The growth and nutritional quality of the African eggplant under abiotic stress

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I hereby declare that this dissertation is all my own work.

Abstract

The African eggplant (*Solanum aethiopicum* L.), a popular indigenous vegetable, is well-suited to local environments, highly nutritious, and has lower water and fertiliser requirements than its close *Solanum* relatives such as the brinjal eggplant or tomato. It might offer a solution to the growing threat of food insecurity arising from the increasing frequency and severity of environmental stresses worldwide, particularly in Africa. However, research on the African eggplant's tolerance to abiotic stress is lacking, limiting its full potential.

The primary aim of this project was to investigate how the African eggplant responds to drought, salinity, and heat, both individually and in combination. Conducted in the UK between 2020 and 2023 using controlled environments and glasshouses, the studies examined morphological, physiological, and biochemical responses of the African eggplant at different growth stages. Data collection was performed on potted plants throughout their growth and analyses were conducted on leaves and fruits in the laboratory.

When comparing four cultivars under salinity or drought, similar responses suggested shared response pathways for the cultivars used. Group differences were noted, however, with heat waves being well tolerated by a Shum cultivar during the vegetative stage while a reduction in leaf production was observed in a Kumba cultivar at a lower temperature. Combining heat stress with salinity or drought generally resulted in one stressor being predominant. In contrast, an additive effect of salinity and drought was observed for most of the recorded characteristics, causing significant plant damage. Biostimulants from the UK did not effectively mitigate these damages, limiting their applicability as a quick and short-term solution in the field.

The outputs of this PhD project agree with the use of the African eggplant in regions prone to heat stress, especially for cultivars from the Shum group. While no specific soil salinity threshold was established, the research uncovered some tolerance pathways, such as enhanced potassium uptake and quick assimilation rate recovery, valuable for researchers focusing on salinity tolerance. The knowledge generated is also relevant for breeders to provide different ways for testing tolerance, a key trait in newly developed seeds.

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Abbreviations

ϕ2	Photosystem II quantum yield.
ϕNPQ	Quantum yields of non-photochemical exciton quenching.
Fv/Fm	Maximum photochemical efficiency of photosystem II.
A	Assimilation rate; amount of CO_2 assimilated per leaf area and time.
AMF	Arbuscular mycorrhizal fungi.
ANOVA	Analysis of variance.
BBSRC	Biotechnology and Biological Sciences Research Council.
BSA	Bovine serum albumin.
Car	Carotenoids.
CAT	Catalase.
Chl a	Chlorophyll a.
Chl b	Chlorophyll b.
CO_2	Carbon dioxide.
DAHS	Days after the start of the heat stress.
DASH	Days after the start of the salinity and heat stress.
DASS	Days after the start of the salinity stress.
dS	DeciSiemens.
DTP	Doctoral Training Programme.
EC	Electrical conductivity of a solution.
EC 1:5	Electrical conductivity of a 1 soil over 5 water mass ratio.
ECe	Electrical conductivity of a soil saturated paste.
EL	Electrolyte leakage.
FAO	Food and Agriculture Organization.
GAE	Gallic acid equivalent.
Gluc.	Glucose.
gs	Stomatal conductance.
H_2O_2	Hydrogen peroxide.
HS	High salinity.
ICP-MS	Inductively coupled plasma mass spectrophotometry.
IPCC	Intergovernmental Panel on Climate Change.
IVs	Indigenous vegetables.
kPa	Kilopascal.
LER	Leaf expansion rate.
LRWC	Leaf relative water content.

