent nutritional properties, such as being rich in minerals and phytochemicals. The aim of this study was to evaluate plant performance and selected nutritional properties of five halophyte species (glasswort, quinoa, garden orache, scurvy grass and palm kale) in saline indoor farming with different salinities. In particular, we evaluated morphological parameters, as well as, carotenoids, chlorophylls, glucosinolates, plant hormones by LC-MS, and anion composition by IC. The five halophyte species showed differences in plant growth performance and nutritional properties depending on the salinity level. For example, we found a total carotenoid content ranging from 1581.4 ± 180.4 ng mg^{-1} DW (scurvy grass) to 188.3 ± 48 ng mg^{-1} DW (glasswort; no salt) and a 40-times higher increase of β-carotene (200 mM salt) for glasswort, compared to the other halophytes. In summary, we observed that the phytochemical content varied with salinity and that halophytes require species-specific growing conditions to enhance valuable metabolites.

1. Introduction

By 2050, freshwater resources will be almost entirely exhausted and phenomena such as soil salinization, fresh water scarcity, environmental disasters and biodiversity loss already threaten food security (FAO 2022; Kanianska, 2016). This inevitably leads to the question of how food security can be managed in the future.

Agriculture must adapt to these challenges and thus sustainable food production is essential (Willett et al., 2019). Therefore, alternative food sources from sustainable and resilient agrifood systems are in demand for sustainable diets (Preiss et al., 2022; Parodi et al., 2018). Since resource-saving water use is one of the key requirements for sustainable agrifood systems, saline agriculture is of great interest due to its potential to conserve fresh water resources (Ladeiro, 2012). Since most people worldwide (76%) live in urban areas, according to the OCED's Urban Studies (OECD, 2020), the development of local indoor farming systems is necessary and leads to a reduction in agricultural land use, the possibility of circular resource-efficient systems, and reduced food transportation distances, thus increasing the sustainability of agrifood systems (van Delden et al., 2021). Combining this needs, saline indoor farming offers the possibility for the production of alternative food sources, such as salt-tolerant plants (halophytes), derived from a sustainable, urban agrifood system.

Since a plant-rich diet is not only more sustainable, but also offers health benefits, halophytes are of particular interest (Willett et al., 2019; Xu et al., 2021). Their potential to serve as alternative vegetables has been demonstrated in several studies, for instance for species of *Salicornia* and *Sarcocornia* (Ventura and Sagi, 2013; Ladeiro, 2012) or

Abbreviations: PSM, plant secondary metabolites; ROS, reactive oxygen species; GLS, glucosinolates.

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https://doi.org/10.1016/j.jfca.2023.105435
Received 26 March 2023; Received in revised form 24 May 2023; Accepted 1 June 2023
Available online 3 June 2023

Chenopodium quinoa (quinoa) (Chaudhary et al., 2023). In terms of the international food market, quinoa has the largest market for halophyte species to date. Other halophyte species are also entering the food market, such as *Salicornia* species as gourmet vegetables (Centofanti and Bañuelos, 2019). More research is needed to optimize their cultivation conditions and to realize their full market potential. Additionally, halophytes have been shown to have excellent nutritional properties, such as mineral composition or bioactive compounds (Zanella and Vianello, 2020; Lopes et al., 2021; Castañeda-Loaiza et al., 2020). For palm kale, quinoa, garden orache and scurvy grass the leaves are the edible parts and for quinoa and garden orache also the seeds can be used (Chaudhary et al., 2023; D'antuono and Neri, 2003; De Vos, 2013; O'Leary et al., 1985). While for quinoa most studies focus on quinoa seeds, the greens also have a valuable nutritional profile with bioactive compounds such as phenols, flavonoids, and carotenoids (Pathan and Siddiqui, 2022) and are in the focus of this study. For glasswort the whole aboveground as well as the seeds can be used (Araus et al., 2021).

Due to its richness in plant secondary metabolites (PSM), plant-based diets are linked to a reduction in various health risks such as cardiovascular diseases, diabetes, or cancer. Carotenoids and chlorophylls are important due to their anti-carcinogenic and chemo-protective properties (Mitra et al., 2021; Milani et al., 2017; Hayes and Ferruzzi, 2020). In addition, β-carotene has provitamin A activity, and lutein and zeaxanthin are related to eye-health (Eisenhauer et al., 2017; Fiedor and Burda, 2014). Glucosinolates (GLSs) are mainly found in *Brassicales* (scurvy grass and palm kale), and their breakdown products are associated with health-promoting effects, in particular with anti-carcinogenic properties (Traka and Mithen, 2009).

In addition to a desirable high level of PSMs, due to the salt-accumulating properties of halophytes, potentially harmful compounds must also be considered. In this context it is important to consider high chloride and nitrate contents as potential health risks. High intake of sodium chloride can contribute to hypertension and cardiovascular diseases (Strohm et al., 2018). Nitrate content is assessed as problematic because nitrates can form reactive metabolites such as nitrite, nitric oxide and *N*-nitroso compounds, which are associated with health risks (EFSA, 2008). Further anti-nutrients can be found in halophytes, such saponins, tannins, oxalate or alkaloids. While oxalate and saponins may be problematic in *Amaranthaceae* (quinoa, glasswort, and garden orache) (Patel, 2016), the tropane alkaloid cochlearine might accumulated in scurvy grass (Brock et al., 2006).

Halophytes have different salt tolerance, due to their different salt tolerance mechanism, namely salt-excluding (SEL), salt-excreting (SER) and salt-accumulating (SAL) mechanisms (Hasanuzzaman et al., 2014). These mechanisms allow the halophytes to grow in higher salinity than non-halophytic plants (Flowers and Colmer, 2008). Besides the salt tolerance mechanisms, abiotic and biotic factors, such as developmental stage, salt composition, climate conditions or salt availability, may influence the salt tolerance. Therefore, salt tolerance in indoor farming might differ from values described in the literature, which mainly focus on greenhouse or open field cultivation (De Vos, 2013; Glenn and O'leary, 1984; Breckle, 2002). It is crucial to identify the optimal salt levels in indoor farming, since halophytes can be also affected by salt stress. Salt stress not only affects yields, but also the plant's metabolism and thus nutritional quality, for example plant secondary metabolites (PSMs). In response to mild salt stress reactive oxygen species (ROS) are formed, which can activate the antioxidative system leading to accumulation of PSMs (Hasanuzzaman et al., 2020). However, under severe salt stress, the damage by ROS cannot be compensated by the antioxidant system, leading to necrosis and decrease in PSMs (Hasanuzzaman et al., 2020). It is shown that the growth conditions have an effect on the nutritional composition of halophytes (Martins-Noguerol et al., 2022). While there is an evaluation of mineral and nutrient composition of halophytes, for example in O'Leary et al. (1985), little emphasis is placed on chloride and nitrate as food components. In addition to the effect on carotenoids and chlorophylls in halophytes and glycophytes (Kim et al., 2008; Yang et al., 2009), photosynthesis is also affected by salinity (Bose et al., 2017).

In this study we focused on five different halophytes, namely glasswort (*Salicornia europaea*), quinoa (*Chenopodium quinoa*), garden orache (*Atriplex hortensis*), scurvy grass (*Cochlearia officinalis*), and palm tree kale (*Brassica oleracea* var. *palmifolia*). The first three belong to the family *Chenopodiaceae*, the latter two to the family *Brassicaceae*. Since different salt tolerance levels have been reported for the halophyte species we have chosen different concentrations from no salt to 1200 mM being slightly above the maximum described tolerance level of glasswort of 1000 mM (Lv et al., 2012). The lowest salt tolerance is assumed for palm kale based 90 mM reported for wild cabbage (Flowers and Al-Azzawi, 2023). The reported tolerance levels for scurvy grass, quinoa and garden orache are between 200 and 600 mM (De Vos, 2013; Adolf et al., 2013; Glenn et al., 2012; Calone et al., 2021). Therefore, the aim of this study was to evaluate the influence of the saline indoor farming system and different salinity levels on plant performance and nutritional properties of the five selected halophyte species.

2. Material and methods

2.1. Plant material and sampling

Seeds of glasswort (*Salicornia europaea* L.) were obtained from Rühlemann's Kräuter & Duftpflanzen (Horstedt, Germany) and seeds of quinoa (*Chenopodium quinoa*), scurvy grass (*Cochlearia officinalis* L.), garden orache (*Atriplex hortensis* L.) and palm kale (*Brassica oleracea* L. var. *palmifolia* DC.) were obtained from Magic Garden Seeds (Regensburg, Germany). The plants were germinated on soil (substrate *type P*, 70% raised bog peat (degree of decomposition: H2-H5), 30% clay; Einheitserdewerke Werkverband e.V., Sinntal-Altengronau, Germany; nutrient composition: Table S1) until two leaves had fully developed and were then transferred to pots (diameter 8 cm) containing one third soil (substrate *type T*, 70% raised bog peat (degree of decomposition: H2-H5), 30% clay; Einheitserdewerke Werkverband e.V., Sinntal-Altengronau, Germany; nutrient composition: Table S1), one third fine quartz sand (grain size 0.5–1 mm), and one third coarse quartz sand (grain size 2–3 mm) (Euroquarz GmbH, Laußnitz, Germany). The experiment was performed twice (exp. 1 and 2) in locally different but technically identical climate chambers, to minimize site-specific related effects on the plants, we randomized the pots once a week. In one experiment per salt treatment twelve plants per species were grown. Of these twelve plants, three plants were pooled for one biological sample. Thus, for further metabolite analysis four biological replicates per salt treatment, plant species and experiment were analyzed. The plants were harvest after three weeks of salt treatment and six to nine weeks after sowing, depending on the plant species. For quinoa and garden orache the main leaves, for scurvy grass and palm kale all leaves (except cotyledons) were harvested and for glasswort the aboveground part was harvested. After harvesting the leaves were snap-frozen in liquid nitrogen and stored at −50 °C until lyophilization. For further analysis the samples were homogenized (3–5 times for 50 s with 3–5 metal beads [diameter 9 mm] at 25 Hz) with a Retsch mill (Retsch MM 400; Retsch GmbH, Haan, Germany) (Fitzner et al., 2023).

2.2. Saline indoor farming system

The indoor farming system was set up in a climate chamber (Vötsch Industrietechnik GmbH, Balingen-Frommern, Germany) with the following environmental conditions: temperature, 22 °C/18 °C (day/night); photon flux density (PFD), 380 μmol m^{-2} s^{-1} (Clean Ace™ R MT400DL/BH YE; EYE Lighting Europe Ltd, Uxbridge, United Kingdom); photoperiod, 14 h/10 h (day/night); air humidity, 65%. Plant pots were placed in gutters equipped with a fleece through which the nutrient solutions (Table S2) were pumped in intervals of ½ hour according to a NFT (Nutrient Film Technique) system. The density in the

gutters was 7.9 plant pots per m^2 (pots diameter 8 cm). After seven days of acclimatization to the NFT-system the plants were treated for three weeks with the following five salt treatments: no salt, 50 mM, 200 mM, 600 mM, and 1200 mM sodium chloride (salt).

2.3. Determination of morphological parameters and moisture content

The fresh weight was determined at the harvest time point by weighing the plants and the leaves. Dry weight and the moisture content were determined after lyophilization. The growth distance was calculated by measuring stem length at the beginning and end of the salt treatment using ImageJ Software 1.53e (GitHub, Public license) (Schneider et al., 2012). The leaf number increase was determined by counting the leaves at the beginning and end of the salt treatment. For glasswort the number of stems was counted.

2.4. Analysis of carotenoid and chlorophyll content

Carotenoids and chlorophylls were extracted as previously described in Frede et al. (2019) with slight modifications. In brief, 5 mg of homogenized, lyophilized plant material was extracted five times with 0.5 mL methanol/tetrahydrofuran (1:1, v/v). The analysis was performed using an Agilent Technologies 6530 UHPLC-DAD-QToF-MS (Agilent Technologies Sales & Services GmbH & Co. KG, Waldbronn, Germany) according to Frede et al. (2017). Identification was achieved using mass spectra and UV/VIS spectra according to the literature (Fig. S1, Table S3) (Britton, 2004; Clementson and Wojtasiewicz, 2019). Quantification was achieved using external calibration from carotenoid standards (CaroteNature GmbH, Munsingen, Switzerland) of *all-trans*-isomers from β-carotene, lutein and zeaxanthin and 9-*cis*-neoxanthin as well as chlorophyll *a* and *b* (Sigma Aldrich Chemie GmbH, Taufkirchen, Germany) at the wavelength of 450 nm. Neoxanthin and violaxanthin contents are each shown as the sum of two isomers.

2.5. Analysis of glucosinolate content

Glucosinolates were analyzed and quantified with UV/VIS at a wavelength of 229 nm as desulfo-GLSs according to Witzel et al. (2015). Sinalbin (Phytolab GmbH & Co.KG, Vestenbergsgreuth, Germany) was used as an internal standard. GLSs were identified with mass spectra according to literature (Fig. S2, Table S4) (Blažević et al., 2020; Olsen et al., 2016).

2.6. Analysis of nitrate and chloride content

Chloride and nitrate contents were determined by ion exchange chromatography according to Fitzner et al. (2023): 10 mg of the freeze-dried plant material was dissolved in 1 mL of ultrapure water, and 500 μL of sodium bromide (0.6 mg mL^{-1}) was added as an internal standard. The solution was sonicated on ice for 10 min and centrifuged for 5 min (4500 *g*, 4 °C). Next, 1.2 mL of the sample solution were transferred to a tube and diluted with ultrapure water depending on the salt concentration according to the range of chloride calibration curve. Measurements were performed using an ion chromatograph 930 Compact IC Flex (Metrohm AG, Herisau, Switzerland) equipped with a conductivity detector with suppression system. The injection volume was set to 20 μL at a flow rate of 0.7 mL min^{-1} and an eluent consisting of Na_2CO_3 (3.2 mM) and $NaHCO_3$ (1 mM) was used as the mobile phase. A Metrosep A Supp5–250/4.0 column (Metrohm AG, Herisau, Switzerland) was used. The final concentration of the anions was quantified with external calibration from standards of chloride, nitrate and bromide ions (Sigma Aldrich Chemie GmbH, Taufkirchen, Germany).

2.7. Analysis of plant hormones

Abscisic acid (ABA) extraction, as well as salicylic acid (SA) and jasmonic acid (JA), was performed as previously described in Fitzner et al. (2023) with slight modifications. First, 10 mg of freeze-dried plant material was extracted with 200 μL methanol/water (60:40, v/v) and deuterated standards of ABA, SA, and JA ((+)-d6-ABA, d4-SA, d5-JA (mixture of diastereomers, (-)-trans major)) were added as internal standards solution. The samples were sonicated for 15 min on ice and centrifuged for 10 min (12,298 *g*, 4 °C). The extraction was repeated twice, and the supernatants collected. The combined supernatant was filtered through a PTFE-filter tube (0.2 μm, Thermo Fischer Scientific Inc., Wilmington, USA), centrifuged for 7 min (12,298 *g*, 4 °C) and inserted into a HPLC vial and diluted 1:2 with ultrapure water containing 0.1% acetic acid. The analysis was performed using an Agilent Technologies 1260 Infinity HPLC coupled with a triple quadrupole, Q-Trap® 6500-MS/MS system (AB Sciex LLC, Framingham, Massachusetts, USA). A Zorbax Eclipse Plus C18 (1.8 μm, 2.1 mm × 50 mm; Agilent Technologies, Waldbronn, Germany) column was used for chromatographic separation with a column temperature of 30 °C. The mobile phase comprised ultrapure water (+ 0.1% acetic acid) and acetonitrile (+ 0.1% ultrapure water). The separation was performed in a gradient mode (flow rate: 650 μL min^{-1}). For MS, electro spray ionization was used in the negative mode and multi reaction monitoring (MRM) (MS parameters and MRM transition, Table S3). Identification and quantification were achieved with ratios of analytes and internal standards of the MRM transitions (Table S5). Quantification was performed using external calibration curves of standards of ABA (± , ≥98%), SA (≥99%) and JA (±) (Sigma Aldrich Chemie GmbH, Taufkirchen, Germany) with internal standards (Toronto Research Chemicals, North York, Ontario, Canada).

2.8. Statistical analysis

Statistical differences were tested using Sigma Plot (14.0; Systat Software GmbH, Frankfurt am Main, Germany) with a one-way ANOVA followed by post-hoc Tukey's test with a significance level of $p \geq 0.05$ when normal distribution and equal variance were present. When either normal distribution or equal variance failed, a Kruskal-Wallis one- way ANOVA on ranks was performed, followed by Tukey test or Dunn's Method. Normal distribution was tested with the Shapiro Wilks normality test and equal variance with the Brown-Forsythe equal variance test. Data are presented in means ± standard deviation (SD), unless otherwise stated, of the two experimental setups separately (exp. 1/2). Fold change is shown for significant results, unless otherwise stated, and with respect to the no salt treatment.

3. Results

3.1. Changing nutritional profiles of halophytes in reaction to salt treatment

To evaluate the nutritional profile as well as the stress status of the plants, we analyzed the content of selected PSMs, chloride and nitrate, and hormones in selected halophytes (facultative halophytes: palm kale, scurvy grass, quinoa, garden orache, and obligate halophyte: glasswort). All the plant growth experiments were carried out twice (exp. 1 and 2) in locally different but technically identical climate chambers. While the absolute values showed slight differences between the two experiments due to biological and site-specific differences, the main results related to salt treatment and halophyte species were the same in both experiments.

3.1.1. Salinity alters the content of plant secondary metabolites

Total chlorophyll content, as well as chlorophyll *a* and chlorophyll *b* content, was highest in no salt treatment for facultative halophytes. At 1200 mM salt, no total chlorophyll, nor chlorophyll *a* or *b* were detected,

since all the plants were necrotic (Table 1). The highest content of total chlorophyll was found in quinoa with 9.7 ± 1.4 μg mg^{-1} DW (exp. 2) followed by scurvy grass, palm leaf kale, glasswort, and lowest in garden orache with 3.7 ± 0.1 μg mg^{-1} DW (exp. 2) (Table 1). At 600 mM salt, we observed a reduction in chlorophyll content in all halophyte species (Table 1). Furthermore, in garden orache and scurvy grass chlorophyll content decreased at 200 mM salt. In glasswort, the total chlorophyll content was highest at 50 mM salt and lowest in the no salt treatment, whereas the chlorophyll a/b ratio was highest at 200 mM and lowest at no salt, which was due to higher chlorophyll b content at 50 mM salt (Table 1).

The investigated carotenoids were found in all plant species (Fig. S1). Total carotenoid content ranged in the no salt treatment from 1581.4 ± 180.4 ng mg^{-1} DW (scurvy grass, exp. 2) to 188.3 ± 48 ng mg^{-1} DW (glasswort, exp. 2) (Table 2). In scurvy grass, garden orache, and palm kale, the total carotenoid content decreased at 200 mM salt (Table 2). Quinoa showed a significant decrease in total carotenoid content only at 600 mM salt, but also a trend of reduction starting at 200 mM salt (Table 2). Glasswort displayed the highest content of total carotenoids at 50 mM salt in exp. 2 and 50 mM and 200 mM in exp. 1 and lowest at 600 mM salt and no salt in both exp. (Table 2). The carotenoid with the highest content in all plant species was lutein and the highest concentration was found in palm kale in the no salt treatment with 872.7 ± 42.9 ng mg^{-1} DW (exp. 1) (Table 2). Considering the reaction to salt treatment, lutein showed the same pattern as total carotenoids. In contrast, β-carotene content increased in all halophyte species at 50 mM salt in at least one experimental setup compared to no salt. Among them, glasswort showed the highest increase (41.3-fold) at 200 mM salt. Zeaxanthin was the carotenoid with the lowest content of all carotenoids and showed an increased content at moderate salinity (50 mM/200 mM) in all halophyte species (Table 2).

GLSs were only present in two of the five halophyte species, scurvy grass and palm kale. The identified GLSs were found in the plants according to Figure S2. The two plant species showed a different composition of indole and aliphatic GLSs, with the indole GLSs in the palm kale accounting for about 50% of the total GLSs, but only 5% in the scurvy grass. This is due to the dominating content of the aliphatic (*1 S*)−1-Methylpropyl GLS (sBu) in scurvy grass, which accounted for up to 98% of the total GLSs. The content of total GLSs was significantly higher in scurvy grass (18.1 ± 2.7/18.7 ± 7.0 μg mg^{-1} DW) than in palm kale (1.3 ± 0.3/1.7 ± 0.5 μg mg^{-1} DW) (Table 3). Both the total GLS content and the ratio of indole and aliphatic GLSs were influenced by salinity (Table 3). In scurvy grass, the total GLS content was reduced at 200 mM and 1200 mM salt compared to no salt treatment (Table 3). Indole GLSs tended to increase at 200 mM salt (1.1/1.6-fold) and decrease (0.8–0.9/0.6–0.9-fold) at the higher salt concentrations. The aliphatic GLSs showed a decrease from 200 mM (0.8/0.8-fold) to 1200 mM salt, which was decreased due to sBu (Table 3). Palm kale showed a significant reduction in total GLSs at 600 mM and 1200 mM salt (Table 3). We observed a stronger decrease in indole GLSs than in aliphatic GLSs at 600 mM and 1200 mM salt, which led to increased ratio between aliphatic and indole GLSs (Table 3).

3.1.2. Chloride and nitrate accumulation in leaves

To assess a potential negative impact for human nutrition, the chloride and nitrate content in leaves were determined by ion chromatography. In particular, palm kale had a 20-fold increased chloride content in 200 mM salt, which doubled at 600 mM salt, but only significantly increased at 1200 mM salt (Table 4). Scurvy grass showed the lowest chloride contents of all halophyte species up to 200 mM salt and then a significant increase at 600 mM and 1200 mM salt. Garden orache and quinoa exhibited an increased chloride content from 200 mM salt. Glasswort displayed constantly increasing chloride content from 50 mM salt, however, this increase was less pronounced than in the other plant species, as they showed an up to 100-fold increase in chloride content and glasswort only a 2.3–7.9-fold increase compared to no salt. This is attributed to a 10-fold higher starting chloride content in the no salt treatment of glasswort compared to the other plant species.

Nitrate levels ranged from 0.9 ± 0.7–108.6 ± 6.0 mg g^{-1} DW in total (Table 4). We observed a trend of decreased nitrate content with increasing salinity for all plant species. Garden orache and scurvy grass showed a 1.5–2-fold decrease in nitrate content from 200 mM to 1200 mM salt compared to no salt. Palm kale, quinoa and glasswort already showed decreased nitrate content at 50 mM salt.

3.2. Halophyte performance in saline indoor farming

To evaluate the plant performance, and in particular plant stress status in a saline indoor farming cultivation system and determine the salt tolerance range of the halophytes, we analyzed morphological parameters (growth, as measured by growth distance, and leaf mass increase), yield in fresh weight, and content of plant hormones with respect to salt treatment. Increasing salinity showed strong effects on the phenotype of the plants; at 1200 mM salt all plants were withered, i.e. necrotic (Fig. 1, Fig. S3, Table S6).

For all facultative halophytes we observed a reduced fresh weight starting from 600 mM salt (Fig. 1). For palm leaf kale growth reduction correlated with increasing abscisic acid (ABA) levels at 200 mM, indicating an optimal growth condition up to 50 mM salt. For scurvy grass optimal growth conditions up to 200 mM salt was observed. Quinoa and garden orache showed a significant decrease in growth distance and leaf mass increase at 600 mM (Table S6), associated with an increase in ABA concentrations, leading to the assumption that the salt tolerance limit is exceeded.

For the obligate halophyte glasswort 50–200 mM salt were identified as optimal growth conditions, expressed in the highest fresh weight and growth distance (Fig. 1E). At 600 mM salt, we measured the same amount in fresh weight as in no salt treatment, but a lower increase in stem number and less growth (Table S6).

3.2.1. Altering ABA content in response to salt treatment

Above these optimal growth conditions, the plants showed increased ABA contents, with palm kale, quinoa and garden orache at 600 mM salt (Fig. 2A, C, D). For glasswort, we observed a 0.4–0.6-fold decrease in ABA content at 50 mM, 200 mM and 600 mM salt compared to no salt and 1200 mM salt, in exp. 2 even an increase at 1200 mM salt compared to no salt (Fig. 2E). Scurvy grass only showed an altered ABA content in one experimental setup (Fig. 2B).

Additionally salicylic (SA) and jasmonic acid (JA) were analyzed. They showed mostly the same pattern by SA increasing with higher salt concentrations and JA decreasing with higher salt concentrations (Fig. S4, S5). Although scurvy grass showed a response in SA by a decreased content at 600 mM (0.5/0.5-fold) and 1200 mM (0.2/0.4-fold) salt compared to no salt and increased content at 50 mM and 200 mM salt compared to 600 mM and 1200 mM salt (Fig. S4B).

4. Discussion

4.1. Assessment of plant performance and growing conditions in saline indoor farming

For evaluating plant performance of halophytes grown in saline indoor farming, we analyzed yields, morphological parameters, and anions. The concentration of the hormone ABA was determined as signaling molecule in plant's stress reaction. We observed different plant performances between the facultative (palm kale, scurvy grass, quinoa and garden orache) and obligate (glasswort) halophytes in their reaction to salt treatment in terms of yield and plant hormone status. For the facultative halophytes, increasing salinity correlated with increased ABA content and decreased growth and yield. But the obligate halophyte, showed an increased fresh mass correlating with decreased ABA levels, within the moderate salinity levels. In addition to the biomass

Table 1
Content of chlorophylls in leaves of 6 or 9 week-old plants depending on the plant species. Means ± SD of two individual experimental setups (1) or (2), n = 4 per experiment. DW, dry weight. Letters indicate significant differences between treatments within one experiment and one plant species in alphabetic order from highest to lowest ($p \leq 0.05$).

	Exp.	Salt [mM]	Palm kale				Scurvy grass				Quinoa				Garden orache				Glasswort			
Chlorophyll *a* [µg mg^{-1} DW]	(1)	0	5.78	±	0.61	a	7.09	±	0.33	a	6.85	±	0.35	a	3.19	±	0.19	a	0.62	±	0.55	c
		50	5.29	±	0.40	a	6.23	±	0.42	a	7.05	±	0.31	a	2.74	±	0.15	a	3.49	±	0.27	a
		200	4.50	±	0.73	b	4.96	±	0.16	b	5.10	±	0.64	b	2.16	±	0.20	b	3.22	±	0.28	a
		600	<LOD				1.01	±	0.59	c	0.04	±	0.06	c	0.26	±	0.19	c	1.77	±	0.21	b
		1200	<LOD				<LOD				<LOD				<LOD				0.03	±	0.03	c
	(2)	0	5.70	±	1.53	ns	6.91	±	0.80	a	7.05	±	0.16	a	3.02	±	0.07	a	1.31	±	0.30	b
		50	4.93	±	0.75	ns	6.34	±	0.48	a	6.23	±	2.45	a	2.87	±	0.25	a	2.81	±	0.47	a
		200	4.43	±	0.39	ns	4.97	±	0.42	b	4.81	±	0.52	a	1.90	±	0.20	b	2.35	±	0.09	a
		600	<LOD				1.21	±	0.75	c	0.06	±	0.03	b	<LOD				1.18	±	0.10	b
		1200	<LOD				<LOD				<LOD				<LOD				0.03	±	0.02	c
Chlorophyll *b* [µg mg^{-1} DW]	(1)	0	2.73	±	0.20	a	2.35	±	0.11	a	2.13	±	1.07	ab	0.6	±	0.06	a	0.55	±	0.12	b
		50	2.49	±	0.11	a	2.08	±	0.20	a	2.17	±	0.11	a	0.51	±	0.05	a	1.09	±	0.09	a
		200	1.93	±	0.35	b	1.73	±	0.09	b	1.49	±	0.23	ab	0.38	±	0.04	b	0.94	±	0.07	a
		600	0.00	±	0.00	c	0.38	±	0.19	c	0.02	±	0.02	b	0.06	±	0.05	c	0.52	±	0.06	b
		1200	<LOD				<LOD				<LOD				<LOD				0.01	±	0.00	c
	(2)	0	2.40	±	0.72	a	2.41	±	0.26	a	2.32	±	0.10	a	0.65	±	0.05	a	0.56	±	0.12	bc
		50	2.39	±	0.29	a	2.37	±	0.30	a	2.31	±	0.37	a	0.57	±	0.04	a	0.91	±	0.09	a
		200	1.66	±	0.17	a	1.62	±	0.10	b	1.42	±	0.14	a	0.34	±	0.02	b	0.67	±	0.03	b
		600	0.01	±	0.01	b	0.55	±	0.31	c	0.05	±	0.02	b	<LOD				0.41	±	0.01	c
		1200	<LOD				<LOD				<LOD				<LOD				0.01	±	0.00	e
Total chlorophylls [µg mg^{-1} DW]	(1)	0	8.51	±	0.71	a	9.44	±	0.44	a	8.97	±	0.42	ab	3.79	±	0.2	a	1.17	±	0.65	c
		50	7.78	±	0.441	b	8.31	±	0.61	a	9.22	±	0.41	a	3.26	±	0.19	a	4.58	±	0.36	a
		200	6.43	±	1.071	c	6.69	±	0.25	c	6.59	±	0.87	ab	2.54	±	0.24	b	4.16	±	0.34	a
		600	0	±	3E-04	d	1.39	±	0.79	c	0.06	±	0.08	ab	0.32	±	0.24	c	2.29	±	0.27	b
		1200	<LOD				<LOD				<LOD				<LOD				0.03	±	0.03	d
										a												
	(2)	0	8.1	±	2.243	a	9.32	±	1.06	a	9.37	±	0.23	a	3.67	±	0.11	a	1.87	±	0.4	c
		50	7.32	±	1.033	a	8.71	±	0.77	b	7.96	±	3.64	a	3.45	±	0.28	b	3.72	±	0.55	a
		200	6.09	±	0.561	a	6.59	±	0.46	c	6.23	±	0.66	a	2.23	±	0.22	c	3.02	±	0.12	b
		600	0.01	±	0.008	b	1.76	±	1.06	c	0.11	±	0.05	b	<LOD				1.59	±	0.11	c
		1200	<LOD				<LOD				<LOD				<LOD				0.04	±	0.02	d
Moisture content [%]	(1)	0	89.49	±	0.61		91.00	±	1.41		90.21	±	0.32		91.31	±	1.27		92.91	±	0.09	
		50	90.11	±	0.11		91.11	±	3.78		90.83	±	0.46		90.42	±	0.63		94.49	±	0.15	
		200	89.73	±	0.81		85.81	±	1.90		89.67	±	0.31		88.76	±	0.63		93.37	±	0.30	
		600	36.32	±	21.33		44.25	±	27.83		33.12	±	4.63		53.28	±	32.05		89.14	±	0.54	
		1200	34.09	±	6.67		31.82	±	6.96		27.78	±	6.79		12.96	±	6.42		51.17	±	19.86	
	(2)	0	89.85	±	0.84		92.38	±	0.39		89.81	±	1.23		91.61	±	0.50		93.02	±	0.54	
		50	89.64	±	0.73		91.36	±	0.84		90.34	±	0.28		90.69	±	0.57		93.50	±	0.99	
		200	84.19	±	9.43		86.44	±	1.04		89.11	±	1.62		88.70	±	0.47		93.03	±	0.13	
		600	32.01	±	8.58		52.83	±	12.17		36.38	±	13.26		26.34	±	1.88		89.98	±	0.82	
		1200	26.04	±	5.05		43.45	±	19.74		22.57	±	5.42		12.00	±	12.16		28.22	±	7.79	

ns, not significant; LOD, limit of detection.

Table 2
Content of individual carotenoids in leaves of 6 or 9 week-old plants depending on the plant species. Means ± SD of two individual experimental setups (1) or (2), n = 4 per experiment. DW, dry weight. Letters indicate significant differences between treatments within one experiment and one plant species in alphabetic order from highest to lowest ($p \leq 0.05$). Moisture content according to Table 1.

	Exp.	Salt [mM]	Palm kale				Scurvy grass				Quinoa				Garden orache				Glasswort			
Total carotenoids [ng mg^{-1} DW]	(1)	0	1543.63	±	42.28	a	1532.37	±	77.18	a	1302.36	±	157.95	ab	713.87	±	73.99	a	188.28	±	48.41	c
		50	1488.39	±	44.28	a	1429.11	±	86.92	a	1497.82	±	28.73	ab	546.06	±	52.44	b	684.21	±	80.83	a
		200	1224.01	±	216.67	b	1201.60	±	47.59	b	1153.22	±	170.04	b	490.88	±	32.35	b	746.97	±	35.23	a
		600	30.88	±	2.55	c	274.26	±	161.28	c	39.44	±	10.31	c	81.61	±	57.21	c	267.59	±	193.22	b
		1200	32.86	±	4.64	d	35.01	±	2.04	d	46.47	±	11.88	c	32.31	±	3.36	c	37.31	±	2.49	d
	(2)	0	1346.37	±	289.12	a	1581.43	±	180.44	a	1412.18	±	72.94	a	664.58	±	25.87	a	304.02	±	37.19	c
		50	1339.23	±	123.48	a	1526.09	±	119.10	a	1575.35	±	214.60	a	585.37	±	63.27	a	649.31	±	77.99	a
		200	1124.19	±	92.82	ab	1198.32	±	28.56	b	1030.56	±	130.82	a	399.38	±	38.22	b	524.75	±	15.78	b
		600	36.34	±	2.00	ab	311.46	±	213.86	c	36.03	±	3.02	b	33.20	±	1.33	c	265.64	±	46.01	c
		1200	31.95	±	1.36	c	31.39	±	0.56	c	32.15	±	2.17	b	34.35	±	1.52	c	33.22	±	5.36	d
Lutein [ng mg^{-1} DW]	(1)	0	872.72	±	42.95	a	751.17	±	35.53	a	625.07	±	27.41	a	249.92	±	19.53	a	106.38	±	47.84	b
		50	795.22	±	40.79	ab	682.16	±	61.15	ab	691.18	±	21.84	a	204.40	±	17.79	b	347.35	±	36.60	a
		200	573.11	±	111.10	b	607.48	±	23.04	b	518.84	±	50.98	b	177.28	±	13.78	b	341.65	±	13.30	a
		600	<LOD				194.72	±	13.77	c	5.34	±	5.65	c	48.57	±	19.32	c	199.87	±	13.84	ab
		1200	<LOD				<LOD				17.22	±	9.30	c	4.32	±	2.55	c	<LOD			
	(2)	0	849.36	±	200.68	a	836.59	±	72.57	a	670.18	±	27.32	a	259.78	±	13.07	a	3.89	±	2.38	c
		50	732.04	±	71.44	ab	771.99	±	80.88	ab	756.79	±	109.03	a	235.04	±	21.96	a	109.71	±	21.91	a
		200	530.56	±	54.70	b	616.13	±	28.93	b	525.24	±	42.62	a	164.85	±	12.69	b	139.21	±	8.36	a
		600	<LOD				176.06	±	133.50	c	3.20	±	2.16	b	<LOD				70.61	±	17.47	b
		1200	<LOD				<LOD				2.51	±	1.55	b	<LOD				<LOD			
β-Carotene [ng mg^{-1} DW]	(1)	0	266.29	±	25.20	b	226.01	±	6.70	b	213.28	±	13.53	ns	156.94	±	13.69	a	3.89	±	2.38	c
		50	291.79	±	17.64	a	295.32	±	11.99	a	261.34	±	14.18	ns	153.38	±	13.15	a	109.71	±	21.91	a
		200	252.67	±	37.81	a	277.30	±	18.23	a	281.53	±	39.74	ns	146.33	±	9.94	a	139.21	±	8.36	a
		600	0.29	±	0.50	c	71.10	±	22.56	c	1.20	±	2.39	ns	31.67	±	8.81	b	70.61	±	17.47	b
		1200	<LOD				<LOD				<LOD				<LOD				<LOD			
	(2)	0	217.39	±	49.68	ns	205.01	±	35.68	b	153.66	±	12.66	ns	120.23	±	3.26	b	6.91	±	2.84	b
		50	259.80	±	29.50	ns	309.14	±	22.21	a	286.49	±	45.92	ns	154.26	±	14.08	a	125.73	±	16.51	a
		200	252.82	±	33.03	ns	232.29	±	8.25	b	210.34	±	29.87	ns	128.20	±	16.17	a	88.50	±	2.33	ab
		600	<LOD				39.26	±	37.86	c	<LOD				<LOD				51.86	±	4.69	ab
		1200	<LOD				<LOD				<LOD				<LOD				<LOD			
Neoxanthin [ng mg^{-1} DW]	(1)	0	248.84	±	27.13	a	213.20	±	12.86	a	142.08	±	95.36	ab	84.10	±	8.14	a	42.64	±	3.17	b
		50	216.82	±	16.52	a	204.92	±	16.52	a	203.02	±	7.11	a	63.03	±	4.84	ab	95.59	±	7.98	a
		200	174.94	±	32.67	b	130.46	±	22.21	b	140.30	±	23.16	ab	52.37	±	5.27	ab	88.55	±	5.01	a
		600	14.82	±	1.32	c	40.92	±	14.29	c	16.43	±	0.76	b	19.15	±	2.98	ab	51.52	±	4.69	b
		1200	15.82	±	1.95	c	16.98	±	0.97	c	16.42	±	0.40	b	14.60	±	0.66	b	18.50	±	1.84	c
	(2)	0	253.05	±	63.19	a	228.34	±	26.16	a	208.28	±	10.72	a	84.17	±	3.01	a	79.60	±	7.29	a
		50	202.75	±	16.04	a	214.80	±	19.75	a	208.96	±	31.79	a	62.67	±	6.17	ab	86.08	±	10.97	a
		200	166.97	±	10.09	b	162.84	±	4.93	b	132.67	±	12.92	a	48.30	±	3.99	ab	75.31	±	2.72	ab
		600	17.79	±	1.12	c	48.91	±	25.55	c	16.13	±	0.41	a	15.73	±	0.09	b	43.19	±	3.20	ab
		1200	15.48	±	0.65	c	15.20	±	0.29	c	14.89	±	0.64	b	16.61	±	0.74	b	16.17	±	2.63	b
Violaxanthin [ng mg^{-1} DW]	(1)	0	155.78	±	53.11	c	341.99	±	29.33	a	345.66	±	16.24	a	222.91	±	40.01	a	36.35	±	4.58	bc
		50	184.56	±	37.34	b	246.71	±	18.91	b	342.28	±	31.26	a	125.25	±	18.07	b	131.56	±	17.12	ab
		200	223.29	±	45.66	a	186.34	±	15.01	c	212.56	±	59.46	b	114.90	±	7.46	b	177.56	±	11.24	a
		600	15.84	±	1.42	d	33.99	±	12.38	d	17.81	±	1.90	c	22.34	±	5.20	c	74.77	±	26.00	bc
		1200	16.97	±	2.13	d	18.03	±	1.07	d	17.13	±	0.12	c	15.55	±	0.70	c	18.81	±	1.29	c

(*continued on next page*)

Table 2 (*continued*)

	Exp.	Salt [mM]	Palm kale				Scurvy grass				Quinoa				Garden orache				Glasswort			
	(2)	0	35.41	±	20.57	c	311.48	±	53.98	a	380.06	±	24.33	a	200.39	±	12.47	a	31.20	±	3.75	b
		50	144.64	±	10.08	b	230.17	±	25.50	b	323.12	±	32.07	ab	133.41	±	21.45	b	83.14	±	17.63	a
		200	173.84	±	12.67	a	187.06	±	51.19	b	162.31	±	45.41	bc	58.03	±	6.68	c	92.60	±	20.91	a
		600	18.55	±	0.88	c	47.23	±	17.48	c	17.50	±	0.49	c	17.47	±	1.30	d	32.55	±	5.26	b
		1200	16.47	±	0.71	c	16.19	±	0.27	d	16.01	±	0.59	c	17.74	±	0.78	d	17.04	±	2.72	b
Zeaxanthin [ng mg^{-1} DW]	(1)	0	49.32	±	21.48	ns	7.87	±	3.21	b	6.37	±	1.68	b	6.24	±	1.47	b	0.92	±	1.29	b
		50	43.38	±	14.04	ns	18.03	±	5.66	b	6.79	±	3.32	b	15.26	±	1.51	a	7.95	±	1.55	a
		200	28.41	±	8.99	ns	38.49	±	4.08	a	14.18	±	4.17	a	18.60	±	4.42	a	3.30	±	0.55	b
		600	<LOD				35.98	±	12.02	a	<LOD				7.099	±	4.35	b	1.31	±	0.96	b
		1200	<LOD				<LOD				<LOD				<LOD				<LOD			
	(2)	0	29.15	±	19.42	b	5.42	±	1.82	b	5.58	±	1.18	b	9.31	±	6.11	ns	13.02	±	3.59	a
		50	80.02	±	9.25	a	23.16	±	8.01	a	23.62	±	3.02	a	11.55	±	4.27	ns	5.27	±	2.33	b
		200	44.26	±	9.06	b	34.82	±	4.70	a	16.46	±	0.92	ab	16.52	±	6.63	ns	6.21	±	0.46	b
		600	<LOD				7.77	±	7.03	b	<LOD				<LOD				5.11	±	0.88	b
		1200	<LOD				<LOD				<LOD				<LOD				<LOD			
Moisture content [%]	(1)	0	89.49	±	0.61		91.00	±	1.41		90.21	±	0.32		91.31	±	1.27		92.91	±	0.09	
		50	90.11	±	0.11		91.11	±	3.78		90.83	±	0.46		90.42	±	0.63		94.49	±	0.15	
		200	89.73	±	0.81		85.81	±	1.90		89.67	±	0.31		88.76	±	0.63		93.37	±	0.30	
		600	36.32	±	21.33		44.25	±	27.83		33.12	±	4.63		53.28	±	32.05		89.14	±	0.54	
		1200	34.09	±	6.67		31.82	±	6.96		27.78	±	6.79		12.96	±	6.42		51.17	±	19.86	
	(2)	0	89.85	±	0.84		92.38	±	0.39		89.81	±	1.23		91.61	±	0.50		93.02	±	0.54	
		50	89.64	±	0.73		91.36	±	0.84		90.34	±	0.28		90.69	±	0.57		93.50	±	0.99	
		200	84.19	±	9.43		86.44	±	1.04		89.11	±	1.62		88.70	±	0.47		93.03	±	0.13	
		600	32.01	±	8.58		52.83	±	12.17		36.38	±	13.26		26.34	±	1.88		89.98	±	0.82	
		1200	26.04	±	5.05		43.45	±	19.74		22.57	±	5.42		12.00	±	12.16		28.22	±	7.79	

ns, not significant; LOD, limit of detection.

Table 3
Content of individual glucosinolates in leaves of palm kale and scurvy grass of 6 or 9 week-old plants depending on the plant species. Means ± SD of two individual experimental setups (1) or (2), n = 4 per experiment. DW, dry weight. Letters indicate significant differences between treatments within one experiment and one plant species in alphabetic order from highest to lowest ($p \leq 0.05$). Moisture content according to Table 1.

	Peak number*	Salt [mM]	Palm kale								Scurvy grass							
Exp.			(1)				(2)				(1)				(2)			
Total GLS [mg g^{-1} DW]		0	1.33	±	0.29	a	1.68	±	0.47	a	18.72	±	6.97	a	18.11	±	2.74	a
		50	1.09	±	0.33	ab	1.02	±	0.51	ab	13.26	±	7.69	ab	16.81	±	9.14	ab
		200	0.59	±	0.37	bc	0.99	±	0.36	ab	3.31	±	3.12	b	4.78	±	1.35	b
		600	0.31	±	0.11	c	0.26	±	0.07	b	6.24	±	1.47	ab	8.19	±	0.66	ab
		1200	0.38	±	0.12	c	0.18	±	0.03	b	3.98	±	0.77	b	5.73	±	0.63	b
Ratio aliphatic/indole		0	1.00	±	0.21	ab	1.05	±	0.45	ab	1.03	±	0.46	ab	1.02	±	0.22	ab
		50	0.55	±	0.20	b	0.84	±	0.65	ab	0.60	±	0.23	ab	0.78	±	0.32	ab
		200	0.99	±	0.80	ab	0.68	±	0.20	b	0.14	±	0.16	b	0.29	±	0.08	b
		600	38.26	±	10.85	ab	20.54	±	6.57	a	1.34	±	0.26	ab	1.37	±	0.63	ab
		1200	61.91	±	32.83	a	28.28	±	20.59	ab	3.13	±	1.87	a	3.36	±	1.38	a
3MSOP [µg g^{-1} DW]	1	0	11.62	±	5.42	ns	12.58	±	4.96	ns	n/a				n/a			
		50	9.30	±	1.50	ns	16.65	±	14.61	ns								
		200	11.42	±	2.03	ns	5.40	±	2.39	ns								
		600	10.40	±	1.55	ns	10.66	±	7.10	ns								
		1200	13.48	±	9.45	ns	3.01	±	0.06	ns								
2OH2MP [µg g^{-1} DW]	2	0	217.41	±	73.77	ns	320.55	±	112.97	a	19.35	±	3.46	ab	n/a			
		50	104.99	±	34.61	ns	119.69	±	22.47	ab	58.60	±	48.80	ab				
		200	104.04	±	42.29	ns	130.69	±	20.21	ab	79.08	±	23.97	a				
		600	132.00	±	52.42	ns	108.94	±	29.54	ab	6.64	±	10.37	ab				
		1200	167.69	±	48.08	ns	73.95	±	9.55	b	1.08	±	0.67	b				
4MSOB [µg g^{-1} DW]	3	0	255.01	±	71.21	a	375.91	±	153.30	a	18.48	±	5.56	ns	23.16	±	3.12	ns
		50	126.55	±	6.70	ab	157.37	±	34.21	b	54.12	±	40.97	ns	102.55	±	87.67	ns
		200	63.87	±	51.62	b	171.15	±	97.69	b	71.41	±	53.73	ns	93.53	±	18.80	ns
		600	153.36	±	77.85	ab	129.97	±	34.51	b	29.43	±	14.34	ns	92.54	±	42.67	ns
		1200	188.96	±	63.75	ab	88.65	±	11.33	b	26.08	±	28.12	ns	77.46	±	41.56	ns
iPr [mg g^{-1} DW]	5	0	n/a				n/a				5.09	±	2.40	a	4.99	±	1.30	a
		50									4.06	±	2.20	ab	5.65	±	3.76	ab
		200									1.50	±	1.30	ab	1.36	±	0.09	ab
		600									1.67	±	0.32	ab	2.35	±	0.13	ab
		1200									1.03	±	0.40	b	1.32	±	0.11	b
4OHI3M [µg g^{-1} DW]	6	0	23.06	±	4.72	a	51.29	±	21.35	a	62.21	±	15.41	a	56.35	±	11.10	ab
		50	14.42	±	3.39	ab	15.01	±	8.11	ab	64.32	±	24.75	a	68.61	±	30.97	a
		200	15.47	±	5.51	ab	38.89	±	15.82	ab	38.59	±	33.97	ab	66.24	±	27.40	a
		600	0.68	±	0.29	ab	0.29	±	0.10	b	2.34	±	1.59	b	12.33	±	20.19	bc
		1200	0.26	±	0.10	b	<LOD			ab	0.41	±	0.36	b	0.15	±	0.18	c
iBu [µg g^{-1} DW]	7	0	n/a				n/a				54.29	±	30.20	ns	33.36	±	9.02	ns
		50									84.93	±	18.86	ns	53.97	±	37.18	ns
		200									26.13	±	30.93	ns	47.18	±	16.92	ns
		600									49.57	±	37.83	ns	24.34	±	6.57	ns
		1200									23.90	±	14.90	ns	21.70	±	2.82	ns
sBu [mg g^{-1} DW]	8	0	n/a				n/a				13.35	±	4.72	a	12.89	±	2.33	a
		50									8.78	±	5.56	ab	10.80	±	5.18	ab
		200									1.71	±	1.98	b	3.12	±	1.21	b
		600									4.38	±	1.11	ab	3.12	±	1.21	ab
		1200									2.88	±	0.64	b	4.27	±	0.61	b
I3M [µg g^{-1} DW]	9	0	726.45	±	149.10	a	772.29	±	283.25	a	10.08	±	4.40	ab	11.68	±	5.97	ns
		50	671.45	±	250.09	ab	554.93	±	357.41	ab	25.38	±	9.34	a	17.42	±	16.20	ns
		200	273.54	±	311.36	abc	500.96	±	257.75	abc	158.20	±	149.73	a	13.83	±	6.56	ns
		600	4.52	±	2.65	bc	13.07	±	3.07	ab	3.54	±	1.44	ab	3.34	±	2.80	ns
		1200	4.70	±	3.96	c	8.46	±	5.62	b	1.03	±	0.80	b	1.78	±	1.33	ns
4MOI3M [µg g^{-1} DW]	10	0	27.12	±	3.62	a	44.65	±	28.68	a	6.36	±	2.09	ab	10.17	±	4.84	ab

(*continued on next page*)

Table 3 (*continued*)

Exp.	Peak number*	Salt [mM]	Palm kale (1)				Palm kale (2)				Scurvy grass (1)				Scurvy grass (2)			
		50	21.05	±	5.65	ab	15.05	±	7.48	ab	10.48	±	4.61	ab	7.78	±	3.91	ab
		200	12.65	±	5.24	b	10.69	±	4.65	ab	12.23	±	3.07	a	19.67	±	2.89	a
		600	1.35	±	0.31	c	1.96	±	0.58	b	7.61	±	3.93	ab	3.62	±	3.06	ab
		1200	1.42	±	1.42	c	1.81	±	1.19	b	1.00	±	0.42	b	2.00	±	1.19	b
1MOI3M [μg g^{-1} DW]	11	0	63.90	±	48.94	ab	98.64	±	31.48	ns	116.47	±	22.88	ab	95.86	±	14.55	a
		50	143.54	±	63.29	a	169.29	±	45.04	ns	119.32	±	52.08	ab	130.10	±	66.51	a
		200	99.04	±	42.86	a	123.19	±	40.60	ns	101.75	±	41.47	b	64.97	±	31.59	ab
		600	3.26	±	2.03	b	1.17	±	0.68	ns	35.44	±	6.05	bc	53.41	±	23.48	ab
		1200	3.60	±	2.59	b	0.98	±	0.08	ns	14.98	±	9.42	c	14.66	±	8.56	b

Aliphatic GLS: 3MSOB, (RS)−3-(methylsulfinyl)-propyl GLS; 2OH2MP, 2-hydroxy-2-methylpropyl GLS; 4MSOB, (RS)−4-(methylsulfinyl)butyl GLS; iPr, 2-propyl GLS; iBu, 2-Methylpropyl GLS; sBU, (1 S)−1-Methylpropyl GLS. Indole GLS: 4OHI3M, 4-hydroxyindol-3-ylmetyl GLS; I3M, Indol-3-ylmethyl GLS; 4MOI3M, 4-Methoxyindol-3-ylmethyl GLS; 1MOI3M, 1-Methoxylindol-3-ylmethyl GLS; *peak number according to Fig. S2. ns, not significant; LOD, limit of detection; n/a, not available.

Table 4

Concentration of chloride and nitrate in leaves of 6 or 9 week-old plants depending on plant species. Means ± SD of two individual experimental setups (1), (2), n = 4 per experiment. Letters indicate significant differences between treatments within one experiment and one plant species in alphabetic order from highest to lowest ($p \leq 0.05$). Moisture content according to Table 1.

	Exp.	Salt [mM]	Palm kale				Scurvy grass				Quinoa				Garden orache				Glasswort			
Chloride [mg g^{-1} DW]	(1)	0	6.28	±	1.16	c	11.82	±	1.07	c	4.58	±	1.12	e	4.52	±	1.12	d	47.49	±	1.31	e
		50	47.37	±	8.70	bc	43.38	±	12.89	cd	65.23	±	13.99	d	37.22	±	2.41	d	157.39	±	6.14	d
		200	135.67	±	14.88	abc	85.43	±	15.20	bc	136.49	±	9.27	c	104.16	±	7.79	c	210.52	±	6.14	c
		600	251.60	±	47.49	ab	132.81	±	45.20	b	204.82	±	26.59	b	162.96	±	8.66	b	278.95	±	27.15	b
		1200	380.12	±	49.37	a	279.19	±	45.36	a	353.34	±	41.71	a	278.96	±	31.90	a	343.50	±	10.22	a
	(2)	0	7.05	±	0.65	b	13.09	±	2.49	c	3.42	±	0.71	d	4.02	±	0.26	d	39.66	±	2.17	d
		50	44.24	±	3.44	ab	38.95	±	3.33	c	58.91	±	13.80	cd	30.66	±	6.52	d	140.72	±	12.48	c
		200	155.11	±	14.01	ab	65.92	±	7.58	c	118.48	±	9.41	c	88.50	±	6.70	c	225.94	±	12.43	b
		600	328.85	±	17.91	ab	248.21	±	71.98	b	325.34	±	36.28	b	141.92	±	28.45	b	351.84	±	31.70	a
		1200	517.14	±	34.15	a	382.82	±	82.15	a	380.95	±	51.75	a	278.83	±	44.94	a	329.49	±	40.08	a
Nitrate [mg g^{-1} DW]	(1)	0	2.73	±	0.20	a	89.81	±	9.73	a	34.37	±	3.94	a	38.38	±	3.67	a	108.58	±	5.96	a
		50	2.49	±	0.11	a	76.36	±	9.05	a	12.34	±	4.87	b	27.08	±	5.63	a	66.25	±	4.40	b
		200	1.93	±	0.35	b	40.64	±	9.90	b	7.04	±	3.13	b	5.93	±	3.21	b	32.73	±	2.65	c
		600	0.00	±	0.00	c	46.26	±	7.29	bc	12.77	±	1.87	b	11.68	±	6.01	b	11.37	±	1.20	d
		1200	<LOD				18.85	±	5.93	c	12.35	±	1.55	b	11.01	±	0.75	b	25.81	±	4.26	c
	(2)	0	45.08	±	8.74	a	66.42	±	6.57	a	32.62	±	11.54	a	32.91	±	2.21	a	90.37	±	9.62	a
		50	10.71	±	4.73	c	54.94	±	6.96	a	13.39	±	7.59	b	16.30	±	7.31	a	52.54	±	8.12	b
		200	2.03	±	1.05	c	12.82	±	9.83	b	0.92	±	0.16	b	0.67	±		b	32.04	±	5.84	c
		600	12.68	±	6.77	bc	20.80	±	12.06	b	6.33	±	3.44	b	2.74	±	0.63	b	9.94	±	2.58	d
		1200	25.17	±	4.94	b	23.49	±	7.25	b	9.13	±	0.46	b	4.43	±	1.42	b	18.55	±	4.88	cd

DW, dry weight.