LS	Low salinity.
MDA	Malondialdehyde.
mM	Millimolar.
MS	Medium salinity.
NaCl	Sodium chloride.
NBI	Nitrogen balance index.
NS	No salinity.
ns	Non significant.
pH	Potential of hydrogen.
PHs	Plant hydrolysates.
PIPS	Professional Internship for PhD Students.
PIs	Principal investigators.
POD	Peroxidase.
ROS	Reactive oxygen species.
rpm	Revolutions per minute.
RRes	Rothamsted Research.
SDGs	Sustainable Development Goals.
SOD	Superoxide dismutase.
SWP	Soil matric water potential.
T27	Temperature treatment with maximum 27°C reached during the day.
T32	Temperature treatment with maximum 32°C reached during the day.
T37	Temperature treatment with maximum 37°C reached during the day.
T42	Temperature treatment with maximum 42°C reached during the day.
TEAC	Trolox Equivalent Antioxidant Capacity.
UV-VIS	Ultraviolet-visible.
WVC	World Vegetable Center.

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Publications

Chapter 1 and Chapter 2

A close derivative of this work was combined and published as: David-Rogeat, N., Broadley, M., and Stavridou, E. (2023). *Solanum* crops with a focus on the African eggplant: The potential of biostimulants to enhance stress tolerance. *International Journal of Plant & Soil Science, 35(18).* doi: 10.9734/ijpss/2023/v35i183411.

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1.1 General introduction

Sustainable, resilient, and reliable food systems are necessary to develop a strong economy and maintain a healthy population. Vegetables and fruits are highly nutritious and diverse crops which contain health-promoting compounds and are low in fats. Vegetable production is, however, facing new challenges around the world due to climate variability as shown by the IPCC (Mbow et al., 2019). The global average temperature warmed by 0.85°C between 1880 and 2012 and many regions in Africa have experienced greater region-scale warming above 1°C (Allen et al., 2018). Extreme conditions, such as heatwaves, long-lasting drought, or varied rainfall patterns, have also an increased likelihood of happening while high-salinity areas are likely to expand in coastal areas (Mbow et al., 2019). These changes are expected to reduce crop productivity and modify the nutrition profile of produced food (Myers et al., 2017).

Abiotic stresses such as drought, heat, and salinity impact negatively every growth stage of many crops by reducing leaf production, photosynthesis, and yield (Giordano et al., 2021). When stress is detected, crops activate a range of responses to survive, which depends on the stress intensity, length, and cultivar involved (Giordano et al., 2021). Despite some common responses to stress, such as the production of reactive oxygen species (ROS) and associated antioxidative response to limit cell damages, crops also display unique traits depending on their tolerance ability (Beacham et al., 2018). In addition, the combination of stresses is often observed in natural conditions and has been suggested to lead to responses by plants not observed under individual stress, adding to the complexity of predicting how current plants will cope in the future (Mahalingam, 2015). For short-term solutions against the damaging effects of abiotic stress, the use of biostimulants and other growth-promoting compounds has often been investigated and shown some positive results, especially when used in combi-

nation (Bulgari et al., 2019). Their application is an environmentally friendly method to enhance crop performance, used mostly on high-value crops such as vegetables (Paradjiković et al., 2019; Petropoulos, 2020).

In Africa, agriculture employed more than 50% of the total population in 2017, most of them smallholders who are at the highest risk of threatened livelihood due to climate change (FAO, 2017). Innovative ways to ensure sustainable and resilient farming are thus needed to ensure food security and reduce poverty. While the Green Revolution was key to improving the yield of grains and staple food, malnutrition is increasing in all parts of the world (FAO et al., 2022). While grains are important to reducing famine, vegetables are key to tackling malnutrition due to their high levels of nutrients and vitamins. Indigenous vegetables (IVs) are of particular importance due to their diversity and the broad genetic pool they offer for breeding purposes (Bokelmann et al., 2022). Indigenous vegetables are widespread in Africa, a source of biodiversity, and adapted to local conditions, often displaying tolerance to environmental stresses (Tadele, 2018). Indigenous vegetables include the African eggplant (*Solanum aethiopicum* L.), Ethiopian mustard (*Brassica carinata* L.), okra (*Abelmoschus esculentus* L.), and legumes such as cowpea (*Vigna unguiculata* L.), and Bambara groundnut (*Vigna subterranea* L.) (Dinssa et al., 2016; Tadele, 2018). Many IVs are nutrient-rich and part of local markets, hence could support and strengthen current agricultural systems to deliver sustainable diets regarding human nutrition and environmental issues (Mabhaudhi et al., 2017). The use of IVs to increase field resilience through crop diversity has been limited to date, however, due to the lack of interest and investment (Akinola et al., 2020). They are under-researched and not used to their full potential, thus not as competitive as main crops such as maize, wheat, or tomato (Akinola et al., 2020). The indigenous vegetable market opportunities are still restricted and the combination of low yields and variable prices due to the locality of products are