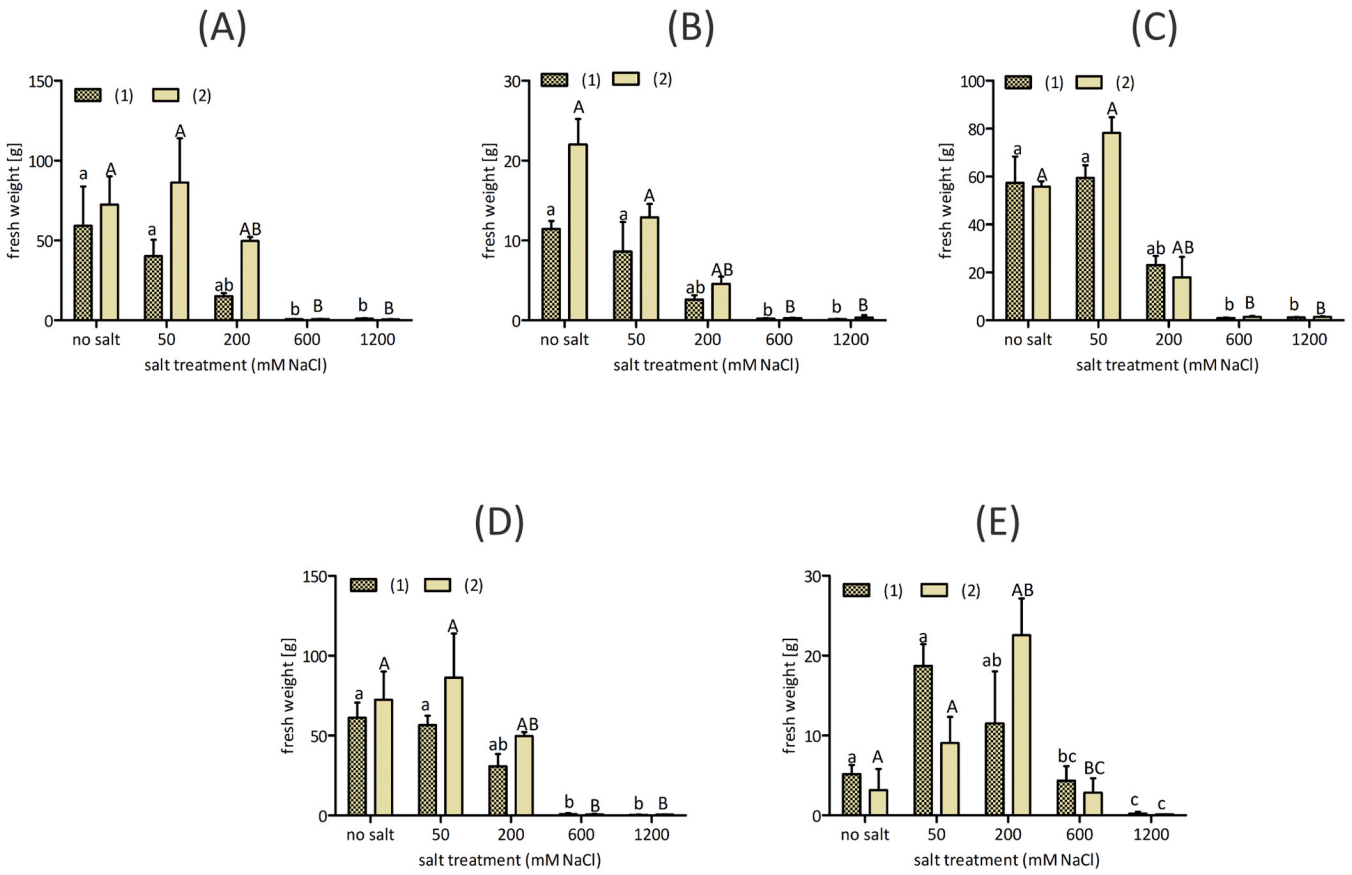

Fig. 1. Effect of salt treatment on the fresh weight of 6 or 9 week-old plants depending on the plant species. (A) Palm kale; (B) scurvy grass; (C) quinoa; (D) garden orache; (E) glasswort. Means ± SD of one experimental setup (1) or (2). Small letters indicate significant differences between treatments within experimental setup 1 in alphabetic order from highest to lowest; capital letters indicate significant differences between treatments in experimental setup 2 in alphabetic order from highest to lowest, ($p \leq 0.05$); n according to Table S2.

produced, other yield assessment parameters such as leaf area index can be used to predict the productivity of a crop production system. Further research is needed to optimize crop management in saline indoor farming, for example by evaluating further yield parameters.

To evaluate plants response to salinity levels, ABA is a key signal molecule in salt stress response, which is regulating distance growth and stomatal closure, among others (Zhu, 2002; Golldack et al., 2014). The halophyte species displayed increased ABA content and decreased growth depending on salinity level. However, the stress response is not mediated by a single plant hormone but rather a crosstalk of several plant hormones. For example, stomata closure under salt or drought stress is mainly regulated by ABA, but influenced at the transcriptional level by JA and SA (Salvi et al., 2021). SA and JA showed a reverse relationship, where higher salt concentrations increased SA and decreased JA levels (Fig. S4, S5).

Based on this, plant performance in the indoor farming system can be evaluated to find an appropriate salinity level for cultivation. For palm kale growth reduction correlated with increasing ABA levels at 200 mM salt, indicating an optimal salinity level of 50 mM. Due to the reduced growth above 600 mM salt in scurvy grass, it can be grown up to 200 mM salt. This is consistent with results in the literature. De Vos (2013) showed that a strong decrease in growth rate occurs in scurvy grass at 400 mM NaCl treatment. Quinoa and garden orache showed a significant decrease in distance growth and leaf increase at 600 mM salt with an increase in ABA concentration, suggesting a suitable salinity level of 200 mM. This is consistent with other studies that found a reduction in growth from 100 mM to 200 mM salt for quinoa (Hariadi et al., 2011) or for garden orache (Kachout et al., 2009). Glasswort showed increased growth at 50 mM and 200 mM salt and decreased ABA concentration compared to control and 1200 mM. This demonstrates that glasswort is an obligate halophyte that requires chloride to maintain turgor and thus optimal growth (Glenn and O'leary, 1984).

4.2. Nutritional properties of edible halophytes

Considering the use of halophytes as alternative vegetables, their protective nutritional effect is of great interest. In particular, PSMs such as GLSs and carotenoids are associated with health-promoting effects, and therefore desirable compounds.

Among carotenoids, for example, β-carotene, lutein and zeaxanthin are of particular interest and scurvy grass provides a good source of β-carotene (3.6 ± 0.6 mg/100 g FW [recommended value of 4.8 mg/day]), lutein and zeaxanthin. In a high lutein/zeaxanthin diet, 11 mg/day lutein + zeaxanthin is recommended, of which 8.7 ± 0.8 mg/100 g FW can be achieved by consuming 100 g of fresh scurvy grass leaves (Wang et al., 2007; DGE, 2020). The halophytes also showed comparable amounts of chlorophylls to common leafy vegetables, such as chard or lettuce. Chlorophylls serve as radical scavengers, intercepting the formation of ROS in the human body, thus protecting against non-transmissible diseases such as cancer (Harttig and Bailey, 1998; Chernomorsky et al., 1999).

The GLS contents in scurvy grass (139.4 ± 73.3 mg/100 g FW (200 mM salt)) are comparable with those in broccoli or kale (Baenas et al., 2019). However, it should be mentioned that the health promoting effects of GLSs are due to the GLSs breakdown products, which can be formed due to enzymatic or thermal degradation. For example,

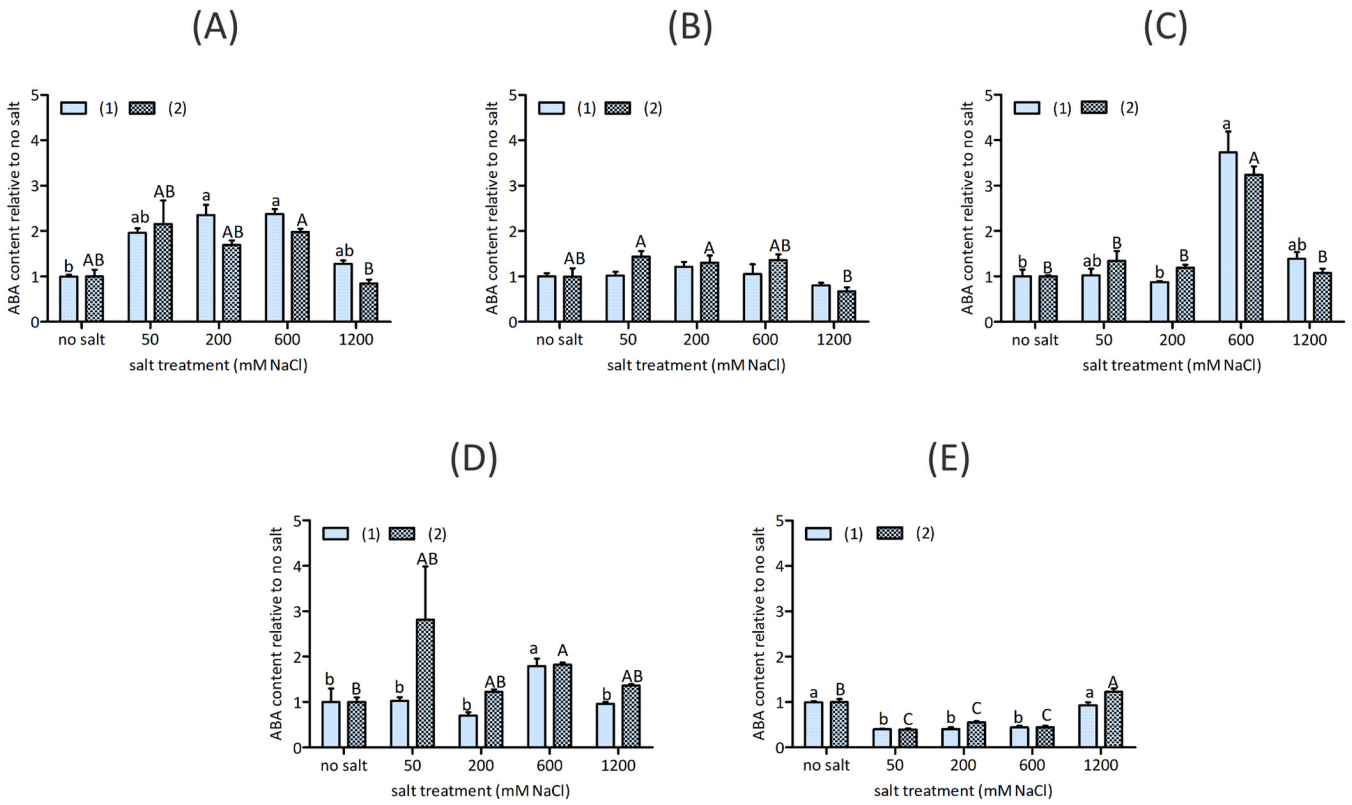

Fig. 2. Effect of salt treatment on abscisic acid content in leaves of 6 or 9 week-old plants depending on the plant species. (A) Palm kale; (B) scurvy grass; (C) quinoa; (D) garden orache; (E) glasswort. Means ± SD of one experiment (1) or (2). Small letters indicate significant differences between treatments within experiment 1 in alphabetic order from highest to lowest; capital letters indicate significant differences between treatments in experiment 2 in alphabetic order from highest to lowest, ($p \leq 0.05$); n = 4 per experiment. ABA, abscisic acid.

indole-3-cabinol, which is the breakdown product of the most abundant GLS in palm kale (I3M), subsequently forms 3,3'-diindolylmethane, which has shown anti-cancerogenic properties in an *in vivo* study (Gao et al., 2020). For *sec*-butyl GLS, which is present in scurvy grass, the breakdown product *sec*-butyl isothiocyanate was found in *Lepidium latifolium* L. (Blažević et al., 2019). However, the bioactivity of this degradation product has not been investigated so far, probably because sec-butyl-GLS is rarely found in the common *Brassica* species.

To gain further insight into these complex metabolic changes and its impact on the nutritional quality of halophytes other relevant bioactives such as polyphenols, betaines or fatty acids as well as anti-nutrients, such as saponins, tannins, oxalate or alkaloids should be investigated in further studies. Considering anti-nutrients further research could focus on the influence of the indoor farming system and salinity on their content in the halophyte species. For example, the light conditions in indoor farming or salinity may influence the content of anti-nutrients, e. g. oxalate or saponins (Wong et al., 2020; Gómez-Caravaca et al., 2012). Furthermore, not only the content of nutrients and anti-nutrients is relevant, but also their bioavailability and bioaccessibility. Further studies on the bioavailability of selected PSMs from halophytes could improve the assessment of their health potential.

Chloride and nitrate contents are also of interest in food but not associated with health benefits. While high nitrate levels appear in leafy vegetables, high salt levels need to be considered in halophytes. Here, special attention must be paid to the glasswort due to its property of accumulating ions in its cell vacuoles. Although sodium and chloride are essential micronutrients in a human diet and important for electrolyte balance and membrane transport mechanisms, excessive consumption of sodium chloride can contribute to the development of cardiovascular diseases and hypertension (Strohm et al., 2018). The World Health Organization (WHO) recommends an intake of 5 g/day for sodium chloride, equating to 3 g/day for chloride (WHO, 2012). The glasswort had an average chloride content of 1.4 ± 0.3 g per 100 g fresh mass in the 200 mM salt treatment group, which is more than one third of the recommended daily intake. This is in agreement with the literature, where 1.3 g per 100 g fresh mass was found in glasswort (Evlash et al., 2021). The average sodium consumption worldwide in 2010 was 4 g/day, which would be equivalent to 9.9 g/day of sodium chloride (Powles et al., 2013). Since the average intake of sodium chloride is above the recommended levels, it is advisable to reduce it. However, as a substitute for salt, halophytes can be consumed fresh or dried as a powder. Considering the powder, a study developed a product "SoleVit Mg" made from the aboveground part of *S. europaea*. In the dried product a chloride content of 10.5 g per 100 g was determined (Evlash et al., 2021). Compared to table salt, which contains about 59.9 g g^{-1} of chloride (Public Health England, 2021), depending on the source, the chloride content of a halophyte salt substitute would be much lower for the same amount of consumption. In addition to sodium chloride, the halophyte salt supplement also contains other desirable minerals such as magnesium, potassium or iodine (Evlash et al., 2021).

Nitrate contents are also of interest in a toxicological context due to associated health risks (EFSA, 2008). The Acceptable Daily Intake (ADI, adult) for nitrate is 3.7 mg/kg body weight/day (EFSA, 2008). For the glasswort a nitrate content in average of 218.1 ± 42.8 mg per 100 g fresh mass in the 200 mM salt treatment group was detected. The ADI for an adult the global average body weight of 62 kg body mass (Walpole et al., 2012) is 229 mg/day nitrate. If 100 g of fresh glasswort is consumed, the intake is slightly below the acceptable limit, but still too high. Nitrate intake is particularly problematic with leafy greens, which often have levels above the recommended levels (Luo et al., 2022; EFSA,

2008; Brkić et al., 2017). Nitrate content can be influenced by plant species, plant development stage, harvest season, plant organ consumed, or light regime (Brkić et al., 2017; Bian et al., 2020; Luo et al., 2022). A common way to alter nitrate contents would be to reduce the nitrate content of the fertilizer (Luo et al., 2022). Changing the lighting conditions can also reduce nitrate contents, such as increasing light intensity or using blue LEDs (Fu et al., 2017; Zheng et al., 2018).

4.3. Influence of salinity levels on plant secondary metabolites

Salinity affects yields, salt accumulation, but also a potentially increases in PSMs. In particular, we were interested in the optimal salinity levels in indoor farming to produce vegetables rich in PSM, while avoiding high concentrations of chloride and nitrate.

While carotenoids are mainly associated with photosynthesis and their function as antioxidants, GLSs are more related to defense against plant pathogens, but are also involved in reactions to abiotic stresses (Del Carmen Martínez-Ballesta et al., 2013; Pang et al., 2012).

A decrease in total carotenoids and chlorophylls were observed depending on salinity level and plant species. While the obligate halophyte, glasswort, showed increasing carotenoid and chlorophyll content with increasing salt treatment (except 1200 mM salt), the facultative halophytes showed a decreasing phytochemical content (Tables 1, 2). A decreased chlorophyll content is consistent with the literature and is most likely related to salt stress-induced over-excitation of the photosynthetic apparatus, and thus to altered content in the photosystems and chlorophyll biosynthesis (Elsayed et al., 2021). Over-excitation of the photosynthetic apparatus leads to increased ROS production, which may result in activation of the plant antioxidant system and also accumulation of ROS-scavenging molecules such as β-carotene and zeaxanthin (Choudhury and Behera, 2001). In summary, the decrease in chlorophyll, lutein, and neoxanthin suggests that selected halophytes reduce photosynthetic activity by accumulating fewer photosynthetically relevant pigments and higher non-photochemical-quenching-(NPQ)-relevant carotenoids (β-carotene, zeaxanthin and violaxanthin) due to an increase in ROS.

For GLS, we observed an influence of salinity on the ratio between aliphatic and indole GLS in palm kale and scurvy grass (Table 3). Several studies have shown that GLS biosynthesis is affected by abiotic stresses such as temperature, salinity, drought, or nutrient availability (Del Carmen Martínez-Ballesta et al., 2013; Pang et al., 2012). Pang et al. (2012), who investigated the effect of salt stress on GLS content and myrosinase activity in *Thellungiella salsuginea*, indicated that altered biosynthesis is the more likely explanation for the changes in GLS content under salt stress, since they could not detect a synergistic effect between altered myrosinase activity and GLS content. Most studies only investigated methionine-derived aliphatic GLS under salinity stress. Here, methionine-derived aliphatic GLSs showed no change, but valine-derived isopropyl GLSs and aliphatic sec-butyl GLSs changed, and also tryptophan-derived indole GLS. This GLS-specific response and the altered ratio between aliphatic and indole GLS lead to the assumption that GLS biosynthesis is specifically influenced by salt stress. Further investigations are necessary to understand the mechanism behind the biosynthesis of indole and aliphatic GLS in salt stress response.

For anions, despite the salt tolerance mechanism, we observed an increasing chloride content with higher salt treatment but a decreasing nitrate content (Table 4). Since reducing nitrate and chloride contents in the diet would be interesting, insights into the plant's uptake mechanism could provide an approach for influencing nitrate and chloride concentrations. Nitrate concentration in leaves was reduced with increasing chloride concentrations in all halophyte species, which is not reflected in the soil (Table S7). One explanation could be an interdependence of nitrate and chloride accumulation. The nitrate transporter NRT1.1 is permeable to chloride, with chloride and nitrate competing for uptake. Influencing transporter activity could be another strategy to reduce nitrate and chloride levels in plant-based foods.

5. Conclusion

The aim of this study was to investigate saline indoor farming of halophytes and to evaluate changes in selected metabolites and minerals, including carotenoids, glucosinolates, chloride and nitrate. Albeit all examined halophyte species were successfully cultivated plant performance and nutritional properties were found to be dependent on the salt concentration. The different halophyte species have different optimal salt concentrations in terms of growth depending on their salt tolerance mechanism. Different responses to salt treatment in fresh mass and plant hormone status were found for the facultative (palm kale, scurvy grass, quinoa, and garden orache) and obligate (glasswort) halophytes. For the facultative halophytes, there was a correlation between increasing salinity, increased ABA content, and decreased distance growth and fresh mass. For PSM, we observed an altered content depending on the salinity concentration. While the obligate halophyte glasswort showed an increasing content of PSMs with rising salinity, the other four halophyte species showed a decreasing content of PSMs. For the chloride content, we observed an increasing content with higher salinity treatment, whereas the nitrate content decreased with higher salinity treatment levels.

In conclusion, halophytes produced in saline indoor farming have the potential to become part of a healthy and sustainable diet in the future, thus contributing to food diversity and security. However, further studies are required to deepen the knowledge about nutrient and anti-nutrient metabolites in halophytes, to make these systems more sustainable, for instance by using alternative water resources (Fitzner et al., 2021), and to evaluate the consumer acceptance.

Funding

This research was funded by the German Federal Ministry of Education and Research, Grant number 031B0730 A, as part of the project food4future.

Declaration of Competing Interest

None.

Data availability

Data will be made available on request.

Acknowledgment

We would like to thank Stefanie Zantopp for helping with the experimental performance and determination of morphological parameters and Andrea Jankowsky for performing anion analysis.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2023.105435.

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Publication I

Supplementary data

Title: Comprehensive characterization of selected phytochemicals and minerals of selected edible halophytes grown in saline indoor farming for future food production.

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Supplemental Table S1 Composition of soil used for halophyte cultivation. Values were provided by the manufacturer.

	N (mg/L)	PO_4^{2-} (mg/L)	K (mg/L)	Mg (mg/L)	pH
Substrate type T	120	120	170	120	5.9
Substrate type P	180	180	260	130	5.9

Supplemental Table S2 Composition of nutrient solution used in the indoor farming system. Values were provided by the manufacturer.

Nutrient solution	
NH_4NO_3 (mg/L)	4.98
$Ca(NO_3)_2$ (g/L)	1.04
KNO_3 (g/L)	0.81
Iron chelate (ppm)	8
KH_2PO_4 (g/L)	0.31
$MnSO_4$ (mg/L)	2.5
$MgSO_4$ (g/L)	0.54
$Na_2[B_4O_5(OH)_4]\cdot 8H_2O$ (mg/L)	3.6
CuSO4 (mg/L)	0.2
Na_2MoO_4 (mg/L)	0.1
$ZnSO_4$ (mg/L)	0.4
pH	6.2

Supplemental Table S3 Identification of chlorophylls and carotenoids. Identification parameters of chlorophylls and carotenoids with UV/Vis and mass spectra. ‘found in literature* and #exemplary according to chromatograms showed in **Fig. S1**. MS, mass spectra; RT, Retention time.

Compound	Ion	MS $[m/z]^+$ ‘	Absorption maxima [nm]‘	peak number	RT [min]	MS $[m/z]^{+\#}$	Absorption maxima of UV/Vis [nm]#
Chlorophyll *a*	$[M+H]^+$	893.54	432	8	22.130	893.5518	432
chlorophyll *b*	$[M+H]^+$	907.52	468	5	17.9626	907.5278	468
9*Z*-Neoxanthin	$[M+H\text{-}H_2O]^+$	583.41	410 434 464	3	11.623	583.4199	412 436 464
Neoxanthin isomer	$[M+H\text{-}H_2O]^+$	583.41	410 434 464	1	8.436	583.4199	412 436 464
all-trans-Violaxanthin	$[M+H]^+$	601.42	419 440 470	2	9.990	601.4334	415 439 468
Violaxanthin isomer	$[M+H]^+$	601.42	419 440 470	4	13.059	601.4375	398 422 448
all-trans-Lutein	$[M+H\text{-}H_2O]^+$	551.43	420 444 472	6	18.683	551.4331	420 444 472
all-trans-Zeaxanthin	$[M+H]^+$	569.43	426 452 478	7	20.516	569.4400	416 438 466
all-trans-β-Carotene	$[M+H]^+$	537.45	424 452 480	9	47.476	537.4565	424 452 478

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Supplemental Table S4 Identification of GLS. Identification parameters for identifying desulfo-glucosinolates with mass spectra [ŧ]found in literature* and [#]exemplary according to chromatograms showed in **Fig. S2**. MS, mass spectra; RT, Retention time.

Compound	MS [m/z][ŧ]	RT [min]	Peak number	MS [m/z][#]
3MSOP	343	2.614	1	343
2OH2MP	311	3.611	2	311
4MSOB	357	4.711	3	357
iPr	283	5.385	5	283
Sinalbin (internal standard)	345	5.231	4	345
4OHI3M	384	6.251	6	384
iBu	295	6.471	7	295
sBu	295	6.871	8	295
I3M	368	8.898	9	368
4MOI3M	398	10.265	10	398
1MOI3M	398	12.425	11	398

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Supplemental Table S5 Multiple reaction monitoring (MRM) transitions, collision gas settings (Collision energy (CE), declustering potential (DP), entrance potential (EP) and cell exit potential (CEP)) for determining abscisic acid (ABA) and deuterated abscisic acid (d6-ABA), salicylic acid (SA) and deuterated salicylic acid (d4-SA), jasmonic acid (JA) and deuterated jasmonic acid (d5-JA). MS parameters: ion source temperature of 500 °C; ion spray voltage of -4,500 V; curtain gas pressure of 50 psi; drying gas 50 psi; nebulizer gas 50 psi; auxiliary gas of 65 psi; MRM dwell time of 0.3781 s.

		MRM Transition	CE [V]	DP [V]	EP [V]	CEP [V]
ABA	quantifier	263 → 153	-15	-20	-5	-15
ABA	qualifier	263 → 203	-40	-20	-5	-15
ABA	qualifier	263 → 122	-48	-20	-5	-15
d6-ABA	quantifier	269 → 159	-15	-20	-5	-15
d6-ABA	qualifier	269 → 209	-40	-20	-5	-15
d6-ABA	qualifier	269 → 128	-48	-20	-5	-15
SA	quantifier	137 → 93	-23	-20	-5	-15
SA	qualifier	137 → 75	-42	-20	-5	-15
SA	qualifier	137 → 65	-40	-20	-5	-15
d4-SA	quantifier	142 → 97	-23	-20	-5	-15
d4-SA	qualifier	142 → 78	-42	-20	-5	-15
d4-SA	qualifier	142 → 69	-40	-20	-5	-15
JA	quantifier	209 → 59	-16	-50	-5	-15
JA	qualifier	209 → 41	-60	-50	-5	-15
JA	qualifier	209 → 109	-28	-50	-5	-15
d5-JA	quantifier	214 → 62	-16	-50	-5	-15

d5-JA	qualifier	214 → 109	-60	-50	-5	-15
d5-JA	qualifier	214 → 42	-28	-50	-5	-15

Supplemental Table S6 Growth distance and leaf number increase in 6- or 9-week-old plants depending on the plant species. Median, 1st quartile and 3rd quartile of two individual experimental setups (1) or (2). Letters indicate significant differences between treatments within one experiment and one plant species in alphabetic order from highest to lowest ($p \leq 0.05$). n, number of plants per treatment.

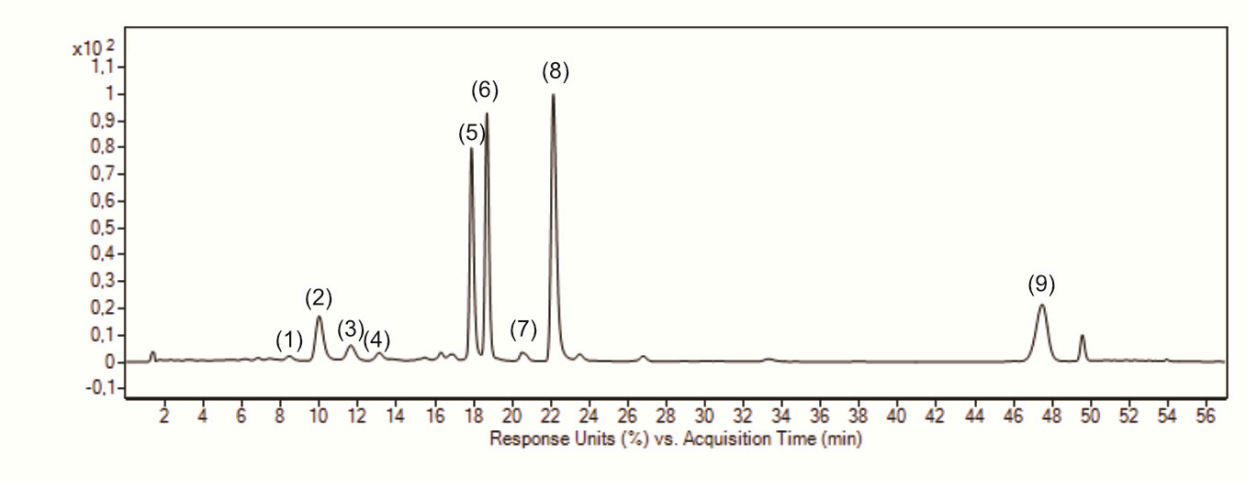
Exp.			(1)					(2)				
		Salt [mM]	Median	3rd Quartile	1st Quartile		n	Median	3rd Quartile	1st Quartile		n
Palm kale	Growth distance	0	27.12	23.82	23.82	a	12	6.40	5.88	5.88	a	12
		50	19.65	18.29	18.29	ab	12	6.15	5.48	5.48	a	12
		200	7.96	7.77	7.77	bc	11	2.80	2.40	2.40	ab	12
		600	0.98	0.57	0.57	c	12	0.00	0.00	0.00	b	12
		1200	0.88	0.63	0.63	c	10	0.00	0.00	0.00	b	12
	Leaf number increase [*N*]	0	8.00	6.00	6.00	a	12	5.00	5.00	5.00	a	12
		50	6.50	5.25	5.25	a	12	7.50	3.75	3.75	a	12
		200	1.00	1.00	1.00	b	11	3.00	2.00	2.00	b	12
		600	1.00	0.00	0.00	b	12	1.50	0.25	0.25	bc	12
		1200	2.00	0.75	0.75	b	10	1.00	0.00	0.00	c	12
Scurvy grass	Growth distance [cm]	0	7.59	8.41	6.80	a	12	12.44	13.32	11.58	a	12
		50	5.93	6.50	4.91	ab	12	9.86	11.34	7.80	ab	12
		200	1.82	2.45	1.56	b	12	2.27	2.72	2.11	b	12
		600	0.00	0.00	0.00	b	12	0.00	0.00	0.00	b	12
		1200	0.00	0.00	0.00	b	12	0.00	0.00	0.00	b	12
	Leaf number increase [*N*]	0	22.50	29.75	11.00	a	12	24.50	35.25	17.00	a	12
		50	11.50	14.25	10.25	a	12	15.50	20.25	10.50	a	12
		200	9.00	10.00	4.50	a	12	10.00	11.75	8.25	a	12
		600	0.00	0.00	0.00	b	12	2.00	2.00	1.00	b	12
		1200	0.00	0.00	0.00	b	12	1.50	2.00	1.00	b	12
Quinoa	Growth distance [cm]	0	27.04	32.01	20.64	a	12	42.20	48.21	32.89	a	12
		50	26.56	28.40	24.09	a	12	39.49	41.54	34.04	a	12
		200	13.67	15.63	12.06	ab	12	10.32	13.34	6.81	ab	12
		600	0.00	0.00	0.00	b	12	0.00	0.00	0.00	c	12
		1200	0.00	0.00	0.00	b	12	0.40	0.93	0.00	bc	12
	Leaf number increase [*N*]	0	63.50	71.75	48.50	a	12	36.50	49.00	32.25	ab	12
		50	51.00	61.75	41.75	a	12	39.50	48.75	35.50	a	12
		200	30.50	39.75	28.00	ab	12	23.50	27.75	13.75	b	12
		600	0.00	0.00	0.00	b	12	1.00	1.00	0.00	c	12
		1200	0.00	0.00	0.00	b	12	2.00	4.00	1.25	bc	12
Garden orache	Growth distance [cm]	0	38.66	47.37	35.98	a	12	49.94	52.39	40.07	ab	12
		50	51.72	62.60	38.83	a	12	62.86	73.74	52.81	a	12
		200	41.63	47.03	37.25	a	12	37.72	43.90	34.11	b	12

		600	0.00	0.00	0.00	b	12	1.38	3.12	0.59	bc	12
		1200	0.00	0.00	0.00	b	12	0.00	0.00	0.00	c	12
	Leaf number increase [*N*]	0	30.00	39.25	23.50	a	12	24.50	30.75	20.25	a	12
		50	25.00	36.25	20.00	a	12	9.00	11.50	7.25	ab	12
		200	24.00	31.00	20.75	a	12	4.00	6.00	4.00	bc	12
		600	3.00	4.00	0.00	b	12	1.00	2.00	0.00	c	12
		1200	2.00	2.75	1.00	b	12	1.50	2.00	1.00	c	12
Glasswort	Growth distance [cm]	0	4.71	5.50	3.56	b	11	4.63	4.84	3.89	ab	9
		50	6.53	7.73	5.46	a	12	4.98	8.13	4.25	a	9
		200	6.22	7.18	5.78	a	11	5.56	7.45	4.14	a	9
		600	3.39	4.35	2.47	b	11	2.76	3.53	2.58	bc	9
		1200	0.00	0.00	0.00	c	11	0.00	0.46	0.00	c	9
	Stem number increase [*N*]	0	52.00	62.00	43.00	ab	11	30.00	45.50	22.50	ab	9
		50	75.50	104.00	67.25	a	12	58.00	68.50	43.50	ab	9
		200	78.00	91.00	64.00	a	11	79.00	89.00	48.00	a	9
		600	33.00	37.00	25.00	bc	11	24.00	32.00	11.00	bc	9
		1200	2.00	3.00	1.00	c	11	1.00	3.00	0.00	c	9

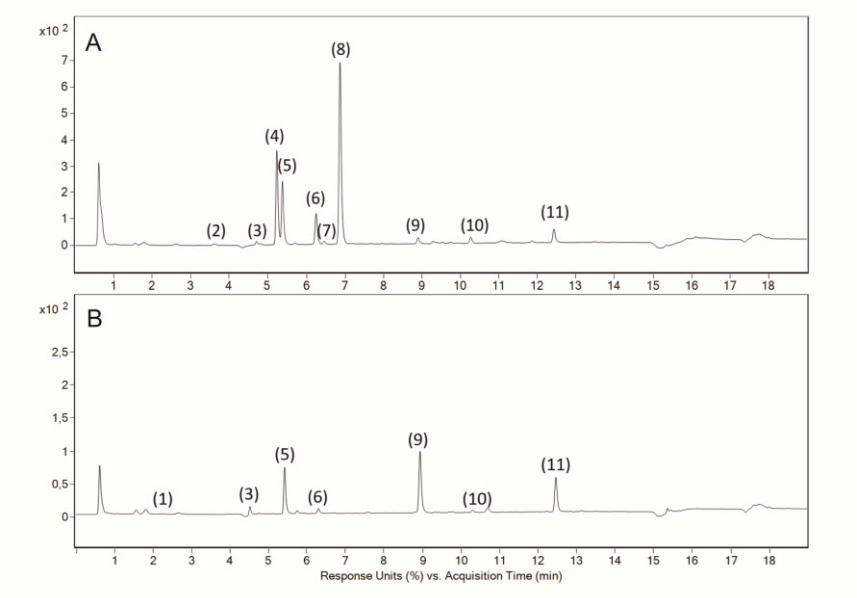
Supplemental Table S7 Concentration of chloride and nitrate in soil of palm kale, scurvy grass, quinoa, garden orache and glasswort. Means ± SD of two individual experimental setups (1), (2), n = 4 per experiment. DW, dry weight. Letters indicate significant differences between treatments within one setup and one plant species in alphabetic order from highest to lowest ($p \leq 0.05$).

	Exp.	Salt [mM]	Palm kale		Scurvy grass		Quinoa		Garden orache		Glasswort	
Chloride [mg g^{-1} DW]	(1)	0	0.26 ± 0.04	b	0.09 ± 0.03	b	0.18 ± 0.05	c	0.17 ± 0.04	b	0.09 ± 0.01	c
		50	4.67 ± 1.13	b	2.08 ± 0.32	b	3.86 ± 0.55	c	2.60 ± 0.86	a	2.33 ± 0.15	c
		200	8.90 ± 0.26	b	6.16 ± 2.76	b	5.39 ± 3.50	c	12.19 ± 1.62	b	7.20 ± 1.19	c
		600	22.86 ± 4.25	b	24.53 ± 2.35	a	20.99 ± 3.23	b	27.03 ± 14.25	b	18.01 ± 4.36	b
		1200	40.09 ± 10.80	a	38.52 ± 14.13	a	71.62 ± 7.24	a	40.56 ± 11.80	a	42.64 ± 10.23	a
	(2)	0	0.21 ± 0.09	d	0.10 ± 0.00	c	0.10 ± 0.04	d	0.20 ± 0.07	b	0.12 ± 0.03	c
		50	5.15 ± 0.57	d	3.25 ± 0.55	c	4.26 ± 1.22	d	4.14 ± 0.98	b	2.64 ± 0.47	c
		200	10.86 ± 3.26	c	7.24 ± 2.16	c	6.79 ± 0.66	c	7.31 ± 1.58	b	7.15 ± 0.68	c
		600	20.82 ± 5.43	b	18.84 ± 3.59	b	17.77 ± 2.26	b	21.25 ± 8.50	a	19.36 ± 5.00	b
		1200	43.45 ± 5.45	a	41.26 ± 4.85	a	29.42 ± 6.42	a	29.94 ± 6.71	a	36.21 ± 9.21	a
Nitrate [mg g^{-1} DW]	(1)	0	0.10 ± 0.04	d	0.34 ± 0.14	b	0.18 ± 0.07	c	0.44 ± 0.33	a	0.24 ± 0.07	b
		50	0.30 ± 0.05	d	0.12 ± 0.04	b	0.04 ± 0.00	c	0.11 ± 0.05	b	0.21 ± 0.11	b
		200	0.36 ± 0.11	c	0.11 ± 0.03	c	0.19 ± 0.04	c	0.27 ± 0.13	b	0.32 ± 0.04	b

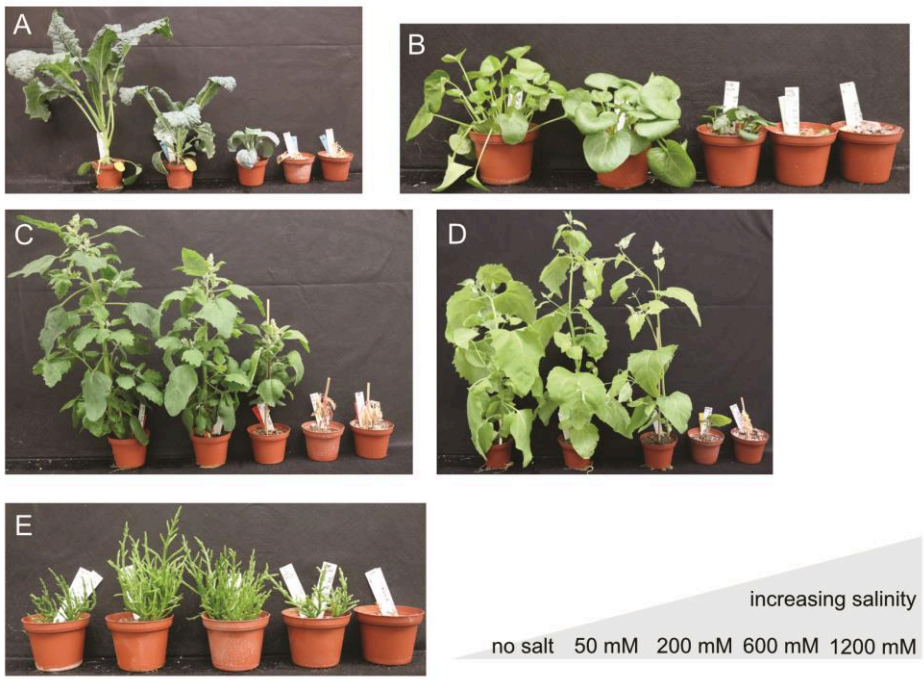
		0.6	0.0		0.6	0.0		0.6	0.0		0.7	0.3		0.4	0.0						
	600	9	± 5	b	5	± 8	a	0	± 6	b	2	± 1	a	9	± 5	a					
		0.7	0.1		0.7	0.1		1.1	0.0		0.7	0.1		0.7	0.1						
	1200	2	± 7	a	9	± 8	a	1	± 8	a	7	± 4	a	4	± 0	a					
		0.3	0.1		0.6	0.1		0.4	0.3	a	1.2	0.3		0.9	0.3	n					
	0	2	± 7	b	0	± 6	a	7	± 6	b	8	± 6	a	9	± 4	s					
		0.2	0.0		0.2	0.1		0.0	0.0		0.4	0.0		0.3	0.1	n					
	50	1	± 6	b	9	± 3	b	4	± 0	b	0	± 7	b	2	± 0	s					
		0.8	0.1		0.4	0.1	a	0.3	0.0	a	0.4	0.0		0.4	0.0	n					
(2)	200	1	± 3	a	1	± 0	b	0	± 6	b	2	± 8	b	0	± 8	s					
		0.6	0.1		0.6	0.1	a	0.6	0.1		0.5	0.1	a	0.6	0.1	n					
	600	4	± 3	a	8	± 8	b	5	± 1	a	1	± 7	b	1	± 2	s					
		0.7	0.0		0.7	0.0		0.5	0.0	a	0.6	0.0	a	0.6	0.0	n					
	1200	7	± 5	a	4	± 7	a	9	± 6	b	0	± 7	b	4	± 9	s					



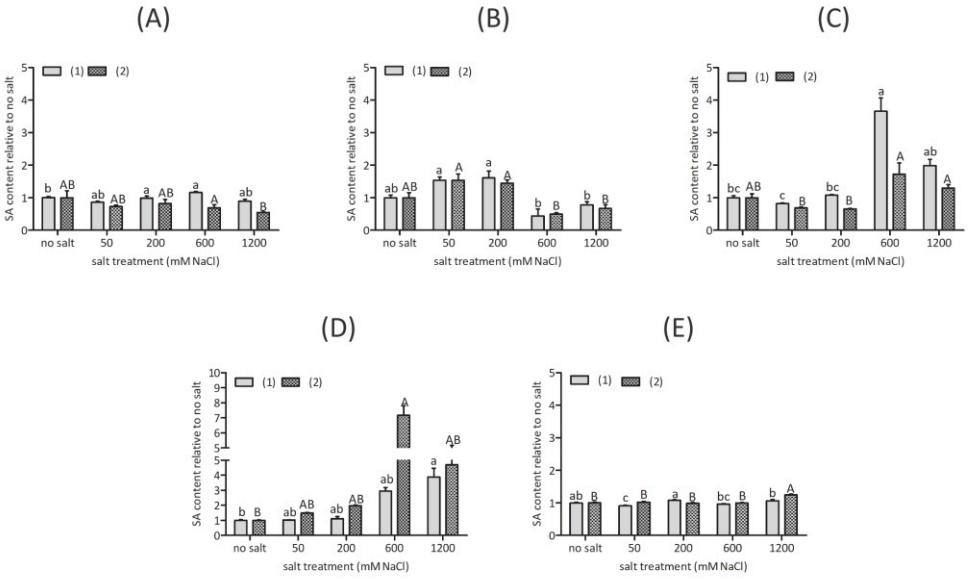
Supplemental Figure S1 Identification of chlorophylls and carotenoids. HPLC-DAD chromatogram at 450 nm showing identified chlorophylls and carotenoids exemplary in leaves of garden orache. (1) Neoxanthin isomer*; (2) *all-trans*-violaxanthin; (3) 9*Z*-neoxanthin; (4) violaxanthin isomer*; (5) chlorophyll *b*; (6) *all-trans*-lutein; (7) *all-trans*-zeaxanthin; (8) chlorophyll *a*; (9) *all-trans*-β-carotene. *Unidentified *cis-trans* isomerism



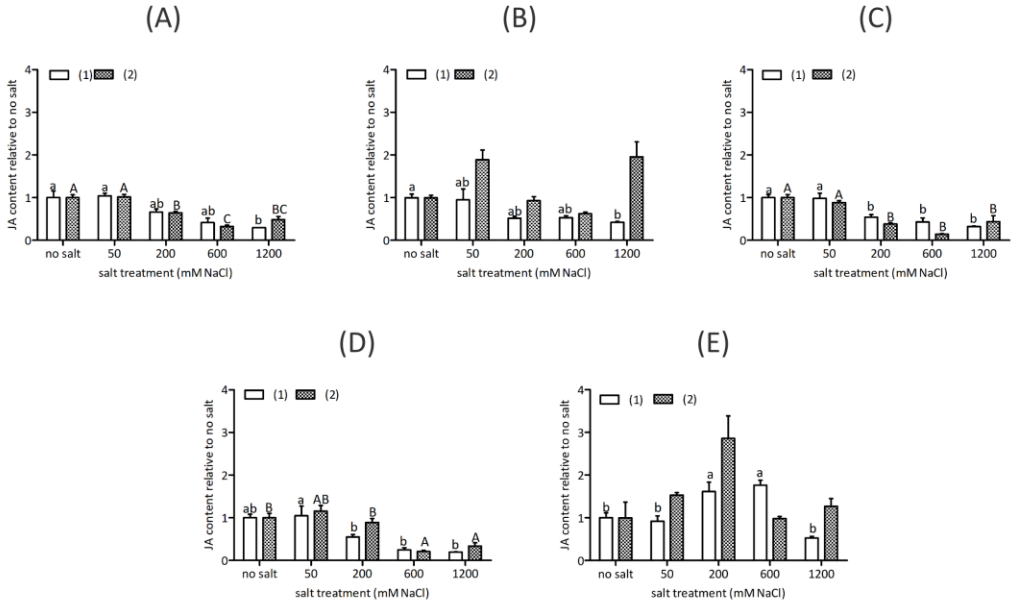
Supplemental Figure S2 Identification of GLSs. HPLC-DAD chromatogram at 229 nm showing the desulfo-glucosinolates identified. Found in leaves of (A) scurvy grass and (B) palm kale: (1) 3MSOB, (R_S)-3-(methylsulfinyl)propyl GLS; (2) 2OH2MP, 2-hydroxy-2-methylpropyl GLS; (3) 4MSOB, (R_S)-4-(methylsulfinyl)butyl GLS; (4) Sinalbin (internal standard); (5) iPr, 2-propyl GLS; (6) 4OHI3M, 4-hydroxyindol-3-ylmetyl GLS; (7) iBu, 2-Methylpropyl GLS; (8) *s*BU, (*1S*)-1-Methylpropyl GLS; (9) I3M, Indol-3-ylmethyl GLS; (10) 4MOI3M, 4-Methoxyindol-3-ylmethyl GLS; (11) 1MOI3M, 1-Methoxylindol-3-ylmethyl GLS.



Supplemental Figure S3 Effect of salt treatment on the plants' phenotypes. (A) Palm kale; (B) scurvy grass; (C) quinoa; (D) garden orache; (E) glasswort. 6 or 9 week-old plants, depending on plant species, after 3 weeks of salt treatment. Increasing salt treatment from left to right.



Supplemental Figure S4 Effect of salt treatment on plant hormone SA in leaves of 6 or 9 week-old plants depending on the plant species. (A) palm kale; (B) scurvy grass; (C) quinoa; (D) garden orache; (E) glasswort. Means ± SD of one experimental setup (1) and (2). Small letters indicate significant differences between treatments within setup 1 in alphabetic order from highest to lowest; capital letters indicate significant differences between treatments in setup 2 in alphabetic order from highest to lowest, ($p \leq 0.05$); n = 4 per experiment. SA, salicylic acid.



Supplemental Figure S5 Effect of salt treatment on plant hormone JA in leaves of 6 or 9 week-old plants depending on the plant species. (A) palm kale; (B) scurvy grass; (C) quinoa; (D) garden orache; (E) glasswort. Means ± SD of one experimental setup (1) and (2). Small letters indicate significant differences between treatments within setup 1 in alphabetic order from highest to lowest; capital letters indicate significant differences between treatments in setup 2 in alphabetic order from highest to lowest, ($p \leq 0.05$); n = 4 per experiment. JA, jasmonic acid.

Publication II

Utilization of regional natural brines for the indoor cultivation of *Salicornia europaea*.

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Published in: *Sustainability*, 2021, 13, 12105
DOI: 10.3390/su132112105

sustainability

MDPI

Communication

Utilization of Regional Natural Brines for the Indoor Cultivation of *Salicornia europaea*

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Abstract: Scaling agriculture to the globally rising population demands new approaches for future crop production such as multilayer and multitrophic indoor farming. Moreover, there is a current trend towards sustainable local solutions for aquaculture and saline agriculture. In this context, halophytes are becoming increasingly important for research and the food industry. As *Salicornia europaea* is a highly salt-tolerant obligate halophyte that can be used as a food crop, indoor cultivation with saline water is of particular interest. Therefore, finding a sustainable alternative to the use of seawater in non-coastal regions is crucial. Our goal was to determine whether natural brines, which are widely distributed and often available in inland areas, provide an alternative water source for the cultivation of saline organisms. This case study investigated the potential use of natural brines for the production of *S. europaea*. In the control group, which reflects the optimal growth conditions, fresh weight was increased, but there was no significant difference between the treatment groups comparing natural brines with artificial sea water. A similar pattern was observed for carotenoids and chlorophylls. Individual components showed significant differences. However, within treatments, there were mostly no changes. In summary, we showed that the influence of the different chloride concentrations was higher than the salt composition. Moreover, nutrient-enriched natural brine was demonstrated to be a suitable alternative for cultivation of *S. europaea* in terms of yield and nutritional quality. Thus, the present study provides the first evidence for the future potential of natural brine waters for the further development of aquaculture systems and saline agriculture in inland regions.

Keywords: carotenoids; glasswort; land-based aquaculture; seawater; phytochemicals; halophytes; salt composition; chlorophylls; artificial salt; saline agriculture

Citation: Fitzner, M.; Fricke, A.; Schreiner, M.; Baldermann, S. Utilization of Regional Natural Brines for the Indoor Cultivation of *Salicornia europaea*. *Sustainability* **2021**, *13*, 12105. https://doi.org/10.3390/su132112105

Academic Editors: Hossein Azadi and Antonio Zuorro

Received: 2 August 2021
Accepted: 28 October 2021
Published: 2 November 2021

1. Introduction

Water scarcity already affects 1.2 billion people worldwide, and this development will be exacerbated by the impacts of climate change in the future [1,2]. Faced with climate change-induced declines in drinking water resources and global population growth, alternative strategies for sustainable water use in agriculture are urgently needed to ensure food security and nutrition in the future [3]. Additionally, urbanization and limited agricultural land has led to a necessity for alternative cultivation systems, such as vertical farming, which offers efficient production sites and is therefore becoming extremely relevant in regard to future cultivation [4]. Importantly, with more than 95% of the world's water resources being saline, aquatic food has been highlighted as one of the seven priorities to end hunger and protect the planet, and thus, saline agriculture is gaining increasing

attention [5–7]. Consequently, the cultivation of salt-tolerant plants (halophytes) is an emerging field both in research and the food industry [8–11]. In this context, indoor cultivation with regional brine waters has three distinct advantages. First, indoor cultivation provides year-round fresh green leafy vegetables that are increasingly needed in today's and tomorrow's diets. Second, short supply chains result in fresh, sustainable products. Finally, the use of brine water in indoor aquatic systems is more cost-effective than the use of artificial seawater, which is also an important factor in terms of the economic potential of indoor aquatic cultivation systems [12].

Halophytes are widely distributed across several plant families. Next to the well-known salt-tolerant varieties of crops, such as quinoa, sugar beet, and barley, less attention has been paid to the herbs and vegetables that have been consumed in coastal regions for centuries [13–15]. Among these are members of the genera *Salicornia* which are found in coastal areas worldwide and are traditionally used as forage and fodder [16]. Given their natural distribution, members of *Salicornia* can be grown on saline soil and are used for its remediation [17–19]. In our research, we focus on *S. europaea* that is widespread on inland salt marshes and European coastal areas and is currently making its way into European supermarkets either as a fresh product or dried herb.

The nutritional value of *S. europaea* is mainly due to its richness in secondary metabolites, such as chlorophylls, carotenoids, saponins, flavonoids and flavanones, or lignans [20]. Epidemiological studies indicate that secondary plant metabolites or plant-based foods can lower the risk of various non-communicable diseases such as diabetes, cardiovascular diseases, eye-related disorders, and several types of cancer [21,22]. This also highlights halophytes as a potential source for new nutraceuticals [23]. Chlorophylls, for instance, can prevent DNA damage and thus have chemo-protective properties. Among the carotenoids, the main focus in green leafy vegetables is on β-carotene content because of its provitamin A activity as well as lutein and zeaxanthin because of their relevance in eye health, as for example they can help to prevent age-related macular degeneration [24,25]. In addition to their health-promoting properties, chlorophylls and carotenoids are essential for plant metabolism as they have a key role in photosynthesis. Therefore, chlorophylls and carotenoids are not only indicators of the nutritional value of food, but are also a part of the plant's adaptation system to changing environmental conditions.

As *S. europaea* is an annual plant and an obligate halophyte with salt tolerance up to 720 mM (4.2%), indoor cultivation with saline water is of great interest [8,26–28]. However, domestically, saline irrigation with seawater or water with added sodium chloride creates the problem of introducing salt into the regional cycle. To overcome these issues, we used regional brine water for cultivation. Natural brine springs are widely distributed and often available in inland areas. As natural brines can have different compositions, we used water from two natural brine locations in Germany.

Previous studies using *S. europaea* have already investigated how saline water affects growth, germination, and seed quality as well as demonstrated successful cultivation with seawater, sodium chloride solutions, brackish, or waste water, thereby highlighting *S. europaea* as a suitable model plant to test natural brines in this case study [8,9,28–32]. However, to date, none of these studies have addressed cultivation with natural brines. Therefore, to test the suitability of natural brine for saline indoor cultivation, (1) we investigated whether *S. europaea* can be cultivated with natural brines, (2) we characterized the basic composition of the salt-enriched nutrient solutions, and (3) we determined the yield and concentration of selected secondary metabolites to assess nutritional quality.

2. Materials and Methods

2.1. Chemicals

Methanol and acetonitrile (Chemsolut for LC/MS; Th. Geyer), tetrahydrofuran (HiPerSolv Chromanorm for LC-MS), and dichloromethane (PESTINORM for GC-capillary analysis) were purchased from VWR International GmbH (Darmstadt, Germany). Isopropanol was obtained from Merck KGaA (Darmstadt, Germany). Ammonium acetate (≥97%),

formic acid (Rotipuran, ≥98%), tert-butyl methyl ether, sodium chloride (plant treatment, >99.8%), and sodium hydrogen carbonate (≥99.5%) were obtained from Carl Roth GmbH (Karlsruhe, Germany). The carotenoid standards were purchased from CaroNature GmbH (Munsingen, Switzerland). Chlorophyll standards (chlorophyll *a* and *b*), sodium carbonate (≥99%), potassium hydrogen phosphate (≥99.0%), sodium bromide (≥99%), sodium chloride (IC, ≥99%), sodium nitrate (≥99%), sodium sulfate (≥99%), sulfuric acid (≥95%), and oxalic acid (≥98%) were purchased from Sigma Aldrich Chemie GmbH (Taufkirchen, Germany).

2.2. Natural Brines

Two different natural brines were used in the present study: brine water from location 1 (BW1: Bad Saarow, Brandenburg (N 52° 17.47 E 14° 3.62); 2.37% salt (Na 8.65 g L^{-1}, Ca 0.52 g L^{-1}, Mg 0.25 g L^{-1}, Cl 14.57 g L^{-1}, SO_4^{2-} 0.29 g L^{-1}, HCO_3 0.24 g L^{-1})) and brine water from location 2 (BW2: Heiligenstadt, Thuringia (51° 22.61 E 10° 8.63); 27.5% salt (Br 96.8 mg L^{-1}, Na 100.8 g L^{-1}, K 1.34 g L^{-11}, Ca 1.53 g L^{-1}, F 4.4 mg L^{-1}, Cl 163.6 g L^{-1}, Mg 1.6 g L^{-1}, S 7.0 mg L^{-1})). Both locations, approximately 360 km apart, are situated in the inner land of Germany and provide natural geothermal brines used in therapeutic spas.

2.3. Plant Material and Cultivation Conditions

The seeds of *Salicornia europaea* were purchased from Rühlemann's Kräuter and-Duftpflanzen (Horstedt, Germany). The plants were germinated and grown on Grodan® delta (4 × 4 × 4.5 cm; rock wool) growth media in a climate chamber with the following settings: light intensity, 200 µmol m^{-2} s^{-1}; temperature, day 20 °C, night 16 °C; CO_2, 400 ppm; photoperiod, 12/12 h (day/night); humidity, 75%. Four weeks after germination, the plants were transferred to pots containing 1/3 soil (substrate type T, pH 5.9, N 180 mg L^{-1}, PO_4^{2-} 180 mg L^{-1}, K 260 mg L^{-1}, Mg 130 mg L^{-1}) and 2/3 fine quartz sand with the grain size of 0.5–1 mm (Euroquarz GmbH [Laußnitz, Germany]) with six plants per pot on average. The plants were irrigated with a nutrient solution (NH_4NO_3 0.6 mmol L^{-1}, $Ca(NO_3)_2$ 1.04 g L^{-1}, KNO_3 0.81 g L^{-1}, iron chelate 8 × 10^{-6}%, KH_2PO_4 0.31 g L^{-1}, $MgSO_4$ 0.54 g L^{-1}, $MnSO_4$ 2.5 mg L^{-1}, $Na_2[B_4O_5(OH)_4]{\cdot}8H_2O$ 3.6 mg L^{-1}, $CuSO_4$ 0.2 mg L^{-1}, Na_2MoO_4 0.1 mg L^{-1}, $ZnSO_4$ 0.4 mg L^{-1}) and enriched with salts of different origin and concentrations for the treatments.

2.4. Experimental Design

To investigate the feasibility of brine water for halophyte cultivation, five different saline water treatments were applied: a control, a sodium chloride solution (2.4% NaCl (24 g L^{-1}), an artificial sea water salt Tropic Marine™ (2.4% TM (24 g L^{-1})), which is commonly used in aquaculture and is more comparable for several experimental approaches, and the two natural brines BW1 (2.37% salt; brine water location 1 (Bad Saarow)) and BW2 (2.4% salt; brine water location 2 (Heiligenstadt)) (Figure 1) [33]. The control condition (1% NaCl (10 g L^{-1}), control group) was applied, as it proved to be the optimal growth condition for *S. europaea* in a preliminary experiment (unpublished data). The salt concentration of 2.4% of the treatment group was chosen regarding to the natural salt concentration of BW1 (Bad Saarow) and is also in the salt tolerance range of up to 4.2% of *S. europaea* [28].

2.5. Experimental Procedure

The treatment started six to seven weeks after germination under cultivation conditions and plants were harvested after three weeks of treatment. Per experiment five biological replicates were in each treatment group and the experiment was repeated three times (experiments 1, 2, and 3). Experiments 1, 2, and 3 (exp. 1, 2, and 3) represent three independent repetitions. Experiment 1 was performed in August to October 2020 and experiments 2 and 3 in September to November 2020 (Figure 1). While harvesting, fresh weight of the aboveground part of the plant was determined and then plants were frozen immediately in liquid nitrogen and freeze dried for one week. For further analysis, samples

were homogenized by grinding (for 3–5 × 50 s with 3–5 metal beads (Ø 9 mm) at 25 Hz) using a Retsch mill (Retsch MM 400; Retsch GmbH, Haan, Germany).

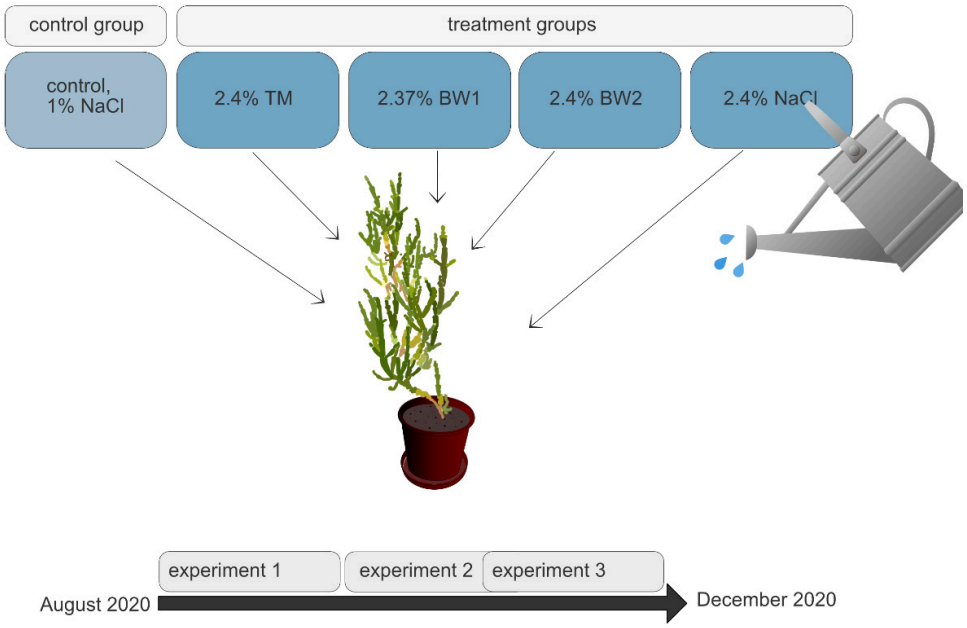

Figure 1. Experimental design to determine feasibility of natural brines for cultivation of *S. europaea*. TM, artificial sea water Tropic Marine; BW1, brine water location 1 (Bad Saarow); BW2, brine water location 2 (Heiligenstadt); NaCl, sodium chloride solution.

2.6. Purchased Salicornia Product

To compare nutritional quality with a purchased product grown under field conditions in seawater, a fresh *Salicornia* was purchased from an online store in 2019. Based on its specifications, the plant was harvested from the Northeast Atlantic FAO 27 IVc where it was grown naturally. The sample was freeze dried for one week and prepared for further analysis as described above.

2.7. Determination of Anion Concentrations in the Saline Solutions

Chloride, nitrate, phosphate and sulfate content in the salt solutions were determined by ion exchange chromatography (IC). For this, 120 µL nutrient solution was diluted up to 12 mL ultrapure water, including 400 µL sodium bromide (0.6 mg mL^{-1}) as an internal standard. The samples were shaken for better homogenization. The measurements were performed with an ion chromatograph 930 Compact IC Flex (Metrohm AG, Herisau, Schweiz) equipped with a conductivity detector with suppression system. The injection volume was 20 µL at a flow rate of 0.7 mL min^{-1} with an eluent consisting of Na_2CO_3 (3.2 mmol L^{-1}) and $NaHCO_3$ (1 mmol L^{-1}) and a Metrosep A Supp5 column (Metrohm AG, Herisau, Schweiz; 250 mm, 4 mm). The final concentration of anions was calculated with an external calibration from standards of chloride, phosphate, nitrate, and sulfate using MagIC Net 3.2 software.

2.8. Determination of Chlorophylls and Carotenoids in the Plants

Carotenoids and chlorophylls were determined as previously described in Frede et al. [34] with slight modifications. First, 10 mg homogenized, lyophilized plant material was extracted three times with 0.5 mL methanol/tetrahydrofuran (1:1, v/v). The samples were evaporated under nitrogen stream until dryness, dissolved in 50 µL dichloromethane and 200 µL isopropanol, and then filtered through PTFE-filter tubes (0.2 µm, Thermo Fischer Scientific Inc., Wilmington, NC, USA) before transferring to HPLC vials. The

analysis was performed with an Agilent Technologies 1290 Infinity UHPLC coupled with a ToF (Agilent Technologies Sales and Services, GmbH & Co. KG, Waldbronn, Germany). The analytes were separated on a C30 Carotenoid column (YMC Co. Ltd., Kyoto, Japan; 100 × 2.1 mm, 3 µm) at 20 °C. Identification was performed based on HR-MS data and UV/VIS spectra. Quantification was achieved by external calibration with carotenoid standards of all-*trans*-isomers from β-carotene, lutein, zeaxanthin, and (9Z)-neoxanthin as well as chlorophyll *a* and *b* standards at the wavelength of 450 nm. The quantification results of individual chlorophylls and carotenoids are cumulated as the total chlorophyll and total carotenoids.

2.9. Statistical Analysis

Statistical analysis was performed with SigmaPlot 14.0. Statistical differences were tested by a one-way ANOVA followed by a post hoc test using the Holm–Sidak method ($p \leq 0.05$) when normal distribution and equal variance were present. When either normal distribution or equal variance was absent, a Kruskal–Wallis one-way ANOVA on ranks was performed, followed by the Tukey test. Normal distribution was tested with the Shapiro–Wilks normality test and equal variance with the Brown–Forsythe equal variance test. Data are presented as means ± standard deviation (SD) of the three independent experiments (experiments 1, 2, and 3).

3. Results

3.1. Composition of the Different Saline Solutions

To determine the differences between the different saline solutions, we analyzed the anion concentrations. The values were very similar in all three experiments and no significant difference was found in anion concentrations at the beginning and the end of each experiment (Table S1 in Supplementary Materials). Exemplary data from experiments 2 and 3 are shown in Figure 2 and data from experiment 1 as well as the end of experiments 2 and 3 can be found in supplemental Table S1. The control group showed the lowest amount of chloride with 6.40 ± 0.02 g L^{-1}. In the treatment groups, the chloride content ranged from highest 15.04 ± 0.04 g L^{-1} (2.4% NaCl) and lowest 12.14 ± 0.06 g L^{-1} (TM), whereas measured differences are due to different compositions of the salt and/or brine water composition. The nitrate content is almost equivalent and ranges between 1.02 ± 0.00 g L^{-1} (2.4% NaCl) and 0.94 ± 0.00 g L^{-1} (2.4% TM). The phosphate content is similar in all solutions (ranged from 0.15 ± 0.01 to 0.20 ± 0.00 g L^{-1}), and slightly decreased in BW1 brine water. TM contained the highest amount of sulfate salts (1.69 ± 0.00 g L^{-1}) within the treatment groups, while 2.4% NaCl contained the lowest amount (0.21 ± 0.00 g L^{-1}). The two brine water solutions fall in between, with BW1 having a higher chloride content (14.44 ± 0.03 g L^{-1}) and lower sulfate content (0.46 ± 0.00 g L^{-1}) than BW2 (Cl^-, 13.02 ± 0.04 g L^{-1}; SO_4^{2-}, 0.62 ± 0.00 g L^{-1}). These findings indicate that the nitrate and phosphate contents originate mainly from the nutrient solution.

3.2. Impact of Treatment on Yield and Phytochemicals

3.2.1. Impact of Treatment on Yield

The fresh weight was strongly affected by the experimental period. Fresh weight of the control was highest in experiment 1 (4.68 g) and almost half as high in experiments 2 (2.56 g) and 3 (2.27 g) (Figure 3). One reason could be the seasonal difference since experiment 1 was sown in August and experiment 2 in late September.

There were no significant differences within treatments. Related to the control, fresh weight was significantly reduced in all treatments, most dramatically with 2.4% NaCl at 0.42-fold (Figure 3). However, all nutrient solutions were suitable for the cultivation of *S. europaea*.

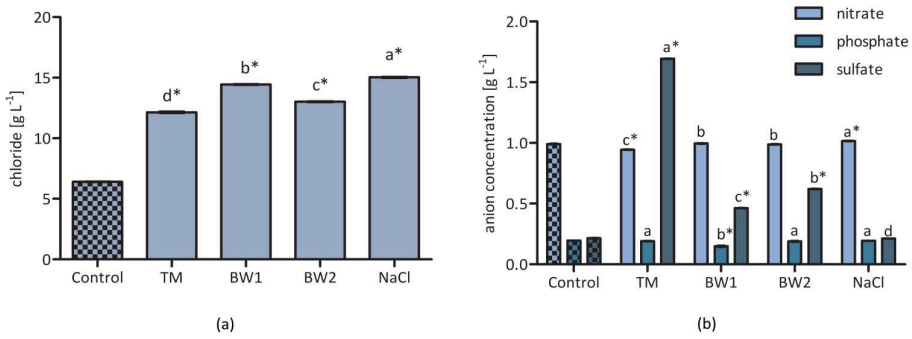

Figure 2. Anion composition of saline solutions. Concentration of (**a**) chloride and (**b**) nitrate, phosphate, and sulfate in salt solutions of the control and treatment groups at the beginning of experiments 2 and 3. Means ± SD of one independent experiment. Asterisks indicate significant differences between individual treatments and the control within one component ($p \leq 0.05$). Small letters indicate significant difference between the treatments within one component ($p \leq 0.05$). TM, artificial sea water Tropic Marine; BW1, brine water location 1 (Bad Saarow); BW2, brine water location 2 (Heiligenstadt); NaCl, sodium chloride solution.

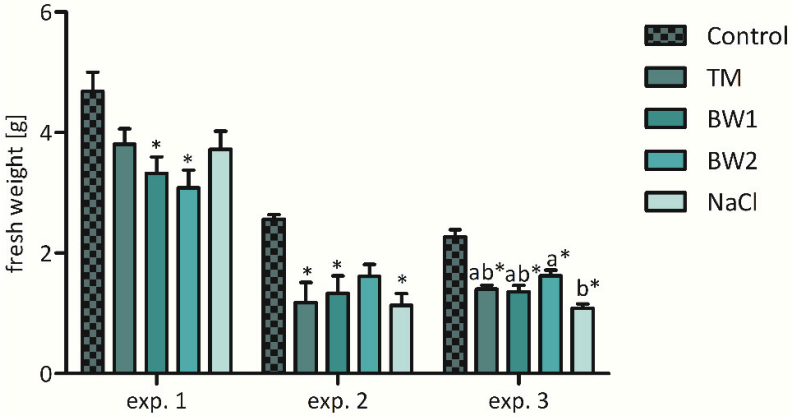

Figure 3. Fresh weight of shoots of *Salicornia europaea* of 10-week-old plants. Means ± SD of three independent experiments (exp.). Asterisks indicate significant differences between individual treatments and the control within an experiment ($p \leq 0.05$). Small letters indicate significant difference between the treatment groups within one experiment ($p \leq 0.05$). TM, Tropic Marine BW1, brine water location 1 (Bad Saarow); BW2, brine water location 2 (Heiligenstadt); NaCl, sodium chloride solution.

3.2.2. Impact of Treatment on the Phytochemical Content

The total chlorophyll content ranged from 1853.27 ng mg^{-1} to 3477.02 ng mg^{-1} (Table 1). There were no significant differences within the treatment group, but the chlorophyll content was significantly reduced (0.1–0.3-fold) in BW1 and BW2 compared to control (optimal growth conditions). The chlorophyll a/b ratio was unaffected (Table 1). The total carotenoid content ranged from the lowest value 356.89 ng mg^{-1} (BW1, exp. 3) to the highest value 551.23 ng mg^{-1} (control, exp. 2) (Table 2). There were no significant differences between the treatments. Compared to the control, only BW2 showed a difference. However, for all treatments, there was a trend toward a slight 0.1–2-fold reduction of total carotenoids. The experiment 3 showed no differences for either the total chlorophyll or the total carotenoid content (Tables 1 and 2) and also had the lowest values of all three experiments. Individual carotenoids showed significant differences within experiments 1 and/or experiment 2, but no particular pattern. Zeaxanthin, however, showed the lowest amounts in the control group.

Table 1. Content of total chlorophylls, chlorophyll a, chlorophyll b and chlorophyll a/b ratio in shoots of *Salicornia europaea* of 10-week-old plants. Means ± SD of one independent experiment (exp.). Asterisks indicate significant differences between individual treatments and the control within an experiment ($p \leq 0.05$). TM, Tropic Marine; BW1, brine water location 1 (Bad Saarow); BW2, brine water location 2 (Heiligenstadt); NaCl, sodium chloride solution; 1, 2, 3, three different experiments; ns, not significant.

Total Chlorophyll (ng mg^{-1} DW)												
	exp. 1				exp. 2				exp. 3			
Control	2843.01	±	156.23		3477.02	±	116.65		2592.50	±	100.07	ns
TM	2430.70	±	161.34		2876.78	±	348.73		2310.38	±	278.75	ns
BW1	2365.12	±	322.58	*	2706.29	±	113.64	*	1853.27	±	188.28	ns
BW2	2251.70	±	210.14	*	2722.68	±	154.77	*	2248.02	±	428.07	ns
NaCl	2499.83	±	313.34		2813.55	±	97.74		2106.85	±	616.33	ns
Chlorophyll a (ng mg^{-1} DW)												
	exp. 1				exp. 2				exp. 3			
Control	2229.06	±	131.31		2755.64	±	85.36		1978.65	±	124.72	ns
TM	1905.73	±	145.10		2299.47	±	274.90	*	1836.28	±	221.58	ns
BW1	1853.07	±	267.48		2149.04	±	106.27	*	1404.60	±	178.87	ns
BW2	1748.37	±	169.81	*	2158.34	±	114.23	*	1725.75	±	384.16	ns
NaCl	1966.04	±	258.95		2225.54	±	69.43	*	1648.19	±	493.74	ns
Chlorophyll b (ng mg^{-1} DW)												
	exp. 1				exp. 2				exp. 3			
Control	613.95	±	35.82		721.38	±	31.52		613.85	±	49.17	
TM	524.96	±	21.17	*	577.32	±	76.08		474.10	±	59.31	*
BW1	512.04	±	56.68	*	557.25	±	16.38	*	448.67	±	20.07	*
BW2	503.33	±	41.23	*	564.34	±	41.09	*	522.27	±	48.43	
NaCl	533.79	±	56.44		588.00	±	28.89		458.66	±	124.38	*
Chlorophyll a/b Ratio												
	exp. 1				exp. 2				exp. 3			
Control	3.63	±	0.18	ns	3.82	±	0.05	ns	3.25	±	0.41	ns
TM	3.63	±	0.20	ns	3.99	±	0.15	ns	3.88	±	0.15	ns
BW1	3.61	±	0.18	ns	3.86	±	0.18	ns	3.13	±	0.37	ns
BW2	3.47	±	0.09	ns	3.83	±	0.09	ns	3.28	±	0.50	ns
NaCl	3.68	±	0.17	ns	3.79	±	0.07	ns	3.58	±	0.23	ns

Table 2. Content of total carotenoids, lutein, β-carotene, and zeaxanthin in shoots of *Salicornia europaea* of 10-week-old plants. Means ± SD of one independent experiment (exp.). Letters indicate significant differences within treatments in one experiment and one component ($p \leq 0.05$). Asterisks indicate significant differences between individual treatments and the control within an experiment ($p \leq 0.05$). Small letters indicate significant difference between the treatment groups within one experiment ($p \leq 0.05$).TM, Tropic Marine; BW1, brine water location 1 (Bad Saarow); BW2, brine water location 2 (Heiligenstadt); NaCl, sodium chloride solution; 1, 2, 3, three different experiment; ns, not significant.

Total Carotenoids (ng mg^{-1} DW)												
	exp. 1				exp. 2				exp. 3			
Control	480.7	±	26.39		551.23	±	17.64		439.7	±	17.38	ns
TM	425.6	±	25.21		488.78	±	67		403.66	±	47.53	ns
BW1	418.17	±	44.57		458.16	±	21.41		356.89	±	11.03	ns
BW2	398.87	±	31.96	*	459.23	±	25.97	*	409.66	±	45.02	ns
NaCl	441.16	±	34.31		468.53	±	20.91		373.19	±	99.08	ns

Table 2. *Cont.*

Lutein												
	exp. 1				exp. 2				exp. 3			
Control	179.23	±	14.58		193.68	±	12.66		170.25	±	17.16	
TM	174.76	±	6.628	a	197.62	±	15.88	a	169.18	±	16.85	
BW1	167.25	±	19.17	a	173.47	±	9.341	ab	144.52	±	12.83	
BW2	140.49	±	9.456	b*	161.62	±	11.59	b*	157.35	±	8.80	
NaCl	156.97	±	7.494	ab	157.5	±	5.648	b	130.87	±	32.07	*
β-Carotene												
	exp. 1				exp. 2				exp. 3			
Control	136.31	±	8.777		158.5	±	6.431		144.66	±	15.72	
TM	123.77	±	6.796		145.89	±	14.67	a	125.31	±	12.11	
BW1	120.02	±	11.08		128.34	±	5.715	*	110.76	±	5.68	
BW2	105.36	±	8.281	*	126.86	±	7.95	*b	124.74	±	12.08	
NaCl	120.4	±	12.88		131.3	±	7.865	*ab	105.1	±	30.47	*
Zeaxanthin												
	exp. 1				exp. 2				exp. 3			
Control	13.532	±	3.347		8.0821	±	1.1	ns	9.116	±	4.29	ns
TM	12.147	±	2.435		21.509	±	6.484	ns	15.683	±	5.28	ns
BW1	15.524	±	3.835		13.795	±	4.066	ns	19.581	±	0.73	ns
BW2	13.854	±	1.839		12.484	±	3.361	ns	16.076	±	2.27	ns
NaCl	21.027	±	2.194	*	22.356	±	15.45	ns	19.746	±	5.24	ns

4. Discussion

Given the global wide distribution of natural brines and their known complex chemical composition, often coupled to local geothermal structures, there is a high potential for their further application to respond to the crop production needs and requirements of regional agricultural [35,36]. In Germany, natural brine springs are widespread and of economic importance for the chlor-alkali electrolysis industry as well as for therapeutic applications in the spa [37,38]. So far, natural brine has not been used for agricultural purposes. Here, the choice of cultivated crop will be crucial, and we predict that the cultivation of halophytes could significantly contribute to resource efficient farming of halophytes or other aquatic organisms as demonstrated for *S. europaea*.

Previous studies have shown that salt concentration and composition can influence the growth of halophytes [9,28–31]. The differences in growth between treatment and control groups are potentially the result of the lower chloride content in the control solution enabling higher biomass production. Even *S. europaea* is an obligate halophyte, yet salt tolerance has a limit at which the plant cannot cope with increasing salinity and responds by inhibiting growth and altering metabolism. Chloride, along with sodium, is the main stressor in salt stress and affects plant growth and photosynthesis. Several studies showed growth inhibition with increasing salinity concentration in halophytes, not only for *Salicornia* species, but also for *Chenopodium quinoa*, *Sarcocornia fructosia*, and *Cochlearia officinalis* [14,29,39–41]. He, Silliman and Cui [9] showed a reduction in the growth of the aboveground part of *S. europaea* at 4 to 10% soil salinity. Moreover, Orlovsky, Japakova, Zhang and Volis [30] investigated the effect of different salt compositions on germination and growth of *S. europaea.* Their study revealed that a mixed chloride-sulfate salt has a positive effect on growth compared to pure chloride salt. Interestingly, no significant differences in growth related to anion composition were observed in the present study, thereby suggesting that the use of natural brine for cultivation of *S. europaea* is both feasible and a suitable alternative for seawater use in the mainland.

Variations in the contents of chlorophylls and carotenoids are of interest as changes in these pigments provide a good indication of oxidative stress. The chlorophyll a/b ratio is an important parameter that, when altered under stress conditions, provides an indication of altered photosystem activity. As the chlorophyll a/b ratio was not changed in the present

study, this indicates that the plants had an unaffected photosynthetic rate. Plants that cannot cope with salinity stress show reduced chlorophyll content and photosynthetic activity. In green bean (*Phaseolus vulgaris* L.) plants for example, ElSayed, et al. [42] showed a decrease in photosynthetic quantum yield and total chlorophyll content already from a salt concentration of 200 mM (1.2%) sodium chloride. Along with the chlorophylls, carotenoids are important pigments in the photosystems and plants can adapt chlorophyll and carotenoid content in their photosystems under suboptimal photosynthetic conditions, which can occur during salt stress [43]. The absence of changes in the total carotenoid content between treatments also indicates that the plants are not suffering from suboptimal photosynthetic conditions. Thus, the altered pigment levels between treatment and control groups are likely due to reduced growth.

Food quality can be influenced by salinity in both glycophytic crops (e.g., *Lactuca sativa*, Eggplant, *Cucumis sativus*, and Solanum lycopersicum) as well as in halophytes (e.g., *Crithmum maritimum* and *Salicornia persica*) [29,44–47]. Chlorophylls and Carotenoids are nutritionally valuable compounds that are associated with health-promoting effects and are particularly abundant in green leafy vegetables. Moreover, carotenoids and chlorophylls have an antioxidant capacity and can prevent DNA damage and lipid peroxidation, and thus, have anti-carcinogenic effect [22,48,49]. In addition, the carotenoids lutein and zeaxanthin have a positive effect on eye health and β-carotene has provitamin-A activity [24,25]. The comparison of the values of total chlorophylls and carotenoids (Tables 1 and 2) of the treatment group with a purchased product of *S. europaea* from the North Sea coast (3–3.5% salt) (Table S2 in Supplementary Materials) showed that the values were in the same range. Furthermore, compared to other vegetables, such as spinach and kale, which are known to be a good sources for carotenoids and chlorophylls, *S. europaea* has a comparable content of carotenoids and chlorophylls [50,51]. Thus, on the basis that no changes in the fresh weight and the selected metabolites were found, we assume that natural brine can be utilized for the production of nutrient-rich vegetables.

In summary, this case study demonstrates the potential of brine water for indoor aquaculture systems. To realize its full potential, further research is needed on the cultivation of halophytes in regional indoor brine water systems and the adaptation of the system to other aquatic organisms such as algae or shrimp, as well as mechanistic approaches to study the influence of brine water on halophytes. Here, initial approaches to determine the optimal growth conditions for halophytes are needed. In detail, lowering the chloride content could be beneficial for yield and as well as the nutrient profile. In this respect, further natural brines should be examined to better assess their regional potential. Finally, among others, (bio-)technological developments, such as wireless senor technologies, automatized irrigation systems, renewable energy, and culture compartments, as well as research unleashing the biodiversity of halophytes as alternative vegetables and mechanistic approaches are required to implement saline food systems [52–54].

5. Conclusions

Due to the freshwater scarcity, new approaches in agriculture are needed to ensure food security in the future. Urban agriculture contributes to the availability of affordable, healthy, fresh food and can improve food security even with less arable land. Additionally, this study highlights that natural brine is a possible alternative to the use of seawater or artificial seawater for the cultivation of the halophyte *Salicornia europaea* and is therefore a promising approach for urban indoor farming without being dependent on shrinkable potable water resources. This is an important step towards more sustainable saline food systems offside coastal regions. Furthermore, the example of *S. europaea* demonstrates that halophytes have a much overlooked potential as a nutritious and health-promoting food source. As such, they can improve the biodiversity of plant-based diets and hence the uptake of plant secondary metabolites and contribute to a healthier diet. Thus, further research aimed at optimizing growth conditions for different halophyte species would

better allow the potential of natural brines for sustainable local solutions to be exploited for aquaculture and saline agriculture.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/su132112105/s1, Table S1: Anion concentrations in nutrient solutions at the beginning and end of the experiments, Table S2: Chlorophyll and carotenoid content of purchased *S. europaea*.

Author Contributions: Conceptualization, S.B., A.F. and M.F.; methodology, S.B. and M.F.; validation, S.B. and M.F.; formal analysis, M.F.; investigation, M.F.; data curation, M.F.; writing—original draft preparation, M.F.; writing—and editing, S.B., A.F. and M.F.; supervision, S.B and M.S.; project administration, S.B. and M.S.; funding acquisition, S.B. and M.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was founded by the German Federal Ministry of Education and Research, grant number 031B0730 A, as part of the project food4future.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the authors.

Acknowledgments: We would like to thank Andrea Jankowsky for performing the anion analysis. We also thank the staff at the thermal baths in Bad Saarow (Axel Walter) and in Heilbad Heiligenstadt (Steffen Menzel) for their support.

Conflicts of Interest: The authors declare no conflict of interest.

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Publication II

Supplementary data

Title: Utilization of regional natural brines for the indoor cultivation of *Salicornia europaea*.

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Supplementary Material

Table S1. Concentrations of chloride, nitrate, phosphate and sulfate in the nutrient solutions used in experiments 1 and 2 and 3 at the beginning (t1) and end (t2) of the treatment period. TM, artificial sea water Tropic Marine; BW1, brine water location 1 (Bad Saarow); BW2, brine water location 2 (Heiligenstadt); ŧ, calculated concentration. BW1*, t2 = no data available

	Experiment 1									
	t1					t2				
	Cl (g l^{-1})	NO_3 (g l^{-1})	PO_4 (g l^{-1})	SO_4 (g l^{-1})	$NaCl^ŧ$ (g l^{-1})	Cl (g l^{-1})	NO_3 (g l^{-1})	PO_4 (g l^{-1})	SO_4 (g l^{-1})	$NaCl^ŧ$ (g l^{-1})
Control 2.4%	6.44	0.93	0.16	0.19	10.61	6.40	0.87	0.12	0.18	10.55
TM 2.4 %	12.40	0.89	0.15	1.64	20.43	12.29	0.80	0.13	1.38	20.26
BW1 2.4%	14.56	0.89	0.15	0.44	24.01					
BW2 2.4 %	13.55	0.96	0.16	0.61	22.34	13.50	0.91	0.12	0.53	22.25
NaCl	15.03	0.93	0.15	0.20	24.78	14.95	0.87	0.13	0.18	24.64
	Experiment 2 and 3									
	t1					t2				
	Cl (g l^{-1})	NO_3 (g l^{-1})	PO_4 (g l^{-1})	SO_4 (g l^{-1})	$NaCl^ŧ$ (g l^{-1})	Cl (g l^{-1})	NO_3 (g l^{-1})	PO_4 (g l^{-1})	SO_4 (g l^{-1})	$NaCl^ŧ$ (g l^{-1})
Control 2.4%	6.40	0.99	0.20	0.22	10.56	6.44	0.96	0.17	0.20	10.61
TM 2.4 %	12.15	0.94	0.19	1.69	20.02	12.21	0.94	0.18	1.68	20.12
BW1 2.4%	14.44	1.00	0.15	0.46	23.79	14.46	0.99	0.09	0.45	23.84
BW2 2.4 %	13.02	0.99	0.19	0.62	21.47	13.04	0.98	0.17	0.61	21.50
NaCl	15.04	1.02	0.19	0.21	24.78	15.20	1.02	0.17	0.21	25.05

Table S2. Content of total chlorophylls and carotenoids, chlorophyll *a*, chlorophyll *b*, lutein and β-carotene in purchased *S. europaea*. Data presented as means ± SD, n=4.

Chlorophylls and carotenoids (ng mg^{-1} DW)			
Total chlorophylls	2865.12	±	258.71
Chlorophyll *a*	539.50	±	53.97
Chlorophyll *b*	2325.62	±	205.29
Total carotenoids	494.99	±	65.73
β-Carotene	125.65	±	6.65
Lutein	192.74	±	17.16

Publication III

The interaction of salinity and light regime modulates photosynthetic pigment content in edible halophytes in greenhouse and indoor farming.

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Published in: *Frontiers in Plant Science.* 2023;14
DOI: 10.3389/fpls.2023.1105162

frontiers | Frontiers in Plant Science

TYPE Original Research
PUBLISHED 04 April 2023
DOI 10.3389/fpls.2023.1105162

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Crop and Product Physiology, a section of the journal Frontiers in Plant Science

RECEIVED 22 November 2022
ACCEPTED 13 March 2023
PUBLISHED 04 April 2023

CITATION
Fitzner M, Schreiner M and Baldermann S (2023) The interaction of salinity and light regime modulates photosynthetic pigment content in edible halophytes in greenhouse and indoor farming.
Front. Plant Sci. 14:1105162.
doi: 10.3389/fpls.2023.1105162

The interaction of salinity and light regime modulates photosynthetic pigment content in edible halophytes in greenhouse and indoor farming

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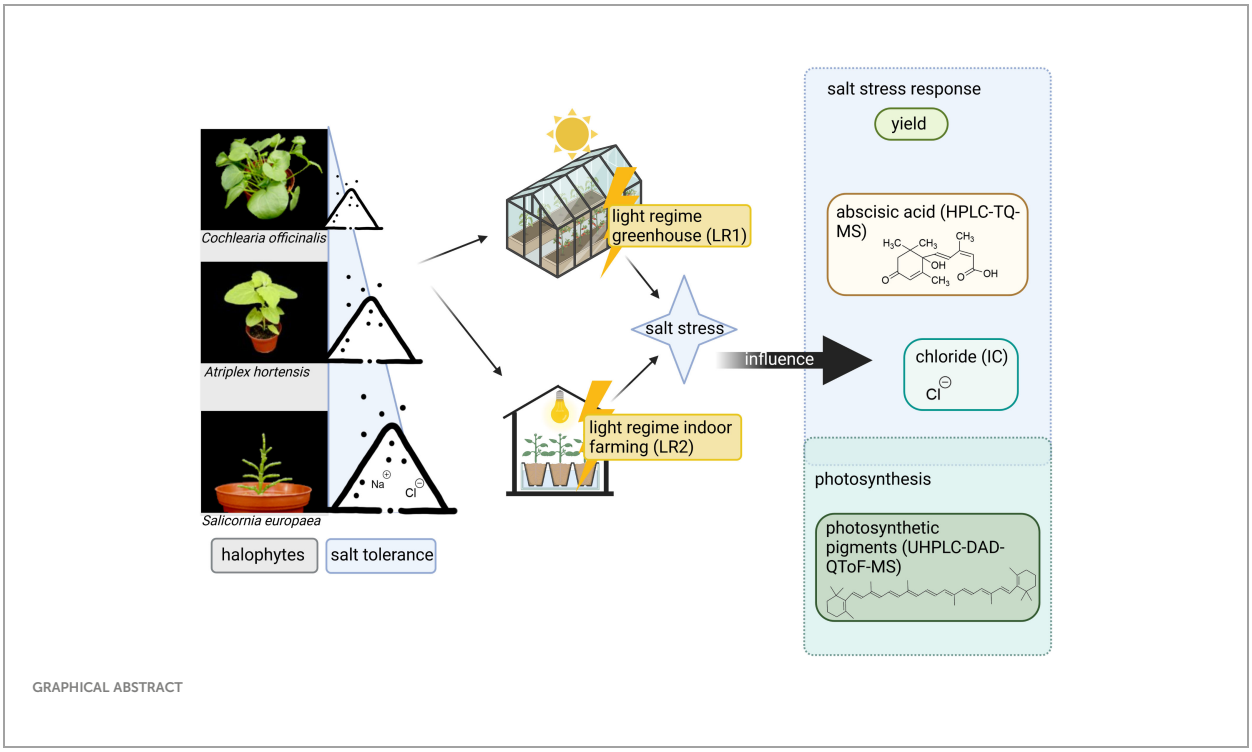
Given its limited land and water use and the changing climate conditions, indoor farming of halophytes has a high potential to contribute significantly to global agriculture in the future. Notably, indoor farming and classical greenhouse cultivation differ in their light regime between artificial and solar lighting, which can influence plant metabolism, but how this affects the cultivation of halophytes has not yet been investigated. To address this question, we studied the yield and content of abscisic acid, carotenoids, and chlorophylls as well as chloride of three halophyte species (*Cochlearia officinalis*, *Atriplex hortensis*, and *Salicornia europaea*) differing in their salt tolerance mechanisms and following four salt treatments (no salt to 600 mM of NaCl) in two light regimes (greenhouse/indoor farming). In particular, salt treatment had a strong influence on chloride accumulation which is only slightly modified by the light regime. Moreover, fresh and dry mass was influenced by the light regime and salinity. Pigments exhibited different responses to salt treatment and light regime, reflecting their differing functions in the photosynthetic apparatus. We conclude that the interaction of light regime and salt treatment modulates the content of photosynthetic pigments. Our study highlights the potential applications of the cultivation of halophytes for indoor farming and underlines that it is a promising production system, which provides food alternatives for future diets.

KEYWORDS

saline agriculture, vertical farming, abiotic stress, vegetables, future food production, light condition

10.3389/fpls.2023.1105162



GRAPHICAL ABSTRACT

Introduction

Reducing land use and water consumption is among the biggest challenges for future food production (FAO, 2020). One approach to overcome these challenges is to combine indoor farming with saline agriculture. Indoor farming holds the capability for year-round uniform product quantity and quality due to controlled environmental conditions. These systems are efficient in resource use through smart climate systems, such as heating, ventilation, and air conditioning (HVAC); fertigation systems, such as the nutrient film technique (NFT); and the use of innovative sensing, modeling, and AI technologies (Asseng et al., 2020; van Delden et al., 2021; Swain, 2022). Saline agriculture offers the possibility to use saline water resources and, thus, minimize the freshwater use of the system to zero (Norton, 2021). Crucially, saline indoor farming could open new opportunities for the exploitation of the vast untapped potential of not only saline water sources but also unused urban areas thereby revolutionizing sustainable agricultural practices and reducing food miles (Norton, 2021). Salt plants (halophytes) are potential candidates for saline indoor farming since they tolerate high salinity levels and still contribute to a healthy diet, due to their content of plant secondary metabolites (PSMs). Nevertheless, the quantity and quality of these alternative vegetables depend on environmental conditions, and thus, it is important to investigate optimal growth conditions for new indoor farming systems.

This study is focused on three different edible halophytes: scurvy grass (*Cochlearia officinalis*), garden orache (*Atriplex hortensis*), and glasswort (*Salicornia europaea*). Halophytes are plants that can grow and reproduce under saline conditions. A key aspect of salinity tolerance of halophytes is the osmotic adjustment, which is different from glycophytes (non-halophytic plants), and also differs among halophyte species (Flowers and Colmer, 2008). Halophytes that tolerate salt, but do not require salt for growth, and therefore grow optimally in non-saline to moderately saline environments, are classified as facultative halophytes (Hasanuzzaman et al., 2014). There are also halophyte species, which require salt for optimal growth and are classified as obligate halophytes (Hasanuzzaman et al., 2014). While *A. hortensis* and *C. officinalis* show optimal growth at no salt or medium salinity (up to 100 mM), *S. europaea* shows optimal growth between 200 and 400 mM of salt (Wilson et al., 2000; Kachout et al., 2009; Lv et al., 2012; de Vos and Broekman, 2013; He et al., 2017). To adapt to salinity, halophytes have evolved different salt tolerance mechanisms, namely, the salt-excluding, salt-excreting, and salt-accumulating mechanisms. The salt-excluding mechanism reduces salt uptake by the roots, the salt-excreting mechanism eliminates salt through salt bladders/glands, and the salt-accumulating mechanism promotes the storage of salt in cell vacuoles (Hasanuzzaman et al., 2014; Chen et al., 2018). *Salicornia europaea* can be assumed to be a salt-accumulating plant since it develops stem succulence (Song and Wang, 2014; Araus et al., 2021). Most *Atriplex* spp., including *A. hortensis*, are salt-excreting plants and form salt bladders (Schirmer and Breckle, 1982; Breckle, 2002; Kachout et al., 2009; Yuan et al., 2016). de Vos and Broekman (2013) classifies *C. officinalis* as an intermediate halophyte but neither as a salt-accumulating plant nor as a plant-forming salt bladder/gland. It can be assumed that *C. officinalis* is a salt-excluding plant even though detailed studies are missing.

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As with glycophytes, salt stress occurs in halophytes above the tolerable salt levels, although these levels vary among halophyte species. Salt stress can be indicated by signaling molecules, such as the plant hormone abscisic acid (ABA). ABA is known to be an essential signaling molecule and regulatory factor in response to salt stress (Zhu, 2002; Golldack et al., 2014). An important effect of ABA is to induce the closure of stomata by guard cells (Tuteja, 2007). This mechanism is essential for plants' water status and involves the sensing of air humidity by the guard cells and water potential by the roots (Julkowska and Testerink, 2015; Ko and Helariutta, 2017). Recent studies suggest differential regulation of ABA metabolism in halophytic and glycophytic guard cells (Ellouzi et al., 2014; Karimi et al., 2021). However, only a few studies have considered both ABA and the salt tolerance mechanism. For example, Ben Hassine et al. (2009) indicate that ABA may be involved in the regulation of salt excretion in *Atriplex halimus*.

There has been a dramatic increase in halophyte research in recent years, including halophyte agriculture for food production (Abdelly et al., 2022). Although many studies focus on halophyte agriculture in the greenhouse or field (Ladeiro, 2012; de Vos and Broekman, 2013; Ventura and Sagi, 2013), very few studies focus on the indoor farming of halophytes (Norton, 2021). A central advantage of indoor farming is that the environmental conditions can be modulated to a full extent. In contrast, greenhouse cultivation still depends on outdoor environmental conditions. For example, there is a great difference in the daily light integral (DLI) in greenhouse cultivation, which is dependent on solar lighting, during the year. Especially in winter months (November–February) in the northern latitudes, the DLI is significantly lower than in summer (Korczynski et al., 2002; Hernandez Velasco, 2021). These indifferences in lighting can lead to inconsistent quality of crops. Artificial lighting in indoor farming, on the other hand, offers a year-round uniform product and the possibility to optimize lighting conditions for yield and nutritional profile. Still, aside from the DLI, the differences between artificial and solar lighting (natural light) also include differences in spectral quality and diurnal changes, which also can affect crop yield and nutritional quality. For instance, Annunziata et al. (2017) grew *Arabidopsis thaliana* plants under natural light and two artificial light sources (fluorescent and LED light), whereby the different light sources resulted in changes in plant metabolism, such as diurnal changes in carbohydrate or amino acid metabolism, which are dependent on the light source.

Similarly, PSMs are influenced by light conditions. Several studies have investigated the influence of light conditions on the composition of PSMs in glycophytes (non-halophytic plants), such as different colored light-emitting diodes (LEDs) or ultraviolet (UV) radiation (Heinze et al., 2018; Naznin et al., 2019; Maina et al., 2021). Notably, the influence of light qualities on PSMs is both species-specific and metabolite-specific. Carotenoids and chlorophylls are of particular interest due to their function as photosynthetically active pigments and their photoprotective properties associated with changing light conditions. Chlorophyll *a* is a light-harvesting molecule that converts light energy into chemical energy (Björn et al., 2009). Chlorophyll *b* is important for the stabilization of the light-harvesting complex (LHC) (Tanaka and Tanaka, 2011). Carotenoids act not only as light harvesters but also as scavengers of reactive oxygen species (ROS). For example, β-carotene protects photosystem II (PSII) from photooxidative damage by quenching singlet oxygen formed in PSII (Choudhury and Behera, 2001; Hideg et al., 2002; Trebst, 2003). Zeaxanthin is known to be involved in non-photochemical quenching (NPQ), which plants use to dissipate excess light energy (Gilmore, 2001). Violaxanthin plays a very important role in plants in dissipation in case the light exceeds the uptake capacity of the photochemical apparatus (photoprotection) (Gilmore, 2001). Since chlorophylls and carotenoids have individual functions in plants, it is important to evaluate them individually. Furthermore, carotenoid metabolism is not only affected by light (Lado et al., 2015; Frede et al., 2018; Frede et al., 2019; Frede and Baldermann, 2022) but also by salinity (Kim et al., 2008). Since salinity-induced changes in PSM levels in halophytes are observed (Aghaleh, 2011), it is likely that the interaction between salinity and light conditions also affects PSMs, as salt stress also influences photosynthesis.

The effect on PSMs is of particular interest because of their health-promoting effects when consumed in the human diet. In particular, carotenoids are crucial components of the human diet as they have been associated with the prevention of non-communicable diseases such as cancer and diabetes. This is attributed to their chemoprotective properties (Fiedor and Burda, 2014). Halophytes, for example, *S. europaea*, exhibit a rich profile of secondary metabolites (Kim et al., 2021).

Indeed, investigating the impact of environmental conditions on yield and PSMs is a key issue for food produced in indoor farming. To address this, our study was designed to compare the light regimes of greenhouse and indoor farming and their effects on salt stress response and photosynthetic pigments in halophytes. Considering this, we evaluated the effect of salt treatment on yield, chloride accumulation, and ABA content as well as individual carotenoids and chlorophylls in the leaves of three different halophyte species (*C. officinalis*, *A. hortensis*, and *S. europaea*) grown in two different light regimes (greenhouse and indoor farming).

The study demonstrates that halophytes adapt species-specific to changing light and salt environments and that these factors mutually influence each other.

Material and methods

Plant material and cultivation

The seeds of *S. europaea* were purchased from Rühlemann's Kräuter & Duftpflanzen (Germany) and the seeds of *C. officinalis* and *A. hortensis* were from Magic Garden Seeds (Germany). The plants were germinated in soil [substrate type P; pH = 5.9; 120 mg L^{-1} of N; 120 mg L^{-1} of PO_4^{2-}; 170 mg L^{-1} of K; 120 mg L^{-1} of Mg; density, 430 kg/m^3; 70% raised bog peat (degree of decomposition: H2-H5), 30% clay; Einheitserdewerke Werkverband e.V., Germany]. When two leaves had fully developed, the plants were transferred to pots

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(diameter, 8 cm) containing one-third of soil [substrate type T; pH 5.9; 180 mg L^{-1} of N; 180 mg L^{-1} of PO_4^{2-}; 260 mg L^{-1} of K; 130 mg L^{-1} of Mg; density, 430 kg/m^3; 70% raised bog peat (degree of decomposition: H2-H5), 30% clay; Einheitserdewerke Werkverband e.V., Germany], one-third of fine quartz sand (grain size 0.5–1 mm), and one-third of coarse quartz sand (grain size 2–3 mm) (Euroquarz GmbH, Germany). The water content of the soil with respect to salt treatment can be found in Table S2. The plants were irrigated with a modified Hoagland solution (Table S1).

Light regimes and salt treatment

Light regimes

The greenhouse cultivation, light regime 1 (LR1), was located at the Leibniz Institute of Vegetable and Ornamental Crops (Grossbeeren, 52°20′5N, 13°18′35.3′′E), and the experiment was conducted in November 2019. The lighting setup consisted of natural light and an additional artificial light source (SON-T Agro 400W; Philips, The Netherlands) for 7 h per day from 05:00 to 12:00 o'clock. Thus, on average, the plants were grown under a light–dark regime of 11 h of light and 13 h of darkness. The intensity of natural light was measured in photosynthetic photon flux density (PPFD) using a PAR sensor (LI-190R Quantum Sensor, LICOR Biosciences GmbH, Germany) on the roof of the greenhouse and calculated based on the light transmittance of the glass (50%). Based on these data, the daily light hours, intensity, and DLI were calculated, taking into account natural and artificial light (Table 1). Since the two replicate experiments were performed simultaneously, their lighting conditions were the same.

The indoor farming system, light regime 2 (LR2), was set up in a climate chamber (Vötsch Industrietechnik GmbH, Germany), also in November 2019. The lighting setup consisted only of artificial lighting (Clean Ace™ R MT400DL/BH YE; EYE Lighting Europe Ltd., United Kingdom), where the plants were grown under a light–dark regime of 14 h of light and 10 h of darkness (Table 1). Replicate experiments were conducted in identical climate chambers at the same time, but light intensity (PPFD) differs slightly and is given for climate chambers 1 and 2 (Table 1).

The remaining adjustable climatic conditions were set the same in both greenhouse and indoor farming: temperature, 22°C/18°C (day/night); humidity, 65%; and CO_2, 400 ppm.

Salt treatment

The plants were irrigated in an NFT system in 0.5-h intervals (Table S1). Plants were acclimated to the NFT system for 1 week prior to salt treatment in both light regimes. Four salt concentrations, no salt, or 50, 200, or 600 mM of sodium chloride, were utilized to study the effect of salt in the NFT system. Salt treatment was initiated by adding the desired salt concentrations to the nutrient solution in a single step. This time point is considered the start of the experiment, and the salt concentrations were monitored from then onwards and adjusted as necessary throughout the experimental period (Figure S4). After 17 days of treatment, the plants were harvested. The chloride content in the substrate was determined at the end of the experiment (Table S2).

Plant sampling and fresh and dry mass

To determine the fresh mass, the 12 plants were cut at the root and then the aboveground part of the plants was weighed as whole plants; then, three plants were pooled and the pooled leaves were weighed separately. For further analysis of the metabolite content, the main leaves of *A. hortensis* and *C. officinalis* were harvested, and the green aboveground part (which is later on referred to as leaves) of *S. europaea* was harvested and pooled into four technical replicates per salt treatment at each experiment, resulting in eight replicates per light regime from two independent experiments. After harvesting, the plants were immediately frozen in liquid nitrogen and then freeze-dried for 1 week until completely dry. Dry mass was determined by weighing the pooled leaves before (FM) and after (DM) freeze drying. Percent dry mass was calculated as DM/FM*100. For further analysis, plant samples were homogenized using a Retsch mill (Retsch MM 400; Retsch

TABLE 1 Light settings of light regime 1 (LR1, greenhouse) and light regime 2 (LR2, indoor farming) (means ± SEM).

		Light hours (h day^{-1})	Light intensity (PPFD) (µmol m^{-2} s^{-1})	Daily light integral (DLI) (mol m^{-2} day^{-1})		
				Average	Min	Max
Light regime 1	Artificial light	7	46.93 ± 1.32			
	Natural light[a]	8.61 ± 0.04	49.47 ± 4.45			
	Combined light sources[b]	11.06 ± 0.04	66.54 ± 6.84	3.18 ± 0.26	1.86 ± 0.14	4.33 ± 0.4
Light regime 2	Climate chamber 1	14	366.23 ± 4.60	18.46 ± 0.23		
	Climate chamber 2		346.93 ± 4.70	17.49 ± 0.24		
	Average[c]		356.58 ± 4.7	17.98 ± 0.24		

PPFD, photosynthetic photon flux density.
[a]Average values calculated for the time period of the experiment (17 days) in light regime 1 with data taken from a sensor on top of the greenhouse.
[b]Values were calculated by including artificial and natural light.
[c]Values were calculated by the average of both climate chambers.

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GmbH, Germany) [three to five times for 50 s with three to five metal beads (diameter, 9 mm) at 25 Hz].

Determination of chloride concentration in the leaves

The chloride content of the sampled leaves was determined by ion chromatography. For this purpose, 10 mg of dried plant material was dissolved in 1 ml of ultrapure water. As an internal standard, 0.5 ml of sodium bromide solution (0.6 mg ml^{-1}) was added. The samples were sonicated on ice for 10 min and then centrifuged for 5 min (4,500×*g*, 4°C). Next, the samples were diluted with ultrapure water, according to the expected salt concentration of the samples, to fit into the calibration range of chloride. Chloride determination was carried out using a 930 Compact IC Flex ion chromatograph (Metrohm AG, Switzerland) equipped with a conductivity detector and suppression system. A Metrosep A Supp 5-250/4.0 column was used with a flow rate of 0.7 ml min^{-1} and an injection volume of 20 µl. Gradient elution was performed using Na_2CO_3 (3.2 mM) and $NaHCO_3$ (1 mM). The final chloride concentration was calculated with external calibration using a chloride standard (>99%; Carl Roth GmbH, Germany).

Determination of ABA content in the leaves

Determination of ABA content was performed as previously described (Errard et al., 2015) with modifications. In brief, 10 mg of the dried plant material was extracted with 0.2 ml of methanol/water (60:40, v/v), and an internal standard [(+)-abscisic acid-d6, Toronto Research Chemicals, Canada] was added. First, the solution was sonicated on ice for 15 min and then centrifuged for 10 min (12,298×g, 4°C). Next, the supernatant was collected in a micro reaction vessel and the extraction steps were repeated twice. Then, the collected supernatant was filtered through a PTFE filter tube (0.2 µm, Thermo Fisher Scientific Inc., USA) and transferred to HPLC vials. Finally, the filtrate was diluted 1:2 with MS water (Supelco, VWR, Germany) + 0.1% acetic acid. The measurement was performed using an Agilent Technologies 1260 Infinity HPLC (Agilent Technologies Sales and Services GmbH & Co. KG, Germany) in combination with a Triple Quadrupole Q-Trap® 6500-MS/MS system (AB Sciex LLC, USA). Chromatographic separation was performed using a Zorbax Eclipse Plus C18 column (1.8 µm, 2.1 mm × 50 mm; Agilent Technologies, Germany), a column temperature of 30°C, a flow rate of 650 µl min^{-1}, and a mobile phase consisting of solvent A: MS water + 0.1% acetic acid and solvent B: acetonitrile + 0.1% ultrapure water. The injection volume was 10 µl. The initial gradient was 90% solvent A for 1 min, reduced to 15% solvent A for 4 min, and then reduced to 0% solvent A for 4 min. The mass spectrometer was operated in negative ionization mode and an electron spray ionization source was used. The MS parameters were set as follows: ion source temperature, 500°C; ion spray voltage, −4,500 V; curtain gas pressure, 50 psi; drying gas pressure, 50 psi; nebulizer gas pressure, 50 psi; auxiliary gas pressure, 65 psi; and multireaction monitoring (MRM) at a dwell time of 0.3781 s. Identification was based on the retention time and MRM transitions of the following: ABA 263 → 153 [quantifier; collision energy (CE), −15 V], 263 → 203 (qualifier; CE, −40 V), and 263 → 122 (qualifier; CE, −48 V) and ABA-d6 269 → 159 (quantifier; CE, −15 V), 269 → 209 (qualifier; CE, −40 V), and 269 → 128 (qualifier; CE, −48 V). The final ABA concentration was calculated from a calibration curve of the quantifier ratios between an ABA standard (≥98.5%, Sigma Aldrich) and the internal standard. Data analysis was performed with the Analyst 1.6.2 software (AB Sciex LLC, USA).

Identification and quantification of chlorophylls and carotenoids in the leaves

The extraction of pigments was performed according to Frede et al. (2019). In brief, 5 mg of plant material was dissolved in 0.5 ml of methanol/tetrahydrofuran (1:1, v/v) and incubated for 5 min in a shaker (1,400 rpm, 20°C) followed by centrifugation for 5 min (4,500×g, 20°C). The supernatant was collected in a vial, and the extraction was performed five times. The solution was evaporated to dryness under a nitrogen stream, dissolved in dichloromethane/isopropanol (1:5, v/v), sonicated (3 min, 20°C), filtered (PTFE filter tubes), and transferred to an HPLC vial. The analysis was performed using Agilent Technologies 6530 QToF-DAD-UHPLC-MS (Agilent Technologies Sales and Services GmbH & Co. KG, Germany) according to Frede et al. (2017). Identification was achieved using mass spectra and UV/VIS spectra (Figure S1), and quantification was achieved using external calibration with carotenoid standards (CaroteNature GmbH, Switzerland) of *all-trans*-isomers from β-carotene, lutein, and zeaxanthin and 9-*cis*-neoxanthin as well as chlorophyll standards (Sigma Aldrich Chemie GmbH, Germany) of chlorophyll *a* and *b* at a wavelength of 450 nm. Data analysis was performed using the TOF Quantitative Analysis (Quant-My-Way) 10.2 (MassHunter, USA).

Statistical analysis

Statistical differences between the light regime and salt treatments were tested using SigmaPlot (14.0) with a two-way ANOVA followed by a Bonferroni *post-hoc* test ($p \leq 0.05$) (*dF*, *F*, and *p*-values represented in Table S3). Data are presented as means ± SEM of two individual experiments per light regime. Twenty-four plants per light regime were used for the determination of fresh mass. For the analysis of the selected metabolites, eight replicates per light regime were used, pooled from three individual plants and two independent experiments.

Results

Characterization of light regimes

To evaluate the variation in both light regimes, LR1 and LR2, the light spectra, light intensity, and daily light hours were measured (Table 1; Figures S2, S3). The major differences

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between both light regimes were detected in the daily light hours and light intensity. To encounter both, the DLI was calculated (Table 1). Light regime 1 showed an average DLI of 3.18 ± 0.26 µmol m^{-2} day^{-1} that was only 18% of the DLI of light regime 2, which was on average 17.98 ± 0.24 µmol m^{-2} day^{-1}. This is due to 3 h less day light and a 290 µmol m^{-2} s^{-1} lower light intensity in light regime 1 (LR1, greenhouse) compared with light regime 2 (LR2, indoor farming). Light regime 1 showed, due to variations in natural light, variations in DLI (Figure S2).

Identifying differences in salt stress response between light regimes

Effect of salt treatment and light regime on fresh and dry mass

To assess whether fresh and dry mass is affected by the salt treatment and whether this response depends on the light regime, we measured the fresh mass of the plants during harvest and determined the dry mass of the leaves after lyophilization. The percent dry mass represents the proportion of dry mass in fresh mass and thus increases with decreasing water content. We found that fresh and dry mass was affected by both the light regime and salt treatment (Figure 1; Table S4). Considering the plant response to salt treatment, we found that *C. officinalis* showed a salt-induced decrease in fresh mass in both light regimes. Considering the plant response to the light regime, this decrease is in LR2 (indoor farming) beginning from 50 mM of salt and in LR1 (greenhouse) from 200 mM (Figure 1A). Accordingly, the highest percent dry mass was found at 600 mM of salt, which was due to the lowest water content in both light regimes (Figure 1D). Similarly, in *A. hortensis*, we observed a decrease in fresh mass, but only at salt treatments greater than 200 mM in both light regimes (Figure 1B). The percent dry mass was also the highest at 600 mM of salt and higher in LR2 (indoor farming) (Figure 1E). *Salicornia europaea* showed an increased fresh mass at 50 and 200 mM of salt in LR2 (indoor farming) and at 200 mM of salt in LR1 (greenhouse) (Figure 1C). The percent dry mass was significantly different only in LR2 (indoor farming) and was also the highest within the 600 mM salt treatment group (Figure 1F). In contrast to the other two halophyte species, the percent dry weight and thus the water content in *S. europaea* changed only by approximately 5%, whereas in *C. officinalis* and *A. hortensis*, these were changed by approximately 30%. We also observed that the plants had a 0.8-fold (*C. officinalis*) to 3.5-fold (*A. hortensis*) higher fresh mass in LR2 (indoor farming) than plants grown in LR1 (greenhouse). The plants showed an interaction between salt treatment and light regime, expressed in a higher fresh mass in LR2 (indoor farming)

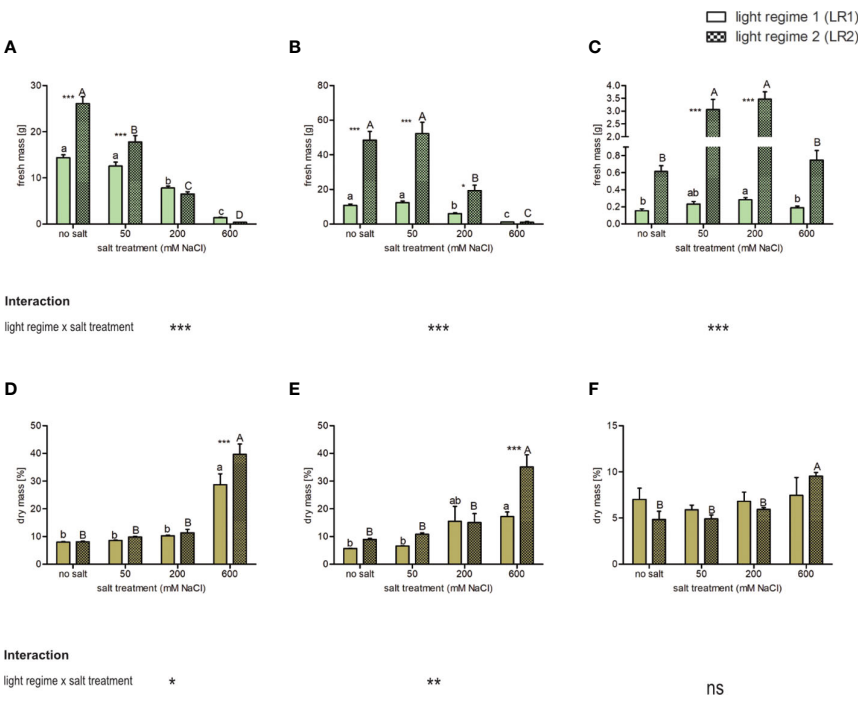

FIGURE 1
Influence of salt treatment and light regime on the fresh mass of plants and percent dry mass (as a percentage of fresh mass) of the leaves of 6- to 9-week-old plants. Fresh mass **(A–C)** and dry mass **(D–F)** of **(A, D)** *Cochlearia officinalis*, **(B, E)** *Atriplex hortensis*, and **(C, F)** *Salicornia europaea*. Fresh mass: the bar represents means ± SEM, n = 24; dry mass: the bar represents means ± SEM of n = 8 pools of three individual plants each from two independent experiments. Small letters indicate significant differences between salt treatments in light regime 1 (LR1, greenhouse) in alphabetical order from highest to lowest; capital letters indicate significant differences between salt treatments in light regime 2 (LR2, indoor farming) in alphabetical order from highest to lowest; asterisks indicate significant differences between LR1 and LR2 in between one salt treatment; interaction shows significantly different interactions between salt treatments and light regimes tested by two-way ANOVA, followed by *post-hoc* Bonferroni test ($p \leq 0.05$) (*≤ 0.05, **≤ 0.01, ***≤ 0.001); ns, not significant.

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in their salt tolerance range, and there were no differences in the salt stress range.

In summary, we observed in the salt tolerance range a significant difference in yield between the two light regimes, but in the salt stress range, the light regime had no effect on yield (except for *A. hortensis* at 200 mM, specific salt tolerance/stress ranges are defined in Section 4.1). *Vice versa*, there was a difference in percent dry mass at the highest salt level (600 mM) between both light regimes and compared with lower salt treatments.

Differences in chloride accumulation in the leaves

To evaluate whether the light regime influences chloride accumulation in the leaves, the chloride concentrations were determined *via* ion chromatography. Since salt affects water uptake and thus water content, chloride content is shown on a dry and fresh mass basis (Figure 2). In all three plant species, chloride concentration was slightly influenced by the light regime and highly influenced by the salt treatment. On a dry mass basis, *C. officinalis* and *S. europaea* showed the lowest and the highest chloride concentrations, respectively, in all salt treatments and in both light regimes. We observed for all plant species a positive correlation between chloride concentration and salt treatment. Comparing the two light regimes, *C. officinalis* showed slightly higher chloride accumulation at 50 and 200 mM of salt and significantly higher chloride accumulation at 600 mM of salt in LR2 (indoor farming). This finding is consistent with the necrotic phenotype of the plants observed at 600 mM of salt in LR2 (indoor farming) (Figure 2A and Figure S5). In contrast, *A. hortensis* showed significantly higher chloride concentration at 50 mM of salt and slightly higher chloride concentration at 200 and 600 mM of salt in LR2 (indoor farming) (Figure 2B). For *A. hortensis*, we observed salt deposition on the leaf and stem surfaces (Figure S6). Interestingly, *S. europaea* showed the same response under both light regimes (Figure 2C). Chloride content on a fresh mass basis showed the same pattern with respect to salt treatment, but with less significant changes (Figures 2D–F). For example, in *A. hortensis*, no significant differences were observed with respect to the light regime, but the contents tended to be higher in LR2 (indoor farming). When comparing the plant species at 600 mM, the chloride content decreased from *A. hortensis* through *C. officinalis* to *S. europaea*. *Salicornia europaea* showed a four to eight times lower increase at 200 and 600 mM of salt compared with no salt and to the other halophytic plant species.

Taken together, these findings indicate that chloride accumulation was less influenced by the light regime than by salt treatment. However, we observed an interaction between the light regime and salt for all treatments for both *C. officinalis* and *A. hortensis*.

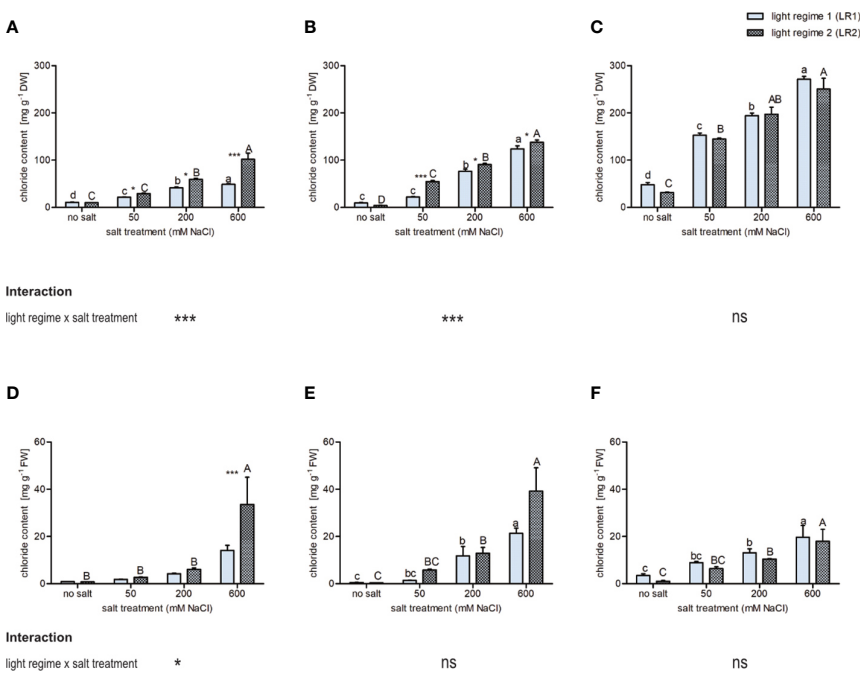

FIGURE 2
Influence of salt treatment and light regime on chloride concentration on **(A–C)** dry mass basis and **(D–F)** fresh mass basis in the leaves of 6- to 9-week-old plants. **(A, D)** *Cochlearia officinalis*; **(B, E)** *Atriplex hortensis*; **(C, F)** *Salicornia europaea*. Bar represents means ± SEM of $n = 8$ pools of three individual plants each from two independent experiments. Small letters indicate significant differences between salt treatments in light regime 1 (LR1, greenhouse) in alphabetical order from highest to lowest; capital letters indicate significant differences between salt treatments in light regime 2 (LR2, indoor farming) in alphabetical order from highest to lowest; asterisks indicate significant differences between LR1 and LR2 in between one salt treatment; interaction shows significantly different interactions between salt treatments and light regimes tested by two-way ANOVA, followed by *post-hoc* Bonferroni test ($p \leq 0.05$) (* ≤ 0.05, *** ≤ 0.001); ns, not significant.

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Response to the light regime and salt treatment on ABA content

As an indicator of salt stress in plants, ABA content in the leaves was measured using HPLC-MS/MS. Considering ABA content, we observed a different response to salt treatment as well as light regimes between obligate and facultative halophytes (Figure 3). The facultative halophytes *C. officinalis* and *A. hortensis* showed a positive correlation between ABA content and salinity but responded differently to salt treatment in LR1 (greenhouse) than in LR2 (indoor farming). *Cochlearia officinalis* showed the highest ABA content at 50 mM of salt in LR2 (indoor farming) and at 600 mM of salt in LR1 (greenhouse) (Figure 3A). *Atriplex hortensis* showed no significant differences in ABA content in LR2 (indoor farming) but showed increased ABA content at 200 and 600 mM of salt compared with no salt in LR1 (greenhouse) (Figure 3B). The obligate halophyte *S. europaea* showed decreased ABA content in the salt treatments compared with the no-salt treatment but showed no changes in ABA content related to light regimes (Figure 3C). On fresh mass, we observed the same trend, but with no significant changes in LR2 (indoor farming) for all plant species (Figures 3D–F). However, *C. officinalis* was found to have significantly higher ABA content at 600 mM of salt compared with the other treatments in LR1 (greenhouse) as well as *A. hortensis* at 200 and 600 mM of salt at LR1 (greenhouse) compared with the 50 mM and no salt.

The facultative halophytes (*C. officinalis* and *A. hortensis*) showed an interaction of salt and light regime (Figure 3) and a different response to salt treatment in the light regimes, while the obligate halophyte (*S. europaea*) showed no interaction and no difference in response to the light regimes.

Influence of light regime and salt treatment on photosynthetic pigment content

To estimate the effect of the light regime on salt treatment on photosynthesis, we analyzed the pigment (carotenoids and chlorophylls) content by UHPLC-DAD-QToF-MS. Chlorophyll *a* and *b*, as well as *all-trans*-isomers of lutein, β-carotene, zeaxanthin, and violaxanthin and the 9-*cis*-isomer of neoxanthin, were detected in all plant species (Figure S1). Both carotenoids and chlorophylls were affected by salt treatment and light regime, and an interaction between these two factors was observed, with different responses for facultative halophytes (*C. officinalis* and *A. hortensis*) and the obligate halophyte (*S. europaea*). Due to the high impact on water content, the metabolites

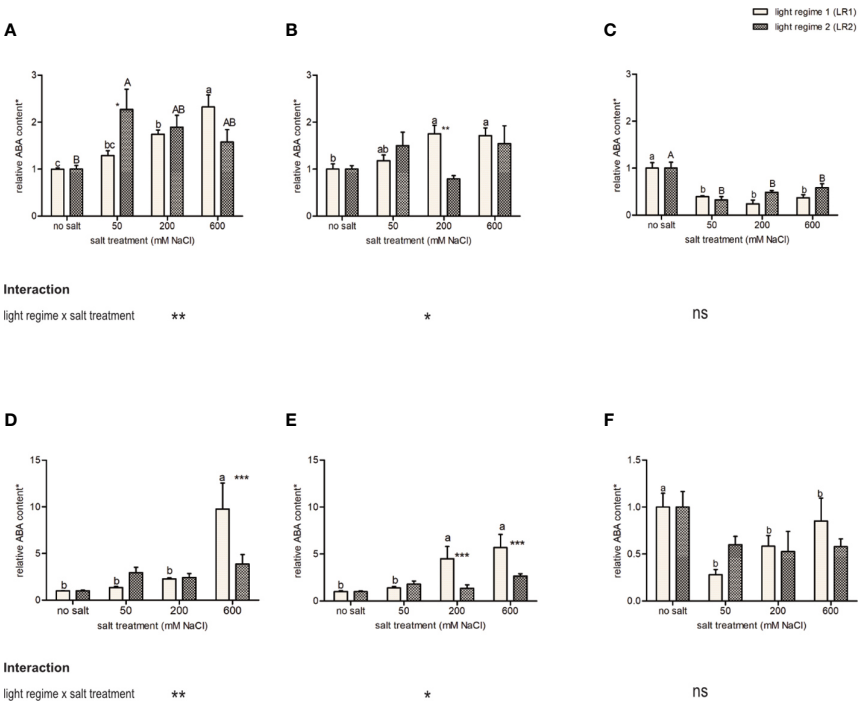

FIGURE 3
Influence of salt treatment and light regime on abscisic acid (ABA) content on **(A–C)** dry mass basis and **(D–F)** fresh mass basis in the leaves of 6- to 9-week-old plants. **(A, D)** *Cochlearia officinalis*; **(B, E)** *Atriplex hortensis*; **(C, F)** *Salicornia europaea*. *Relative data of the respective control of light regime; bar represents means ± SEM of $n = 8$ pools of three individual plants each from two independent experiments. Small letters indicate significant differences between salt treatments in light regime 1 (LR1, greenhouse) in alphabetical order from highest to lowest; capital letters indicate significant differences between salt treatments in light regime 2 (LR2, indoor farming) in alphabetical order from highest to lowest; asterisks indicate significant differences between LR1 and LR2 in between one salt treatment; interaction shows significantly different interactions between salt treatments and light regimes tested by two-way ANOVA, followed by *post-hoc* Bonferroni test ($p \leq 0.05$) (* ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001); ns, not significant.

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are presented on a dry mass basis (Tables 2, 3), while the results based on a fresh mass basis can be found in Tables S5, S6.

Chlorophyll content in the leaves

Halophytes responded differently to salt treatment in the two light regimes, and both regimes affected chlorophyll content (Table 2, dry mass basis; Table S5, fresh mass basis).

Considering the plant response to the light regime, *C. officinalis* exhibited a higher content of chlorophyll *a* and *b* at all salt levels in LR1 (greenhouse) compared with LR2 (indoor farming), which was at no salt 0.2-fold higher and at 600 mM of salt 3.1-fold higher. Considering the plant response to salt treatment, we found that the content of chlorophyll *a* and *b* decreased in both light regimes. This decrease occurred in LR2 (indoor farming) from 200 mM of salt but was only observed in LR1 (greenhouse) at higher salinity (600 mM). However, due to the changes in water content, the results based on the fresh mass basis are different. For instance, the highest content of chlorophylls was found at 600 mM of salt in LR1 (greenhouse) and corresponds to the darker green color of the leaves (Figure S8).

Considering the plant response to the light regime, *A. hortensis* had a 0.2-fold increased content of chlorophyll *a* at no salt in LR2 (indoor farming), and in LR1 (greenhouse), there was an increased content of both chlorophylls at 200 and 600 mM of salt. Considering the plant response to salt treatment, we determined

TABLE 2 Content of chlorophylls on a dry mass basis in the leaves of 6- to 9-week-old plants (means ± SEM).

		Salt treatment (mM NaCl)	Chlorophyll *a* (µg mg^{-1} DM)					Chlorophyll *b* (µg mg^{-1} DM)				
Cochlearia officinalis	Light regime 1	No salt	8.59	±	0.50	a	**	2.81	±	0.15	a	***
		50	8.90	±	0.13	a	***	2.94	±	0.07	a	***
		200	8.25	±	0.18	a	***	2.77	±	0.05	a	***
		600	6.05	±	0.22	b	***	2.17	±	0.09	b	***
	Light regime 2	No salt	7.26	±	0.20	A		2.32	±	0.07	A	
		50	6.65	±	0.10	A		2.13	±	0.05	A	
		200	4.72	±	0.07	B		1.56	±	0.04	B	
		600	1.47	±	0.22	C		0.54	±	0.08	C	
Interaction: light regime × salt treatment							***					***
Atriplex hortensis	Light regime 1	No salt	3.15	±	0.11	a		0.72	±	0.01	a	
		50	3.31	±	0.07	a		0.69	±	0.02	a	
		200	3.14	±	0.06	a	***	0.67	±	0.01	a	***
		600	1.74	±	0.10	b	***	0.41	±	0.04	b	***
	Light regime 2	No salt	3.82	±	0.12	A	***	0.75	±	0.03	A	
		50	3.34	±	0.05	B		0.62	±	0.02	B	
		200	2.50	±	0.15	C		0.49	±	0.03	C	
		600	0.49	±	0.10	D		0.10	±	0.02	D	
Interaction: light regime × salt treatment							***					***
Salicornia europaea	Light regime 1	No salt	0.54	±	0.24	b		0.51	±	0.15	ns	
		50	0.78	±	0.27	ab		0.50	±	0.06	ns	
		200	1.49	±	0.17	a		0.57	±	0.05	ns	
		600	0.91	±	0.07	ab		0.37	±	0.04	ns	
	Light regime 2	No salt	0.62	±	0.20	C		0.44	±	0.07	C	
		50	3.21	±	0.20	A	***	1.01	±	0.04	A	***
		200	2.67	±	0.11	A	***	0.79	±	0.04	B	
		600	1.74	±	0.07	B	**	0.55	±	0.02	C	
Interaction: light regime × salt treatment							***					**

Small letters indicate significant differences between salt treatments in light regime 1 (LR1, greenhouse) in alphabetical order from highest to lowest; capital letters indicate significant differences between salt treatments in light regime 2 (LR2, indoor farming) in alphabetical order from highest to lowest; asterisks indicate significant differences between LR1 and LR2 in between one salt treatment; interaction shows significantly different interactions between salt treatments and light regimes tested by two-way ANOVA, followed by post-hoc Bonferroni test ($p \leq 0.05$) (** ≤ 0.01, *** ≤ 0.001); n = 8 pools of three individual plants each from two independent experiments.
ns, not significant.

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TABLE 3 Content of carotenoids on a dry mass basis in the leaves of 6- to 9-week-old plants (means ± SEM).

		Salt treatment (mM NaCl)	Lutein (ng mg^{-1} DM)					β-Carotene (ng mg^{-1} DM)					Zeaxanthin (ng mg^{-1} DM)					*all-trans*-Violaxanthin (ng mg^{-1} DM)					*9Z*-Neoxanthin (ng mg^{-1} DM)				
Cochlearia officinalis	Light regime 1	No salt	1,019.02	±	47.83	a	***	300.39	±	19.36	b		14.39	±	1.02	ns		204.73	±	40.98	a		245.43	±	16.39	ab	**
		50	1,080.23	±	29.39	a	***	396.59	±	6.95	a		19.39	±	2.54	ns		181.14	±	33.45	ab		268.29	±	7.79	a	***
		200	1,004.56	±	38.25	a	***	428.78	±	8.66	a	***	29.55	±	6.34	ns		156.77	±	19.06	ab		225.08	±	9.76	b	***
		600	534.79	±	30.43	b	***	277.09	±	28.21	b	***	29.93	±	3.35	ns		92.81	±	23.10	b		147.74	±	17.67	c	***
	Light regime 2	No salt	821.81	±	17.80	A		360.76	±	10.52	A	*	10.42	±	0.90	b		218.13	±	30.66	A		201.66	±	4.68	A	
		50	759.90	±	10.42	A		397.04	±	6.43	A		17.15	±	1.02	ab		254.93	±	10.16	A	*	178.75	±	4.53	A	
		200	542.08	±	10.94	B		291.83	±	3.73	B		32.31	±	2.33	a		118.21	±	4.02	B		105.69	±	4.44	B	
		600	197.46	±	31.20	C		81.68	±	14.39	C		19.25	±	3.35	a		27.51	±	4.88	C		34.80	±	7.26	C	
Interaction: light regime × salt treatment							***					***					ns					***					**
Atriplex hortensis	Light regime 1	No salt	291.13	±	3.90	a		155.39	±	4.33	b		6.57	±	0.82	b		123.76	±	16.61	ns		72.80	±	0.98	a	*
		50	304.76	±	6.71	a	*	186.99	±	4.64	ab		12.96	±	3.57	b		121.23	±	5.63	ns		72.52	±	1.75	a	***
		200	286.67	±	6.46	a	***	218.25	±	2.98	a		30.26	±	6.05	a	**	103.88	±	7.97	ns		71.07	±	0.65	a	***
		600	116.97	±	12.89	b	***	115.41	±	15.48	c	***	16.26	±	0.67	b	*	92.92	±	12.97	ns	**	38.73	±	4.10	b	***
	Light regime 2	No salt	326.36	±	12.42	A	*	262.65	±	10.22	A	***	11.15	±	1.16	ns		134.15	±	45.22	A		55.61	±	7.15	A	
		50	272.81	±	3.36	B		250.73	±	3.69	A	***	8.90	±	2.35	ns		100.86	±	24.99	AB		36.45	±	9.14	B	
		200	211.79	±	10.37	C		194.80	±	10.23	B		26.09	±	8.03	ns		58.80	±	5.96	B		35.88	±	6.86	B	
		600	33.71	±	6.39	D		32.99	±	6.50	C		4.96	±	0.67	ns		14.15	±	0.89	C		11.72	±	1.14	C	
Interaction: light regime × salt treatment							***					***					**					*					ns
Salicornia europaea	Light regime 1	No salt	48.68	±	17.69	b		17.95	±	2.26	b		25.54	±	1.68	a	***	21.12	±	4.71	ns		22.81	±	1.78	b	
		50	121.78	±	17.81	ab		32.24	±	9.45	b		6.38	±	1.61	b		21.31	±	3.29	ns		23.81	±	1.81	b	
		200	217.64	±	17.73	a		72.81	±	4.86	a		8.48	±	1.96	b		17.07	±	1.22	ns		46.99	±	3.61	a	
		600	139.13	±	14.68	b		28.29	±	6.58	b		6.19	±	0.60	b		15.95	±	1.02	ns		34.05	±	2.39	b	
	Light regime 2	No salt	55.70	±	11.10	C		8.26	±	1.14	C		0.96	±	0.18	ns		17.88	±	1.13	B		19.39	±	2.40	D	
		50	364.75	±	18.03	A	***	159.50	±	13.59	A	***	5.97	±	0.36	ns		40.86	±	9.52	A	**	77.97	±	4.57	A	***
		200	337.14	±	14.50	A	***	144.03	±	5.53	A	***	6.26	±	0.59	ns		41.75	±	3.98	A	***	65.67	±	2.69	B	***

(Continued)

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TABLE 3 Continued

	Salt treatment (mM NaCl)	Lutein (ng mg^{-1} DM)				β-Carotene (ng mg^{-1} DM)				Zeaxanthin (ng mg^{-1} DM)				*all-trans*-Violaxanthin (ng mg^{-1} DM)				9Z-Neoxanthin (ng mg^{-1} DM)			
			±				±				±				±				±		
	600	207.47		8.07	B	*	82.39		3.43	B	***	4.84		0.60	ns		38.76		3.60	A	**	38.85		3.91	C	
Interaction: light regime × salt treatment						***					***					***					*					***

Small letters indicate significant differences between salt treatments in light regime 1 (LR1, greenhouse) in alphabetical order from highest to lowest; capital letters indicate significant differences between salt treatments in light regime 2 (LR2, indoor farming) in alphabetical order from highest to lowest; asterisks indicate significant differences between LR1 and LR2 in between one salt treatment; interaction shows significantly different interactions between salt treatments and light regimes tested by two-way ANOVA, followed by post-hoc Bonferroni test ($p \leq 0.05$) (* ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001); n = 8 pools of three individual plants each from two independent experiments.
ns, not significant.

that the response was similar to *C. officinalis* in LR1 (greenhouse), expressed in a decreased content at 600 mM of salt in both chlorophylls. In LR2 (indoor farming), however, the decrease of both chlorophylls was already observed at 50 mM of salt. The impact was more pronounced on a fresh mass basis. The treatment with 600 mM of salt resulted in a reduction, independent of the light regime. The effect was even more evident under LR1 (greenhouse) and significantly induced chlorophyll reduction starting from 50 mM of salt.

Considering the plant response to the light regime, *S. europaea* in LR2 (indoor farming) showed a drastically higher chlorophyll *a* content in the salt treatments than without salt. Considering the plant response to salt treatment, we found that the lowest content of both chlorophylls could be measured at no salt and then a steep increase at 50 mM, in both light regimes, but differed in the intensity of the increase. Chlorophyll *a* showed at 50 mM of salt in LR2 (indoor farming) a 10-times higher increase than in LR1 (greenhouse). Although these differences in content between LR1 (greenhouse) and LR2 (indoor farming) decreased with increasing salinity, at 600 mM of salt, the difference in contents had decreased by half. At 50, 200, and 600 mM of salt, we also observed a higher chlorophyll *a* content in LR2 (indoor farming) on a fresh mass basis.

For all halophyte species and both chlorophylls, we observed a significant interaction between the light regime and salt treatment on a dry mass basis. On a fresh mass basis, this interaction was observed for *A. hortensis* and *C. officinalis*, but not for *S. europaea*.

Content of individual carotenoids in the leaves

The individual carotenoids showed differences in their content related to the response to salt treatment and light regime and related to the plant species (Table 3, dry mass basis; Table S6, fresh mass basis).

Lutein displayed a similar response as chlorophylls to salt treatment and light regime for all plant species. Only for *A. hortensis*, we observed, in addition to the higher content at 200 and 600 mM of salt in LR1 (greenhouse) compared with LR2 (indoor farming), also at 50 mM of salt a higher content in LR1 (greenhouse). Likewise, changes on a fresh mass basis were observed, and for all halophyte species, the highest levels were found for 200 or 600 mM of salt, except for *C. officinalis*, where no significant changes were found under LR2 (indoor farming).

β-Carotene showed the same pattern in both *C. officinalis* and *A. hortensis* but with a different intensity. Considering the plant response to the light regime, we found that at no salt both halophytes showed higher content in LR2 (indoor farming), and the content was 0.7-fold higher in *A. hortensis* and 0.2-fold higher in *C. officinalis*. Considering the plant response to salt treatment, we found that both plant species showed in LR1 (greenhouse) an increasing content from no salt to 200 mM of salt and then a decrease again at 600 mM to the no-salt treatment, whereas, in LR2 (indoor farming), the content was the highest in the no salt and 50 mM and then decreased. Considering the plant response to the light regime, *S. europaea* exhibited a higher content of β-carotene at 50, 200, and 600 mM of salt in LR2 (indoor farming) than in LR1 (greenhouse). Considering the plant response to salt treatment, we

found that *S. europaea* showed in LR1 (greenhouse) an increase at 200 mM and in LR2 (indoor farming) a steep increase from no salt to 50 mM and then a decrease at 600 mM again, although at 600 mM, the content was still higher compared with the no salt. Based on fresh mass, the highest β-carotene content under LR1 (greenhouse) was found for *C. officinalis* at 600 mM and for *A. hortensis* at 200 mM of salt, whereas no significant changes were detected for S. *europaea*. In LR2 (indoor farming), 50 mM of salt induced the highest accumulation rate in *C. officinalis*, 50 and 200 mM in *A. hortensis*, and 50 to 600 mM in *S. europaea.* These changes are also reflected in the significant interactions of light regime and salt observed for β-carotene and for all the halophytes (Table S6).

For zeaxanthin, we observed a very indifferent pattern. Considering the plant response to salt treatment, we found that *C. officinalis* only in LR2 (indoor farming) expressed significantly increased content at 200 and 600 mM of salt. Furthermore, *A. hortensis* showed only in LR1 (greenhouse) an increased content at 200 mM of salt. Considering the plant response to the light regime, *A. hortensis* displayed a higher content in LR1 (greenhouse) at 200 and 600 mM of salt. Considering the plant response to the light regime, *S. europaea* exhibited a higher content at no salt in LR1 (greenhouse), and considering the plant response to salt treatment, it showed a decreased content at 50, 200, and 600 mM of salt in LR1 (greenhouse). On a fresh mass basis, the only difference was an increased content at 600 mM of salt in LR1 (greenhouse) for *C. officinalis.*

For both *C. officinalis* and *A. hortensis*, violaxanthin showed a decreasing trend with increasing salinity in both light regimes. Considering the plant response to the light regime, *A. hortensis* showed only at 600 mM a higher content in LR1 (greenhouse) and *C. officinalis* at 50 mM in LR2 (indoor farming). Furthermore, *S. europaea* exhibited an increased content in LR2 (indoor farming) within salinity levels. Considering the plant response to salt treatment, we observed only in LR2 (indoor farming) a significant response, expressed with an increased content from 50 to 600 mM of salt. On a fresh mass basis, *A. hortensis* showed no significant differences, while *C. officinalis* showed a contrasting pattern in LR1 (greenhouse) and the same pattern in LR2 (indoor farming). Also, comparing the light regimes, we observed a higher content at no salt in LR2 (indoor farming) and at 600 mM in LR1 (greenhouse). For *S. europaea*, we observed an increased content at 200 mM in LR2 (indoor farming) compared with all salt treatments.

For both facultative halophytes (*C. officinalis* and *A. hortensis*), neoxanthin presented a strong response to salt treatment and light regime. Considering the plant response to the light regime, the content for both plants was higher at all salt levels in LR1 (greenhouse). Considering the plant response to salt treatment, within increasing salinity, the content decreased, whereby the decrease in LR2 (indoor farming) was much steeper. For the obligate halophyte *S. europaea*, considering its response to the light regime, we observed a higher content in LR2 (indoor farming) at 50 and 200 mM of salt. Based on fresh mass, *A. hortensis* again showed no significant differences, *S. europaea* the same pattern, and *C. officinalis* in LR1 (greenhouse) the highest content at 600 mM, and in LR2 (indoor farming), there were no significant differences.

Taken together, we observed a similar pattern for both facultative halophytes, *C. officinalis* and *A. hortensis*, which was different from *S. europaea*. Also, lutein and neoxanthin showed the same response to salt treatment and light regime, which differed from the response in β-carotene, whereas zeaxanthin and violaxanthin showed the most indifferent pattern. Both lutein and β-carotene showed an interaction between the light regime and salt treatment for all plant species, zeaxanthin only for *A. hortensis* and *S. europaea*, and neoxanthin only for *C. officinalis* and *S. europaea* on a dry mass basis. In contrast, on a fresh mass basis, no interaction was observed for lutein for *A. hortensis* and *S. europaea*.

Impact on the overall metabolite composition

To gain insight into the influence of light regime and salt treatment on the dynamic metabolic variation, a PCA analysis was performed (Figure 4). The greatest influence was due to the difference in salt treatment. For all three halophytes (*C. officinalis*, *A. hortensis*, and *S. europaea*), distinct clusters were found for the treatments with and without salt based on PC1 and PC2 (Figures 4A–C). With respect to the light regime, the effects on the metabolite profiles were less pronounced. However, interactions on individual metabolite levels have been demonstrated, e.g., for carotenoids and chlorophylls (Tables 2, 3).

Discussion

Here, we demonstrated that the interaction of light regime and salt treatment modulates the content of photosynthetic pigments and influences the salt tolerance of halophytes.

Influence of light regime on the response to salt treatment

In the evaluation of salt-tolerant crops, the yield loss in response to the salt concentrations in soil or water is a key aspect to be considered. Considering the salt treatments, a reduction in fresh mass was found at 50/200 mM for *C. officinalis*, at 200 mM for *A. hortensis*, and at 600 mM and no salt for *S. europaea*. Evaluating the effect of the light regime in the non-salt-stressed conditions (*A. hortensis* and *C. officinalis* no salt and for *S. europaea* 50 and 200 mM of salt), the fresh mass is higher in indoor farming (LR2). This suggests that the DLI in the greenhouse (LR1) was too low for optimal growth. However, relative fresh mass still differs in the light regimes at salt treatments 50 and 200 mM for *S. europaea* and *C. officinalis* (Figure S7). Additionally, we observed differences in the influence of salt treatment on the water content, between both the facultative halophytes (*A. hortensis* and *C. officinalis*) and the obligate halophyte (*S. europaea*). Water content was less affected by salt treatment for the obligate halophyte. Succulent halophytes (salt-accumulating), like *Suaeda maritima*, show a different osmotic adjustment and, thus, a different water content under salinity

10.3389/fpls.2023.1105162

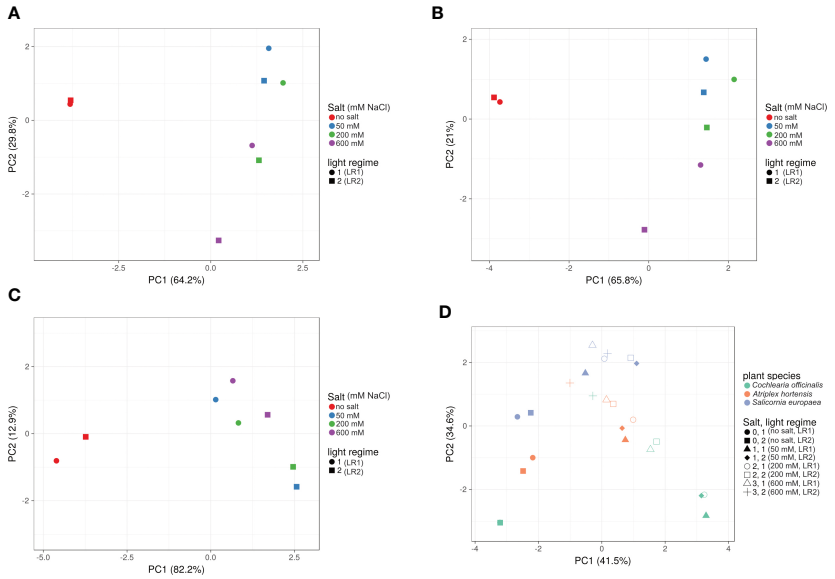

FIGURE 4
Effect of salt treatment and light regime on metabolite profile. PCA plots of **(A)** *Cochlearia officinalis*, **(B)** *Atriplex hortensis*, **(C)** *Salicornia europaea*, and **(D)** all three plant species. PCA plots were generated with ClustVis; *n* = 8 pools of three individual plants each from two independent experiments; unit variance was applied to rows; SVD with imputations is used to calculate principal components; factor loadings are provided in *Appendix* **(B)** Light regime 1 (LR1, greenhouse); light regime 2 (LR2, indoor farming).

(Flowers and Colmer, 2008). To evaluate the influence of light regime in relation to salt treatment, further insights were obtained by studying changes in the ABA and chloride contents. ABA serves as an indicator of salt stress response in halophytes and glycophytes and mediates the stomatal movement of guard cells (Zhang et al., 2006; Karimi et al., 2021). According to previous research, we observed a correlation between increased salt stress (increased ABA content) and yield loss (Breckle, 2002; Metselaar, 2013). However, the response of ABA differs between halophytes and glycophytes, at least with respect to the salt level. A study conducted by Karimi et al. (2021) showed only a short-term response of ABA in *Thellungiella salsuginea* (a halophyte) at 200 mM, while *Arabidopsis thaliana* (a glycophyte) showed a long-term response. Ben Hassine et al. (2009), on the other hand, showed an increased ABA content in the seedlings of the halophyte *Atriplex halimus* in a short- and long-term response to 160 mM of NaCl treatment. This suggests that ABA regulation not only differs among glycophytes and halophytes but also among halophyte species. Aside from sodium content, chloride content also changes with salinity treatment, and accumulation varies between halophyte species. For instance, the ratio of sodium and potassium cations to chloride anions varies among halophyte species, which could influence the external chloride uptake (Flowers and Colmer, 2008) and should be considered in future studies.

In this study, *C. officinalis* showed an increased ABA content correlating with increased chloride and reduced growth already from 50/200 mM. This is in accordance with a lower salinity tolerance (de Vos and Broekman, 2013). In contrast, de Vos and Broekman (2013) observed a higher percent dry weight at 200 mM and a leaf succulence, whereas, in our study, it was only significantly increased at 600 mM. The lowest chloride accumulation in the leaves of *C. officinalis* compared with the two other plant species (*A. hortensis* and *S. europaea*) would support the salt exclusion mechanism if the salt is translocated into the xylem and root (Chen et al., 2018). *Atriplex hortensis* showed an increased ABA content (only in LR1), paired with a reduction in fresh mass and chloride accumulation at 200 mM of salt. This is in accordance with the literature, where a salt tolerance of up to 250 mM of salt was shown for another variety of *A. hortensis* (red orache) (Wilson et al., 2000). Furthermore, we observed excreted salt crystals on the leaf surface, which is typical for halophytes with salt bladders (Schirmer and Breckle, 1982). Therefore, when considering chloride content in *A. hortensis*, it is important to consider the salt deposition on the leaf surface. One possibility is to wash off the salt from the leaves before measurement, but this may not reveal the transport of salt into the leaves, making it more difficult to compare salt tolerance mechanisms. Nevertheless, it would be interesting to distinguish between the salt excreted and the salt accumulated from and in the leaf. Since *S. europaea* is an obligate halophyte, it showed an increase in ABA and a decrease in fresh mass not only at 600 mM but also at no salt, unlike the other plant species, suggesting that this salt concentration and very low salt lead to stress. This can be explained by the fact that in obligate halophytes, salt uptake is essential for maintaining turgor and for optimal growth and is also reflected in the water content, which changes only slightly with salt treatment (Glenn and O'Leary, 1984). Furthermore, when considering the differences between chloride content in shoots based on dry and fresh mass, it can be clearly observed that *S. europaea* has a lower chloride/fresh mass ratio at higher salt treatments compared with the other plant species. This is

due to lower salt-induced water loss, indicating better osmotic regulation in *S. europaea* at higher salinity levels.

Whereas the influence of the light regime on fresh mass was clearly visible, the effect on metabolite profiles was less pronounced. However, the influence of the light regime on metabolite profiles also differed within plant species (Figure 4). Again, different patterns were observed with respect to salinity treatment, suggesting a different adaptation to salinity and a different influence of the interaction between salinity treatment and light regime in different halophyte species. This interaction is particularly interesting for photosynthetic pigments.

The interaction of light regime and salt treatment in influencing photosynthetic pigments

Carotenoids and chlorophylls have multiple functions in plants; for example, carotenoids are accessory pigments, and also they have essential photoprotective properties, while chlorophylls are the main pigments of photosystems. Carotenoid and chlorophyll biosynthesis and metabolism are affected by light, e.g., light quality or light intensity, as well as salinity (Pizarro and Stange, 2009; Tanaka and Tanaka, 2011; Soltabayeva et al., 2021). All the pigments studied are part of the photochemical apparatus but have different functions according to which they can be divided into two groups. First, chlorophyll *a* and *b*, lutein, and neoxanthin, in a simplified way, function as absorbers and converters for the incoming light energy (Choudhury and Behera, 2001). Second, violaxanthin, β-carotene, and zeaxanthin, on the other hand, function as dissipators of excessive light energy (Choudhury and Behera, 2001). This should be taken into account as we observed a different pattern in pigment accumulation between the two light regimes in the salt stress and salt tolerance range.

Salt stress leads to limited activity in several parts of the photosynthetic apparatus (e.g., RuBisCO activity, NADPH oxidase activity) and, thus, increased formation of reactive oxygen species (ROS) (Hasanuzzaman et al., 2020). In high light stress, the high photon flux density leads to excessive light energy that exceeds the capacity of the photosynthetic apparatus, resulting in the formation of ROS, which can cause subcellular damage and photooxidation of pigments (Gilmore, 2001). A study by Simkin et al. (2003) showed that in pepper (*Capsicum annuum* L. cv. Yolo Wonder), the photooxidation of carotenoids already occurs at the transition of light intensity from 150 to 280 μmol m^{-2} s^{-1}. Since we observed a combined effect of light regime and salt treatment and found a strong difference in DLI between the two light regimes, this difference must be taken into account. Higher light energy combined with the salt stress-induced limited activity of the photosynthetic apparatus results in overexcitation of the photosynthetic apparatus and increased ROS formation that exceeds antioxidant capacity (Carillo, 2018). Accordingly, we observed a salt stress-induced reduction of all pigments in indoor farming (LR2) with a higher DLI. In contrast, within salt tolerance ranges, we observed an accumulation of the carotenoids violaxanthin, β-carotene, and zeaxanthin, which act as dissipators of excess light energy and thus scavenge ROS, and a decrease in lutein and neoxanthin and chlorophylls, which act as absorbers and converters of incoming light energy and thus maintain photosynthetic activity (Choudhury and Behera, 2001). These effects are in accordance with the changes in our study in indoor farming (LR2) and resulted in higher levels of violaxanthin, β-carotene, and zeaxanthin during salt stress at lower DLI in greenhouse (LR1) and suggest that DLI affects the carotenoid profiles as a function of salt concentration with respect to their different functions in the photosynthetic process.

Interestingly, the response of the obligate halophyte (*S. europaea*) in salt stress (no salt) is different from the response of the facultative halophytes (*C. officinalis* and *A. hortensis*). For *S. europaea*, we observed particularly low contents of pigments in both light regimes at no salt. An explanation could be a different adaptation of the photosynthetic apparatus to salt. It is suggested that halophytes have the ability to regulate steady chloride concentrations by a different ion (Na^+, Cl^-, and K^+) transport compared with glycophytes (Bose et al., 2017). Since salt is essential for maintaining intracellular pH, altered pH in the thylakoid interior could affect the function of crucial enzymes for photosynthesis, e.g., RuBisCO or NADPH oxidase. This could influence photosynthetic activity, e.g., photooxidation of pigments and biosynthesis of carotenoids and chlorophylls (Glenn and O'Leary, 1984).

Taken together, we observed an interaction of light regime and salt treatment in influencing the performance of the three halophyte species. Therefore, when optimizing the light conditions in indoor farming, the plant species, salt tolerance, and salinity of the cultivation medium must be taken into account. In indoor farming, lighting conditions are not only important for the plants but also for evaluating the profitability and sustainability of a production system. Hence, light efficiency use (LUE) is a factor, considering the consumed electricity of the system, which helps to compare indoor farming and greenhouse cultivation. A study by Jin et al. (2022) pointed out that the average LUE in vertical farming is higher than in greenhouse cultivation. Considering the influence of salt stress and light on yield, assuming LUE is higher under lower DLI (greenhouse) than under higher DLI (indoor farming), therefore, lower light intensity in saline indoor farming could decrease light energy while maintaining yield and, thus, optimize LUE. Nevertheless, the DLI in the greenhouse (LR1) was also too low. Therefore, lower DLI with moderate salinity could lead to optimized resource use and even improved nutritional quality by increasing the amount of PSMs. The implementation of UV-B LEDs or colored LEDs could further enhance the PSM content (Wiesner-Reinhold et al., 2021; Frede and Baldermann, 2022). Further research could aim to study the influence of DLI and salinity on other nutritive compounds, such as polyphenols and vitamins.

Study limitations and perspective

The major limitation of this study is that the effects are assumed to be due to the daily light integral and not due to light quality. An altered light quality, in this case mainly light spectra, has also an influence on the plant metabolism and pigment content (Alrifai

et al., 2019; Frede et al., 2019). For example, different photoreceptors can be activated through changes in the light spectra (Kami et al., 2010). However, our study design aimed to investigate the differences between greenhouse cultivation and indoor farming, and thus, there are differences not only in the light regime but also in light intensity. Since the daily light impact was highly influenced (72% differences between both light regimes) by the light regimes, we focused on this while explaining the results. Nevertheless, it would be beneficial to investigate further influences of different light parameters on the quality of vegetables in indoor farming systems.

Regarding the response of plants to salt stress, it is important to know whether salt was applied in a single step or gradually. If salt is applied in a single step, there is a possibility that plants will suffer from salt shock (Shavrukov, 2012). In our study, salt was applied in a single step. However, salt was applied in an NFT system where the pots were irrigated from below, which resulted in a slower accumulation of salt in the soil. In addition, plants had a long acclimation period of 17 days, during which they could have recovered from the osmotic shock (Shavrukov, 2012). Nevertheless, this is an important point that should be considered in future studies which may affect the tolerance of plants to ionic stress and, hence, their response to varying light regimes.

It would be interesting to study the modification of light conditions with respect to the adjustable salt tolerance of halophytes and the impact of light in relation to the use of different saline water sources. The salt concentration is not only dependent on the water source, e.g., brackish water, wastewater, or brine water, but also on the location (Atkinson and Bingman, 1997). For example, regional brine waters have different salt concentrations and compositions (Fitzner et al., 2021). One option to adjust the salt concentration to the halophyte salt tolerance range is dilution with freshwater. However, freshwater is an exhaustible resource, and in sustainable agriculture, freshwater consumption should be reduced (Gleick, 1993). If there is a way to regulate light intensity, this would be a potential solution.

Further research also could aim to study other halophyte species to broaden the picture of differences between obligate and facultative halophytes and investigate the interaction of the salt tolerance mechanism and the influence of light.

In conclusion, this study highlights the potential applications of halophytes for indoor farming and also hints at the adaptation of photosynthesis during salt stress under different light regimes in halophytes. Furthermore, optimization of indoor farming lighting conditions, taking into account salinity and plant species, could improve resource efficiency and pigment profile. Given the limited land and water use and the changing climate conditions, we argue that indoor farming has a high potential to become a fundamental contributor to global agriculture. In addition to sustainable crop production, healthy and sustainable nutrition will be a valued aspect of future diets. Halophytes are not only suitable for indoor farming but can also be irrigated with saline water, which conserves freshwater resources, and are additionally rich in PSM. Hence, saline indoor farming with halophytes could contribute to food and nutritional security in the future.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

MF performed the experiment, analysis, and data analysis. MF, MS, and SB wrote the article. MF and SB developed and established the methods. MS and SB did the funding acquisition. MS and SB conceived the original research plans. All authors contributed to the article and approved the submitted version.

Funding

This research was funded by the German Federal Ministry of Education and Research (grant number 031B0730 A), as part of the project Food4Future.

Acknowledgments

We would like to thank Stefanie Zantopp for helping with the experimental performance, Andrea Jankowsky for performing the anion analysis, Martin Weiland for performing the analysis of the light spectra, and Brian Cusak for reviewing the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2023.1105162/full#supplementary-material

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Publication III

Supplementary data

Title: The interaction of salinity and light regime modulates photosynthetic pigment content in edible halophytes in greenhouse and indoor farming.

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Supplementary Material

Supplemental Methods

S1.1 Determination of anion content in soil and nutrient solution

Chloride and nitrate content in soil and nutrient solution samples were determined by ion exchange chromatography. Freeze-dried soil samples were grinded (3 times for 2 min, 800 U min^{-1}) in a ceramic vessel using a Planetary Ball Mill (PULVERISETTE 7 classic line; Fritsch, Idar-Oberstein, Germany) to a homogenous powder. For extraction, 200 mg fine powder was dissolved in 14 mL ultrapure water and 500 µl of sodium bromide (0.6 mg mL^{-1}) was added as an internal standard. The sample preparation of nutrient solution samples was done according to Fitzner et al. (2021). Further extraction and measurement was carried out according to "Determination of chloride concentration in leaves" in section 2.4 of the main manuscript.

Supplemental Tables

Supplemental Table S1 Composition of nutrient solution (manufacturer specification) used in the nutrient film technique system in both greenhouse (LR1) and indoor farming (LR2).

Nutrient solution	
NH_4NO_3 [mmol/L]	0.6
Ca(NO3)2 [g/L]	1.04
KNO_3 [g/L]	0.81
Iron chelate [ppm]	8
KH_2PO_4 [g/L]	0.31
$MnSO_4$ [mg/L]	2.5
$MgSO_4$ [g/L]	0.54
$Na_2[B_4O_5(OH)_4]\cdot 8H_2O$ [mg/L]	3.6
CuSO4 [mg/L]	0.2
Na_2MoO_4 [mg/L]	0.1
$ZnSO_4$ [mg/L]	0.4
pH	6.2

Supplementary Material

Supplemental Table S2 Chloride concentrations and water content in the soil of indoor farming (LR2) samples at the end of the experimental period. Means ± SEM; n = 8 from two independent experiments.

		Cochlearia officinalis	*Atriplex hortensis*	*Salicornia europaea*
Chloride [mg g^{-1} DW]	0	0.08 ± 0.01	0.10 ± 0.01	0.09 ± 0.01
	50	3.21 ± 0.70	4.41 ± 0.54	2.58 ± 0.26
	200	6.68 ± 0.96	7.47 ± 0.48	7.02 ± 0.71
	600	18.36 ± 2.64	22.66 ± 3.35	16.03 ± 1.96
Water content [%]	0	18.42 ± 0.78	19.88 ± 0.70	22.17 ± 0.63
	50	17.75 ± 1.75	19.53 ± 0.89	20.05 ± 1.48
	200	18.10 ± 0.72	19.13 ± 1.41	17.83 ± 1.04
	600	18.65 ± 0.79	19.54 ± 0.57	18.08 ± 0.75

Supplemental Table S3 dF, F and p values of Two Way ANOVA's.

			Cochlearia officinalis			*Atriplex hortensis*			*Salicornia europaea*		
			DF	F	p	DF	F	p	DF	F	p
	Fresh weight (g)	Interaction	3	26.73	<0.001	3	16.78	<0.001	3	35.22	<0.001
		Light regime	1	37.56	<0.001	1	95.34	<0.001	1	207.50	<0.001
		Salt treatment	3	206.20	<0.001	3	38.70	<0.001	3	40.50	<0.001
	Fresh weight (FW) (%)	Interaction	3	4.31	0.006	3	0.261	0.853	3	24.60	<0.001
		Light regime	1	26.17	<0.001	1	1.57	0.212	1	73.75	<0.001
		Salt treatment	3	192.21	<0.001	3	70.25	<0.001	3	43.04	<0.001
	Dry weight (DW) (%)	Interaction	3	3.36	0.025	3	4.88	0.005	3	1.76	0.167
		Light regime	1	5.98	0.02	1	11.69	0.00	1	0.52	0.476
		Salt treatment	3	81.37	<0.001	3	19.95	<0.001	3	3.96	0.013
Per DW	Chloride	Interaction	3	11.02	<0.001	3	28.80	<0.001	3	0.50	0.684
		Light regime	1	29.76	<0.001	1	432.68	<0.001	1	2.09	0.154
		Salt treatment	3	63.82	<0.001	3		<0.001	3	148.99	<0.001
	ABA	Interaction	3	4.84	0.005	3	3.60	0.02	3	1.93	0.137
		Light regime	1	0.35	0.555	1	2.01	0.16	1	2.92	0.093
		Salt treatment	3	6.81	0.001	3	3.23	0.03	3	27.96	<0.001
	Chlorophyll *a*	Interaction	3	16.12	<0.001	3	26.10	<0.001	3	12.39	<0.001
		Light regime	1	274.44	<0.001	1	13.50	<0.001	1	68.70	<0.001
		Salt treatment	3	119.82	<0.001	3	179.07	<0.001	3	23.93	<0.001
	Chlorophyll *b*	Interaction	3	18.18	<0.001	3	7.60	<0.001	3	4.73	0.006

		Light regime	1	321.90	<0.001	1	16.34	<0.001	1	18.93	<0.001
		Salt treatment	3	96.72	<0.001	3	65.66	<0.001	3	7.62	<0.001
	Lutein	Interaction	3	6.38	<0.001	3	20.17	<0.001	3	19.49	<0.001
		Light regime	1	2.38	<0.001	1	41.19	<0.001	1	92.27	<0.001
		Salt treatment	3	152.12	<0.001	3	310.97	<0.001	3	75.53	<0.001
	β-Carotene	Interaction	3	32.54	<0.001	3	52.61	<0.001	3	28.25	<0.001
		Light regime	1	43.08	<0.001	1	7.66	0.008	1	131.21	<0.001
		Salt treatment	3	86.77	<0.001	3	136.93	<0.001	3	65.85	<0.001
	Zeaxanthin	Interaction	3	0.73	0.54	3	5.83	0.002	3	17.81	<0.001
		Light regime	1	0.03	0.865	1	0.00	0.991	1	21.39	<0.001
		Salt treatment	3	8.11	<0.001	3	3.92	0.013	3	5.14	0.004
	Violaxanthin	Interaction	3	12.16	<0.001	3	4.10	0.011	3	10.89	<0.001
		Light regime	1	1.37	0.247	1	102.28	<0.001	1	7.28	0.01
		Salt treatment	3	41.84	<0.001	3	55.64	<0.001	3	8.81	<0.001
	9*Z*-Neoxanthin	Interaction	3	5.19	0.003	3	1.51	0.221	3	29.76	<0.001
		Light regime	1	152.00	<0.001	1	65.24	<0.001	1	62.53	<0.001
		Salt treatment	3	71.95	<0.001	3	8.73	<0.001	3	45.11	<0.001
Per FW	Chloride	Interaction	3	3	0.041	3	2.31	0.087	3	0.01	0.998
		Light regime	1	4	0.046	1	4.58	0.037	1	1.47	0.231
		Salt treatment	3	15	<0.001	3	23.88	<0.001	3	13.43	<0.001
	ABA	Interaction	3	5	0.003	3	4	0.02	3	1	0.264
		Light regime	1	2	0.137	1	8	0.006	1	0	0.899
		Salt treatment	3	13	<0.001	3	7	<0.001	3	5	0.004
	Chlorophyll *a*	Interaction	3	32.62	<0.001	3.00	3.37	0.025	3	2.62	0.061
		Light regime	1	11.47	<0.001	1.00	0.01	0.922	1	17.46	<0.001
		Salt treatment	3	11.09	<0.001	3.00	4.10	0.011	3	8.17	<0.001
	Chlorophyll *b*	Interaction	3	26.67	<0.001	3.00	6.48	<0.001	3	1.64	0.192
		Light regime	1	75.43	<0.001	1.00	0.00	0.957	1	1.32	0.256
		Salt treatment	3	13.55	<0.001	3.00	2.31	0.087	3	1.38	0.26
	Lutein	Interaction	3	5.98	0.001	3.00	2.59	0.062	3	2	0.212
		Light regime	1	32.69	<0.001	1.00	0.08	0.775	1	6	0.017
		Salt treatment	3	2.44	0.07	3.00	5.41	0.003	3	14	<0.001
	β-Carotene	Interaction	3	23.10	<0.001	3.00	3.80	0.015	3	3	0.046
		Light regime	1	29.29	<0.001	1.00	0.52	0.473	1	9	0.004

	Salt treatment	3	8.98	<0.001	3.00	5.21	0.003	3	11	<0.001
Zeaxanthin	Interaction	3	2.05	0.12	3.00	3.64	0.019	3	12	<0.001
	Light regime	1	1.79	0.19	1.00	0.23	0.636	1	27	<0.001
	Salt treatment	3	9.99	<0.001	3.00	5.29	0.003	3	5	0.003
Violaxanthin	Interaction	3	12.10	<0.001	3.00	1.48	0.231	3	9	<0.001
	Light regime	1	1.96	0.17	1.00	2.72	0.105	1	1	0.46
	Salt treatment	3	1.10	0.36	3.00	2.57	0.064	3	7	<0.001
9Z-Neoxanthin	Interaction	3	14.45	<0.001	3.00	2.07	0.115	3	5	0.003
	Light regime	1	58.75	<0.001	1.00	3.40	0.071	1	1	0.373
	Salt treatment	3	2.98	0.04	3.00	2.12	0.108	3	14	<0.001

Supplemental Table S4 Fresh and dry mass of leaves of three pooled plants of 6 to 9 week-old plants in light regime 1 (LR1, greenhouse) and light regime 2 (LR2, indoor farming). n = 8 pools of 3 individual plants each from two independent experiments.

		Salt treatment [mM NaCl]	Fresh mass [g]	Dry mass [g]
Cochlearia officinalis	Light regime 1	0	22.91 ± 0.82	1.83 ± 0.07
		50	20.64 ± 2.01	0.96 ± 0.15
		200	14.37 ± 0.85	0.81 ± 0.09
		600	2.22 ± 0.21	0.37 ± 0.18
	Light regime 2	0	43.93 ± 2.86	3.55 ± 0.35
		50	36.55 ± 3.88	1.01 ± 0.38
		200	14.37 ± 1.49	0.47 ± 0.22
		600	0.77 ± 0.08	0.09 ± 0.04
Atriplex hortensis	Light regime 1	0	17.56 ± 1.45	0.99 ± 0.08
		50	21.06 ± 1.23	1.39 ± 0.10
		200	10.92 ± 0.81	1.55 ± 0.48
		600	1.66 ± 0.21	0.27 ± 0.03
	Light regime 2	0	123.49 ± 12.36	11.46 ± 1.13
		50	98.28 ± 16.15	10.23 ± 1.47
		200	50.08 ± 5.29	6.48 ± 0.26
		600	3.80 ± 0.52	1.21 ± 0.14
Salicornia europaea	Light regime 1	0	0.41 ± 0.07	0.03 ± 0.01

		50	0.70	±	0.10			0.04	±	0.01		
		200	0.84	±	0.09			0.05	±	0.01		
		600	0.56	±	0.56			0.04	±	0.04		
		0	1.76	±	0.12			0.09	±	0.02		
	Light regime 2	50	8.34	±	0.84			0.41	±	0.06		
		200	10.45	±	0.60			0.62	±	0.05		
		600	2.24	±	0.25			0.21	±	0.02		

Supplemental Table S5 Content of chlorophylls on fresh mass basis in leaves of 6 to 9 week-old plants. Means ± SEM of n = 8 pools of 3 individual plants each from two independent experiments. Small letters indicate significant differences between salt treatments in light regime 1 (LR1, greenhouse) in alphabetic order from highest to lowest; capital letters indicate significant differences between salt treatments in light regime 2 (LR2, indoor farming) in alphabetic order from highest to lowest, asterisks indicate significant differences between LR11 and LR2 in-between one salt treatment, interaction shows significant different interaction between salt treatments and light regimes, Two-Way ANOVA, followed by *post hoc* Bonferroni Test ($p \leq 0.05$)(* ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001); n = 8 pools of 3 individual plants each. ns, not significant.

		Salt treatment [mM NaCl]	Chlorophyll *a* [ng mg^{-1} FM]					Chlorophyll *b* [ng mg^{-1} FM]				
Cochlearia officinalis	Light regime 1	0	685.90	±	45.03	B		224.45	±	12.68	B	
		50	761.33	±	7.35	B		251.51	±	2.79	B	
		200	846.84	±	27.47	B	***	243.49	±	45.88	B	*
		600	1516.45	±	106.65	A	***	623.95	±	87.44	A	***
	Light regime 2	0	569.19	±	21.91	AB		182.16	±	7.39	ns	
		50	621.00	±	25.53	A		199.34	±	8.87	ns	
		200	482.76	±	57.81	AB		160.36	±	20.61	ns	
		600	363.86	±	108.99	AB		129.42	±	38.44	ns	
	Interaction light regime x salt treatment					***					***	
Atriplex hortensis	Light regime 1	0	179.11	±	8.84	b		40.70	±	0.74	ns	
		50	217.29	±	7.65	b		45.34	±	1.74	ns	
		200	335.91	±	92.02	a		66.61	±	25.40	ns	
		600	281.37	±	25.36	ab		67.22	±	4.95	ns	***
	Light regime 2	0	336.00	±	8.22	ns		65.63	±	1.16	A	
		50	357.12	±	16.83	ns		66.35	±	2.39	A	
		200	356.99	±	74.28	ns		69.92	±	14.37	A	
		600	91.83	±	21.43	ns		19.43	±	4.76	B	
	Interaction light regime x salt treatment						*					***
Salicornia europaea	Light regime 1	0	35.49	±	18.32	ns		33.77	±	12.74	ns	
		50	45.13	±	16.60	ns		31.24	±	3.97	ns	

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	200	90.18	±	9.05	ns		30.71	±	5.85	ns
	600	50.23	±	13.58	ns		22.47	±	5.73	ns
Light regime 2	0	33.94	±	14.79	b		20.37	±	7.46	ns
	50	135.88	±	13.28	a	***	43.65	±	4.75	ns
	200	147.87	±	15.70	a	*	44.02	±	5.10	ns
	600	105.30	±	23.49	a	*	32.90	±	7.28	ns
Interaction light regime x salt treatment						ns				ns

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Supplemental Table S6 Content of carotenoids on fresh mass basis in leaves of 6 to 9 week-old plants. Means ± SEM of n = 8 pools of 3 individual plants each from two independent experiments. Small letters indicate significant differences between salt treatments in light regime 1 (LR1, greenhouse) in alphabetic order from highest to lowest; capital letters indicate significant differences between salt treatments in light regime 2 (LR2, indoor farming) in alphabetic order from highest to lowest, asterisks indicate significant differences between LR1 and LR2 in-between one salt treatment, interaction shows significant different interaction between salt treatments and light regimes, Two-Way ANOVA, followed by *post hoc* Bonferroni Test ($p \leq 0.05$)(* ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001); n = 8 pools of 3 individual plants each. ns, not significant.

		Salt treatment [mM NaCl]	Lutein [ng mg-1 FM]				β-Carotene [ng mg-1 FM]				Zeaxanthin [ng mg-1 FM]				*all-trans*-Violaxanthin (isomer 1) [ng mg-1 FM]				*9Z*-Neoxanthin (isomer 1) [ng mg-1 FM]			
Cochlearia officinalis	Light regime 1	0	81.16	± 3.88	b		24.00	± 1.70	c		1.14	± 0.07	b		6.11	± 0.24	b		19.58	± 1.37	b	
		50	92.24	± 1.38	b		33.99	± 0.99	bc		1.68	± 0.24	b		6.69	± 0.40	ab		22.94	± 0.61	b	
		200	102.92	± 4.09	b	**	44.00	± 1.36	b	*	2.36	± 0.42	b		8.88	± 1.20	ab		23.08	± 1.08	b	**
		600	157.54	± 28.59	a	***	77.19	± 9.24	a	***	8.33	± 2.37	a		10.12	± 1.95	a	***	41.97	± 6.62	a	***
	Light regime 2	0	64.42	± 2.04	ns		28.30	± 1.18	AB		0.80	± 0.05	ns		10.42	± 1.03	a	**	15.86	± 0.75	ns	
		50	70.85	± 2.34	ns		37.01	± 1.22	A		1.62	± 0.14	ns		8.15	± 0.67	a		16.70	± 0.76	ns	
		200	55.40	± 6.24	ns		29.91	± 3.56	AB		2.94	± 0.42	ns		6.46	± 0.84	ab		10.87	± 1.44	ns	
		600	49.72	± 14.94	ns		19.43	± 5.89	B		4.21	± 1.64	ns		2.73	± 0.63	b		6.53	± 1.76	ns	
	Interaction light regime x salt treatment					***				***				ns				***				***
Atriplex hortensis	Light regime 1	0	16.49	± 0.30	ab		8.83	± 0.38	b		0.38	± 0.05	b		4.09	± 0.21	ns		4.13	± 0.11	ns	
		50	20.03	± 0.75	ab		12.29	± 0.49	b		0.86	± 0.25	b		5.63	± 0.25	ns		4.76	± 0.18	ns	
		200	44.21	± 15.26	a		33.96	± 12.10	a		5.15	± 1.95	a	**	10.28	± 4.09	ns		10.96	± 3.81	ns	
		600	18.93	± 1.66	b		18.41	± 2.25	ab		1.49	± 0.42	b		4.56	± 0.57	ns		6.28	± 0.53	ns	
	Light regime 2	0	28.65	± 0.63	AB		23.05	± 0.47	AB		0.99	± 0.10	ns		3.01	± 0.68	ns		5.32	± 0.79	ns	
		50	29.19	± 1.34	AB		26.80	± 1.20	A	**	1.02	± 0.33	ns		6.40	± 0.79	ns		4.18	± 1.14	ns	
		200	30.04	± 5.75	A		27.76	± 5.62	A		1.80	± 0.44	ns		4.54	± 0.97	ns		4.79	± 0.86	ns	

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		600	6.87	±	1.92	B		6.13	±	1.47	B		2.99	±	0.45	ns		2.96	±	0.51	ns		3.70	±	1.02	ns	
	Interaction light regime x salt treatment						ns					*					*				ns					ns
Salicornia europaea	Light regime 1	0	3.23	±	1.03	b		1.06	±	0.08	ns		1.71	±	0.05	a	***	1.38	±	0.20	ns		1.43	±	0.15	ns	
		50	7.30	±	1.87	ab		1.92	±	0.70	ns		0.36	±	0.04	b		1.29	±	0.27	ns		1.46	±	0.34	ns	
		200	15.39	±	2.07	a		4.91	±	0.81	ns		0.34	±	0.11	b		2.22	±	0.41	ns		3.36	±	0.32	ns	*
		600	8.36	±	3.13	b		1.75	±	1.20	ns		0.49	±	0.05	b		1.33	±	0.06	ns		2.02	±	0.37	ns	
	Light regime 2	0	2.58	±	1.38	b		0.27	±	0.12	b		0.05	±	0.36	ns		0.65	±	0.46	B		0.60	±	0.24	c	
		50	15.80	±	1.03	a	***	6.69	±	0.61	a	***	0.27	±	0.11	ns		2.83	±	0.13	A	***	3.32	±	0.10	ab	***
		200	18.75	±	2.98	a		7.96	±	0.49	a	*	0.37	±	0.07	ns		1.48	±	0.15	B		3.60	±	0.56	a	
		600	13.07	±	2.10	a		5.09	±	0.67	a		0.33	±	0.18	ns		0.88	±	0.22	B		2.08	±	0.44	b	
	Interaction light regime x salt treatment						ns					*					***				***					**

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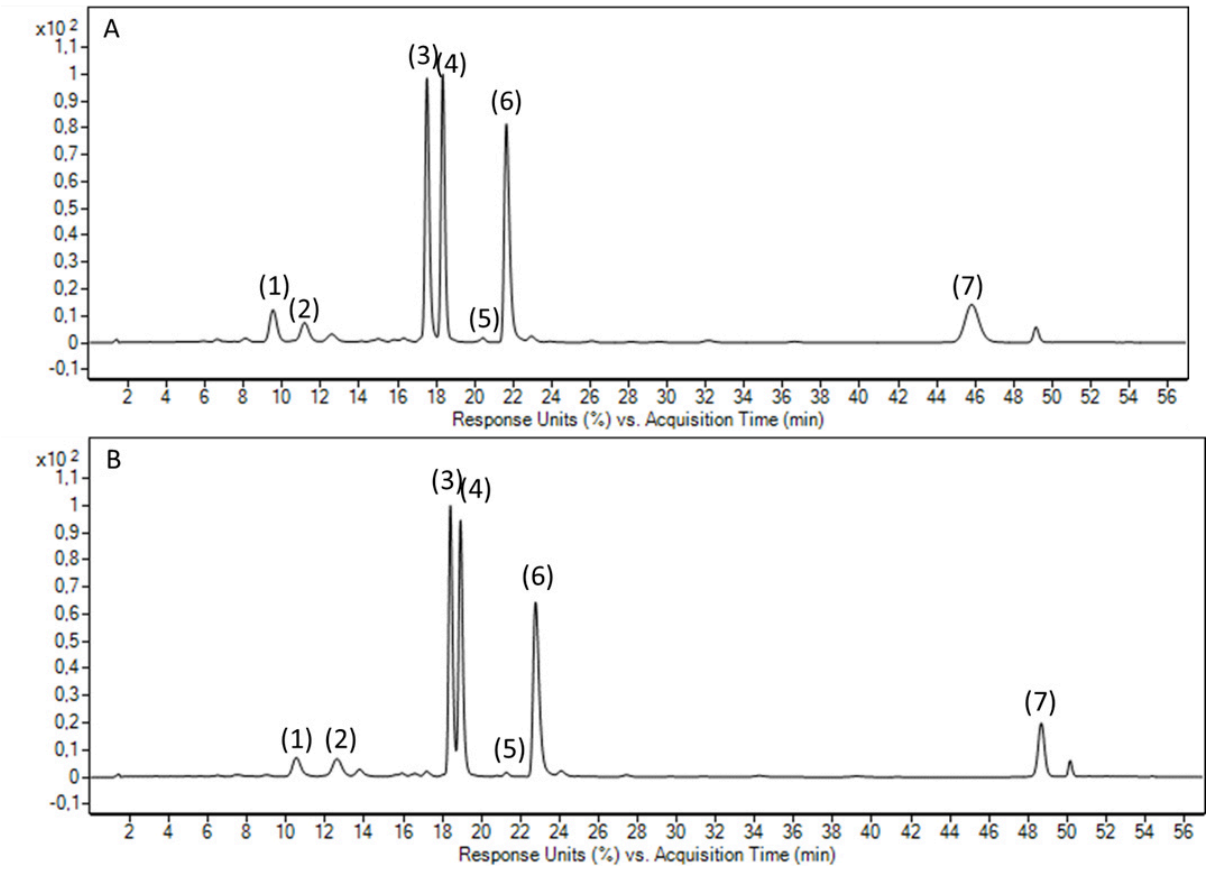
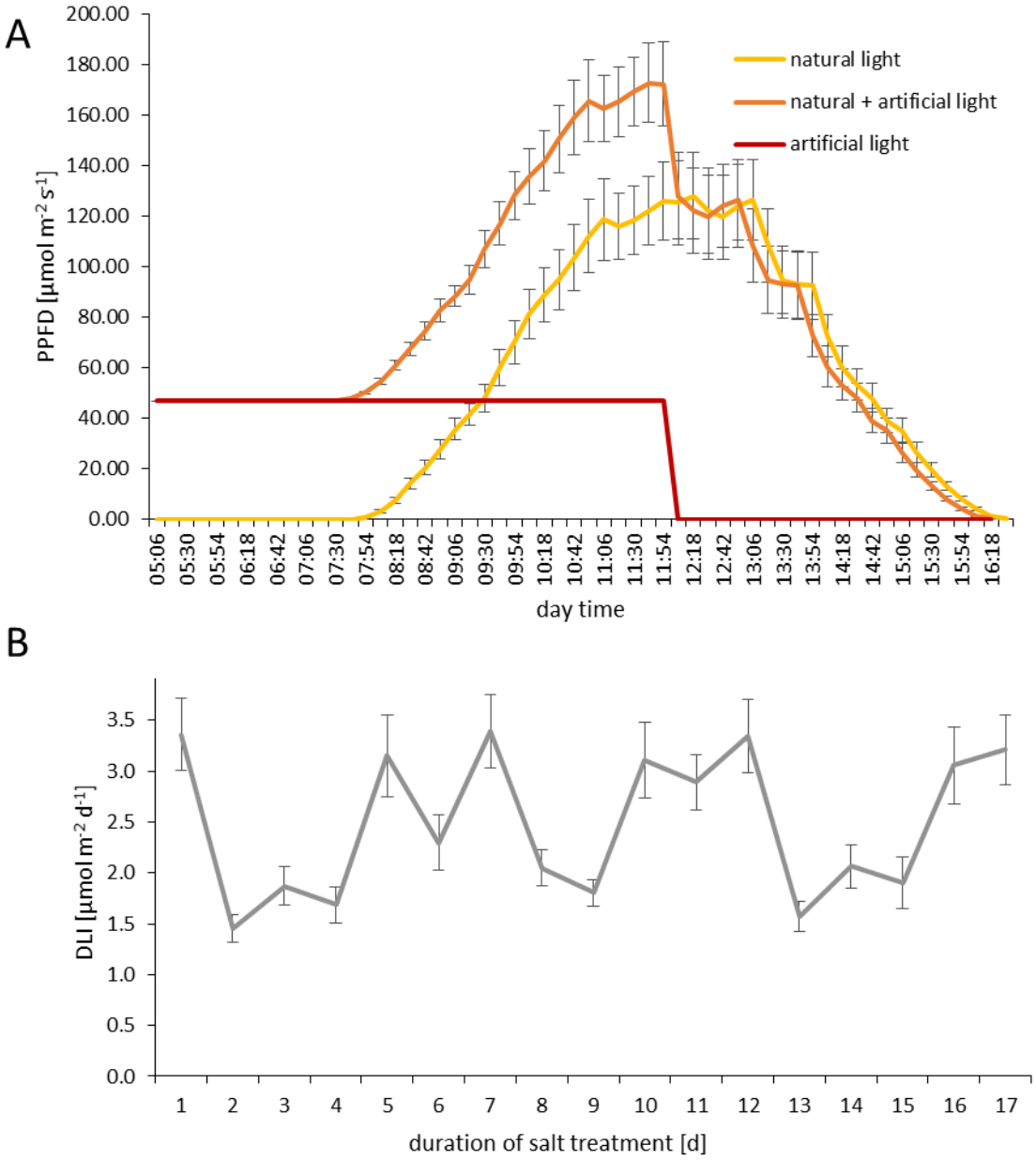
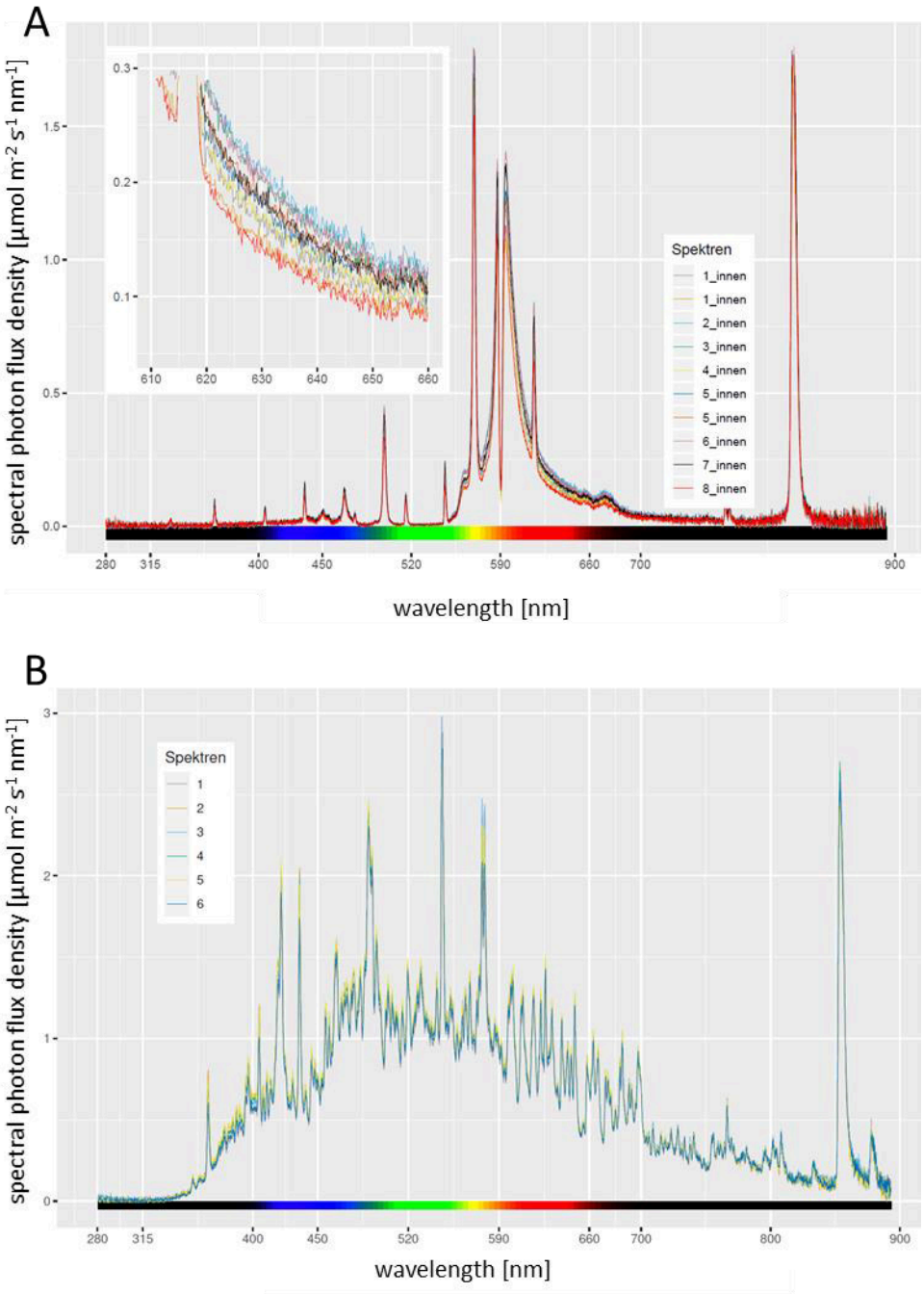
Supplemental Figures



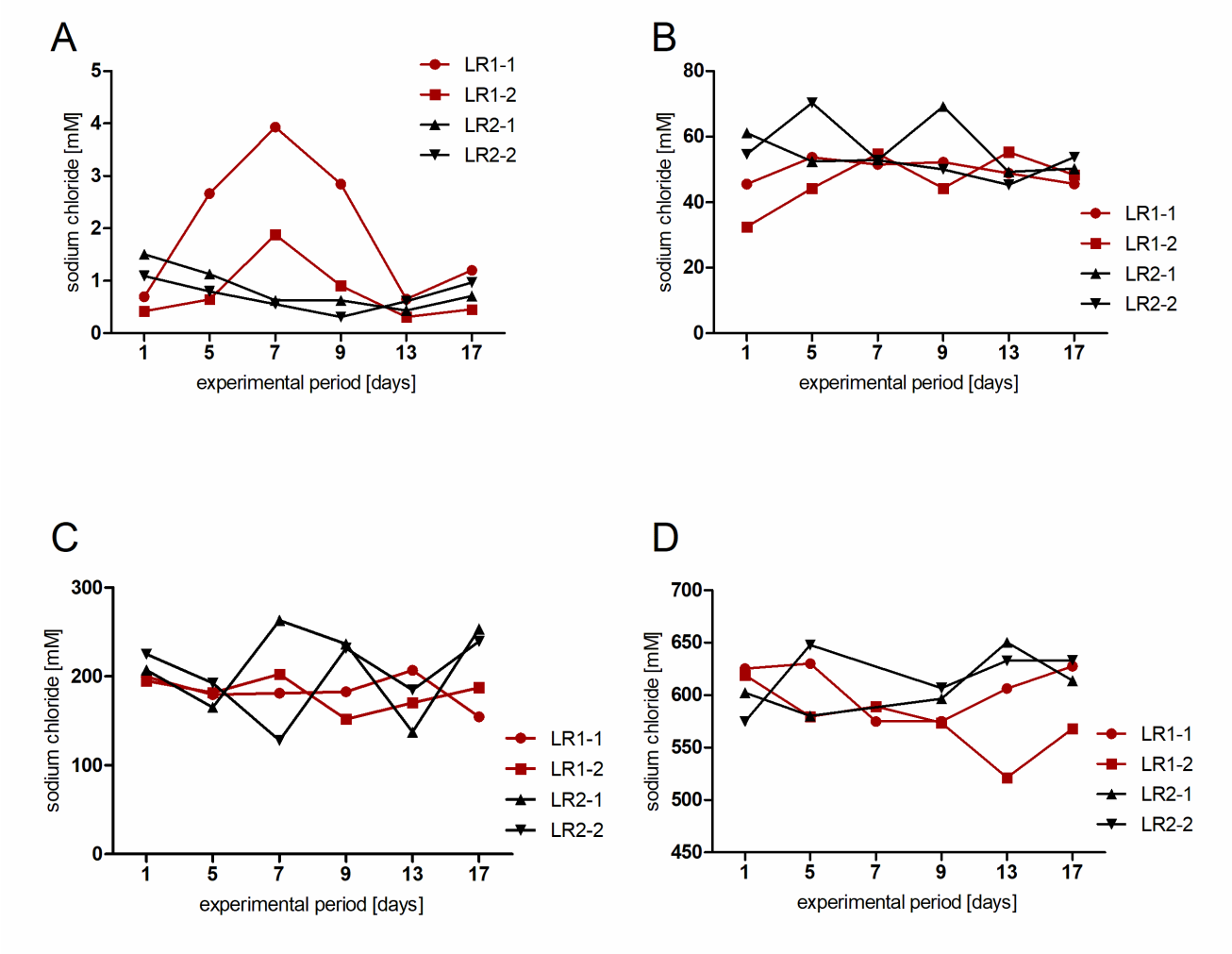
Supplemental Figure S1 HPLC-DAD chromatogram at 450 nm showing identified chlorophylls and carotenoids exemplary in leaves of *Cochlearia officinalis* grown in (A) greenhouse (LR1) and (B) indoor farming (LR2). (1) *all-trans*-violaxanthin; (2) 9*Z*-neoxanthin; (3) chlorophyll *b*; (4) *all-trans*-lutein; (5) *all-trans*-zeaxanthin; (6) chlorophyll *a*; (7) *all-trans*-β-carotene.



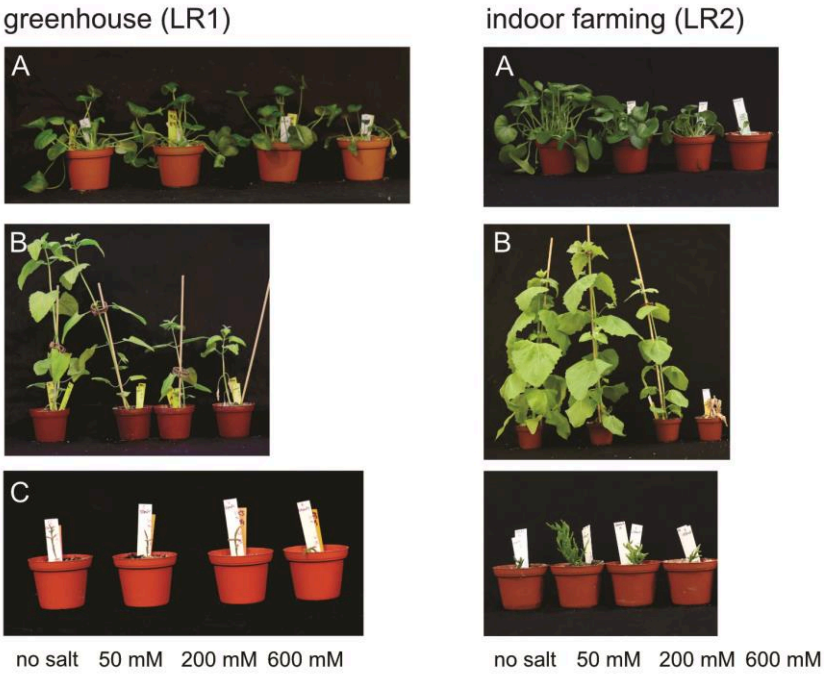
Supplemental Figure S2 Average light distribution in greenhouse (LR1). (A) Average light intensity over the day of natural and artificial light represented in photosynthetic photon flux density (PPFD). (B) Fluctuations of the daily light integral (DLI) over the salt treatment period. Means ± SEM.



Supplemental Figure S3 Light spectra in (A) greenhouse (LR1) and (B) indoor farming (LR2) measured with a spectrophotometer (Ocean Insight, US).

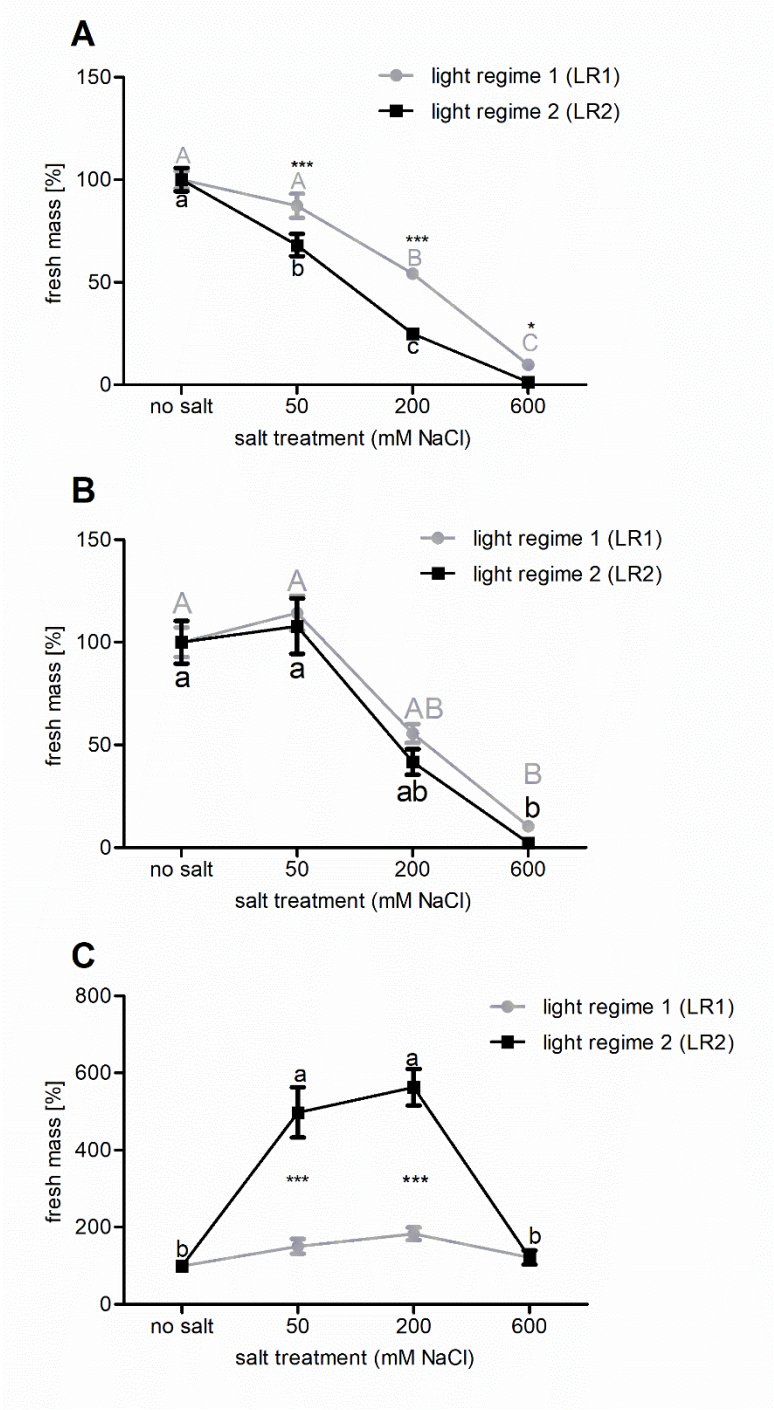


Supplemental Figure S4 Sodium chloride concentrations in nutrient solution during the experimental period of 17 days in greenhouse (LR1) and indoor farming (LR2). (A) No salt; (B) 50 mM; (C) 200 mM and (D) 600 mM. LR1-1, replicate experiment 1 (greenhouse); LR1-2, replicate experiment 2 (greenhouse); LR2-1, replicate experiment 1 (indoor farming); LR2-2, replicate experiment 2 (indoor farming).

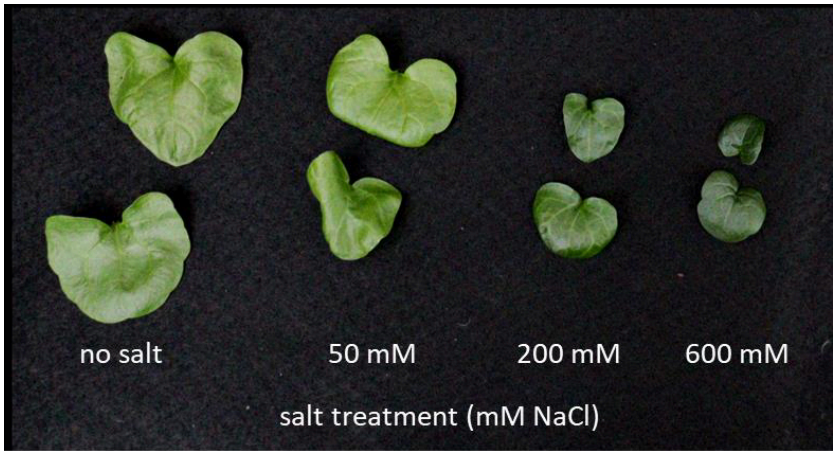


Supplemental Figure S5 Effect of salt treatment on the phenotypes of (A) *Cochlearia officinalis*; (C) *Atriplex hortensis*; (C) *Salicornia europaea* in greenhouse (LR1) and indoor farming (LR2) 6 or 9 week-old plants depending on plant species, after 3 weeks of salt treatment. Increasing salt treatment (in mM NaCl) from left to right.

Supplemental Figure S6 Salt bladders/glands. Magnification (1.4-fold) of (A) leaves and (B) stem of *Atriplex hortensis*; at 600 mM salt treatment in indoor farming (LR2) after 5 days of salt treatment.



Supplemental Figure S7 Effect of salt treatment and light regime on fresh weight calculated as percent of no salt. (A) *Cochlearia officinalis*; (C) *Atriplex hortensis*; (C) *Salicornia europaea* in greenhouse (LR1) and indoor farming (LR2). Means ± SEM of n = 24. Small letters indicate significant differences between salt treatments in light regime 1 in alphabetic order from highest to lowest; capital letters indicate significant differences between salt treatments in light regime 2 in alphabetic order from highest to lowest, asterisks indicate significant differences between light regime 1 and 2 in-between one salt treatment, interaction shows significant different interaction between salt treatments and light regimes tested by Two-Way ANOVA, followed by post hoc Bonferroni Test ($p \leq 0.05$)(* ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001).



Supplemental Figure S8 Influence of salt treatment on leaves of *Cochlearia officinalis*. Six week-old plants after 17 days of salt treatment in the greenhouse (LR1).

Publication IV

Between eustress and distress: UVB induced changes in carotenoid accumulation in halophytic *Salicornia europaea*

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Published in: *Journal of Plant Physiology*. 2023.
DOI: 10.1016/j.jplph.2023.154124

Journal of Plant Physiology 291 (2023) 154124

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.com/locate/jplph

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ARTICLE INFO

Keywords:
Glasswort
Photosynthetic pigments
Reactive oxygen species
Abscisic acid
Indoor farming
LED

ABSTRACT

Halophytes are potential future crops with a valuable nutritional profile. Produced in indoor farming, they are considered to contribute to sustainable and resilient food systems. Indoor farms operate using artificial light. In this context narrowband and low dose UVB radiation can be used to increase plant secondary metabolites, such as carotenoids, and provide an improved nutritional profile for a human diet. UVB radiation can cause eustress or distress in the plant depending on the lighting situation. The aim of this study was to identify the doses of UVB that lead to either eustress or distress and to analyze these responses in *Salicornia europaea*. Therefore, *S. europaea* plants were exposed to different UVB radiation levels, low, medium and high, and analyzed for reactive oxygen species (ROS), plant hormones, amino acids, and photosynthetic pigments. High UVB treatment was found to affect phenotype and growth, and the metabolite profile was affected in a UVB dose-dependent manner. Specifically, medium UVB radiation resulted in an increase in carotenoids, whereas high UVB resulted in a decrease. We also observed an altered oxidative stress status and increased SA and decreased ABA contents in response to UVB treatment. This was supported by the results of menadione treatment that induces oxidative stress in plants, which also indicated an altered oxidative stress status in combination with altered carotenoid content. Thus, we show that a moderate dose of UVB can increase the carotenoid content of *S. europaea*. Furthermore, the UVB stress-dependent response led to a better understanding of carotenoid accumulation upon UVB exposure, which can be used to improve lighting systems and in turn the nutritional profile of future crops in indoor farming.

1. Introduction

Current food systems will not be able to ensure food security in the near future. One of the major challenges for future agricultural systems is the increasing scarcity of water (FAO, 2020; Gosling and Arnell, 2016; EEA, 2019). Fresh water resources are threatened by extensive overuse and pollution, mainly related to agricultural systems (FAO, 2021). Therefore, new food systems that do not put a strain on freshwater resources are of great interest. Salt-tolerant crops (halophytes) can be irrigated with saline water and produced as an alternative vegetable crop. Edible halophytes are grown in greenhouses and open fields for commercial use in several countries, including the Netherlands and Israel (Aronson, 1985; Panta et al., 2014). *Salicornia europaea* is a prominent halophyte candidate for food production (Ventura and Sagi, 2013).

Indoor farming offers season-independent production of vegetables (Ladeiro, 2012), and *Salicornia europaea* has been shown to be suitable for indoor farming (Fitzner et al., 2023a). Research into optimizing vertical farming for food production has increased in recent years (van Delden et al., 2021). An advantage of indoor farming is that the light conditions can be fully modified to suit the plant species and desired nutrient profile. Recent developments in indoor farming research are concerned with the use of an additional low dose of UVB light to improve growth and content of plant secondary metabolites (PSMs). For example, a study by Wiesner-Reinhold et al. (2021) Badmus et al. (2022b) showed that UVB induced changes in the carotenoid profile. Nevertheless, the effect of UVB light on plants depends on several factors, such as wavelength, duration, intensity, and most importantly, the plant species (Tossi et al., 2019). Thus, the effect of UVB light must be

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https://doi.org/10.1016/j.jplph.2023.154124
Received 5 July 2023; Received in revised form 13 October 2023; Accepted 27 October 2023
Available online 28 October 2023

studied specifically for each plant species and experimental setup.

A balanced profile of PSMs is desirable because of their health-promoting effects and their environmental interaction as photosynthetic pigment. For example, carotenoids are known for their chemoprotective properties (Fiedor and Burda, 2014). *S. europaea* shows a rich profile of PSMs, including carotenoids, which have been shown to be present in amounts comparable to other vegetables (Kim et al., 2021; Fitzner et al., 2021).

The effect of UVB stress can mainly be divided into eustress and distress. Eustress occurs at lower stress levels and leads to the activation of stress signaling and defense mechanisms, such as the activation of the antioxidant system and accumulation of antioxidants such as carotenoids (Hideg et al., 2013). Distress occurs at high levels of stress. Stress signaling is also activated, but the antioxidant system is not sufficient to cope with the stress related damage, leading to cell death (Nawkar et al., 2013). Eustress develops a reversible phenotype, such as shortened root growth, whereas distress develops an irreversible phenotype, such as the formation of necrosis (Blum, 2015).

A key mechanism in UVB perception in plants, is the photoreceptor UV RESISTANCE LOCUS 8 (UVR8). The action of UVR8 depends on the wavelength of the UVB light and has been shown to have an absorption maximum at 280 nm and a maximum photon efficacy between 290 nm and 300 nm in *Arabidopsis thaliana* (Díaz-Ramos et al., 2018). UVB light activates the protein and induces UVB signaling, which includes increased DNA repair and protection of the photosynthetic apparatus through the accumulation of antioxidants, the so-called "sunscreen" metabolism, where UV-absorbing metabolites, such as carotenoids and flavonoids are accumulated (Ulm and Jenkins, 2015). In addition, UVB light causes oxidative stress through the formation of reactive oxygen species (ROS), including the formation of hydrogen peroxide (H_2O_2) in photosystems II (Vass, 2012). The oxidative stress status, which is influenced by changes in ROS levels and the productivity of antioxidant enzymes such as superoxide dismutase (SOD), is thought to be related to changing levels of photosynthetic pigments (Bouvier et al., 1998). Other signaling molecules, such as plant hormones and amino acids, are involved in the response to UVB stress. Abscisic acid (ABA) and salicylic acid (SA) have been shown to be involved in the UVB stress response in glycophytes, and SA, for example, is responsible for the upregulation of antioxidant enzymes and accumulation of anthocyanins and tocopherol (Bandurska and Cieślak, 2013; Vishwakarma et al., 2017). However, most of the studies have been performed with glycophytes (non-halophytic plants), such as *Arabidopsis thaliana*.

While some studies have already investigated the effect of UVB light on carotenoids in glycophytes, this is an almost unexplored area of research in halophytes. The aim of this study was to identify UVB eustress- and distress-induced changes in the carotenoid profile of *S. europaea* in indoor farming. For this purpose, *S. europaea* was exposed to different UVB doses (based on preliminary experiments) and carotenoid accumulation, chlorophylls as well as photosynthetically active pigments, signaling molecules, such as plant hormones and amino acids, and oxidative stress markers, H_2O_2 and SOD activity were analyzed. Subsequently we selected a middle UVB dose corresponding to eustress for further analyses, and compared these results to menadione treatment, which induces oxidative stress in plants.

2. Materials and methods

2.1. Plant material and cultivation

Salicornia europaea seeds were purchased at Rühlemann's Kräuter & Duftpflanzen (Germany) and germinated on grodan cubes (Grodan® delta (4 × 4x4.5 cm; rock wool)) in a climate cabinet (polyklima, Freising, Germany). Cultivation parameters were set to a light intensity of 150 µmol m^{-2} s^{-1} PPFD, a temperature of 22 °C day and 20 °C night, a photoperiod of 14 h daylight and 10 h night, and a humidity of 65%. White light was provided by light emitting diodes (LEDs) at 6500 K. Plants were irrigated with nutrient solution (composition: Table S1) three weeks after germination. Salt acclimatization was initiated one week before the start of the treatment by enriching the nutrient solution with 200 mM NaCl, which was identified as the optimal growing condition for *S. europaea* in indoor farming in a previous experiment (Fitzner et al., 2023b).

2.2. Treatments

2.2.1. UVB treatment

UVB treatment was carried out using UVB LEDs in the climatic cabinet, which could be used additionally with the white light LEDs. The UVB LEDs emitted light at the wavelength of 285 nm.

For the first experimental setup (UVB I), three different UVB doses were tested, resulting in the following four treatments: control (no UVB), low UVB (1.25 kJ m^{-2} d^{-1}), medium UVB (2.5 kJ m^{-2} d^{-1}), and high UVB (5 kJ m^{-2} d^{-1}). For the second experimental setup (UVB II), no (control) and medium UVB (2.5 kJ m^{-2} d^{-1} 18 days in both experiments, with a radiation duration of 14 h per day, according to the photoperiod.

2.2.2. Menadione treatment

Menadione (MND) treatment comprised a control condition and a menadione condition, both cultivated without UVB in the same climate cabinet as the UVB experiments. Menadione (Sigma Aldrich, Taufkirchen, Germany, 315 µM) was dissolved in 1% ethanol/water (+ 0.01% Tween20) and applied as a spray on the green tissue. Control conditions consisted of 1% ethanol/water without menadione. Treatment was applied five times over 18 days with at an average of 0.8 mL per biological replicate/date. The treatment was always applied 1 h after irrigation and the last time 24 h before harvest. The experimental setup was carried out twice, with seven biological replicates in first experiment (MND I) and eight biological replicates in the second experiment (MND II). The total amount of menadione applied was the same between the two experiments, but the amount per date was slightly different (Fig. S1). For this reason, the data were analyzed individually and are presented as MND I and MND II.

2.3. Harvest and determination of fresh weight gain

For all experiments, plants were harvested by cutting off the aboveground part. For the UVB II experiment and menadione experiment, samples for SOD, H_2O_2, and metabolite analysis were harvested separately by cutting the plants of a grodan cube into small pieces and then dividing them. All samples were immediately frozen in liquid nitrogen and stored at −80 °C until further analysis.

Fresh weight gain was determined by weighing the plants before and after treatment and calculating the difference.

2.4. Analysis of oxidative stress markers

To evaluate the plant oxidative stress status, the antioxidative enzyme SOD and H_2O_2 content were determined. Since *S. europaea* is not a foliage plant, the methods for measuring SOD activity and H_2O_2 content had to be adapted as described below.

2.4.1. Determination of SOD activity

Sample preparation was optimized following Alici and Arabaci (2016) for *in vitro* measurements of SOD activity. In a first step, approximately 100 mg of frozen plant material was ground (2 × 3 mm beads, 2 × 50 s, 25 Hz) using a Retsch mill (Retsch MM 400; Retsch GmbH, Haan, Germany). Then, 500 µl PBS buffer (pH = 7) was added immediately. Next, the samples were centrifuged (7 min, 22 °C, 4500×*g*), the supernatant was collected and diluted with PBS buffer (1:10). A superoxide dismutase activity assay KitC (Sigma Aldrich, Taufkirchen, Germany) was used for the analysis following the manufactures instructions. The reaction is based on the formation of

superoxide anions (SO) by xanthine oxidase interacting with the supplied dye, resulting in the formation of a formazan dye measured with a microplate reader (Tecan Infinite 200 PRO) at a wavelength of 450 nm. The amount of SO depends in a negative correlation on the SOD activity. The final amount of SOD activity was calculated from a calibration curve. The measurements were conducted in triplicate.

2.4.2. Determination of H_2O_2 content

For sample preparation, several method optimizations were performed in advance regarding sample storage time and sample handling (data not shown) (Gerna et al., 2020). For the H_2O_2-Assay, approximately 170 mg frozen plant material was used. The measurement was performed one day after the plants were harvested to ensure that the H_2O_2 content had not been degraded. For the sample preparation, 500 µl PBS buffer (pH = 7.4) was added to the frozen plant material and the solution was shaken (1 min, 1500 rpm, 22 °C) to temper and homogenize the sample. Activated carbon (2 ± 0.5 mg) was then added to absorb interfering substances. The solution was filtered through a Clear Spin Filter (cellulose acetate, 0.22 µm; Kikser Biotech, Steinfurt, Germany) and centrifuged (2 min, 22 °C, 4500×*g*). The analysis was performed using a Fluorimetric Hydrogen Peroxide Assay Kit (Sigma Aldrich, Taufkirchen, Germany) following the manufactures instructions. For this purpose, 50 µL of the plant sample solution was used. The determination of H_2O_2 content is based on the conversion of H_2O_2 to water catalyzed by a horseradish peroxidase while the red peroxidase substrate is oxidized to a fluorescent product. The increasing absorbance of the fluorescent product was measured using a microplate reader (Tecan Infinite 200 PRO) at an excitation wavelength of 540 nm and an emission wavelength of 590 nm. Each sample was measured in duplicate.

2.5. Analysis of metabolites

The metabolite profiles of amino acids, plant hormones and pigments were analyzed to evaluated changes due to UVB and oxidative stress. The frozen plant material was freeze-dried for seven days and then ground twice for 50 s at 25 Hz with 2 metal beads (diameter 3 mm) using a Retsch mill (Retsch MM 400; Retsch GmbH, Haan, Germany).

2.5.1. Determination of carotenoid and chlorophyll content

The determination of carotenoids and chlorophylls This was performed as described in Fitzner et al. (2021). Identification was based on mass spectra and UV/VIS spectra according to the literature (Fig. S2). Quantification was achieved using external calibration from carotenoid standards (CaroteNature GmbH, Munsingen, Switzerland) of *all-trans*-isomers from β-carotene, lutein, and zeaxanthin and 9*Z*-neoxanthin as well as chlorophyll *a* and *b* (Sigma Aldrich Chemie GmbH) at a wavelength of 450 nm. For both violaxanthin isomers the content was calculated using a 9*Z*-neoxanthin calibration curve.

2.5.2. Determination of plant hormones

Plant hormones were determined according to a method developed in our laboratory (Fitzner et al., 2023a). Identification and quantification were performed using an internal calibration with standards for ABA (±, ≥98%), SA (≥99%) and JA (±) (Sigma Aldrich Chemie GmbH, Taufkirchen, Germany) and internal standards ((+)-d6-ABA, d4-SA, d5-JA (mixture of diastereomers, (−)-trans major)) (Toronto Research Chemicals, North York, Canada).

2.5.3. Determination of amino acids

Amino acids were determined as previously described in Ziegler et al. (2015) with modifications. In brief, 5 mg of freeze-dried plant material was extracted with 200 µL methanol/water (60:40 v/v). Samples were shaken for 5 min (1500 rpm, 20 °C) and then centrifuged (10 min, 20 °C, 4500×*g*). From the collected supernatant 25 µl were used for derivatization. To this, 50 µL of a borate buffer (pH = 7.9) and 100 µl Fmoc-Cl (1.4 mg/mL, acetone) (9-Fluorenylmethoxycarbonyl chloride 97%; Sigma Aldrich, Taufkirchen, Germany) were added. Samples were incubated for 5 min while shaking (1500 rpm, 20 °C). Then, the samples were extracted with 500 µL n-pentane three times and the organic layer was removed and discarded. Next, 500 µL acetonitrile/water (5:95, v/v) was added. An SPE MULTI 96-well plate containing CHROMABOND C18 (100 mg, 45 µm; Macherey-Nagel, Düren, Germany) in combination with a vacuum manifold (CHROMABOND Mulit 96 vacuum manifold, Macherey-Nagel, Düren, Germany) was used for sample cleanup and SPE. The SPE was performed as described in Ziegler et al. (2015). The eluate was evaporated until dryness overnight using a Speedvac (Savant SPD111V; Thermo Fischer Scientific Inc., Wilmington, USA). Next, the samples were dissolved in 100 µl acetonitril/water (30:70 v/v) and 100 µL water (+ 0.1% acteic acid). Then, the samples were sonicated (10 min, 20 °C) and centrifuged (2 min, 20 °C, 4500×*g*), and filtered trough a Clear Spin Filter (cellulose acetate, 0.22 µm; Kikser Biotech, Steinfurt, Germany). Finally, the sample were transferred to an HPLC vial and measured immediately. The measurement was performed using an Agilent Technologies 1260 Infinity HPLC coupled with a triple quadrupole, Q-Trap® 6500-MS/MS system (AB Sciex LLC, Framingham, USA). Chromatographic separation was achieved using a Zorbax Eclipse Plus C18 (1.8 µm, 2.1 mm × 50 mm; Agilent Technologies, Waldbronn, Germany) and a mobile phase composed of solvent A: ultrapure water (+ 0.1% acetic acid) and solvent B: acetonitrile (+ 0.1% ultrapure water). The flow rate was set to 650 µL min^{-1}, and a gradient elution was used. Quantification was performed by external calibration with standards of 24 amino acids (Sigma Aldrich, Taufkirchen, Germany) and the internal standards of serine (L-Serine-d3), glutamine (L-Glutamic Acid-d5), tyrosine (L-Tyrosine-d4) and proline (L-Proline-d3) (Toronto research chemicals, North York, Canada).

2.6. Data evaluation and statistical analysis

Data analysis was performed for pigments using TOF Quantitative Analysis (Quant-My-Way) 10.2 (Mass Hunter, USA), for amino acids and plant hormones using Analyst 1.6.2 software (AB Sciex LLC, USA) and further with Excel (Microsoft).

Statistical differences between treatments were tested using Sigma Plot (14.0), with either a Student's t-test, a one way ANOVA followed by post-hoc Tukey's test, or a Two-Way-ANOVA followed by a Bonferroni post-hoc test ($p \leq 0.05$) applied to the data. Correlation analyses were performed using Pearson's method. Data are presented as means ± SEM.

3. Results

3.1. Different doses of UVB lead to eustress or distress in S. europaea (UVBI)

3.1.1. Effect of UVB treatments on phenotype and growth

To assess which UVB treatment leads to eustress or distress, the stress status of plants was evaluated based on plant growth and phenotype in response to different UVB doses. UVB treatment appeared to have a dose-dependent effect on the plant (Fig. 1). The high dose of UVB (5 kJ m^{-2} d^{-1}) resulted in chlorosis and reduced growth. The medium dose of UVB (2.5 kJ m^{-2} d^{-1}) resulted in slight colorless spots, and the low dose of UVB (1.25 kJ m^{-2} d^{-1}) resulted in no phenotypic or growth changes. Thus, primarily the high UVB treatment affected the growth and phenotype of *S. europaea*.

3.1.2. Influence of different UVB doses on metabolite profiles

To determine whether UVB induced eustress or distress, and to define this response, we analyzed amino acids and the plant hormones abscisic acid (ABA), salicylic acid (SA), and jasmonic acid (JA) by LC-MS/MS and photosynthetically active pigments, carotenoids, and chlorophylls, by LC-ToF-MS. The changes in each metabolite were calculated as a fold change compared to the control condition (no UVB). Pigments

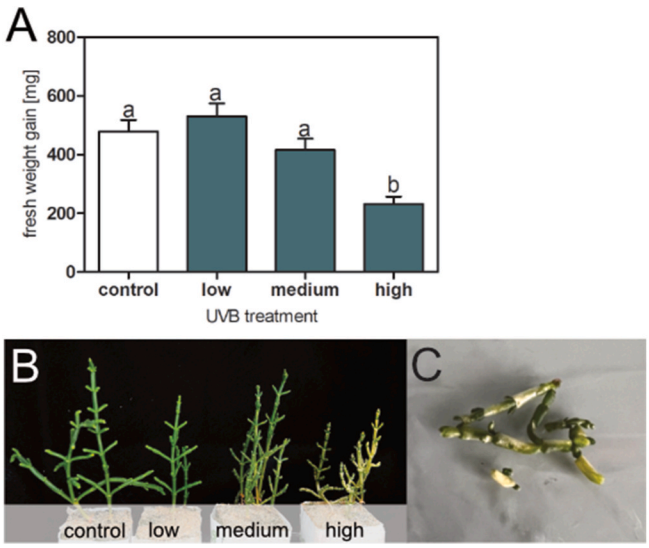

Fig. 1. Influence of different UVB doses (UVB I) on growth and phenotype. A, fresh weight gain during 18 days of UVB treatment. Means ± SEM, letters indicate significant differences between UVB treatments ($p \leq 0.05$, $n = 27$ from 3 experimental replications); B, plant phenotypes. Representative picture from one experiment; C, magnification of chlorosis at high UVB. UVB treatments (285 nm): Low UVB, 1.25 kJ m^{-2} d^{-1}; medium UVB, 2.5 kJ m^{-2} d^{-1}; high UVB, 5 kJ m^{-2} d^{-1}].

were found to be affected by UVB treatment depending on the dose. Medium UVB resulted in an increase in total carotenoids by 0.1-fold and high UVB resulted in a 0.1-fold decrease (Fig. 2A). Total chlorophylls showed a 0.1-fold increase at low and medium UVB and a 0.2-fold decrease at high UVB (Fig. 2B).

The three measured plant hormones responded differently to UVB light, although the response within the three UVB doses differed only in the intensity of the change. ABA content showed a decreased content in all UVB treatments by 0.3-fold at the low and medium UVB, and by 0.4-fold at the high UVB (Fig. 2C). SA content increased 1.7-fold at medium UVB and 7.3-fold at high UVB (Fig. 2D). The JA content increased 1-fold at the medium UVB (Fig. S3A).

Amino acids were mainly affected by medium and high UVB, and only histidine was also affected by low UVB doses (Fig. 2, Table S2). Medium UVB resulted in a 0.3- to 0.7-fold decrease in the levels of tryptophan, tyrosine, valine, threonine, arginine, proline, asparagine, histidine, glycine, phenylalanine, aspartic acid, citrulline, cysteine, and alanine (Table S2). High UVB treatment also affected amino acids, mainly those showing a 0.2- to 0.7-fold decrease with medium UVB (Table S2) (tryptophan, tyrosine, valine, threonine, methionine, glycine, phenylalanine, cysteine and alanine). Notably, the content of glutamine and thus the gln/glu-ratio was increased, as the content of glutamic acid decreased (Fig. 2E and F; Table S2).

In summary, UVB treatment induced dose-dependent changes in the plants' metabolite profile. High UVB induced a decrease in pigment content, amino acids and ABA and an accumulation of SA. Medium UVB resulted in an increase in pigment content, but also in a decrease in ABA content, an increase in SA content, and a decrease in most of the amino acids. Finally, low UVB only slightly affected the metabolite profile, as reflected by a decreased ABA content and an increased content of total chlorophylls. In particular, the plant hormones showed greater changes at high UVB compared to medium and low UVB.

3.2. *Eustress-related carotenoid accumulation under medium UVB treatment (UVB II)*

To evaluate UVB signaling during eustress, plants were exposed to medium UVB treatment and signaling molecules (ABA, SA, and JA), oxidative stress markers (H_2O_2 and SOD activity), and pigment contents were analyzed (Fig. 3/S3). Fold changes showed significant differences between control and UVB treatment, although significant differences between experimental repetitions were also observed.

SOD activity and H_2O_2 negatively correlated in the first repetition with a 0.21-fold increase in SOD activity and a 0.25-fold decrease in H_2O_2 content, while in the second and third repetition no significant changes were observed (Fig. 3A and B). ABA showed decreased levels in response to UVB in all three experiments, with up to 0.6-fold in repetition 2 (Fig. 3C). SA showed increased levels in response to UVB in all three repetitions, up to 4.94-fold in repetition 2 (Fig. 3D). JA showed no differences in response to UVB stress (Fig. S3B). Total carotenoids showed a significant increase with UVB treatment in repetitions 2 and 3, 0.16-fold in repetition 2, but no significant changes in repetition 1 (Fig. 3H). This was also reflected at the level of individual carotenoids, which showed significant or tendential increased contents in response to UVB treatment in repetitions 2 and 3 (Fig. 3E, G, S4A-D). Except for *all-trans*-violaxanthin, the highest increase in response to UVB in repetition 1 was 0.66-fold (Fig. 3F). Among the other carotenoids, zeaxanthin showed the highest increase in repetition 3 at 0.88-fold (Fig. 3E). Total chlorophylls showed only a small but significant increase of 0.06-fold in repetition 3, which was also reflected in the individual chlorophylls, but chlorophyll *b* showed an additional significant increase in repetition 2 (Figs. S4E–G).

In conclusion, we observed changes in the oxidative stress status and carotenoid profile during UVB treatment. The plant hormones ABA and SA showed opposing responses to UVB, with an increase in SA and decrease in ABA.

3.3. *Oxidative stress-related carotenoid accumulation*

To determine whether the induction of carotenoid metabolism under UVB exposure was related to oxidative stress, plants were treated with menadione, which induces oxidative stress in plants. Again, signaling molecules (ABA and SA), oxidative stress markers (H_2O_2 and SOD), and pigments were analyzed. Both menadione treatments resulted in an altered phenotype, reflected by local necrotic spots on the stems, which appeared approximately 10 days after the start of treatment (Fig. S5). Menadione treatment involved applying a measured volume to the plants' shoots, to evaluate the overall amount of treatment applied (Fig. S1). Significant differences between the two experimental regimes (MND I and MND II) were observed, and between menadione treatment and controls, but not between the controls (Fig. 4, Fig. S6).

In the first menadione regime MND I, SOD activity was unaffected by menadione treatment, but H_2O_2 levels showed an increase by 0.36-fold (Fig. 4A and B). In contrast, in the second menadione regime MND II, SOD activity increased by 0.29-fold and H_2O_2 content decreased by 0.16-fold. ABA levels decreased by 0.6-fold under MND I and increased by 0.47-fold under MND II regimes, while SA levels remained unchanged under both regimes (Fig. 4C and D). Total carotenoid content increased by 0.2-fold under MND I and decreased by 0.10-fold under MND II regimes (Fig. 4H). As with the UVB treatment, individual carotenoids showed the same pattern, except for *all-trans*-violaxanthin, which showed no significant changes in response to menadione treatment (Fig. 4F; Figs. S6A–D). The total chlorophyll content increased under MND I by 0.14-fold and decreased by 0.13-fold under MND II treatment regimes, which was also reflected in the individual chlorophylls (Figs. S6E–G). JA showed no difference in response to MND treatment (Fig. S3C).

Correlation analysis between total carotenoid content and SOD activity, H_2O_2 content, ABA content, and SA content of UVB II as well as MND I and II experiments, revealed a significant positive correlation between carotenoids and H_2O_2 ($r = 0.905$, $p = 0.035$) and a tendential negative correlation between SOD activity and carotenoid content ($r = -0.861$, $p = 0.061$) (Fig. S7A). This was reflected in the individual carotenoids, with the exception of *all-trans*-violaxanthin (Table S3).

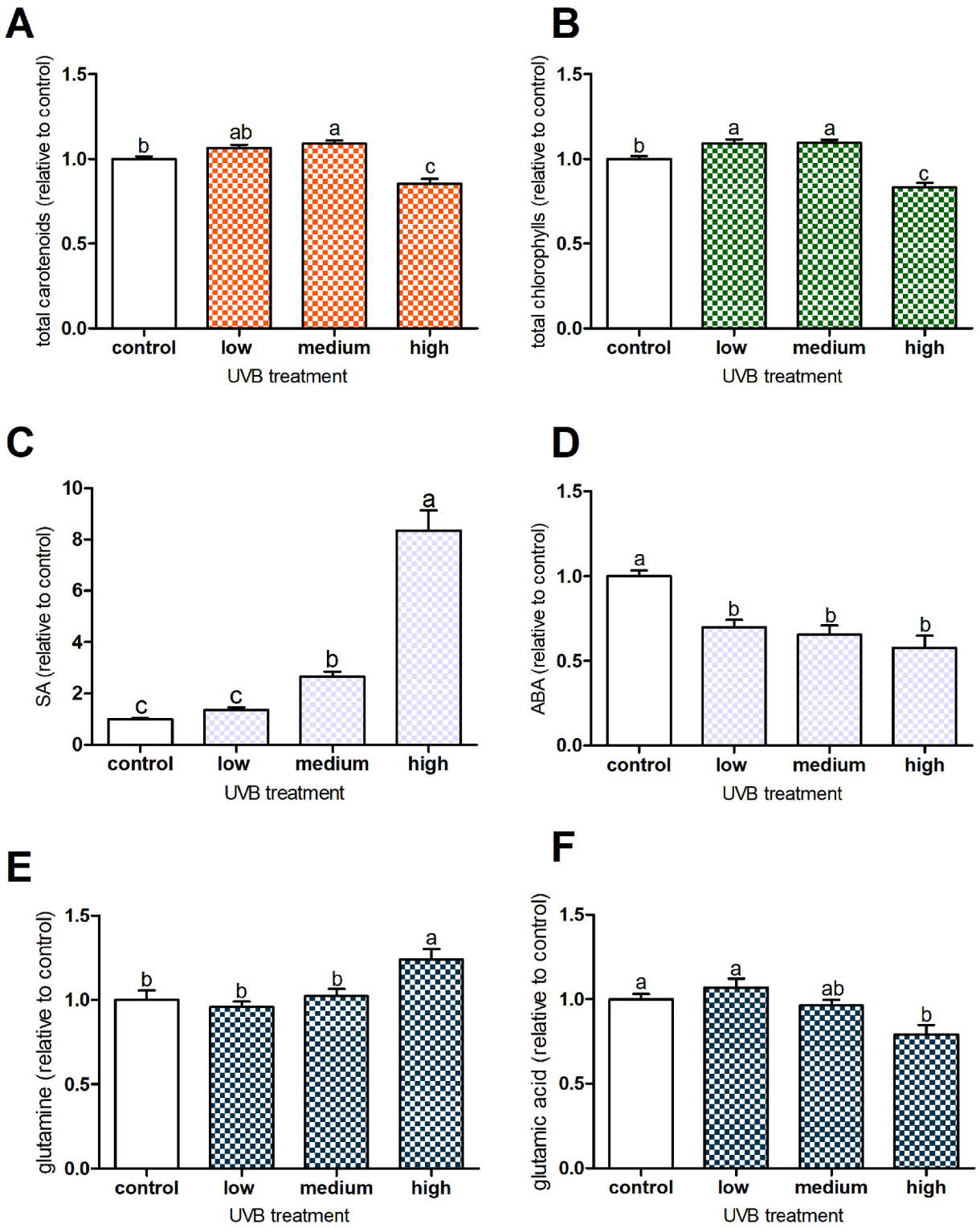

Fig. 2. Influence of different UVB doses (UVB I) on metabolite changes. A, total carotenoid content; B, total chlorophyll content; C, SA content; D, ABA content; E, glutamine content; F, glutamic acid content. Data normalized to respective controls. Means ± SEM, letters indicate significant differences between UVB treatments ($p \leq 0.05$, $n = 27$). Gln/Glu ratio can be found in Table S2. UVB treatments (285 nm): Low UVB, 1.25 kJ m^{-2} d^{-1}; medium UVB, 2.5 kJ m^{-2} d^{-1}; high UVB, 5 kJ m^{-2} d^{-1}. ABA, abscisic acid; SA, salicylic acid.

Furthermore, a significant negative correlation was found between total carotenoid content and ABA content ($r = -0.918$, $p = 0.03$) and a tendential positive correlation between ABA content and SOD ($r = 0.857$, $p = 0.064$); ABA and H_2O_2 showed a negative correlation ($r = -0.773$ $p = 0.125$), but this was not significant (Figs. S7B and C). SA showed no correlation to carotenoids, SOD or H_2O_2 (Fig. S7D).

In summary, menadione treatment affected the oxidative stress status as well as carotenoids and chlorophylls similarly to what was seen in the UVB experiments. ABA also responded to menadione treatment, while SA did not.

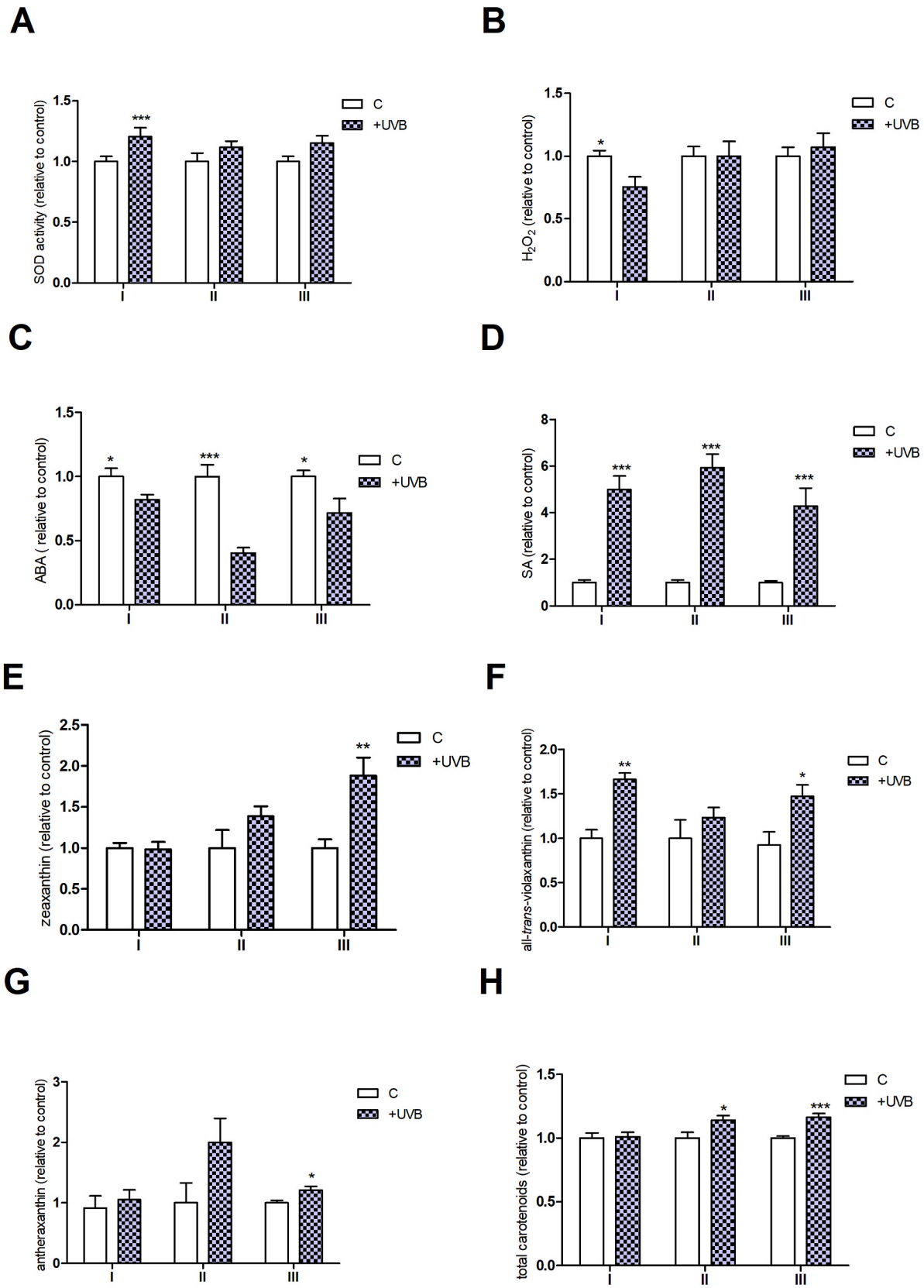

Fig. 3. Influence of medium UVB treatment (UVB II) on ROS, plant hormones and pigments. A, SOD activity; B, H_2O_2 content; C, ABA content; D, SA content; E, zeaxanthin content; F, *all-trans*-violaxanthin content; G, antheraxanthin content; H, total carotenoid content. Data normalized to respective controls. Means ± SEM; asterisks indicate significant differences between UVB treatment and controls (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$; $n = 9$). I, II, III, experimental repetitions. Medium UVB, 2.5 kJ m^{-2} d^{-1}; H_2O_2, hydrogen peroxide; SOD, superoxide dismutase; ABA, abscisic acid; SA, salicylic acid.

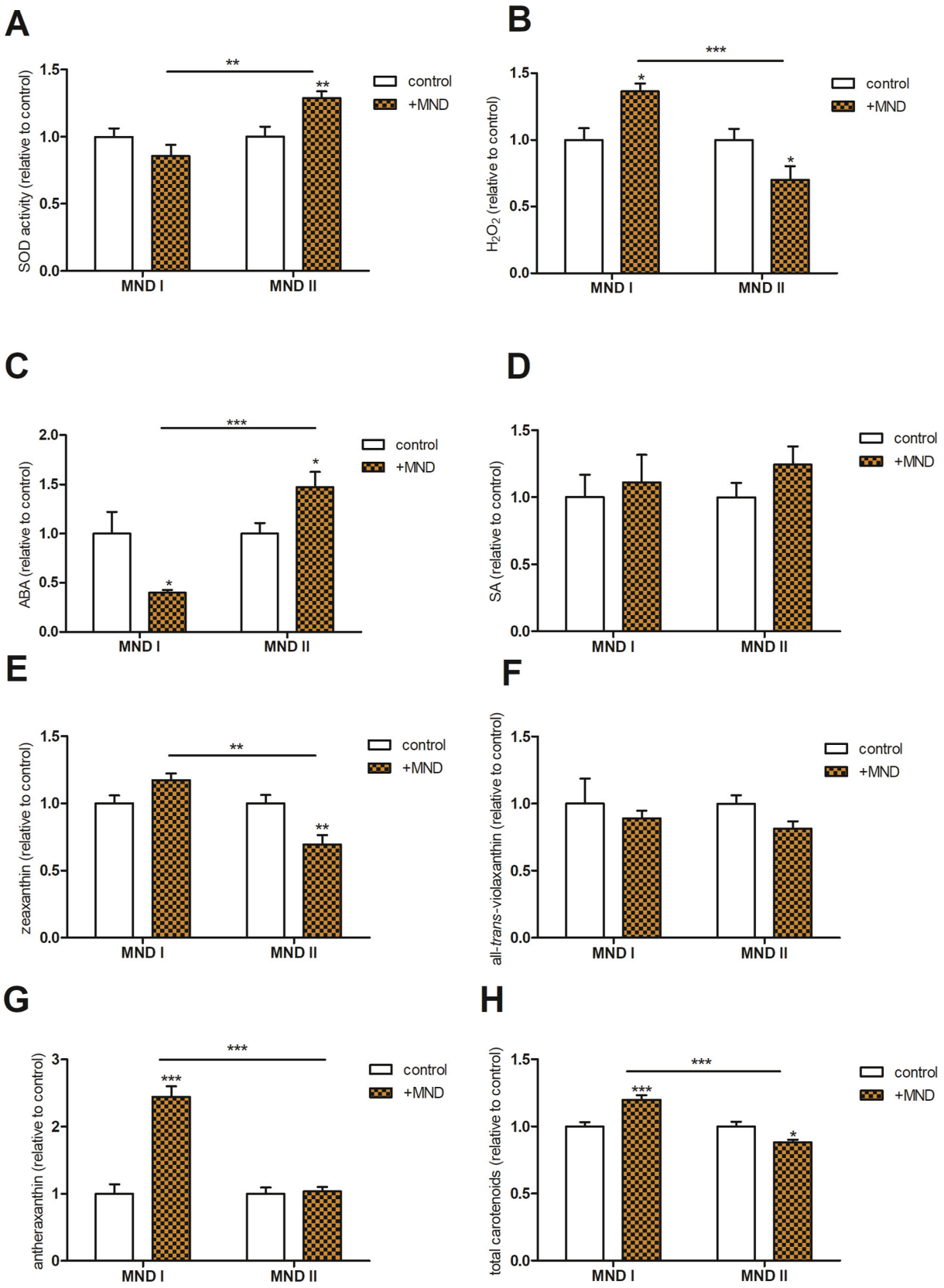

Fig. 4. Influence of menadione treatment on ROS, plant hormones and pigments. A, SOD activity; B, H_2O_2 content; C, ABA content; D, SA content; E, zeaxanthin content; F, *all-trans*-violaxanthin content; G, antheraxanthin content; H, total carotenoid content. Data normalized to respective controls. Means ± SEM, asterisks indicate significant differences between UVB treatment and control, line + asterisks indicate significant differences between time points (*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001; n = 7–8). MND I, first menadione experimental regime; MND II, second menadione experimental regime; H_2O_2, hydrogen peroxide; SOD, superoxide dismutase; ABA, abscisic acid; SA, salicylic acid.

4. Discussion

Many studies have focused on *Arabidopsis thaliana*, but responses to UVB light have been shown to vary among plant species, for example, in *Vitis vinifera* or *Solanum lycopersicum*, among others (Tossi et al., 2019). Thus, it is important to evaluate each plant species individually. Therefore, we aimed to shed light on the UVB response of the halophytic *S. europaea* in controlled environments.

In this study, as a first step, we demonstrated a dose-dependent UVB response reflected in altered carotenoid accumulation and identified ABA and SA as stress markers in the UVB response of *S. europaea*. This confirms that UVB LEDs can be used in indoor farming to evoke eustress or distress in *S. europaea*. Secondly, we continued to analyze individual carotenoids paired with the identified stress markers plus additional stress markers for oxidative stress in response to medium levels of UVB treatment. This revealed a correlation between carotenoid accumulation and oxidative stress.

4.1. UVB-induced eustress and distress

Two pathways in the response to UVB light are related to low/medium or high levels of UVB radiation, and thus with eustress or distress (Hideg et al., 2013). The UVB eustress response is mediated by UVR8, whereas the UVB distress response is mediated by ROS (Hideg et al., 2013). UVR8 is a photoreceptor that detects and is activated by UVB light, leading UVB-related gene expression through multiple interactions with the transcription factors *COP1* (constitutively photomorphogenic 1) and *HY5* (elongated hypocotyl 5) (Hideg et al., 2013; Tossi et al., 2019). This UVB-related gene expression induces, among others, signaling pathways of SA or ABA (Hideg et al., 2013). This leads to the accumulation of antioxidant molecules, such as carotenoids, the increased activity of antioxidant proteins to scavenge ROS, and the activation of DNA-repair enzymes (Nawkar et al., 2013). We demonstrated this in our study at medium UVB, observing slight changes in the phenotype paired with an accumulation of SA and carotenoids.

In UVB distress, high radiation levels result in H_2O_2-induced regulation of gene expression that is independent of *UVR8* (Hideg et al., 2013). UVB exposure leads to insufficient antioxidant capacity to cope with the ROS generated, resulting in DNA damage, chloroplast and mitochondrial damage, and necrosis (Hideg et al., 2013; Nawkar et al., 2013). Here, we observed the occurrence of chlorosis and necrosis, growth inhibition, and a decreased content of photosynthetically active pigments at a high dose of UVB. Furthermore, the altered glutamine/glutamate ratio coupled with the reduced chlorophyll content indicates lower photosynthetic activity, which in turn results in less oxidation of ferredoxin. This reduces glutamate synthase (GOGAT) activity, which is subsequently reflected in lower glutamate and higher glutamine levels (Carillo, 2018). Reduced photosynthetic activity might be explained by photosystem damage caused by UVB light-induced ROS in chloroplasts (Vass, 2012).

Since the low dose of UVB showed almost no differences in the UVB response compared to the controls, it is assumed that it does not cause UVB-induced stress. Therefore, the levels between UVB-induced eustress or distress were determined as between medium UVB treatment (2.5 kJ m^{-2} s^{-1}) and high UVB treatment (5 kJ m^{-2} s^{-1}) at 285 nm UV radiation wavelength in a controlled environment. Even though, the high UVB treatment of 5 kJ m^{-2} s^{-1} is comparable to a UV dose in the natural habitat of *S. europaea* (Costa et al., 2006), it must be considered that a UV treatment in a controlled environment is not comparable to outdoor conditions for instance in regard to intensity and irradiation duration. Additionally, narrow-banded LED radiation (Fig. S9) differs from the UV sunlight spectra, and hence the biologically active dose as well (Table S4). A study in a controlled environment showed effects for a low broad band UVB dose of 1.4 kJ m^{-2} d^{-1} for leafy vegetables (Heinze et al., 2018). Indeed, it should be noted that the UVB dose in combination with the intensity and duration of irradiation, as well as other environmental conditions, varies greatly between a controlled and a natural environment, which may influence the effect of UVB light. For example, in our study UVB light was applied continuously over a period of 18 days, whereas differences in UVB irradiance can arise in the natural habitat due to cloudy weather. In addition, in our study we used narrow-band UVB radiation at 285 nm, whereas in a natural environment UVB radiation covers there a spectrum between 280 nm and 315 nm, with higher intensities from 300 to 315 nm. Since the absorption maximum of UVR8, a specific UV receptor in plants, is approximately at 280 nm, the selected wavelength likely triggers UVR8-related UVB signaling (Díaz-Ramos et al., 2018). Furthermore, not only is UVR8 involved in the osmotic stress response and has been shown to respond to changing salinity, but halophytes have also show that different adaptation mechanisms of the photosynthetic apparatus to salinity stress also influence their response to UVB (Fasano et al., 2014). This suggests that *S. europaea* may show a different UVB response in a natural environment, as it will receive a different UVB spectrum as well as an expected lower biologically effective dose. Further studies should aim to investigate the influence of different narrow-band UVB wavelength on *S. europaea* stress response and metabolism.

4.2. Differentiation of UVB and oxidative stress-mediated signal transduction under eustress conditions

The UVR8-mediated UVB eustress response is very complex and differs among species. To shed more light on UVB signaling in halophytes, we evaluated the response of *S. europaea* to UVB in terms of plant hormone signaling, oxidative stress markers, and photosynthetic pigments. To determine the ROS-related response in UVB signaling, we examined the effects of an oxidative stress-inducing menadione treatment without UVB radiation (Maurino and Flügge, 2008; Noctor et al., 2016). Menadione is a redox active quinone that induces non-specific oxidative stress in plants (Sweetlove et al., 2002).

The plant hormones we analyzed were abscisic acid (ABA), salicylic acid (SA) and jasmonic acid (JA), where ABA and SA are considered to be involved in UVB responses in glycophytes (Bandurska and Cieślak, 2013; Vishwakarma et al., 2017). JA showed significant changes in response to UVB treatment in the first experimental setup (UVB I), but no response to medium UVB treatment in the second experimental setup (UVB II) or to either menadione treatment regime (MND I, II) (Fig. S3). Therefore, it is not included in further discussion.

During UVB stress, ABA regulates the NADPH oxidase, increases H_2O_2, and increases nitric oxide. Our results, in contrast to the literature, show decreased ABA levels in response to medium UVB (Vishwakarma et al., 2017). For example, two studies by (Rakitin et al.)showed increased ABA levels after 24 h of UVB irradiation in *Arabidopsis thaliana* (Rakitin et al., 2008a, 2008b). However, this work mainly focused on glycophytes and it is possible that UVB signaling is different in glycophytes and halophytes. For instance, *UVR8* is also expressed in the context of other abiotic stresses, such as salinity, chilling or drought in different species (Tossi et al., 2019).

ABA is also a key regulator in salinity stress and is important for maintaining osmotic balance and regulating stomatal conductance, among other functions (Karimi et al., 2021). In halophytes, stomatal aperture is associated with maximum photosynthetic rates while minimizing water loss (Lovelock and Ball, 2002). Salinity leads to water stress and thus to higher ABA levels and lower stomatal aperture in *Arabidopsis thaliana* and *Thellungiella salsuginea* (Karimi et al., 2021). It is assumed that medium UVB is associated with a higher rate of photosynthesis due to higher chlorophyll levels, while water loss is unaffected. This leads to a shift in photosynthetic rates to which stomatal aperture must adapt. Since higher ABA levels are associated with lower stomatal aperture (Lovelock and Ball, 2002), it can be assumed that lower ABA levels are associated with higher stomatal aperture.

An interaction between salinity and UVB in ABA signaling is supported by our observations combining UVB and salt stress. In response to

high salt (600 mM), ABA levels increased. However, at both medium and high salinities (200 and 600 mM), ABA levels decreased in response to UVB treatment compared to no UVB (controls; Fig. S8). Furthermore, menadione treatment also led to a decrease in ABA levels. This suggests that the ABA pathway under UVB light is regulated by ROS rather than by *UVR8* in *S. europaea*. Further research is needed to further elucidate the ABA signaling pathway under UVB light in halophytes.

SA is, for example, responsible for the upregulation of antioxidant enzymes, as well as the accumulation of anthocyanins and tocopherol in response to UVB light (Bandurska and Cieślak, 2013; Khan et al., 2015). In our study, SA increased in content in response to UVB treatment, which is consistent with the literature. A study by Bandurska and Cieślak (2013) showed an increase in SA content in response to UVB treatment in barley. Since SA neither responded to menadione treatment nor correlated with carotenoids, SOD and H_2O_2, it suggests that the SA pathway is *UVR8*-mediated and involved in the UVB response of *S. europaea*, but not related to carotenoid accumulation (Fig. S7).

4.3. UVB and oxidative stress related carotenoid accumulation under eustress conditions

The results of our study suggest that carotenoid accumulation is related to oxidative stress. In addition, our correlation analysis revealed a correlation between carotenoid content, H_2O_2, SOD and ABA (Fig. S8), suggesting that the ABA pathway, oxidative stress status, and carotenoid accumulation are linked. The oxidative status changes during UVB stress, for example, due to the formation of H_2O_2 in the photosystem II (Vass, 2012). Since, carotenoids, especially zeaxanthin, are able to scavenge ROS and thus protect the photosystems from damage, a positive correlation between carotenoids and H_2O_2 levels is not surprising. Overexpression of the *S. europaea* phytoene synthase (*PSY*), a key enzyme in carotenoid biosynthesis, in *A. thaliana* resulted in increased salinity tolerance, which was exhibited by lower levels of oxidative stress, and an increased photosynthetic rate and photosystem II activity (Han et al., 2008). Also, halophytes are known to send rapid signals through H_2O_2 (Bose et al., 2014). Since we propose that ABA is negatively related to photosynthetic rates, and ABA is also related to carotenoid biosynthesis (Lovelock and Ball, 2002; Cazzonelli, 2011), a correlation between carotenoids and ABA is also not surprising.

ABA is also involved in the regulation of ROS signaling (Tuteja, 2007). In contrast, increasing SOD activity negatively correlated with H_2O_2 and carotenoid content, which could be related to a daily shift in SOD activity. A study by Köhler et al. (2017) showed a daily difference in SOD activity in response to UVB light, which peaked at midday (12:00 h) and then decreased to control levels. In contrast, peroxidase (POD) activity peaked in the afternoon and then slowly declined. Whereas SOD converts superoxide to H_2O_2, PODs reduce H_2O_2 to water (Hasanuzzaman et al., 2020; Bindoli and Rigobello, 2013). Many enzymes reduce H_2O_2, such as the ascorbate peroxidase or catalase, but class III PODs have been suggested to be the main H_2O_2-scavenging enzyme under UVB stress (Köhler et al., 2017).

In our first experimental replicate (medium UVB), we observed higher SOD activity combined with lower H_2O_2 levels in response to UVB, whereas in the second and third repetitions, we observed lower SOD activity, thus tended to observe higher H_2O_2 levels than in the first replicate. This suggests that in the first replicate, SOD activity was higher due to an activated antioxidant system, resulting in lower H_2O_2 levels, while in the second and third replicates SOD is less active, resulting in rising H_2O_2 levels. Since POD activity peaks later in the day, the degradation of H_2O_2 has not yet occurred. This is also consistent with the increasing accumulation of carotenoids in the second and third repetitions, which correlates with increasing H_2O_2 levels.

Notably, when looking at individual carotenoids, *all-trans*-violaxanthin showed increased levels in all replicates (being significant in two replicates). *All-trans*-violaxanthin has been identified as the only carotenoid that responds to UVB stress in many cases (Badmus et al., 2022a); however its role in the UVB response remains uncertain. However, it assumed not to be related to protecting the photosynthetic machinery (Badmus et al., 2022b). This corresponds with no correlation between ROS and *all-trans*-violaxanthin, while zeaxanthin showed a tendency to correlate with ROS (Table S3). A possible explanation could be different regulation of carotenoid metabolism within the xanthophyll cycle. This is supported by the finding that *all-trans*-violaxanthin content does not change with menadione treatment, whereas zeaxanthin and antheraxanthin, as intermediates of the xanthophyll cycle, increase with increased H_2O_2. This suggests that the accumulation of zeaxanthin, which is related to scavenging H_2O_2, is ROS mediated and the accumulation of *all-trans*-violaxanthin, is mediated by UVR8. In conclusion, combination of UVR8-and ROS-mediated regulation of carotenoid biosynthesis under UVB eustress is most likely. Further research is needed to unravel the UVB induced changes in xanthophyll cycle pigments.

5. Conclusion

In conclusion, this study revealed levels of UVB doses (at 285 nm) that lead to eustress and distress in *S. europaea* in a controlled environment and draws a link between carotenoid accumulation and oxidative stress. The study provides insights into how UVB signaling functions in halophytes and how it differs from glycophytes. Further studies could aim to investigate UVB wavelengths more closely related to sunlight spectra to draw a link between natural and controlled environments in UVB response. A better understanding of UVB-related carotenoid accumulation as well as the metabolic crosstalk between different biosynthetic pathways and the analysis of other antioxidant metabolites, especially flavonoids, as they have been shown to be increased under UVB light, can contribute to improving the nutritional profile of halophytes grown with UVB LEDs in indoor farming. This may be important when considering future agrifood systems that need to be adapted to the needs and demands of a changing world.

Funding

Federal Ministry of Education and Research [Grant number 031B0730 A], as part of the project food4future.

Compliance with ethics requirements

This article does not contain any studies with human or animal subjects.

CRediT authorship contribution statement

Maria Fitzner: Data curation, Investigation, Methodology, Writing – original draft. **Monika Schreiner:** Conceptualization, Supervision, Funding acquisition, Writing – review & editing. **Susanne Baldermann:** Conceptualization, Supervision, Methodology, Funding acquisition, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

We would like to thank Martin Weiland for helping with the setup of

the UVB treatment.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jplph.2023.154124.

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Publication IV

Supplementary data

Title: Between eustress and distress: UVB induced changes in carotenoid accumulation in halophytic *Salicornia europaea*

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Supplemental Material

Supplemental Table S1 Composition of nutrient solution used in the experiments. Values were provided by the manufacturer.

Nutrient solution	
NH_4NO_3 (g L^{-1})	4.98
$Ca(NO_3)_2$ (g L^{-1})	1.04
KNO_3 (g L^{-1})	0.81
iron chelate (ppm)	8
KH_2PO_4 (g L^{-1})	0.31
$MnSO_4$ (mg L^{-1})	2.5
$MgSO_4$ (g L^{-1})	0.54
$Na_2[B_4O_5(OH)_4]\cdot 8H_2O$ (mg L^{-1})	3.6
$CuSO_4$ (mg L^{-1})	0.2
Na_2MoO_4 (mg $L^{-1}L$)	0.1
$ZnSO_4$ (mg L^{-1})	0.4
pH	6.2

Supplemental Table S2 Influence of different UVB doses (UVB I) on the content of amino acids. Means ± SEM, color indicates significant differences between the UVB treatments ($p \leq 0.05$, $n = 27$) relative to controls. Green indicates a decreased fold change; purple indicates an increased fold change. UVB treatments (285 nm): Low UVB, 1.25 (kJ m^{-2} d^{-1}); medium UVB, 2.5 (kJ m^{-2} d^{-1}); high UVB, 5 (kJ m^{-2} d^{-1}). GABA, γ-aminobutyric acid; gln, glutamine; glu, glutamic acid.

UVB treatment	Control			Low			Medium			High		
Tyrosine	1.00	±	0.18	0.59	±	0.13	0.27	±	0.04	0.43	±	0.16
Tryptophan	1.00	±	0.09	0.84	±	0.11	0.38	±	0.05	0.30	±	0.06
Valine	1.00	±	0.14	0.64	±	0.12	0.33	±	0.04	0.32	±	0.08
Threonine	1.00	±	0.05	0.98	±	0.09	0.65	±	0.04	0.61	±	0.05
Serine	1.00	±	0.04	0.96	±	0.07	0.83	±	0.04	0.85	±	0.05
Arginine	1.00	±	0.06	0.97	±	0.10	0.53	±	0.05	1.00	±	0.16
Proline	1.00	±	0.05	0.78	±	0.09	0.67	±	0.04	1.00	±	0.13
Asparagine	1.00	±	0.04	1.02	±	0.08	0.76	±	0.04	0.82	±	0.05
Methionine	1.00	±	0.03	1.06	±	0.06	0.96	±	0.04	0.76	±	0.05
Histidine	1.00	±	0.07	0.69	±	0.08	0.59	±	0.05	0.75	±	0.10
GABA	1.00	±	0.08	0.99	±	0.10	0.92	±	0.08	1.09	±	0.11
Glycine	1.00	±	0.07	0.83	±	0.10	0.45	±	0.04	0.38	±	0.05
Phenylalanine	1.00	±	0.06	0.98	±	0.08	0.64	±	0.05	0.52	±	0.04
Aspartic acid	1.00	±	0.05	1.16	±	0.09	1.06	±	0.07	0.83	±	0.09
Citrulline	1.00	±	0.09	1.25	±	0.12	1.28	±	0.14	1.33	±	0.20

Cysteine	1.00	±	0.05	0.93	±	0.08	0.67	±	0.04	0.57	±	0.04
Alanine	1.00	±	0.06	1.00	±	0.08	0.69	±	0.04	0.54	±	0.04
Glutamine	1.00	±	0.06	0.96	±	0.03	1.02	±	0.04	1.24	±	0.06
Glutamate	1.00	±	0.03	1.07	±	0.05	0.96	±	0.03	0.79	±	0.06
Gln/Glu ratio	1.02	±	0.07	0.95	±	0.05	1.07	±	0.04	1.67	±	0.12
	0.3 - 0.45			0.45 - 0.65			0.65 - 0.85			1.5 - 2.0		
	1.2 - 1.5			1.0 - 1.2								

Supplemental Table S3 Correlation analysis between individual carotenoids and SOD activity and H_2O_2 content, of UVB II and MND I, II. Pearson correlation. H_2O_2, hydrogen peroxide; SOD, superoxide dismutase.

	SOD		H_2O_2	
	Pearson *r*	*p*-value	Pearson *r*	*p*-value
Antheraxanthin	-0.958	0.010	0.903	0.036
9*Z*-Neoxanthin	-0.756	0.140	0.868	0.057
9*Z*-Violaxanthin	-0.641	0.244	0.844	0.073
β-Carotene	-0.921	0.026	0.955	0.011
Lutein	-0.798	0.105	0.849	0.069
Zeaxanthin	-0.820	0.089	0.868	0.057
All-trans-violaxanthin	0.206	0.739	-0.257	0.676

Supplemental Table S4 Biologically effective dose (BED) of UVB treatments with narrow band LED lamps.

UVB treatment	BED [kJ m^{-2}]*
Low (1.25 kJ m^{-2} d^{-1})	3.0
Medium (2.5 kJ m^{-2} d^{-1})	9.2
High (5 kJ m^{-2} d^{-1})	25.5

*Calculated after Caldwell

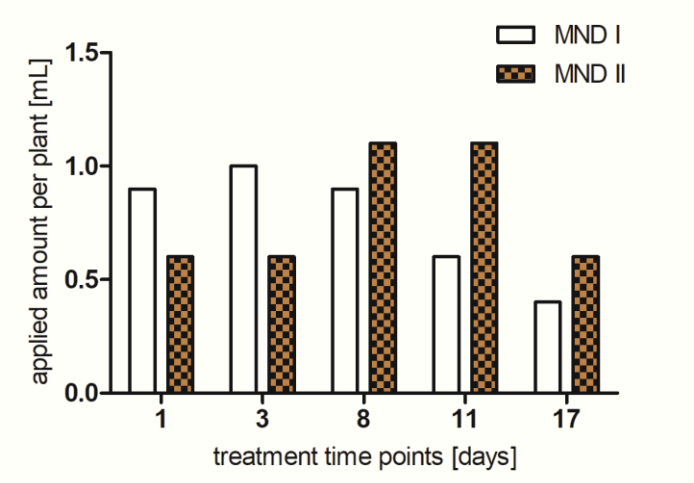

Figure S1 Menadione (MND, 315 µM) application in the first (MNDI) and second (MNDII) experimental regime. Applied amounts shown in amount of menadione solution applied per plant per treatment time point.

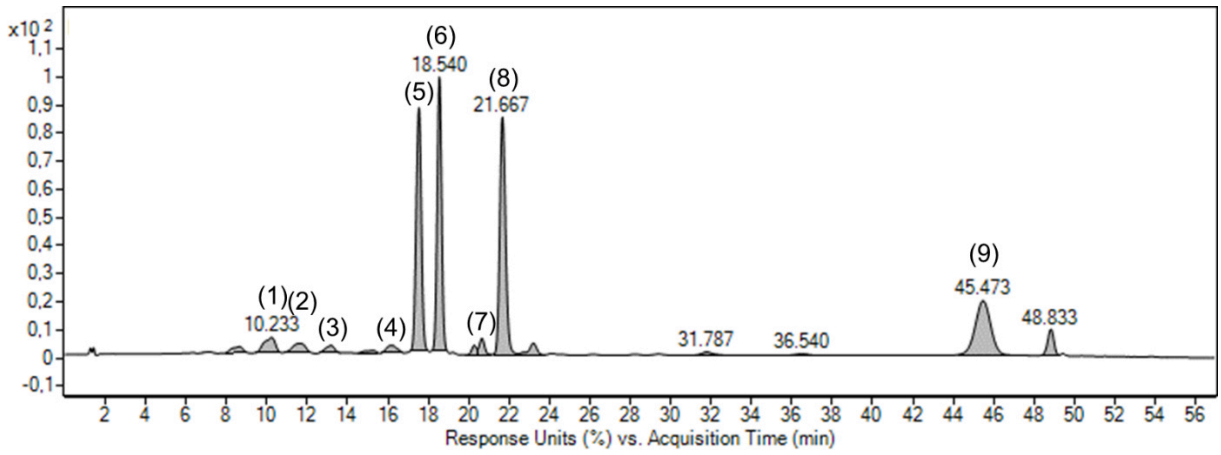

Figure S2 HPLC-DAD chromatogram at 450 nm showing identified chlorophylls and carotenoids in *Salicornia europaea.* (1) *all-trans*-violaxanthin; (2) 9*Z*-neoxanthin; (3) 9*Z*-violaxanthin; (4) antheraxanthin; (5) chlorophyll *b*; (6) lutein; (7) zeaxanthin; (8) chlorophyll *a*; (9) β-carotene.

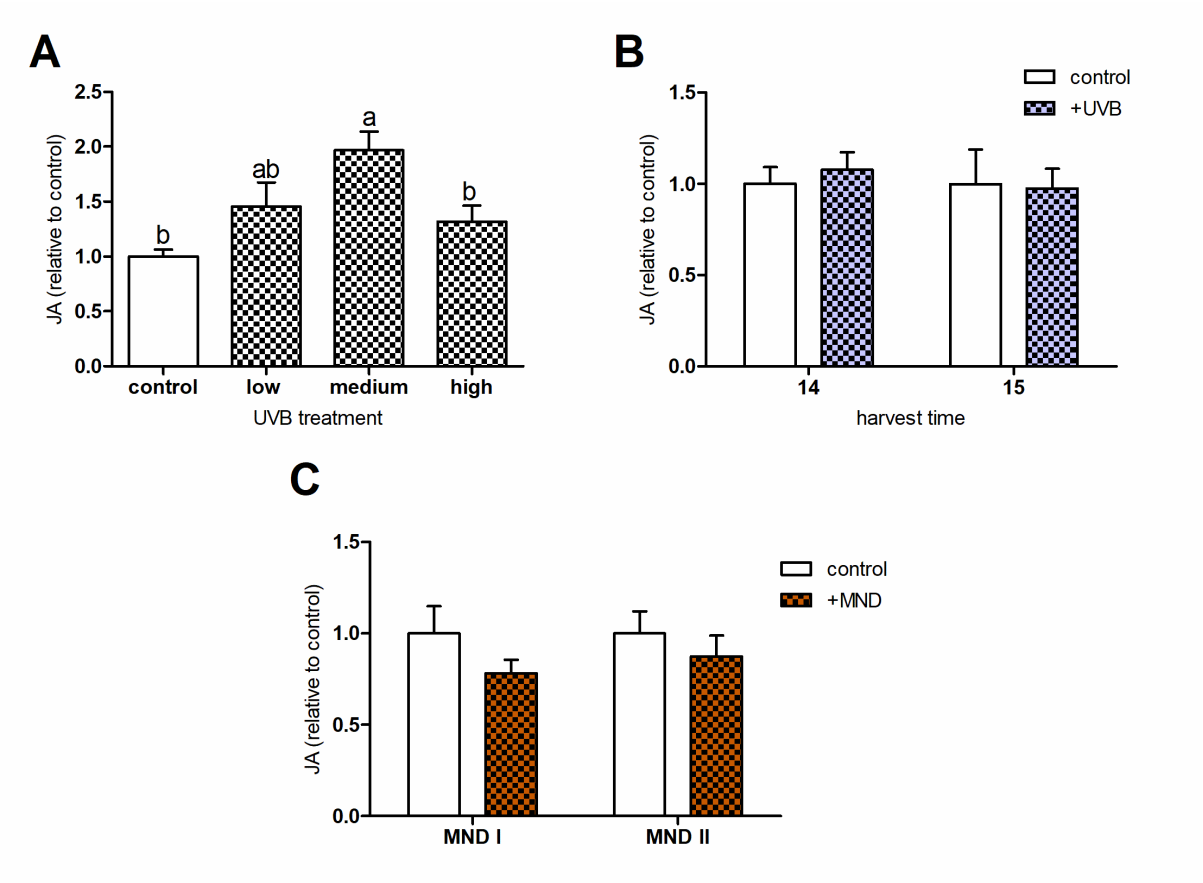

Figure S3 Influence of UVB treatment on jasmonic acid (JA) content. A, influence of different UVB doses (UVB I), means ± SEM, letters indicate significant differences between UVB treatments ($p \leq 0.05$, n = 27) and control; B, influence of moderate UVB treatment (UVBII), means ± SEM, C, influence of menadione treatment, means ± SEM. MND I, first menadione experiment; MND II, second menadione experiment; JA, jasmonic acid; UVB treatments (285 nm): Low UVB, 1.25 (kJ m^{-2} d^{-1}); medium UVB, 2.5 (kJ m^{-2} d^{-1}); high UVB, 5 (kJ m^{-2} d^{-1}).

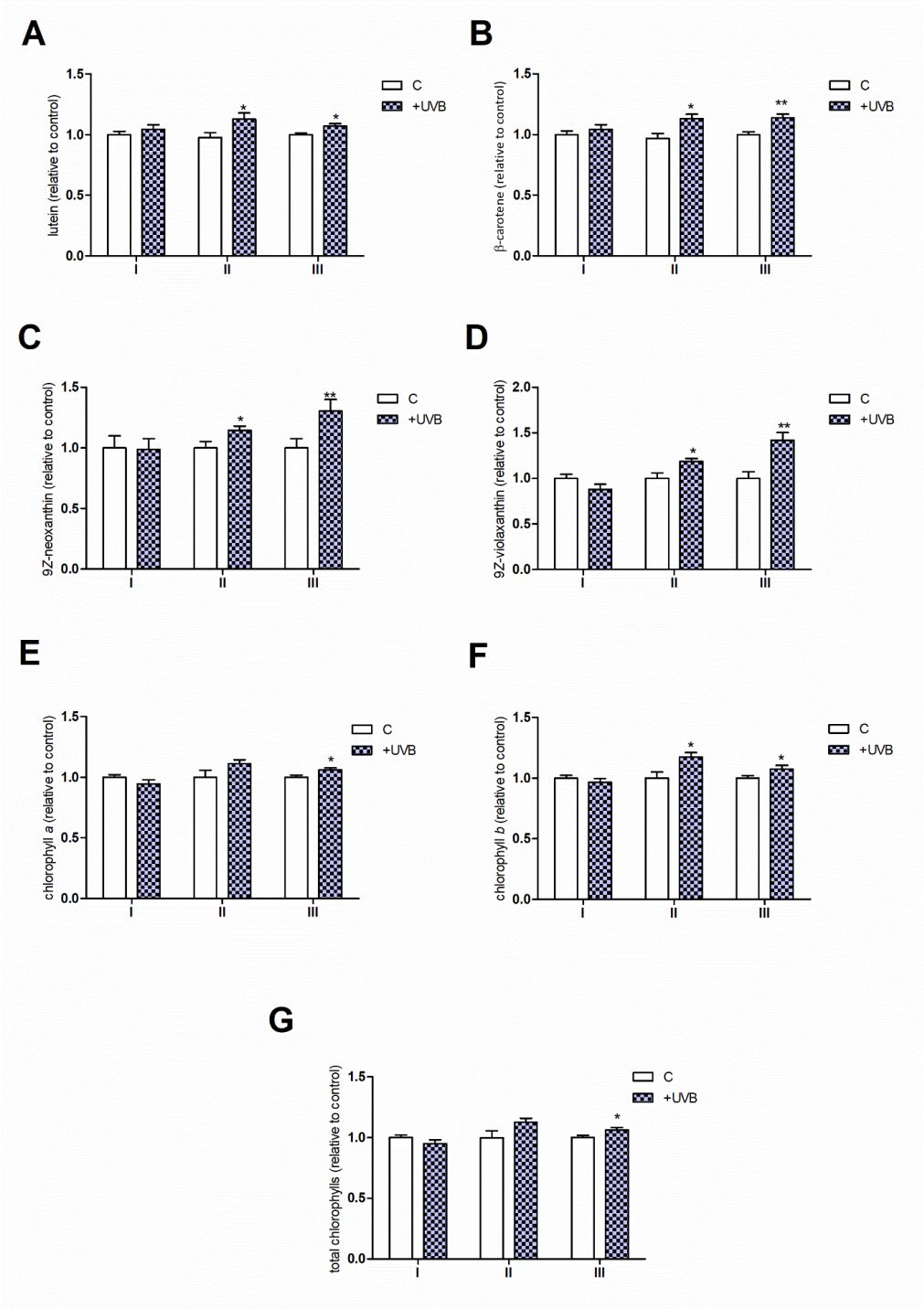

Figure S4 Influence of moderate UVB treatment (UVB II) on individual carotenoids and chlorophylls. A, lutein content; B, β-carotene content; C, 9*Z*-neoxanthin content; D, 9*Z*-violaxanthin content; E, chlorophyll *a* content; F, chlorophyll *b* content; G, total chlorophyll content. Data normalized to respective control. Means ± SEM, asterisks indicate significant difference between UVB treatment and control in one experimental repetition (*p ≤ 0.05, **p ≤ 0.01, ***p≤ 0.001, n = 9). I, II, III, experimental repetition. UVB treatment (285 nm): medium UVB, 2.5 (kJ m^{-2} d^{-1}).

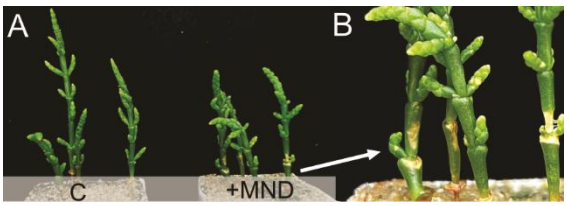

Figure S5 Influence of menadione on 9-week-old plants after 18 days of treatment in MND I. A, control and menadione (MND) treatment; B, magnification of A (+MND) showing morphological changes induced by menadione.

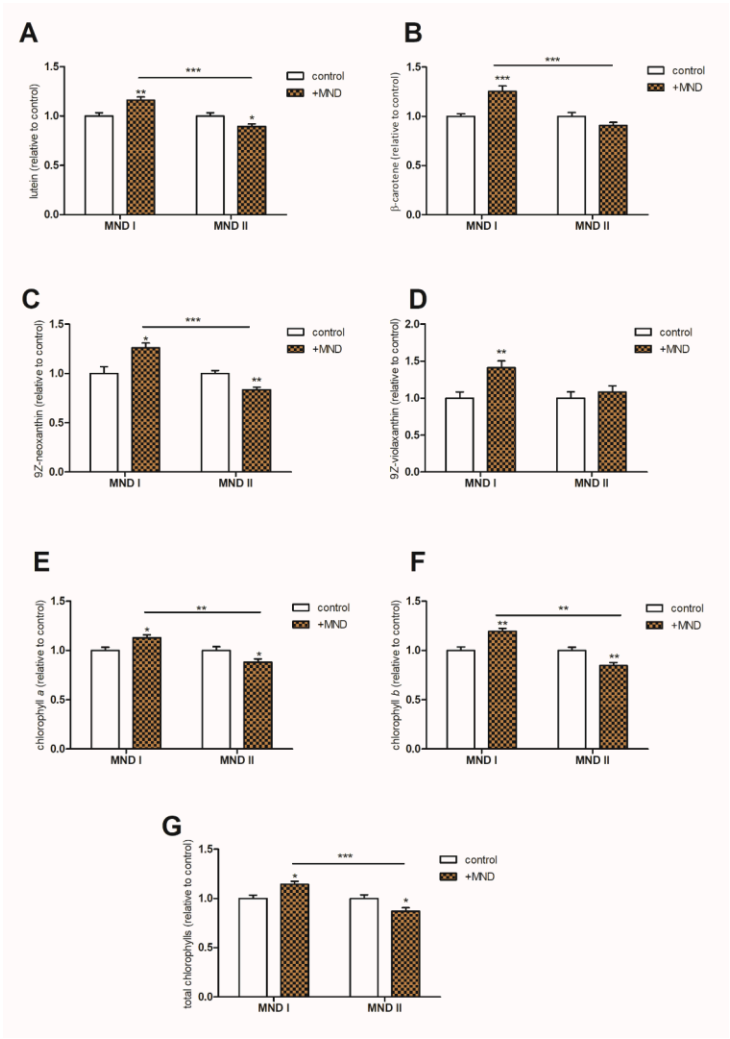

Figure S6 Influence of menadione treatment on individual carotenoids and chlorophylls. A, lutein content; B, β-carotene content; C, 9*Z*-neoxanthin content; D, 9*Z*-violaxanthin content; E, chlorophyll *a* content; F, chlorophyll *b* content; G, total chlorophyll content. Data normalized to respective controls. Means ± SEM, asterisks indicate significant differences between UVB treatment and controls, asterisks and line indicate significant differences between MND I and MND II (*p ≤ 0.05, **p ≤ 0.01, ***p≤ 0.001, n = 7-8). MND I, first menadione experimental regime; MND II, second menadione experimental regime.

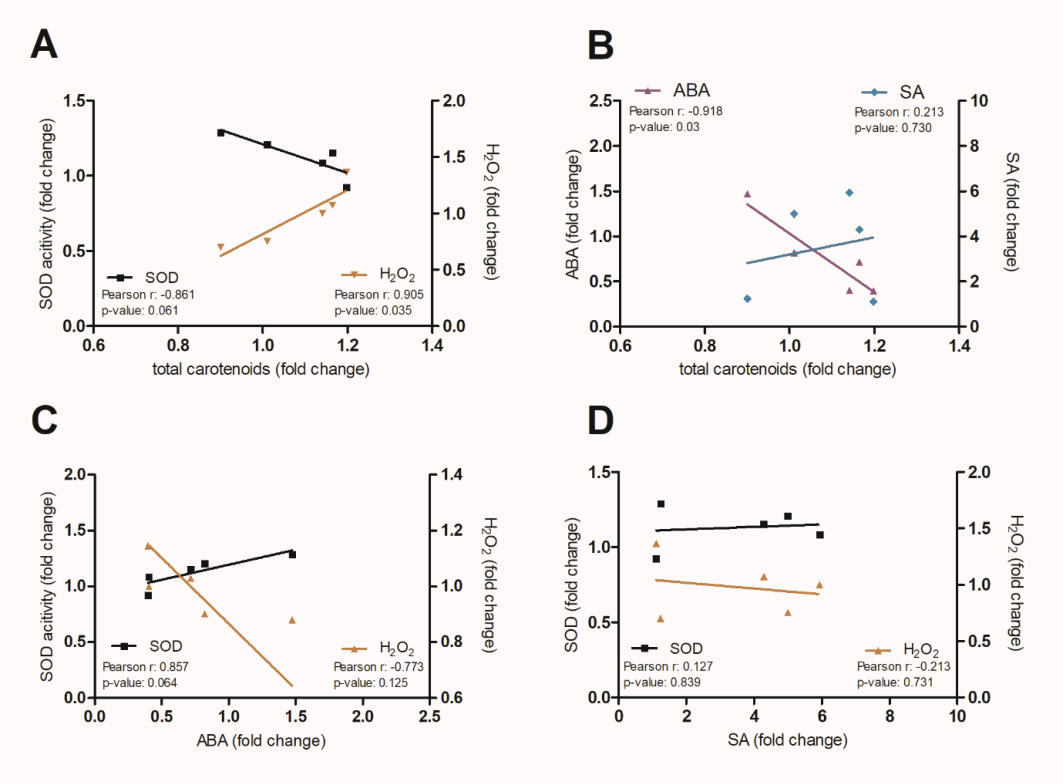

Figure S7 Correlation analysis between total carotenoid content, SOD activity, H_2O_2 content, ABA content and SA content after UVB II and MND I, II treatment. Data are presented as means of fold change. Pearson correlation. A, total carotenoid content to SOD activity and H_2O_2 content; B, total carotenoid content to ABA content and SA content; C, ABA content to SOD activity and H_2O_2 content, and D, SA content to SOD activity and H_2O_2 content. H_2O_2, hydrogen peroxide; SOD, superoxide dismutase; ABA, abscisic acid; SA, salicylic acid. *Pearson correlation for individual carotenoids can be found in Table S3.

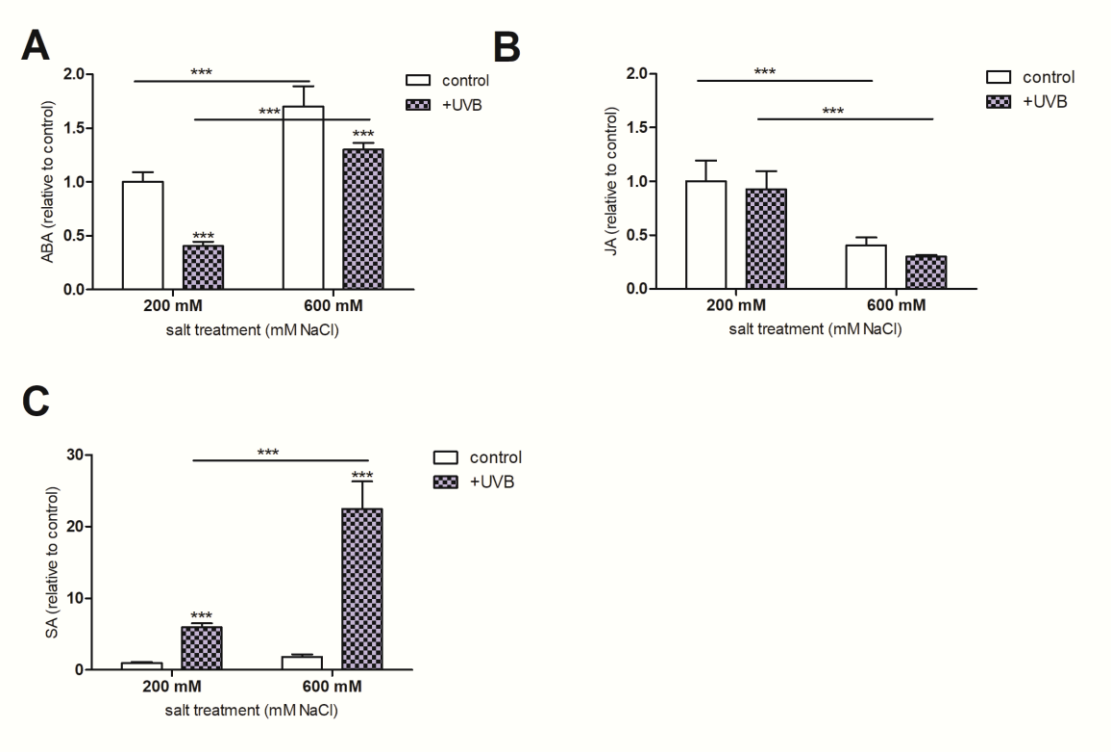

Figure S8 Influence of salt and UVB treatment on *S. europaea.* Effect of salt (200 mM and 600 mM NaCl) and UVB treatment (control and medium UVBII) on plant hormones after 18 days of treatment. Means ± SEM, asterisks indicate significant differences between UVB treatment and controls, asterisks and line indicate significant differences between 200 mM and 600 mM salt (***p≤ 0.001, n = 9). UVB treatment (285 nm): medium UVB, 2.5 (kJ m^{-2} d^{-1}); ABA, abscisic acid; SA, salicylic acid; JA, jasmonic acid.

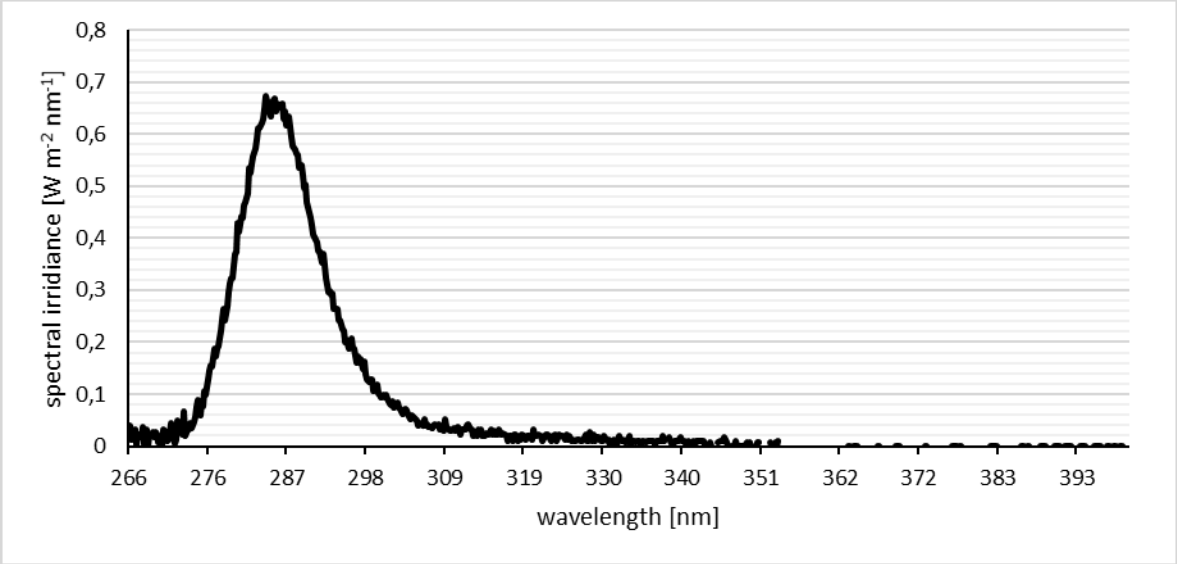

Figure S9 Spectra of narrow-banded (285 nm) UVB LED lamps measured with a spectrophotometer (Ocean Insight, US).

Discussion

1. Outline

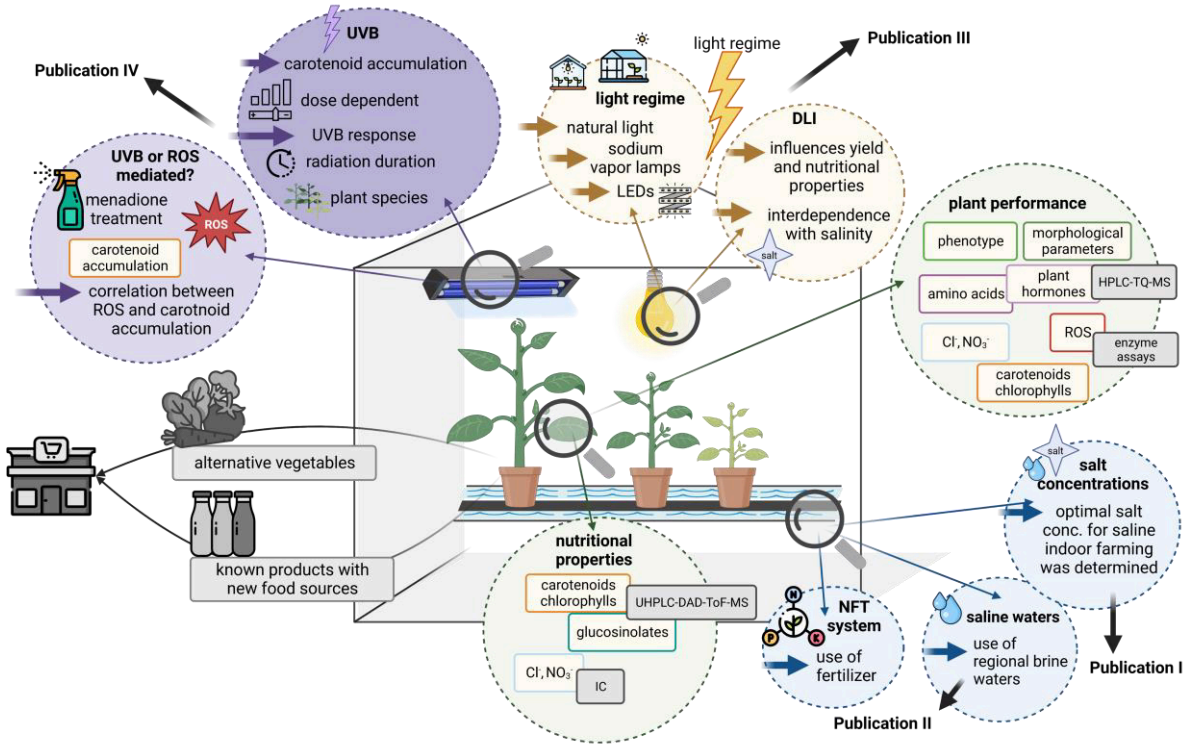

Figure 5 Schematic overview of aspects considered in this thesis about saline indoor farming with halophytes. IC, ion chromatography. UHPLC-DAD-ToF-MS, ultra-high performance liquid chromatography with diode array detection coupled to time-of-flight mass spectrometry. HPLC-TQ-MS, high performance liquid chromatography coupled to a triple quadrupole mass spectrometer. LEDs, light emitting diode lamps. ROS, reactive oxygen species; DLI, daily light intake; conc., concentrations. This figure was created using BioRender.com.

In this thesis, the feasibility of five different halophyte species for saline indoor farming to produce alternative vegetables with an improved nutritional profile for human consumption was investigated in four plant studies. Therefore, different methods were optimized for the determination of phytohormones, amino acids, ROS and anions. HPLC-MS$^{(3)}$, enzymatic methods and ion chromatography were used. In addition to chloride and nitrate, which are of interest from a nutritional point of view, the plant physiologically relevant sulfate and phosphate concentrations were also determined in nutrient solutions, soil and plant material. However, no significant results or interesting patterns were observed and thus not further subject to the discussion.

The subject of the first plant study was the suitability of halophytes as alternative vegetables in a saline indoor farming system and the influence of salinity on nutritional quality. The feasibility of halophytes for saline indoor farming was demonstrated for the first time (Publication I). The optimal salt concentrations for growth and nutritional profile were determined. The influence of salt concentration on nutritional properties was shown to be species specific and related to the salt tolerance mechanism. An interdependence of nitrate and chloride levels was shown (2.1.2 Fertilization and 3.1.3.1 Influence of salt). This study demonstrates the potential of halophytes as alternative vegetables produced in saline indoor farming (2.2 Halophytes as alternative vegetables). It also shows the need to consider the salt tolerance mechanism when introducing new halophyte species in terms of their performance in the system and their nutritional properties.

In the second study, the feasibility of brine water for saline indoor farming of halophytes was evaluated using the halophyte *S. europaea* (Publication II). The use of regional brine water was tested as a regional saline water resource to improve the sustainability of the indoor farming system (2.1.1 Sustainability). The brine water was shown to have a different salinity composition, but no negative effects on growth and nutritional quality in terms of pigment content were observed. This is the first study on halophyte indoor farming using brine waters and demonstrates the potential of brine water for halophyte cultivation and saline agriculture.

The third study focused on the interaction between salinity and light regime and its influence on halophyte growth and nutritional quality (Publication III). The interaction between light regime and salinity was demonstrated. This interaction was also shown to influence photosynthetic pigment content and profile and was observed to be species specific (3.1.1.2 Influence of light regime). This study demonstrates that light and salinity interact in influencing halophytes and that the different salt-tolerance mechanisms could play an important role in salt-stress induced adaptation of photosynthesis (3.2 Interaction of salt, PAR and UVB light).

The fourth study investigated the possibility of increasing the carotenoid content of halophytes using narrow-band UVB LEDs (Publication IV). It was shown that UVB light can be used to increase carotenoid content in the halophyte *S. europaea* (3.1.1.2 Influence of light regime). In addition, the response of halophytes to UVB light was shown to be dependent on plant species, UVB dose and UVB irradiation duration (2.2.2 Use of UVB light in halophyte cultivation). Furthermore, the study revealed a relationship between changes in ROS homeostasis and carotenoid accumulation under eustress UVB exposure. It is suggested that there is a difference between a ROS-mediated and a UVB-mediated UVB response and gave an indication that the UVB response in halophytes may be different from that in glycophytes.

2. Saline indoor farming with halophyte crops for future food production

2.1 Saline indoor farming system

The system was optimized in terms of temperature, humidity, fertilization and lighting. Using a hydroponic system, like an NFT system, has several advantages and it is the most popular indoor farming system (Wong et al., 2020). Halophytes have shown to be feasible for cultivation in an NFT system (Publication I). The automation of the system offered advantages to soil-based systems in terms of manageability. However, in a saline system, there is a possibility of salt accumulation. Depending on the system setup, water evaporation and subsequent salt accumulation in the water tanks may occur. Therefore, a setup with minimal water evaporation and constant monitoring of the salt concentration is necessary (Publication II, Fig. S4).

Indoor farming is a very energy-intensive production. The main cost of indoor farming is the electricity (Pennisi et al., 2019), and since the cost of electricity has been an emerging issue in Europe and especially in Germany in the last year (Shaffer, 2022), more efficient energy use is one of the main targets in developing a sustainable and resilient indoor farming system.

2.1.1 Sustainability

Sustainable development is stated in the UN Report "Our Common Future" as: "Sustainable development seeks to meet the needs and aspirations of the present without compromising the ability to meet those of the future." (World Commission on Environment and Development, 1987). A sustainability assessment of a product or technology is very complex and involves several analyses of aspects such as a life cycle assessment (LCA) or a carbon footprint (Hou and O'Connor, 2020). Since a sustainability assessment is beyond the scope of this thesis, only some aspects of how to improve the sustainability of the system will be discussed. As mentioned above, energy use efficiency (EUE) is critical to improve sustainability and profitability. Considering the cost of electricity, lighting is one of the most energy consuming parts and therefore the lighting system needs to be carefully evaluated (van Delden et al., 2021). Looking at the energy requirements of a hydroponic facility, 40% of the energy is consumed by artificial lighting, 42% by heating, ventilation and air conditioning (HVAC), and 17% by water and air pumping (Gillani et al., 2023). To optimize light use efficiency (LUE), light intensity, photoperiod and light sources can be modified, but also spectral quality can affect LUE (van Delden et al., 2021). For example, the photosynthetic photon number efficacy (PPNE) is a term used to rate the effectiveness of a light

source for its photosynthetic active radiation. The PPNE is higher for LED lamps than for fluorescent lamps (Pennisi et al., 2019). Therefore, the use of LED lamps is a system improvement in terms of LUE (2.1.3 Lighting). Another important factor for a sustainable agrifood system is water use efficiency (WUE). This is already higher in indoor farming compared to conventional agriculture (Pennisi et al., 2019), and can be further increased with the use of saline water. By using local brine water, additional freshwater is conserved and additional salt is not introduced into the local ecosystem through salt-enriched water (Publication II). WUE can be influenced by light regime and salinity, for example through changes in stomatal aperture, which affects the effective use of water by plants (Pennisi et al., 2019). The use of a NFT system comes with a minimal water use, but also improves the EUE, due to an optimal plant density and growth (2.1.2 Fertilization) (Olympios and Choukr-Allah, 1999, Gillani et al., 2023). For example, the NFT system showed a higher EUE for growing lettuce compared to deep water culture (DWC) (Gillani et al., 2023). The growing medium is also a factor that affects the sustainability of the system. Initially, the plants were grown in a mixture of soil and quartz sand, which was disadvantageous due to the flushing of small particles that can clog filters. After testing a selection of materials, Rockwool cubes were chosen. Rockwool is widely used in greenhouse horticulture because of its ability to distribute the nutrient solution. However, it is very expensive and not recyclable and thus unsustainable (Olympios and Choukr-Allah, 1999). New materials, such as biodegradable polymers from cellulose and polylactic acid, can be used to create new composite materials for cultivation systems (Fricke et al., 2022).

2.1.2 Fertilization

In terms of sustainability, fertilizer use is relevant in the context of production and pollution. Fertilizer use can be evaluated with nutrient use efficiency (NUE). The NUE can be improved with a hydroponic system, especially with NFT systems (Pennisi et al., 2019, Olympios and Choukr-Allah, 1999). However, NUE is also influenced by abiotic factors such as the light regime (Pennisi et al., 2019). Furthermore, we observed a correlation between chloride and nitrate content in the plant (Fig. S1; Publication I, Table 4), which is related to an ionic antagonism of chloride and nitrate. For equally charged ions, suppression of one ion by another can occur in membrane transport (Schubert, 2017). This was observed for chloride and nitrate, at the nitrate transporter NRT1.1 for model plants in *Arabidopsis thaliana* (Liu et al., 2020). NRT1.1 is permeable to chloride and subsequently nitrate and chloride compete for its uptake. Furthermore, it has been shown that high salinity in the root zone affects the nitrate uptake rate (Rubinigg et al., 2003). At

the optimal salt concentration for *S. europaea* (200 mM), we observed a lower nitrate content in the plant compared to no salt conditions (Publication I, Table 4). When considering nitrate levels, it is important to consider the nitrate content of the fertilizer. A study showed that the nitrate content in leafy vegetables, such as green and red amaranth or Chinese cabbage, is dependent on the applied fertilizer (Luo et al., 2022). Since the halophytes studied also belong to these plant families (*Brassicaceae* and *Amaranthaceae*), a comparable effect can be expected. As nitrate is not only an environmental pollutant but also an undesirable component of food, a reduction is also desirable in terms of healthy nutrition. Nevertheless, nitrate is also an essential nutrient for plant growth and yield improvement. A comparison of two different fertilizers within this thesis showed that a lower nitrate content did not have a negative effect on growth (Fig. S2; Table S3). However, differences in other minerals, such as sulfate or phosphate, could have also an effect on growth. This is in agreement with the literature where different fertilizer solutions for hydroponic systems were compared for *A. thaliana* (van Delden et al., 2020). Nevertheless, the nitrate requirement of a plant varies from one plant species to another and is influenced by several factors such as the stage of development or the availability of nutrients. Therefore, further research is needed to find the optimal nitrate levels considering salt concentration and light regime.

2.1.3 Lighting

Lighting affects the cost of electricity and therefore the profitability of the system, as well as yield and nutritional characteristics (Appolloni et al., 2022, Pennisi et al., 2019, Annunziata et al., 2017, Wong et al., 2020). For technical reasons, the saline indoor farming system was set up in a climatic chamber and a greenhouse equipped with halogen-sodium vapor lamps, while the experiments with UVB light were conducted in climate cabinets equipped with LED lamps (Fig. S4). As mentioned above, LED lamps have a higher PPNE than fluorescent lamps, and therefore presumably than sodium vapor lamps (2.1.1 Sustainability). By optimizing the daily light integral (DLI), light intensity and photoperiod the LUE could be improved and thus electricity costs saved, which also could favor the nutritional properties (3.1.1.2 Influence of light regime). Nevertheless, the use of LED lamps in indoor farming is beneficial in terms of energy use and, as it did not show any negative effect on carotenoid content, it is advisable to implement it in the saline indoor farming system (Wong et al., 2020). To further reduce the energy use of LED lamps, a pulsed LED lighting could be used instead of a continuous lighting (Olvera-Gonzalez et al., 2021). Considering the use of additional UVB LEDs (2.2.2 Use of UVB light in halophyte cultivation) an alternating circuit is imaginable.

2.2 Halophytes as alternative vegetables

2.2.1 Halophytes in saline indoor farming system

Halophytes showed to be feasible for saline indoor cultivation and the optimal salt concentration for each halophyte species could be determined (Publication I). The proposed mechanism of salt tolerance could be confirmed by evaluation of chloride accumulation, phenotype and morphological parameters (Publications I and III). For example, salt bladders were observed in *C. quinoa* and *A. hortensis*, which is in agreement with the literature as shown in 1.3.3.4 *Atriplex hortensis* L. and 1.3.3.3 *Chenopodium quinoa* L. (Fig. 1D, Fig. S3; Publication III, Fig. S6). Or, in *S. europaea*, an increased fresh weight was observed while growth stagnation was observed at higher salt levels, highlighting stem succulence caused by salt accumulation; which is in agreement with the literature as shown in *1.3.3.5 Salicornia europaea* L. (Fig. 1E; Publication I). For more details, see Publication I, Section 4.1.

Furthermore, the use of brine water was shown to be feasible for halophyte indoor cultivation despite the differences in salt composition (Publication II).

Since most studies on halophyte cultivation have focused on greenhouses or open fields (Ventura and Sagi, 2013, Panta et al., 2014, O'Leary, 1985, de Vos, 2013, Ladeiro, 2012), the saline indoor farming system was evaluated in comparison to an identical system setup in the greenhouse, aside from the lighting conditions. Differences between greenhouse cultivation and indoor farming were found in response to salt and yield (Publication III). Advantages of indoor farming are year-round cultivation, this was shown in the study for a fall month, where in indoor farming a higher yield was achieved depending on the salt concentration compared to the greenhouse cultivation (Publication III, Fig. 1). This is in accordance to literature, which states a higher yield and productivity in indoor farming, for example for wheat (Asseng et al., 2020). Besides yield, indoor farming offers advantages, such as reduced fertilizer and pesticide use, resilience to natural disasters, or resource efficiency in energy, water, and light use, as discussed in 2.1 Saline indoor farming system (van Delden et al., 2021, Benke and Tomkins, 2017).

2.2.2 Use of UVB light in halophyte cultivation

White light LED lamps mostly contain the PAR portion of the light spectrum (Fig. S4). However, the spectrum of sunlight also includes UV light. Since plants have a photoreceptor that recognizes UVB light (*UVR8*), we know that UVB light can affect for example, plant growth or accumulation

of PSM, and even at high UVB levels necrosis (Hideg et al., 2013, Nawkar et al., 2013, Blum, 2015). Thus, in this thesis the use of UVB light to modulate carotenoid content was evaluated (Publication IV). Since the use of UVB LEDs is a new field of research anyway, the literature on halophytes exposed to UVB LEDs to modulate the metabolite profile is zero to date. Therefore, to study the effect of UVB light on halophytes, the UVB LEDs were integrated into the white light LEDs to expose the plants to continuous UVB light according to the photoperiod. In this context, it was found that the effect of UVB light depends on the plant species, the dose and the duration of UVB radiation.

In order to study the influence of UVB light on the different plant species and to select a candidate for further experiments, the three different halophyte species (*C. officinalis*, *A. hortensis* and *S. europaea*) were exposed to different doses of UVB light. Due to major differences between the plant species in their growth and pigment content in response to UVB light, further studies were focused on *S. europaea* (Fig. S5). *A. hortensis* showed a poor performance under the higher UVB doses and was almost dead after 3 days (Fig. S6A, B). And *C. officinalis* did not show any strong response to the applied UVB doses (Fig. S6C). As with other abiotic stresses, UVB stress is expected to have different plant responses to UVB stress, however, there are many studies on the influence of UVB on plant metabolites focusing either on *A. thaliana* or on other single plant species (Badmus et al., 2022b, Badmus et al., 2022c, Heinze et al., 2018, Yeo et al., 2022, Sakalauskaitė et al., 2013, Neugart et al., 2020, Mátai et al., 2019), there are fewer that consider different plant species and if mostly from one plant family (Wiesner-Reinhold et al., 2021, van de Staaij et al., 2002).

3. Nutritional properties of halophytes

Considering halophytes as alternative vegetables for future nutrition, their contribution to a healthy diet is important. As mentioned above, healthy diets should contribute to overall well-being, but they depend on the individual nutritional needs of a person (2. Nutritional properties of healthy diets in human nutrition). However, plant-based diets, and in particular, their health-promoting PSMs, are associated with positive nutritional properties and thus with a healthy diet (2.1 Nutritional properties of plant-based diets). In addition to health-promoting components, anti-nutritional components must also be considered. When evaluating halophytes as alternative vegetables, the first step is to compare their nutritional properties with those of common leafy vegetables. The selected halophyte species have comparable levels of health-promoting PSMs to common glycophyte green leafy vegetables, such as kale, spinach, lettuce or cabbage (Publication

I). Considering carotenoids, for instance, the highest content of β-carotene can be found in *C. officinalis*, with ~3.6 mg 100 g^{-1} fresh mass, grown under optimal salinity, which is comparable to lettuce or spinach (Table S1). The lowest content of β-carotene with ~0.7 mg 100 g^{-1} fresh mass was found in *S. europaea*, which is comparable to Chinese or savoy cabbage. β-carotene not only has pro-vitamin A activity, but also several other health-promoting properties. (Table 1). For lutein and zeaxanthin, comparable levels to spinach were found in *C. officinalis* ~8.7 mg 100 g^{-1} fresh mass and *B. oleracea* var. *palmifolia* ~8.5 mg 100 g^{-1} fresh mass (Table S1). Lutein and zeaxanthin, for example, have been linked to reduced risk of age-related macular degeneration (AMD) (Table 1). For a more detailed discussion on the nutritional properties of edible halophytes, see Publication I, Section 4.2.

For GLS, *B. oleracea* var. *palmifolia* contains a comparably low content of total GLS with ~11 mg 100 g^{-1} fresh mass, while *C. officinalis* is mid-range with ~63 mg 100 g^{-1} fresh mass (Table S2). Since the major GLSs in *C. officinalis* are not the common GLSs found in *Brassica* vegetables, comparing the contents of individual GLSs is only interesting for *B. oleracea* var. *palmifolia* (Table S2). For example, *B. oleracea* var. *palmifolia* shows comparable concentrations to savoy or white cabbage of glucobrassicin (~5.3 mg 100 g^{-1} fresh mass) and glucoraphanin (~1.4 mg 100 g^{-1} fresh mass), which breakdown products are associated with anticancer properties (Gao et al., 2020, Zhang, 2007). For a more detailed discussion on the nutritional properties of edible halophytes, see Publication I, Section 4.2.

In addition, halophytes have been shown to contain other bioactive compounds such as polyphenols or fatty acids (Pathan and Siddiqui, 2022, Patel, 2016, Zanella and Vianello, 2020, Lopes et al., 2021, Castagna et al., 2022). Apart from the health-promoting PSMs, halophytes have antinutritive compounds such as saponins or alkaloids, which may be a risk factor for consumption and should be the subject of further research. In *C. officinalis* the tropane alkaloids cochlearine (0.02% DW) and its breakdown products calystegine A5, B2, and B3 are found (Brock et al., 2006). To date, there are no regulatory limits for cochlearine and calystegine. However, calystegine is toxic, which is related to its affinity to bind to glycosidases and thus causes the inhibition of the carbohydrate metabolism (Binaglia et al., 2019). In the *Amaranthaceae* sp. (*C. quinoa*, *A. hortensis* and *S. europaea*), saponins and oxalate are found (Patel, 2016, Oakenfull, 1981). For example, oxalate reduces the bioavailability of calcium and other minerals (Dolan et al., 2010). Saponins are very diverse in their structure and thus they are related to health concerns, e.g. effects on absorption of micronutrients and minerals and hemolytic ability. However, they also have health-promoting properties related to their cytotoxicity and thus anticancer agents (Podolak et al., 2010, Elekofehinti

et al., 2021, Sharma et al., 2023). Further research is needed to unravel the full potential of bioactive compounds in halophytes.

Studies on halophytes tend to discuss their health-promoting properties, but often do not take into account their risk potential due to salt and nitrate intake. Chloride levels were found to be significantly higher compared to glycophyte vegetables (Table S4; Publication I). However, since a reduced intake of chloride and sodium is recommended by the WHO (WHO, 2012), one potential use of halophytes is as a salt substitute (Evlash et al., 2021). For a more detailed discussion, see Publication I, section 4.2.

Nitrate levels were found to be comparable or lower than in glycophyte vegetables (Table S4; Publication I). In addition, nitrate levels are particularly problematic in green leafy vegetables and are recommended to be reduced (Luo et al., 2022, EFSA, 2008). As mentioned in 2.4 Minerals, the ADI for nitrate is 3.7 mg kg^{-1} body weight, depending on body weight, the studied halophytes show values below and above these intake limits (Table S4; Publication I). For example, for an average man in Germany, the intake limit is 323 mg day^{-1} (2.4 Minerals), the nitrate levels are within this limit (at 200 mM salt, Table S4). For an average child on the other hand, the intake limit is 69 mg day^{-1} (2.4 Minerals), the nitrate levels in *S. europaea* and *B. oleracea* var. *palmifolia* are well above the intake limit. For a more detailed discussion, see Publication I, Section 4.2. This is possible by adjusting fertilization or light regime as discussed in sections 2.1.2 Fertilization and 3.1.3.2 Influence of light regime. In addition, nitrate and chloride levels were found to be interdependent as discussed in 3.1.3.1 Influence of salt (Publication I).

Among minerals, iodine is also of interest. While chloride and nitrate are more associated with their limits, iodine supply is in demand. The iodine supply in Germany is sufficient, however, considering the WHO recommendations at a lower level (Thamm et al., 2007). *S. europaea* can take up iodine if it is supplied in the nutrient solution. For a dietary supplement of *S. europaea*, a dried product, an iodine content of 43 µg 100 g^{-1} was found (Evlash et al., 2021). On the other hand, the iodine content can also be a risk if it is too high, as has been reported for other *Salicornia* species and algae (Alfheeaid et al., 2022, Dujardin, 2023). Depending on the iodine, measured as iodide, content of the nutrient and saline solution, the plants could be enriched with iodide, but as chloride and nitrate, the iodide content would need to be monitored.

In addition to minerals, heavy metals may also pose a health risk in halophytes due to their accumulation in some halophyte species, such as *S. europaea* (Khalilzadeh et al., 2021a).

Monitoring and reduced accumulation is favored in indoor farming due to the controlled environment, including amounts in fertilizer and growing materials.

3.1 Influence of cultivation conditions on selected nutritional properties of halophytes

In a second step, the influence of the cultivation system and thus the cultivation conditions in the saline indoor farming system on selected nutritional properties was evaluated. The aim of this thesis was to favor the accumulation of desired components, such as carotenoids and GLS. This showed that salt concentration affects the nutritional properties of halophytes in terms of pigments, GLS, and chloride and nitrate content (Publication I, Publication II). Also, an influence of the light regime was observed (Publication III; Publication IV).

3.1.1 Carotenoids and chlorophylls

3.1.1.1 Influence of salt

Carotenoids and chlorophylls are desirable food components because of their multiple health-promoting properties (Table 1). It was mentioned above that the values are comparable to those of common leafy vegetables (3. Nutritional properties of halophytes), but it was investigated how they can be further increased due to cultivation conditions. In general, carotenoid content showed to be highest at optimal or eustress salt concentrations (Publication I, Table 2). This was dependent on the halophyte species. However, halophyte species showed to differ in their pigment content also at optimal salt level (Table S1). Salt composition, on the other hand, did not appear to have any effect on the pigment content (Publication II, Table 2). In addition, the fertilizer had no effect on pigment content (Fig. S2). Taken together, the pigment content was more influenced by the salt concentration, then the salt or fertilizer composition, in the experimental setup and within the evaluated parameters. Considering halophytes as alternative crops, the main advantage is that they can be grown at higher salinities than glycophytes crops. However, comparing the carotenoid content of glycophyte vegetables is challenging because glycophytes affected by salinity show very different responses in terms of carotenoid content (Saini and Keum, 2018), and most studies are focused on low salinity stress. For example, a study by Borghesi et al. (2011) showed a 2-3-fold increase in carotenoid content in tomato plants with a salt treatment of 5.5 dS m^{-1} (equivalent to 88 mM). However, compared to halophytes, glycophytes such as tomato or lettuce show a decrease in yield at lower salinities (Borghesi et al., 2011, Kim et al., 2008a). Thus, halophytes can be grown at higher salinities while maintaining their positive nutritional properties.

3.1.1.2 Influence of light regime

In addition to salinity, light has a major impact on photosynthetic active pigments. Carotenoid biosynthesis is regulated by light signals transduced by photoreceptors such as phytochromes (Saini and Keum, 2018). Important players in the transcriptional regulation are, for example, the antagonistic transcription factors *PIF1* (phytochrome-interacting factor 1, repressor) and *HY5* (elongated hypocotyl 5, activator), which are able to bind to the same promotor element of the key enzyme of carotenoid biosynthesis *PSY* (phytoene synthase) influencing its gene expression (Stanley and Yuan, 2019). The xanthophyll cycle (conversion of zeaxanthin via antheraxanthin to violaxanthin) is also affected by light (Saini and Keum, 2018). Therefore, light can be used in controlled environments to induce carotenoid accumulation and thus improve the nutritional properties of a crop.

For example, the DLI influences the pigment content in an interdependent manner with the salt concentration (Publication III, Table 3). While salt stress was shown to increase carotenoids related to ROS scavenging (e.g. zeaxanthin, β-carotene), higher PAR intensity was shown to affect carotenoids (and chlorophylls) related to light absorption (e.g. lutein, neoxanthin). Since a reduced pigment content was observed at the combination of salt stress and higher PAR light intensity, it is assumed that the stress combination leads to an overexcitation of the photosynthetic apparatus and thus to photooxidation of pigments (Fig. 6). Photooxidation of carotenoids by high light has also been demonstrated in pepper (*Capsicum annuum* L. cv. Yolo Wonder), for example (Simkin et al., 2003). For more details, see Publication III, Section “The interaction of light regime and salt treatment in influencing photosynthetic pigments”.

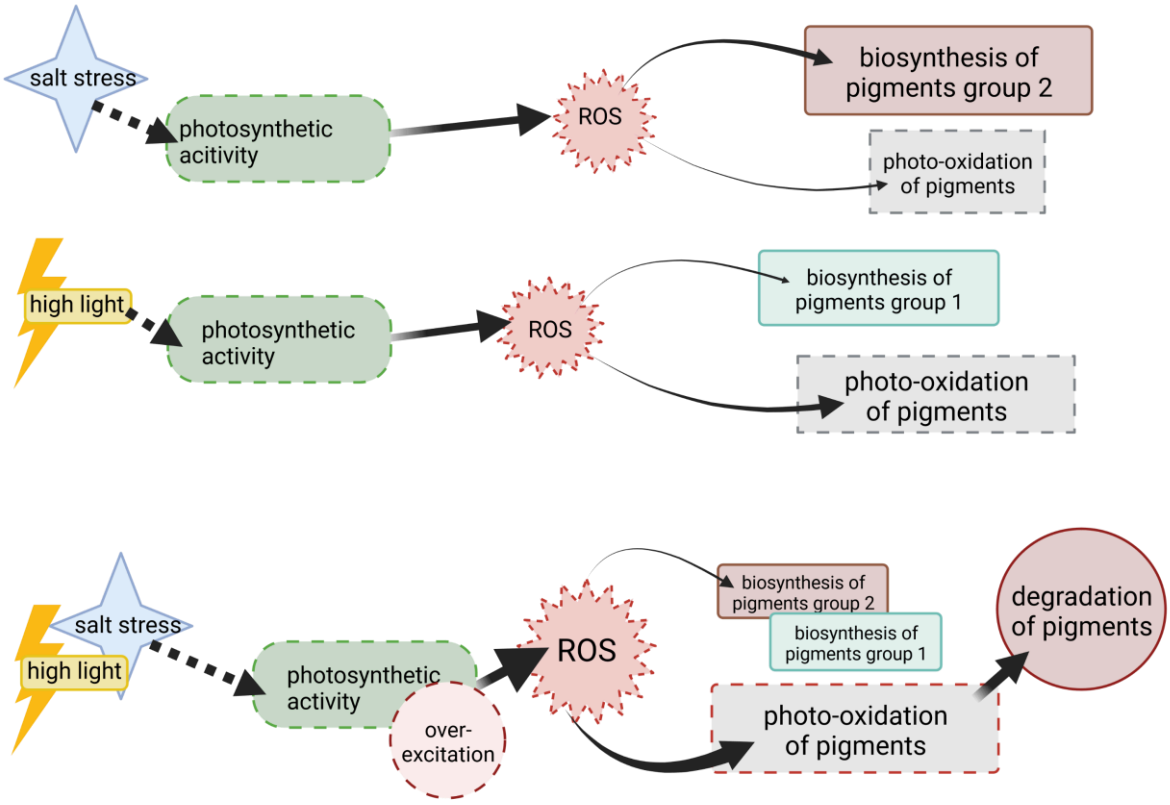

Figure 6 Effect of salt stress and light regime on photosynthetic pigments. Group 1 pigments: chlorophyll a and b, lutein, 9*Z*-neoxanthin; Group 2 pigments: ß-carotene, zeaxanthin, (all-E)-violaxanthin. ROS, reactive oxygen species. This figure was created using BioRender.com.

Furthermore, when looking at the different light qualities of the lamps, it is noticeable that the white LED lamps in the climate cabinets have a higher irradiance in the blue and green wavelengths compared to the sodium vapor lamps in the indoor farming system (Fig. S4; Publication III, Fig S3). Spectral quality affects carotenoid content, among others (Wong et al., 2020). For example, Frede and Baldermann (2022) showed that the addition of blue light to white light LED lamps increased the carotenoid content in *Brassica rapa* ssp. *chinensis*. Another study showed a positive effect on carotenoid content of supplemental blue light in greenhouse cultivation of *Brassica campestris* ssp. *chinensis* var. *communis cv* (Zheng et al., 2018). A higher content of carotenoids was observed in *S. europaea* grown under LED lamps compared to the other light regimes (Table S5). One possible explanation could be the higher blue light proportion in white LED lamps (Fig. S4). However, the experimental setup was different, and other factors, such as light intensity or photoperiod may also influence the carotenoid content.

Besides plant species, UVB radiation dose is the most important factor in pigment accumulation (Publication IV). A dose that increases carotenoid content has been identified for *S. europaea*. Besides the dose, several characteristics of the UVB treatment, such as duration or continuity of exposure, may influence the UVB response and thus the carotenoid accumulation. The study of different irradiation durations (4 h, 24 h and 18 d) revealed differences in the metabolite profiles (unpublished data). According to the literature, a higher carotenoid content was found after a longer period of UVB treatment (Badmus et al., 2022b). Interestingly, a correlation between carotenoids and ROS was found, except for (all-*E*)-violaxanthin. The (all-*E*)-violaxanthin showed only a UVB-induced response, suggesting that ROS-mediated and UVB-mediated responses differ between carotenoids. This is consistent with the literature where a UVB-induced increase is reported for violaxanthin in *A. thaliana* (Badmus et al., 2022a). While most studies identify only violaxanthin, in this study (all-*E*)-violaxanthin and a 9*Z*-isomer were identified. Since differences were observed between the two isomers, it could be speculated that (all-*E*)-violaxanthin may play an important role that has not yet been discovered. Furthermore, it is important to consider the salinity tolerance levels of halophytes, as UVB light-induced pigment accumulation is inhibited at higher salinities (Fig. S7). Therefore, the interaction of UVB light and salinity stress must be taken into account when working with halophytes, since the nutritional properties, such as reduced carotenoid content, are influenced. Taken together, different light intensities, light qualities and UVB have been shown to influence carotenoid content and thus nutritional properties. Therefore, further research could aim to further improve the carotenoid profile through different light parameters.

3.1.2 Glucosinolates

GLS and their breakdown products, especially ITCs, are desired food components due to their health-promoting properties (2.3 Glucosinolates). GLS showed to be influenced by the salt concentration and showed the highest contents at no salt. In addition to salt concentration (Publication I, Table 3), GLS content was also shown to be influenced by light regime (Fig. S8). This is in agreement with the literature where it is stated that GLS can be influenced by light quantity and quality (Wong et al., 2020, Zhou et al., 2023, Zheng et al., 2018, Gao et al., 2021). Interestingly, in both plant species (*C. officinalis* and *B. oleracea* var. *palmifolia*), only aliphatic GLS are affected by the light regime. For *C. officinalis* this is reflected in the major GLSs, glucocochlerian (*s*Bu GLS) and glucopurtanjivin (iPr GLS), and for *B. oleracea* var. *palmifolia* in glucoraphanin (4MSOP GLS). However, the two plant species showed opposite responses, *C. officinalis* showed a higher content in the greenhouse, while *B. oleracea* var. *palmifolia* showed a

higher content in the indoor farming light regime. This could be due to different biosynthetic ways, while glucocochlerian is derived from isoleucine, glucopurtanjivin from valine, glucoraphanin is derived from methionine (Fig. 4) (Blažević et al., 2020). A study by Zhou et al. (2023) showed a difference between the ratio of aliphatic/indole/aromatic GLSs in *Brassica rapa* L. ssp. *pekinensis* depending on the light intensity. A higher light intensity shifted the ratio to lower aliphatic GLS levels and higher indole GLS levels. However, for glucoraphanin, they showed an increased content within higher light intensities. Furthermore, they showed an influence of light intensity on biosynthetic genes of GLS pathway. Influenced by light, *HY5* is involved in GLSs biosynthesis, as well as, carotenoid biosynthesis, (Li et al., 2013). *HY5* is regulating the *MYB* transcriptions factors, which are involved in GLSs biosynthesis. There are different *MYB* factors for aliphatic and indole GLS biosynthesis. The differences in the response of aliphatic and indole GLSs could be related to differences in the expression levels of the *MYB* factors (Zhou et al., 2023).

Changes in GLSs distribution also alters the amount of the health-promoting GLS breakdown products. For example, the breakdown products of glucoraphanin are either sulforaphane or sulforaphane nitrile (Basten et al., 2002). Sulforaphane has been linked to cancer prevention and is therefore the preferred form, while the nitrile is less desirable (Zhang, 2007). The induction of the formation of a breakdown product is regulated by specifier proteins, for example, an epithiospecifer protein (EPS) has been shown to influence the formation of sulforaphane or sulforaphane nitrile from glucoraphanin in broccoli (Matusheski et al., 2006). The expression of these specifier proteins may be influenced by several factors, such as ecotype or the light regime, but much remains unknown (Kissen et al., 2012). Therefore, it is possible that the light regime also influences the formation of the GLS degradation product and thus impact the nutritional properties. Further, also UVB light showed to influence GLSs content in *Brassica* vegetables (Wiesner-Reinhold et al., 2021, Heinze et al., 2018). Possibly UVB light could also be used to enhance GLS content in halophytes. However, more research is needed to unravel the influence of the light on the biosynthesis of GLSs and their breakdown products.

3.1.3 Minerals

3.1.3.1 Influence of salt

Nitrate and chloride are both essential to plants, but as described above, not necessarily desired food components (2.4 Minerals). Especially leafy vegetables show nitrate values above the recommended levels. Considering the salt concentration, a higher salinity led to a higher chloride

content in all investigated halophyte species (Publication I). However, the chloride level is still below the recommend level in 100 g fresh product (Table S4; Publication I, Table 4) (WHO, 2012). As mentioned above, chloride correlates with nitrate (2.1.2 Fertilization.). Therefore, higher chloride content is associated with lower nitrate content and *vice versa*. This must be considered when modulating the fertilizer to reduce nitrate levels. The composition of the salt may have an effect on chloride and nitrate due to differences in their concentrations in the salt solution (Publication II, Fig. 1). These results correspond to results achieved from greenhouse grown *S. europaea*. In cooperation with a local agricultural project (Innovationsprojekt "Salzpflanzen aus Sachsen-Anhalt") *S. europaea* plants were analyzed for their anion composition during a growing season (Fitzner et al., 2023). Again, a correlation between nitrate and chloride content was observed (Fig. S9C, D). Further, it was observed, that the chloride and nitrate content vary between different harvests in one growing season (Table S6). The nitrate and chloride content of halophytes in relation to food is an underrepresented area of research. However, a study investigating the influence of growing season on nitrate content in the glycophytic lettuce (*Lactuca sativa* L. var. *acephala*), showed differences between nitrate content in spring and summer (Fallovo et al., 2009). Furthermore, they showed that different fertilizer concentrations (with different EC values) also have an effect on nitrate content, supporting that differences in anions or minerals in the nutrient solution (e.g. fertilizer, salt concentration or composition) can affect the nitrate content.
In conclusion, the results show that chloride and nitrate levels need to be monitored during the growing season as they may increase (Table S6, Fig. S9A). Further research could aim at the correlation between nitrate and chloride content in different halophyte species, considering their salt tolerance mechanism, fertilizer, salt composition and harvest in the growing season.

3.1.3.2 Influence of light regime

Nitrate levels can also be influenced by the light regime. *HY5* (elongated hypocotyl 5) is a light-dependent transcription factor that activates the expression of nitrate reductase (NR)-related genes, which promote nitrate assimilation and thus reduce nitrate levels in the plant (Bian et al., 2020). Nitrate is assimilated by nitrite to ammonium, which is involved in the formation of glutamine by glutamine-synthase (GS) (Fig. 7). Thus, light stimulates nitrate reduction, which is closely related to photosynthesis and CO_2 fixation (Bian et al., 2020). It is shown that a higher light intensity reduces the nitrate content (Fu et al., 2017). Consistent with this, a higher nitrate content (lower DLI) was observed in the greenhouse for *A. hortensis* and *S. europaea*, as a function of salt concentration (Table S7). Chloride content, on the other hand, showed a higher value in indoor

farming (Publication III, Fig. 2). Chloride uptake has received less attention in halophyte research than, for example, sodium or potassium uptake. However, recent studies show that the CLC (chloride channel) family may also be involved in chloride transport in halophytes (Nedelyaeva et al., 2022). Since CLCs were shown to be influenced by light regime in *A. thaliana* (Jossier et al., 2010), it is likely that they are also influenced by light in halophytes. However, this is a largely unknown area of research where further research is needed to shed light on chloride uptake in halophytes. Since chloride and nitrate levels are correlated, higher chloride levels in indoor farming would result in even lower nitrate levels (Fig. S1). However, higher levels of chloride are also toxicologically relevant and therefore desirable to be reduced in the plant. Correlation analysis between nitrate and chloride content in greenhouse and indoor cultivation showed no or a tendentially positive correlation for *C. officinalis* and *A. hortensis*, but the same negative correlation for *S. europaea* (Fig. S10). This suggests that nitrate uptake and assimilation are regulated differently in these halophyte species, which may be related to their salt tolerance mechanism (1.3.2.2 Salt tolerance mechanisms; Fig. 2). Regarding LED lamps, the addition of blue light to white light LEDs had a positive effect on nitrate reduction combined with increased levels of phytochemicals, e.g. carotenoids (Zheng et al., 2018). This is consistent with the observation that a higher proportion of blue light in white light LED lamps corresponds to a higher carotenoid content.

In addition, UVB light has been shown to affect nitrate reductase (NR), which is related to GOGAT activity (Bian et al., 2020). A study by Schwalbe et al. (1999) showed that lower GOGAT activity is associated with lower NR activity, while higher GOGAT activity is associated with higher NR activity. Under UVB light, dose-dependent changes were observed in the glutamine/glutamate ratio, which is related to GOGAT activity (Fig 7) (Publication IV, Fig. 2, Table S2). Assuming reduced GOGAT activity due to a higher glutamine/glutamate ratio in UVB distress would also result in reduced NR activity and thus higher nitrate. On the other hand, under UVB eustress, photosynthetic pigments and thus presumably photosynthetic activity are increased, which would lead to higher nitrate assimilation and thus lower levels; suggesting, UVB light could reduce the nitrate content. However, further research is needed to unravel the influence of spectral quality including UVB light on the nitrate content. This could contribute to reduction of the nitrate content in the plant and thus improve the nutritional properties.

3.2 Interaction of salt, PAR and UVB light

Considering plants grown in controlled environments, the stress of abiotic factors can be minimized to almost zero, in natural environments however, the influence of several abiotic stressors occurs more often. Due to anthropogenic effects the number and intensities of these stressors is increasing (Zandalinas and Mittler, 2022). Thus, the combination of different biotic and abiotic stressors can influence plant growth and survival, but also nutritional quality.

For example, in saline indoor farming the influence of interaction of salt, PAR and UVB light can be relevant. The use of natural saline water with different salinity levels could lead to an imbalance of this system, so it is important to understand how PAR and UVB intensity interact with salinity to be able to adjust the system. Also due to the fact that "low-level" stresses can lead in combination to a decline in plant growth and survival, while individually they are harmless (Zandalinas and Mittler, 2022). In this thesis, an interaction between light regime and salt on growth and pigment content of halophytes was observed, as well as between UVB light and salt (Table S8; Publication III). As the influence of multifactorial stressors is an emerging field of research, there is little research to date, especially within halophyte species.

3.2.1 Plant hormones as stress signaling molecules – specific for a stress?

Plant hormones act as signaling molecules for various abiotic stressors. In the individual plant studies, plant hormone levels were measured to assess plant performance and plant stress status. This is essential to evaluate the feasibility of a system for plant cultivation, but also to assess eustress and distress levels, which influence the nutritional quality. However, when comparing different stressors across studies, some hormones may be more responsive to one stress than others. ABA is shown to be a clear indicator of salt stress, independent from altering light regime or UVB, it increases with salt stress condition (Publication I, Publication II, and Publication IV). Several studies show the increase of ABA in response to salt stress, for example for the model plant *A. thaliana* or the halophyte *Atriplex halimus* (Karimi et al., 2021, Ben Hassine et al., 2009). For more details, see Publication III, Section "Influence of light regime on the response to salt treatment". Due to ABA's importance for maintaining water balance by regulating stomatal opening, it is crucial in salinity stress (Golldack et al., 2014, Tuteja, 2007). However, an interaction between light regime and salt impacting ABA content of *A. hortensis* and *C. officinalis* was observed (Table S8; Publication III, Fig. 3). In response to UVB, there was altered ABA content, however no interaction with salt (Table S8; Publication IV, Fig. S8). The changes in ABA levels in response to

UVB are thought to be related to changes in oxidative status. Therefore, different regulatory mechanisms of ABA during UVB and salt stress are likely. SA was shown to be an indicator of UVB stress (Publication IV, Fig. 1), this is in accordance to literature, for example also reported for barley (Bandurska and Cieślak, 2013). For more details, see Publication IV, Section 4.2. While less interaction with the light regime was observed (Table S8). With respect to the salt treatment, SA showed an increased level only at high salinity and was observed contrary to JA (Publication I, Fig. S5). JA showed a decreased content with salt treatment, but no interaction with UVB, but an interaction with light regime (Table S8; Publication I, Fig. S5; Publication IV, Fig. S3). However, the response of a plant to an individual stress or two stressors cannot predict the response to a certain combination of stressors (Zandalinas et al., 2021).

3.2.2 How the interaction of salt, PAR and UVB light could influence photosynthesis

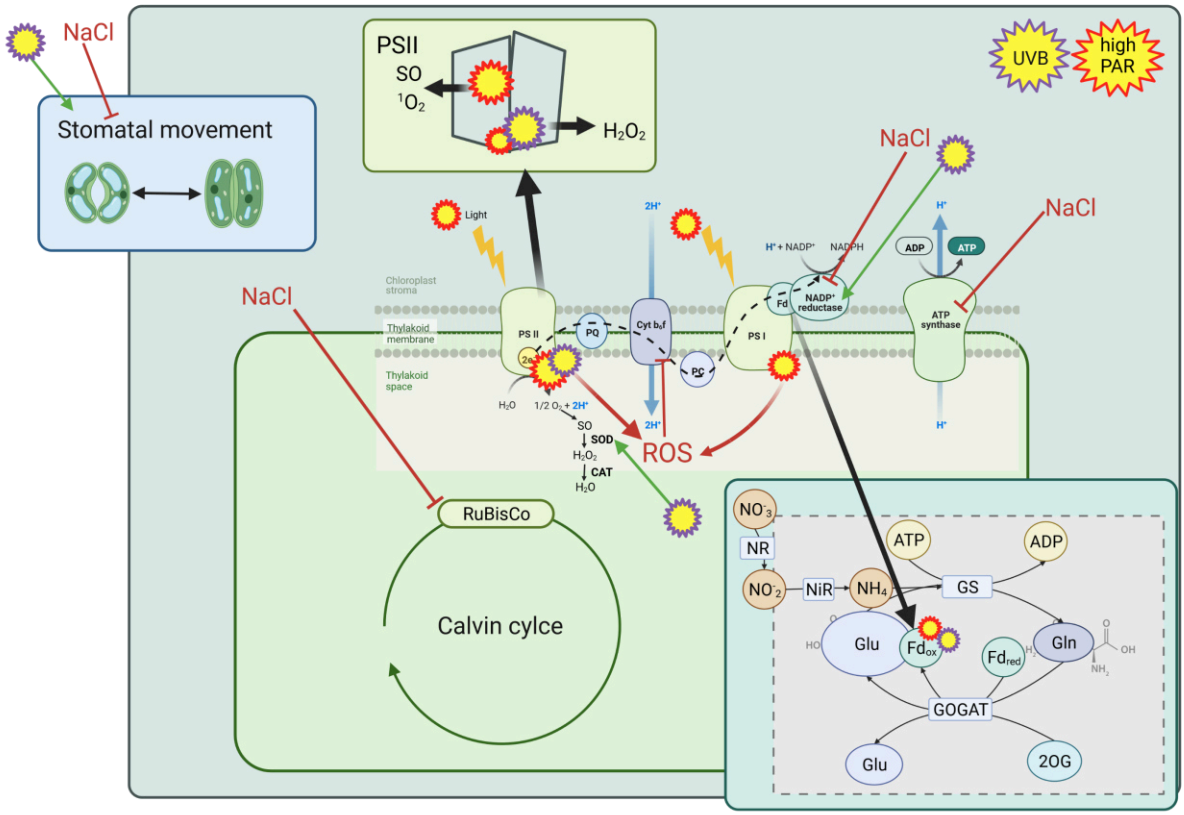

Figure 7 Effect of salinity, high light and UVB radiation on photosynthesis. According to Hasanuzzaman et al. (2020), Carillo (2018), Vass (2012) and Bian et al. (2020). Red lines indicate an inhibition; green lines indicate an induction. High PAR, influence of high light intensity in the PAR region of the light spectrum; UVB, influence of UVB radiation; NaCl, influence of salt stress; SOD, superoxide dismutase; CAT, catalase; SO, superoxide; PS II, photosystem II, PS I, photosystem I; PQ, plastoquinone; $_1O^2$, singlet oxygen; H_2O_2, hydrogen peroxide; GOGAT, glutamine synthase; Fd_{ox}, ferredoxin oxidized; Fd_{red}, ferredoxin reduced; 2OG, 2-oxoglutarate; glu, glutamic acid; gln, glutamine. This figure was created using BioRender.com.

Understanding the impact on the photosynthetic apparatus is essential for plant performance, but also for pigment content. Since the goal is to increase carotenoid content for human nutrition, it is important to understand this. As described above, salt, PAR and UVB light stress have an effect on several aspects of photosynthesis and can lead to limitations (Fig. 7). Because halophytes have special coping mechanisms to deal with salinity stress, their stress tolerance to salt stress is higher compared to glycophytes. However, exposure to high light and salt stress can still lead to overexcitation and thus to cell death. This is mainly due to the reactive oxygen species that are formed during stress. However, it is difficult to distinguish between the ROS produced by a single stressor; there are types that are more likely to be produced by high PAR or UVB radiation due to the different targets. At high PAR, singlet oxygen is formed in photosystem II (PSII) and superoxide radicals are formed by the Mehler reaction in photosystem I (PSI). In addition to singlet oxygen, hydrogen peroxide can also occur at high PAR, but is not the dominant ROS (Fig. 7) (Hasanuzzaman et al., 2020). During UVB irradiation, the main target is PSII, where hydrogen peroxide, superoxide and hydroxyl radicals are formed (Hasanuzzaman et al., 2020, Vass, 2012). During salt stress, superoxide and hydrogen peroxide can be formed (Hasanuzzaman et al., 2020), which can affect carotenoid biosynthesis; for example, during UVB exposure a correlation between increased hydrogen peroxide and carotenoid accumulation was overserved (Publication IV, Fig. S7). Thus, a difference ROS-mediated and UVB-mediated carotenoid accumulation were hypothesized (Publication IV). This becomes even more complex, when another influencing factor, such as salt, is added. This is important because different carotenoids show differences in their ability to scavenge reactive oxygen species (Gunathilake et al., 2018). In addition, the carotenoid geometric isomers also have different scavenging abilities (Zhang et al., 2014, Böhm et al., 2002).

Conclusion and Outlook

This thesis demonstrates the feasibility of halophytes for saline indoor farming and their potential as alternative vegetables. By using regional brine water, as an alternative saline water source, the sustainable water use of the system can be improved by reducing freshwater consumption and demonstrate its potential for saline agriculture. Furthermore, the influence of salt concentration and composition as well as light regime on the nutritional properties of the halophytes were investigated. While salt concentration was found to have a significant effect on the nutritional properties, salt composition did not. Furthermore, light regime and salinity were shown to have an interdependent influence on the growth and nutritional properties of halophytes, which was observed to be species specific and related to the salt tolerance mechanism of the halophyte species. In addition, an enhancement of the nutritional properties due to an accumulation of carotenoids by UVB radiation could be achieved. Considering the modulation of the cultivation conditions, the saline indoor farming system has the potential to become a new agrifood system producing halophytes as alternative vegetables.

Future research could target the influence of the saline indoor farming system on other bioactives (e.g. polyphenols) and anti-nutritive compounds (e.g. saponins, alkaloids). Furthermore, studying the influence of UVB light on other halophyte species could help to elucidate the UVB response in halophytes and possibly link it to the salt tolerance mechanism. The influence of UVB light on GLS, and chloride and nitrate would also be useful for improving nutritional properties. Furthermore, reducing nitrate content by optimizing fertilizer and light intensity could improve nutritional properties. Also, studying the influence of spectral quality on carotenoids, could improve nutritional quality. Studying the combined effect of UVB, salinity and PAR intensity on plant growth and nutritional properties could provide more insight into the stress response of halophytes to multiple stressors and improve yield and food quality.

A new agrifood system should primarily contribute to improving food security in a sustainable and healthy way. The system has the potential to improve sustainable water use, which is one of the key aspects of a sustainable agrifood system and contributes to SDG 6: “Safe water”, through the use of saline water instead of fresh water and the use of local brine water. An indoor farming system is more resilient to weather, natural disasters, or droughts, which improves food security. By using halophytes as new food sources, biodiversity is improved, contributing to SDG 15: “Life on land”. This is not only good for respecting planetary boundaries, but also for healthy diets. Healthy diets prevent malnutrition, which improves food security and contributes to SDG 2 “Zero hunger”. Other

positive nutritional properties of halophytes, such as their richness in PSM, contribute to a healthy diet. Overall, saline indoor farming can contribute to ensure food security in the future, given the challenges such as water scarcity, urbanization, and population growth that agricultural systems face, while aligning with the food4future vision of sustainable, healthy, and resilient agrifood systems.

However, before this new agrifood system can be implemented, there are still a number of aspects that need to be considered. In addition to plant performance and the nutritional quality of a new food, the economics of the system, consumer acceptance of the product and, from a nutritional perspective, the influence of the food matrix on bioavailability are critical considerations. Higher yield is important for the profitability of a new agrifood system, but so is consumer acceptance. By upscaling to vertical farming, a higher yield could be achieved and the RUE could be further improved (van Delden et al., 2021). However, energy use will increase, and to be sustainable, the way electricity is produced must be considered. Consumer acceptance of a new food is influenced by food choice, implementing aspects such as gender or religion, but the production system can also influence consumer acceptance (Jürkenbeck et al., 2019). One way to introduce a new or underutilized food source to the food market is to incorporate it into known products. As mentioned above, it is important to consider regulatory requirements such as the Novel Food Regulation. To test this with an example, we created a smoothie containing fresh *S. europaea* as a new food source and combined it with common fruits such as kiwi and mango. This resulted in high acceptance (8 out of 10 points) in a consumer survey. Apart from the described effects of the indoor farming system on PSM and anions, toxic and sensory components can also be influenced e.g., by the light regime. The sensory profile can also be influenced by growing under artificial light. For example, for basil grown indoors, the light regime has been shown to affect the volatile compounds and thus the aroma profile (Pennisi et al., 2019, Carvalho et al., 2016). The influence of the plant matrix of halophytes on the bioactivity and bioavailability of PSMs could be the subject of future research. Furthermore, it would be valuable to study the influence of the plant matrix of halophytes on the bioavailability of sodium and chloride in order to assess the risk of consuming halophytes. In the long run, this could not only be a gain for research, but also improve consumer acceptance of new food sources.

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Appendix

Supplementary Tables

Supplementary Table S1 Carotenoid content of common green leafy vegetables[#] and studied halophytes, at their optimal salt level according to Publication I (*B. oleracea* var. *palmifolia*, 50 mM; *C. officinalis*, *C. quinoa, A. hortensis* and *S. europaea*, 200 mM), in µg per 100 g fresh mass. n/a, no data available.

	β-Carotene	Lutein + Zeaxanthin
Kale	5927[1]	8198[1]
	3130[2]	n/a
	5200[3a]	n/a
Spinach, mature	5626[1]	12198[1]
	8295[2]	n/a
	4800[3b]	n/a
Lettuce, gr leafy	4443[1]	1730[1]
	60[2]	n/a
	1100[3c]	n/a
Lettuce, Iceberg	299[1]	299[1]
Cabbage, savoy	600[1]	77[1]
	45[3d]	n/a
Cabbage, Chinese	190[1]	48[1]
	70[2]	n/a
	426[3e]	n/a
B. oleracea var. *palmifolia*	2820	8460
C. officinalis	3550	8690
C. quinoa	1980	5410
A. hortensis	1550	2120
S. europaea	770	2100

[#]Most consumed leafy vegetables in Germany according to a study by the Federal Ministry of Food and Agriculture (BMEL), https://www.bmel-statistik.de/ernaehrung-fischerei/versorgungsbilanzen/obst-gemuese-zitrusfruechte-schalen-und-trockenobst; [1](USDA, 2016); [2](Public Health England, 2021); [3]Souci (1994); (a) p. 832, (b) p. 871, (c) p. 837, (d) p. 881, (e) p. 821

Supplementary Table S2 Glucosinolate content of common green leafy vegetables# and studied halophytes, at their optimal salt level according to Publication I (*B. oleracea* var. *palmifolia*, 50 mM; *C. officinalis*, *C. quinoa, A. hortensis* and *S. europaea*, 200 mM), in 100 g fresh mass. n/a, no data available.

	Total glucosinolates [mg]	Glucobrassicin [mg]	Glucoraphanin [mg]	Neoglucobrassicin [µg]
Kale	n/a	n/a	6.0[1a]	700[1a]
	54[2]	2.4[2]		
Cabbage, savoy	151[1b]	0.046[1b]	1.0[1b]	n/a
Brussel sprout	n/a	n/a	5.2[1c]	1000[1c]
Cabbage, white	80[1d]	1.4[1d]	2.2[1d]	829[1d]
B. oleracea var. *palmifolia*	11	5.3	1.4	1500
C. officinalis	63	0.6	0.7	1000

[#]Most consumed leafy vegetables in Germany according to a study by the Federal Ministry of Food and Agriculture (BMEL), https://www.bmel-statistik.de/ernaehrung-fischerei/versorgungsbilanzen/obst-gemuese-zitrusfruechte-schalen-und-trockenobst. [1]Souci (1994); (a) p. 834, (b) p. 882, (c) p. 856, (d) p. 879 – 880; [2]Baenas et al. (2019)

Supplementary Table S3 Anion contents in vegetable nutrient solution and algae nutrient solution. Means ± SD, n = 3. Anions analyzed by ion chromatography. VNS, vegetable nutrient solution; ANS, algal nutrient solution.

Anions [g L^{-1}]	VNS	ANS
Chloride	64.05 ± 0.17	65.27 ± 0.44
Nitrate	9.91 ± 0.06	0.65 ± 0.02
Sulfate	2.16 ± 0.03	0.73 ± 0.03
Phosphate	1.96 ± 0.01	0.76 ± 0.03

Supplementary Table S4 Nitrate and chloride contents in common green leafy vegetables# and studied halophytes at their optimal salt level according to Publication I (*B. oleracea* var. *palmifolia*, 50 mM; *C. officinalis*, *C. quinoa, A. hortensis* and *S. europaea*, 200 mM), in mg per 100 g fresh mass.

	Chloride	Nitrate
Kale	60[1a]	101[1a]
Spinach, mature	54[1b]	166[1b]
Lettuce, gr leafy	57[1c]	219[1c]
Cabbage, savoy	29[1d]	48[1d]
Cabbage, Chinese	n/a	112[1f]

B. oleracea var. *palmifolia*	458	251
C. officinalis	1055	41
C. quinoa	1352	42
A. hortensis	1086	37
S. europaea	1370	218

[1]Souci (1994); (a) p. 832, (b) p. 871, (c) p. 837, (d) p. 881, (e) p. 821

Supplementary Table S5 Total carotenoid content of *S. europaea* at different experimental setups and light regimes at 200 mM salt. Carotenoid analysis was performed as described in publications III and IV.

Publication number	DLI	Lamp type				
IV	10.1	LED	989.56	±	33.87	b
IV	10.1	LED	618.55	±	19.74	d
IV	7.6	LED	1067.02	±	24.11	a
IV	7.6	LED	758.08	±	21.36	c
IV	7.6	LED	666.68	±	22.15	dc
III	3.2	Natural light/Sodium vapor lamps	398.31	±	19.86	d
III	18.0	Sodium vapor lamps	633.60	±	27.93	f
II	8.6	Sodium vapor lamps	480.70	±	11.80	e
II	8.6	Sodium vapor lamps	551.23	±	7.89	de
II	8.6	Sodium vapor lamps	439.70	±	7.77	ef

Supplementary Table S6 Chloride and nitrate contents in *S. europaea* plants grown in a greenhouse with rock salt during a growing season from May to September in 2021. Analysis by ion chromatography. Rock, rock salt; cattle, salt used in livestock farming.

Anions [mg 100 g^{-1} FM]		Rock			Cattle		
Chloride	Harvest 1	937.7	±	84.7	1910.2	±	155.7
	Harvest 2	2617.3	±	181.8	2141.5	±	207.4
	Harvest 3	2515.7	±	144.4	2266.9	±	96.0
Nitrate	Harvest 1	300.2	±	60.0	142.4	±	6.6
	Harvest 2	72.9	±	28.5	33.6	±	5.4
	Harvest 3	7.1	±	2.5	28.5	±	2.7

Supplementary Table S7 Nitrate content in leaves of 6- to 9-week-old plants. Means ± SEM of n = 8 pools of 3 individual plants each from two independent experiments. Small letters indicate significant differences between salt treatments in light regime 1 (greenhouse) in alphabetical order from highest to lowest; capital letters indicate significant differences between salt treatments in light regime 2 (indoor farming) in alphabetical order from highest to lowest, asterisks indicate significant differences between light regimes within a salt treatment, interaction indicates significantly different interaction between salt treatments and light regimes; analyzed by two-way ANOVA, followed by post hoc Bonferroni test ($p \leq 0.05$)(* ≤ 0.05, *** ≤ 0.001). Analysis performed with ion chromatography. Cultivation conditions according to Publication III. ns, not significant.

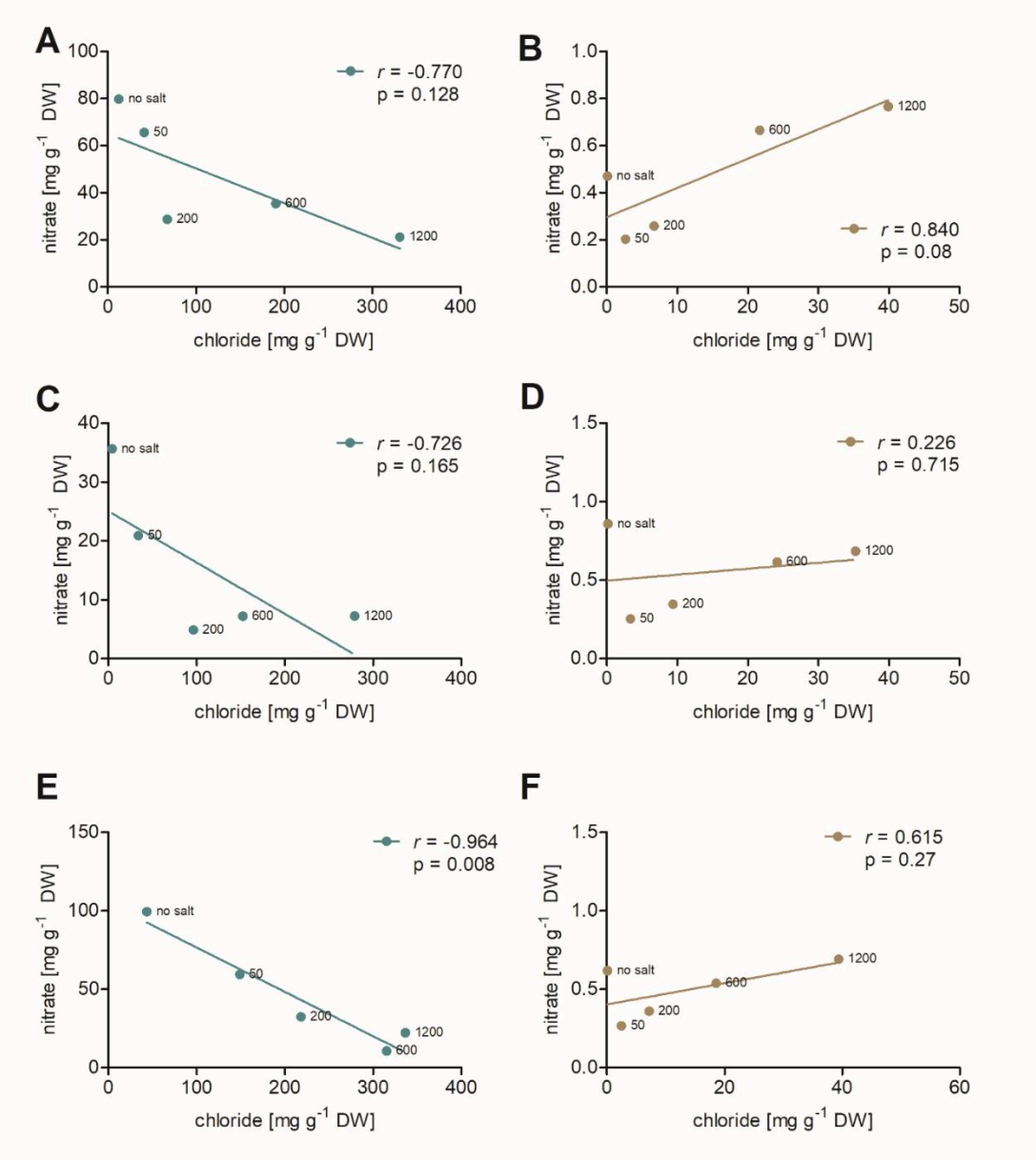
		Salt treatment [mM NaCl]	Nitrate [mg g^{-1} DW]				
C. officinalis	Light regime 1	0	7.68	±	2.13	b	
		50	1.19	±	0.25	b	
		200	0.89	±	0.08	b	
		600	34.81	±	1.95	a	***
	Light regime 2	0	50.30	±	9.41	A	***
		50	10.47	±	4.25	B	
		200	1.02	±	0.19	B	
		600	3.32	±	0.65	B	
	Interaction light regime x salt treatment		***				
A. hortensis	Light regime 1	0	43.23	±	4.97	b	***
		50	27.24	±	4.94	c	***
		200	8.01	±	3.03	d	
		600	53.20	±	4.92	a	***
	Light regime 2	0	17.48	±	2.28	ns	
		50	2.28	±	0.71	ns	
		200	0.66	±	0.00	ns	
		600	1.36	±	0.00	ns	
	Interaction light regime x salt treatment		***				
S. europaea	Light regime 1		90.74	±	5.61	a	
			34.94	±	3.18	b	
			31.59	±	2.11	b	
			21.83	±	1.81	b	*
	Light regime 2		81.81	±	7.00	A	
			41.87	±	2.36	B	
			22.62	±	3.19	C	

	6.50 ± 0.80	C
Interaction light regime x salt treatment		no

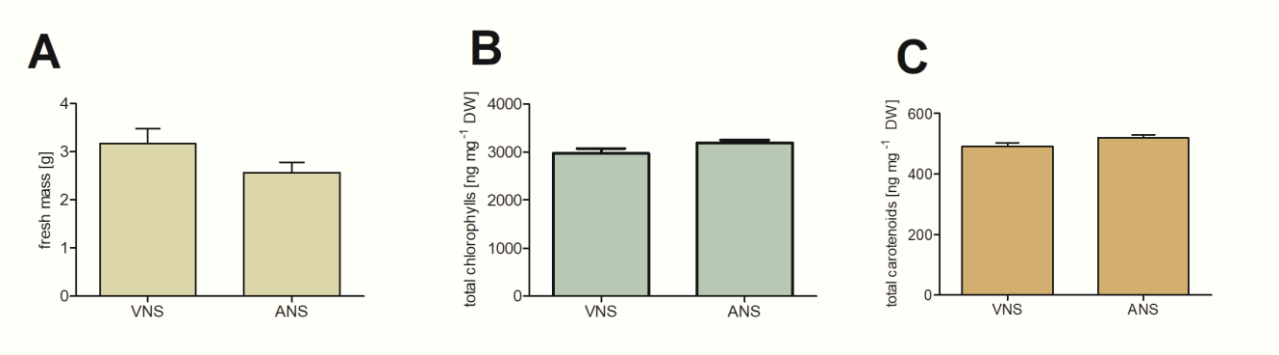
Supplementary Table S8 Interaction of light and salt and UVB and salt on growth and metabolites in different halophyte species. Asterisks indicate significant interactions between treatments analyzed by two-way ANOVA, followed by post hoc Bonferroni test ($p \leq 0.05$) (* ≤ 0.05, *** ≤ 0.01, ** ≤ 0.001). aGLS, aliphatic GLS; iGLS, indole GLS; n/a, parameter not present because not measured, not present in plant species, or plant species was not included in the experiment; ns, not significant.

		Plant species		
Stress combination	Parameter	*C. officinalis*	*A. hortensis*	*S. europaea*
Light x salt	aGLS	**	n/a	n/a
Light x salt	iGLS	ns	n/a	n/a
Light x salt	JA	**	***	**
Light x salt	SA	**	ns	ns
Light x salt	ABA	**	*	ns
Light x salt	Total carotenoids	***	***	**
Light x salt	Total chlorophylls	***	***	***
Light x salt	Fresh mass	***	***	***
Light x salt	Dry mass	*	**	ns
Light x salt	Nitrate	***	***	*
Light x salt	Chloride	***	***	ns
UVB x salt	SOD	n/a	n/a	***
UVB x salt	H_2O_2	n/a	n/a	ns
UVB x salt	Total carotenoids	n/a	n/a	**
UVB x salt	Total chlorophylls	n/a	n/a	*
UVB x salt	ABA	n/a	n/a	ns
UVB x salt	SA	n/a	n/a	***
UVB x salt	JA	n/a	n/a	ns
UVB x salt	Total carotenoids	ns	n/a	ns
UVB x salt	Total chlorophylls	ns	n/a	*
UVB x salt	Biomass	ns	n/a	ns
UVB x salt	Biomass	ns	ns	ns
UVB x salt	Total carotenoids	ns	***	**
UVB x salt	Total chlorophylls	ns	***	**

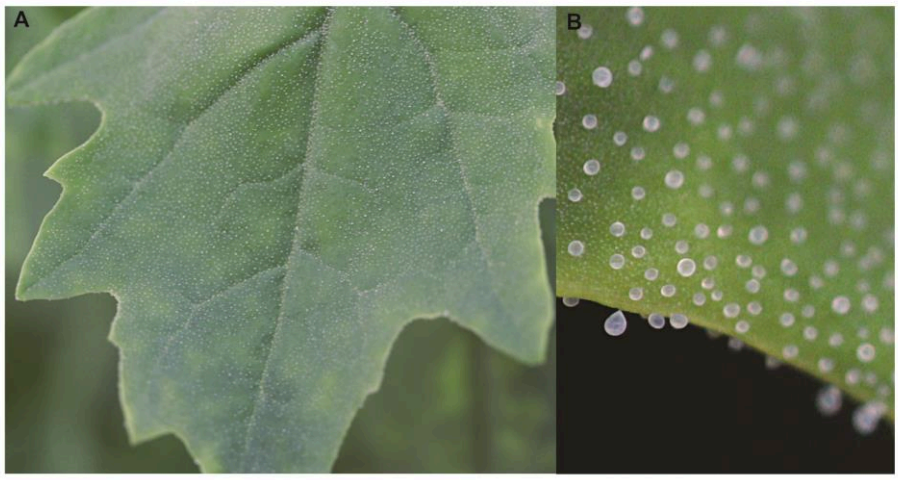
Supplementary Figures



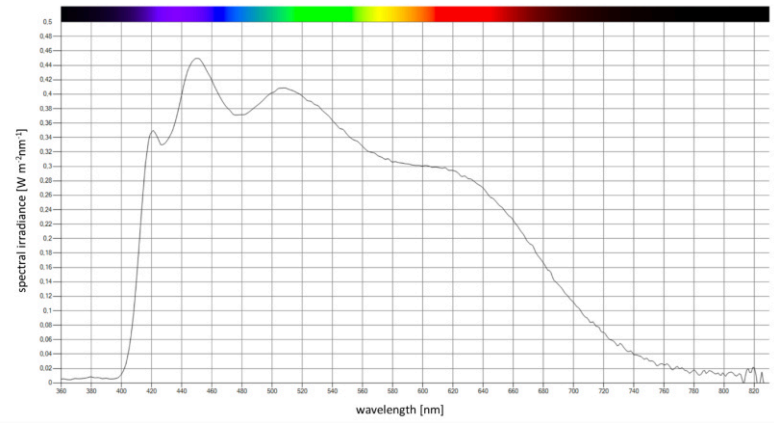
Supplementary Figure S1 Correlation between chloride and nitrate content in (A, C, D) plant and (B, D, F) soil of (A, B) *C. officinalis*, (C, D) *A. hortensis*, and (E, F) *S. europaea* in saline indoor farming system with no salt (0 mM), 50 mM, 200 mM, 600 mM, and 1200 mM salt (sodium chloride) treatments (according to Publication I). Means, n = 8 from two single experiments. Lines indicate linear regression (confidence interval = 95%). Correlation tested by Pearson correlation (Pearson *r*, *p*-value). Analysis performed by ion chromatography. DW, dry weight.



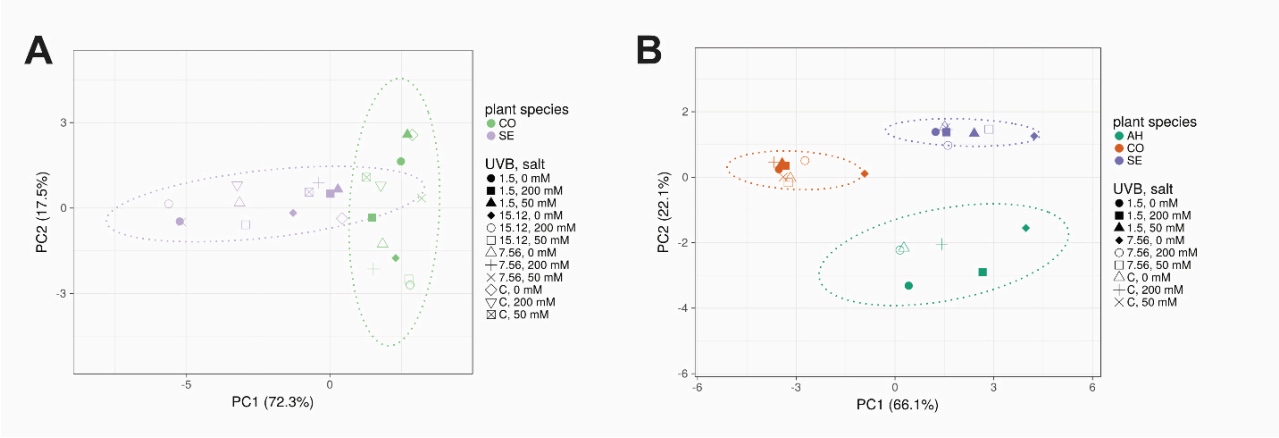
Supplementary Figure S2 Influence of nutrient solution on growth and pigment content of *S. europaea* in 10-week-old plants. (A - C) Comparison of vegetable nutrient solution and algal nutrient solutions. (A) Fresh mass after 3 weeks of treatment. (B) Total chlorophyll content. (C) Total carotenoid content. Letters indicate significant differences between saltwater treatments tested by one-way ANOVA, followed by post hoc Tukey's test ($p \leq 0.05$). Fresh mass determined by weighing and pigment content analyzed by UHPLC-DAD-ToF-MS. Cultivation conditions according to publication II. VNS, vegetable nutrient solution; ANS, algal nutrient solution; DW, dry weight.



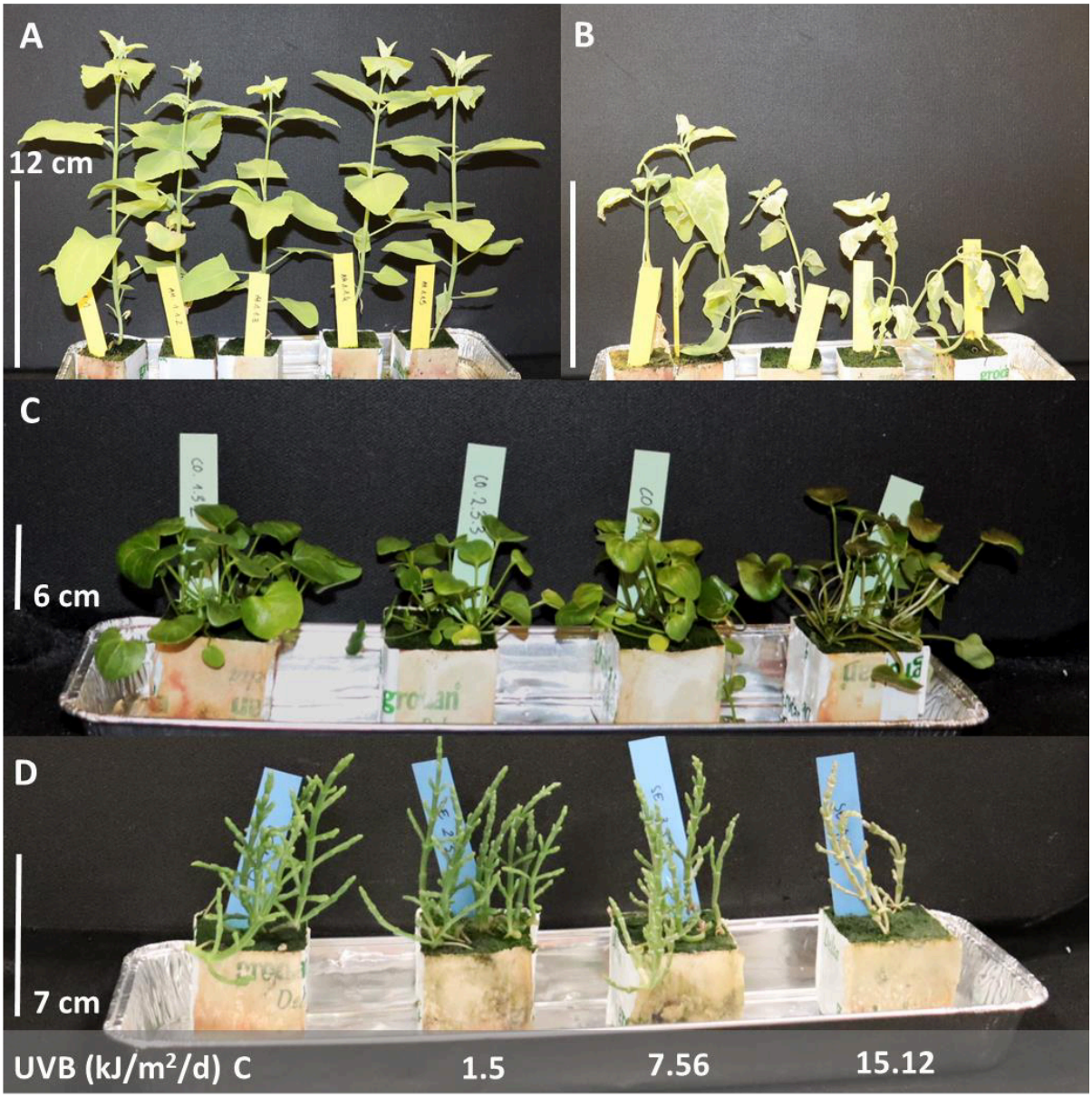
Supplementary Figure S3 Salt bladders on leaves of *C. quinoa*. Magnification (A) 1.4 times and (B) 11.2 times.



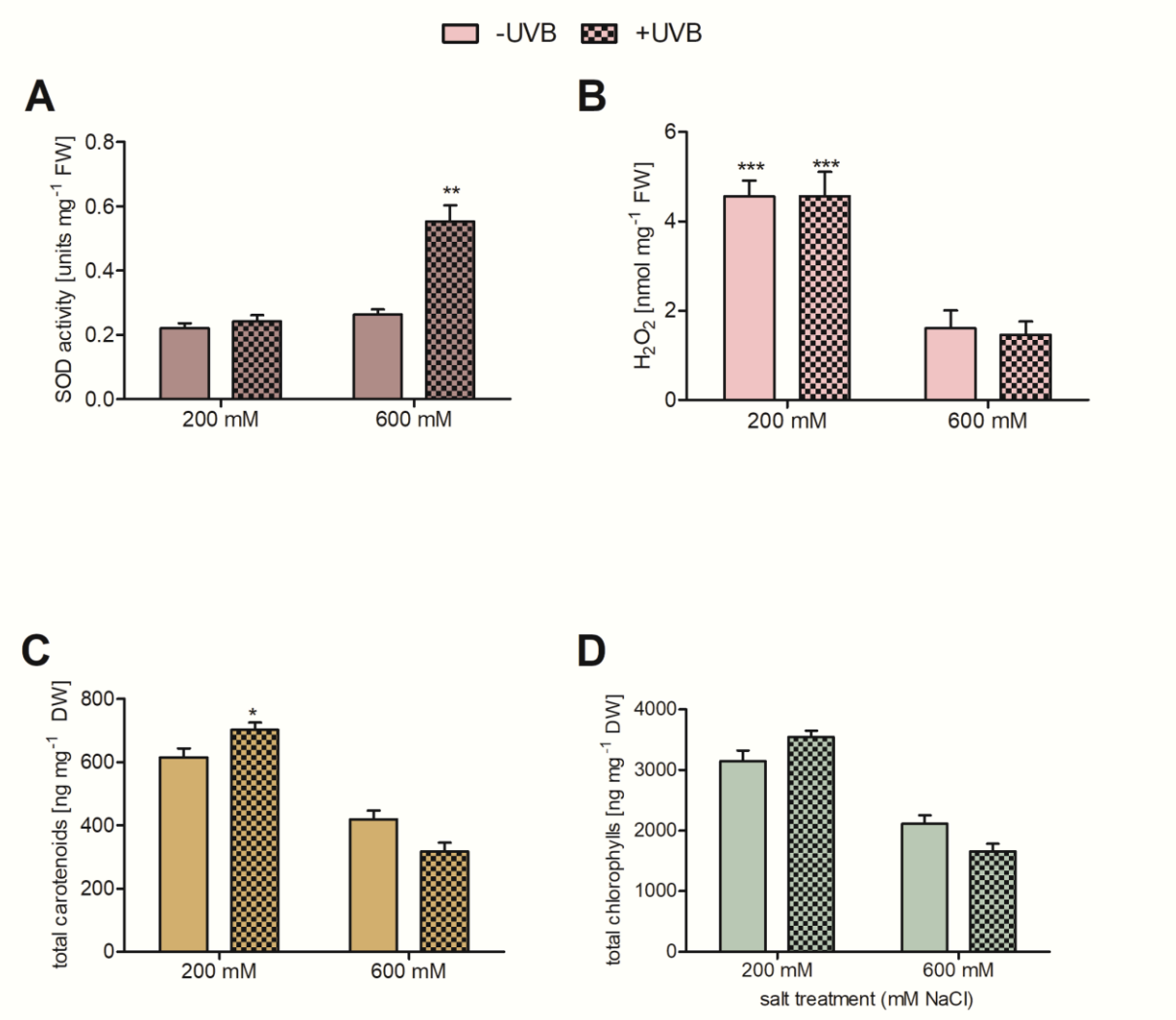
Supplementary Figure S4 Light spectra of white light LED lamps (6500 K) measured with a spectrophotometer (Ocean Insight, US).



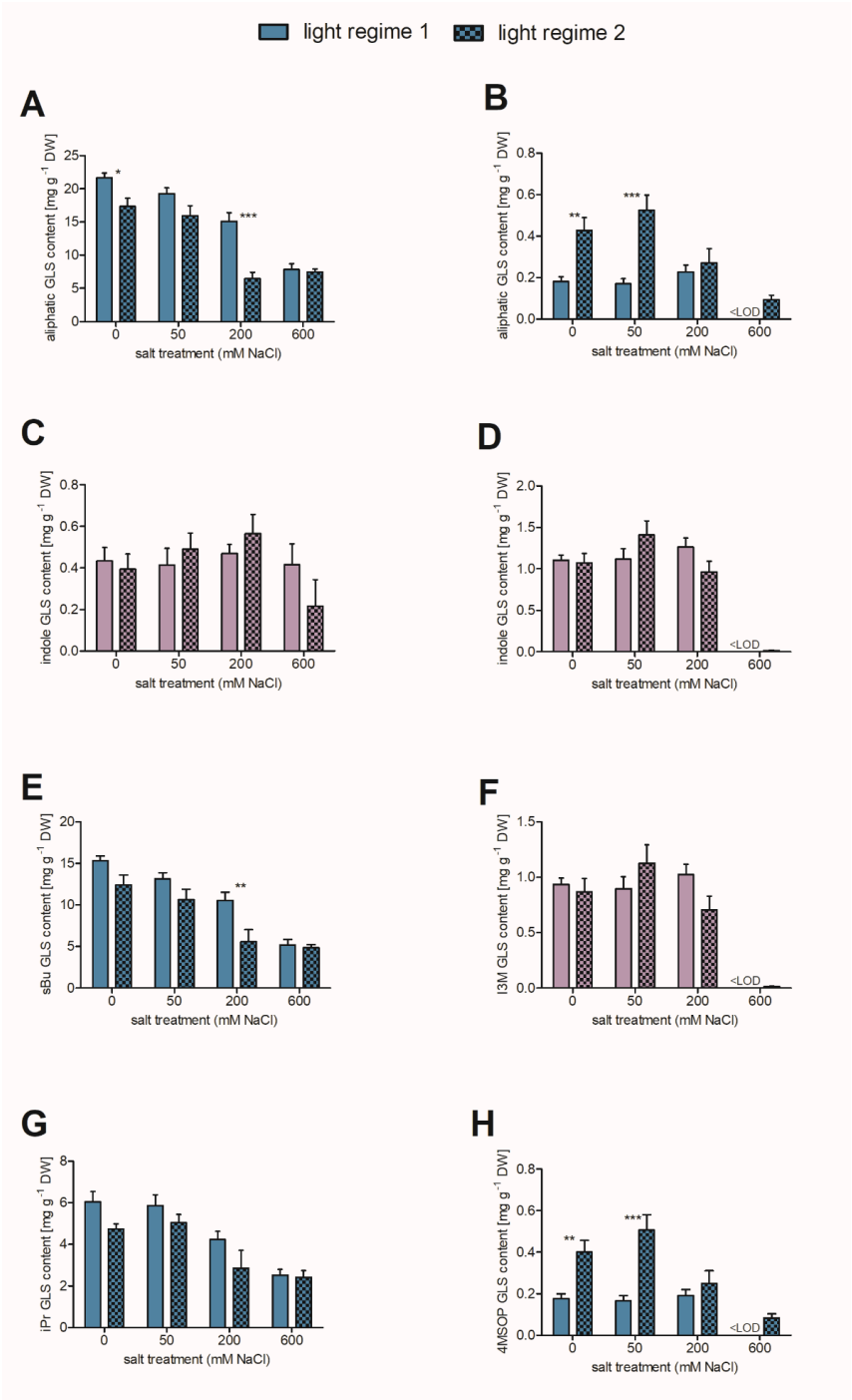
Supplementary Figure S5 Influence of UVB and salt treatment on growth and pigments of *A. hortensis*, *C. officinalis* and *S. europaea*. (A) First experiment: *C. officinalis*, *S. europaea*, salt treatment: 0, 50 and 200 mM NaCl solution; UVB treatment (285 nm): Control, 0 kJ m^{-2} d^{-1}, 1.5 kJ m^{-2} d^{-1}, 7.56 kJ m^{-2} d^{-1}, 15.12 kJ m^{-2} d^{-1}. (B) Second experiment: *C. officinalis*, *A. hortensis*, *S. europaea*, salt treatment: 0, 50 and 200 mM NaCl solution; UVB treatment (285 nm): Control, 0 kJ m^{-2} d^{-1}, 1.5 kJ m^{-2} d^{-1}, 7.56 kJ m^{-2} d^{-1}. PCA plots generated with ClustVis [(A) unit variance scaling is applied to the rows, SVD with imputation is used to calculate the principal components, x- and y-axis show principal component 1 and principal component 2, which explain 66.1% and 22.1% of the total variance, respectively, prediction ellipses are such that with probability 0.95, a new observation from the same group will fall inside the ellipse, n = 24 data points; (B) unit variance scaling is applied to the rows, SVD with imputation is used to compute principal components, x- and y-axis show principal component 1 and principal component 2, which explain 72.3% and 17.5% of the total variance, respectively, prediction ellipses are such that, with probability 0.95, a new observation from the same group will fall inside the ellipse, n = 24 data points]. Growth: fresh weight; pigments: total carotenoid content, lutein, β-carotene, all-*E*-violaxanthin, 9*Z*-violaxanthin, 9*Z*-neoxanthin, zeaxanthin, total chlorophyll content, chlorophyll *a*, and chlorophyll *b*. CO, *Cochlearia officinalis*; SE, *Salicornia europaea*; AH, *Atriplex hortensis*.



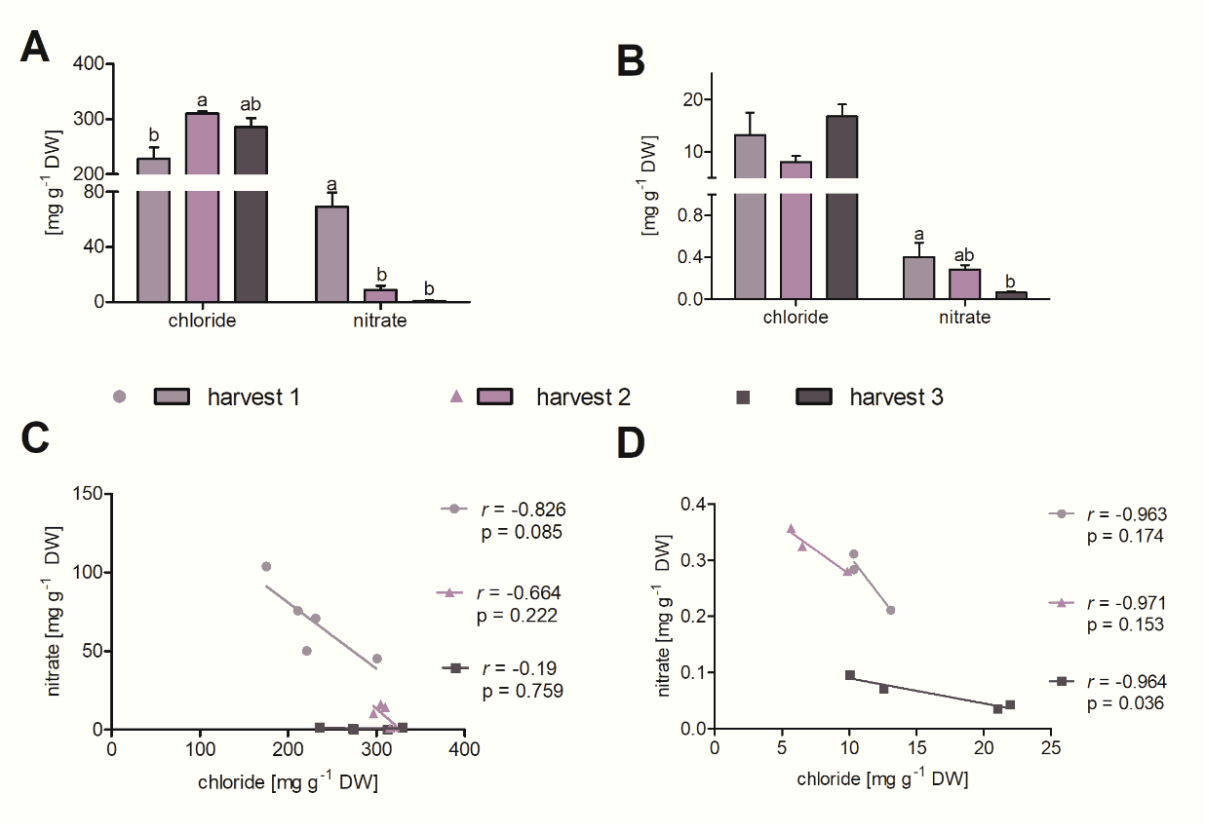
Supplementary Figure S6 Influence of the UVB dose on the phenotype of (A, B) *A. hortensis*, (C) *C. officinalis*, (D) *S. europaea*. (A, B) After three days of (A) control and (B) 15.12 kJ m^{-2} d^{-1} UVB treatment. (C, D) After 18 days of the same UVB treatment. UVB, 285 nm; control, 0 kJ m^{-2} d^{-1}. Plants were cultivated in a climate cabin under the following conditions: humidity, 65%; light intensity, 200 µmol m^{-2} s^{-1}; temperature, 20°C day, 18°C night; photoperiod, 14 h daylight, 10 h darkness; CO_2, 400 ppm.



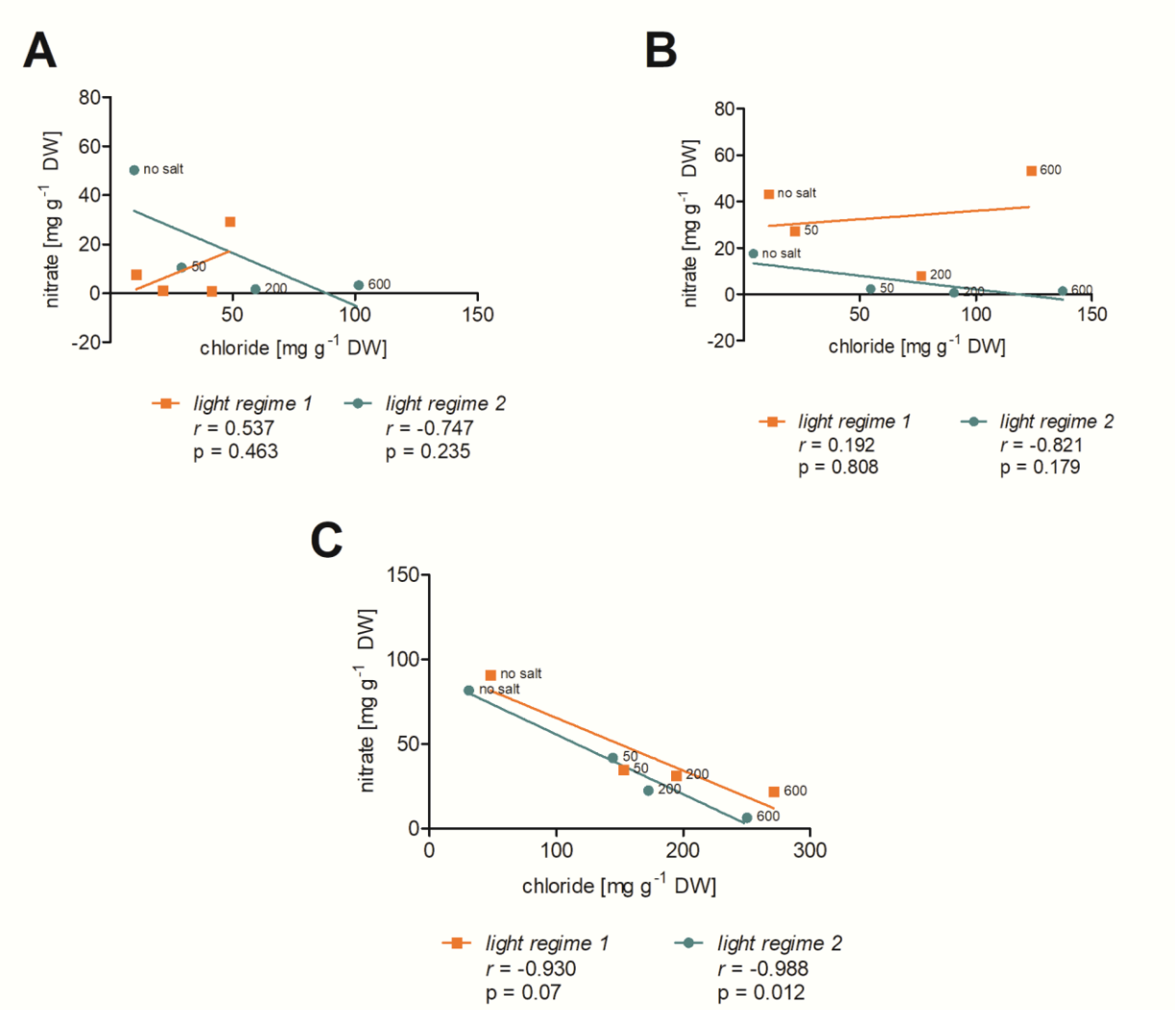
Supplementary Figure S7 Effect of salinity and moderate UVB treatment on pigment content and ROS in *S. europaea*. (A) SOD activity; (B) H_2O_2 content; (C) total carotenoids; (D) total chlorophylls. SOD and H_2O_2 were measured by a photometric method in fresh plant tissues. Pigments were analyzed by UHPLC-DAD-ToF-MS in freeze-dried plant tissue. Bars represent the mean ± SEM of n = 9. Asterisks indicate significant differences between UVB treatments within a salt treatment tested by two-way ANOVA, followed by post hoc Bonferroni test ($p \leq 0.05$) (* ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001). Cultivation conditions according to Publication IV. UVB treatment (285 nm); -UVB, 0 kJ m^{-2} d^{-1}; +UVB, 2.5 kJ m^{-2} d^{-1}; SOD, superoxide dismutase; H_2O_2, hydrogen peroxide; FW, fresh weight; DW, dry weight.


light regime 1
light regime 2
A
aliphatic GLS content [mg g-1 DW]
salt treatment (mM NaCl)
B
aliphatic GLS content [mg g-1 DW]
<LOD
salt treatment (mM NaCl)
C
indole GLS content [mg g-1 DW]
salt treatment (mM NaCl)
D
indole GLS content [mg g-1 DW]
<LOD
salt treatment (mM NaCl)
E
sBu GLS content [mg g-1 DW]
salt treatment (mM NaCl)
F
I3M GLS content [mg g-1 DW]
<LOD
salt treatment (mM NaCl)
G
iPr GLS content [mg g-1 DW]
salt treatment (mM NaCl)
H
4MSOP GLS content [mg g-1 DW]
<LOD
salt treatment (mM NaCl)

Supplementary Figure S8 Effect of salinity and light regime on glucosinolate content in leaves of 6- to 9-week-old plants. (A, C, E, G) *C. officinalis* and (B, D, F, H) *B. oleracea* var. *palmifolia.* (A, B) aliphatic GLS; (C, D) indole GLS; (E) *s*Bu GLS (aliphatic); (F) I3M GLS (indole); (G) iPr GLS (aliphatic) and (H) 4MSOP GLS (aliphatic). Glucosinolates were analyzed by UHPLC-DAD-ToF-MS. Cultivation conditions were as described in Publication III. Bar represents mean ± SEM of n = 8 pools of 3 individual plants each. Asterisks indicate significant differences between light regimes 1 and 2 within a salt treatment tested by two-way ANOVA, followed by post hoc Bonferroni test ($p \leq 0.05$) (* ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001). Light regime 1, greenhouse; light regime 2, indoor farming; <LOD, below the limit of detection; I3M, indole-3-ylmethyl GLS; iPr, 2-propyl GLS; sBu, (1S)-1-methylpropyl GLS; 4MSOP, (R_S)-4-(methylsulfinyl) butyl GLS; DW, dry weight.



Supplementary Figure S9 Chloride and nitrate content in (A) plant and (B) soil and correlation between chloride and nitrate in (C) plant and (D) soil. *S. europaea* plants grown in a greenhouse with rock salt during a growing season from May to September in 2021. Letters indicate significant differences between harvest times. Correlation tested by Pearson correlation (Pearson *r*, *p* value). Analysis performed by ion chromatography.



Supplementary Figure S10 Correlation between chloride and nitrate in (A) *C. officinalis* (B) *A. hortensis*, and (C) *S. europaea* in greenhouse (light regime 1, orange line) and indoor farming (light regime 2, green line). Means, n = 8 from two single experiments. Lines indicate linear regression (confidence interval = 95%). Correlation tested by Pearson correlation (Pearson *r*, *p* value). Analysis performed by ion chromatography. DW, dry weight. Cultivation conditions as described in Publication III.

Acknowledgements

I would like to express my gratitude and appreciation to all those who have contributed to the successful completion of this thesis.

First and foremost, I am sincerely grateful to my two supervisors, Susanne Baldermann and Monika Schreiner, for their unwavering support, invaluable guidance, and expertise throughout this research endeavor. I appreciate their profound knowledge, constant encouragement, and constructive feedback.

I would like to express my sincere gratitude to Annett Platalla, Andrea Jankowsky, Andrea Maikath, and Elke Büsch for their assistance and support. I would like to thank the entire Food Chemistry and Nutrition Research Group for the insightful discussions, the exchange of ideas, and the pleasant working atmosphere within the research group, which helped to improve the quality of this work. Especially I would like to thank Anna Fricke for her support and insights throughout the whole time. Further, I would like to thank all project members of the food4future project for the great cooperation.

I would like to thank all the graduate students in my department, but especially Vanessa, Max, Matthias, and Amy, for the great time, constant support, and helpful discussions.

Furthermore, I would like to thank Ken Dohrmann and the Innovationsprojekt “Salzpflanzen aus Sachsen-Anhalt” for the great cooperation work.

Furthermore, I would like to express my gratitude to the Federal Ministry of Education and Research (BMBF) for the financial support that made this research possible.

Last but not least, I would like to acknowledge the support and encouragement of my family and friends.

To all those who have contributed in ways seen and unseen, I extend my heartfelt thanks. I am grateful for your presence on my academic path.

Scientific contributions

Publications

Harbart V, Frede K, Fitzner M, Baldermann, S. Regulation of carotenoid and flavonoid biosynthetic pathways in *Lactuca sativa* var *capitate* L. in protected cultivation. Frontiers in Plant Science. 2023;14.

Raffeiner M, Üstün S, Guerra T, Spinti D, Fitzner M, Sonnewald S, et al. The Xanthomonas type-III effector XopS stabilizes CaWRKY40a to regulate defense responses and stomatal immunity in pepper (*Capsicum annuum*). *The Plant Cell*. 2022.

Fricke A, Psarianos M, Sabban J, Fitzner M, Reipsch R, Schlüter OK, et al. Composite materials for innovative urban farming of alternative food sources (macroalgae and crickets). *Frontiers in Sustainable Food Systems.* 2022;6.

Talks

Invited Talks

Baldermann S., Fitzner M., Fricke A., Gusovius HJ., Schlüter O., Schreiner M. - Kultivierung und Aufbereitung alternativer Nahrungspflanzen am Beispiel von Hanf, Halophyten und Makroalgen. GDL-Webinar Wiederentdeckte und neue pflanzliche Rohstoffe und deren Potenzial für die Lebensmittelproduktion (online), 2023

Fitzner M., Fricke A., Kühnhold H. - Makroalgen, Halophyten und Medusen: Lebensmittel der Zukunft? Bioremediation in the Baltic Sea (BaMS e.V.) (online), 2021

Talks on Conferences and Seminars

Fitzner M., Dohrmann K., Schreiner M., Baldermann, S. – Einfluss verschiedener Salzquellen auf den Mineralstoffgehalt von *Salicornia europaea* im kommerziellen Anbau während eines

Erntejahres. Arbeitstagung der Regionalverbände Nord und Nordost der Lebensmittelchemischen Gesellschaft (Fachgruppe in der GDCh) (Hannover, Germany), 2023

Fitzner M., Schreiner M., Baldermann S. – Kultivierung von Halophyten. 4. Status seminar food4future (Potsdam, Germany), 2023

Fitzner M., Schreiner M., Baldermann, S. – Zwischen Eustress und Distress - UV-B Signaltransduktion in Halophyten. Status seminar - Agricultural systems of the future (online), 2022

Fitzner M., Schreiner M., Baldermann, S. - Exploiting the potential of halophytes for enhancing biodiversity and improving future diets. 3rd Workshop Nutritional Security Working Group: Future-proofed crops to address the nutritional security goal in Europe (Lecce, Italy), 2022

Fitzner M., Schreiner M., Baldermann S. - Influence of salinity and different light regimes on growth and metabolite profiles of halophytes. (Elevated Pitch Talk); Plant Biology Europe (online), 2021

Fitzner M., Schreiner M., Baldermann S. – Kultivierung von Halophyten. 2. Status seminar food4future (online), 2021

Poster

Fitzner M., Dohrmann K., Schreiner M, Baldermann S. - Einfluss verschiedener Salzquellen auf den Mineralstoffgehalt von *Salicornia europaea* im kommerziellen Anbau während eines Erntejahres. 4. Status seminar food4future (Potsdam, Germany), 2023

Fitzner M., Schreiner M., Baldermann S. - Halophytes as alternative source of carotenoids in our diet. 3rd International Conference on Food Bioactives & Health (Parma, Italy), 2022, (Best Poster Presentation Award)

Fitzner M., Schreiner M., Baldermann S. - Einsatz von UV-B Strahlung zur gezielten Steigerung ausgewählter Inhaltstoffe. 3. Status seminar food4future (online), 2022

Fitzner M., Schreiner M., Heidebach T., Mäder J., Baldermann S. -Halophytes – Future Food? Introducing Alternative Crops for Food Production. XXI EuroFoodChem (online), 2021

Fitzner M., Schreiner M., Baldermann S. - Kultivierung ausgewählter Halophyten zur Optimierung des Inhaltsstoffprofils. 2. Status seminar food4future (online), 2020

Fitzner M., Schreiner M., Baldermann S. - Understanding and optimizing the cultivation of halophytes in artificial environments. N² Network of Doctoral Researcher Event, Berlin, 2019

Curriculum vitae

The personal data has been removed for the publication of this dissertation.

Eigenständigkeitserklärung

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Arbeit selbstständig und ohne Benutzung von anderen als angegebenen Hilfsmitteln und Quellen angerfertigt habe.

Die Arbeit wurde an keiner anderen Universität eingereicht.

Bamberg,

_____

Maria Fitzner