adding to their limited usage (Arumugam et al., 2022).

The African eggplant, also referred to as scarlet eggplant, has received increasing scientific attention due to its high nutritional value, taxon diversity, and market presence in several countries (Nyadanu & Lowor, 2015). It is liked for its yield and nutritional quality and requires less input than the tomato and brinjal eggplant in terms of water and fertiliser, making it an environmentally friendly crop easier to grow without a developed setup. Despite being already widely used by small-scale farmers, its stress response pathways have not often been explored, especially under the combination of stress, limiting its promotion and wider use.

1.2 The African eggplant - *Solanum aethiopicum*

1.2.1 Taxonomy and genetics of the African eggplant

The African eggplant originates from Africa and has been domesticated from *S. anguivi* Lam., still found in the wild (Page et al., 2019). It is a close relative to the common brinjal eggplant from Asia (*S. melongena* L.) and tomato (*S. lycopersicum* L.) and is also a relative of other indigenous *Solanum* such as the Gboma eggplant (*S. macrocarpon* L.) (Aubriot & Daunay, 2019). The African eggplant is a complex hermaphrodite species consisting of four groups distinct both morphologically and in their use: Shum, Gilo, Kumba, and Aculeatum (Han et al., 2021; Taher et al., 2017) (Fig 1.1).

Only the small, hairless leaves from Shum plants are commonly eaten, while their highly bitter small fruits growing in clusters are used to collect seeds (Yang & Ojiewo, 2013). Shum varieties are found in the higher rainfall zones of West and Central African countries, or grown in swamps during the dry season

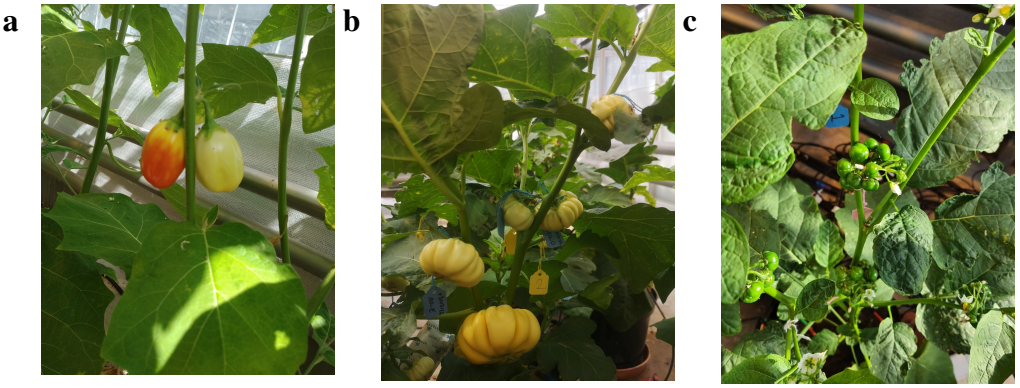


Figure 1.1: African eggplant **(a)** Gilo, cv. DB3 **(b)** Kumba, cv. Meketan **(c)** Shum, genotype E11 grown at NIAB East Malling, UK.

(Page et al., 2019). Due to the recurrent shoot and leaves harvest for selling, plants do not grow tall and will develop instead a much-branched architecture with weak stems and many small laves, preferred by the consumers (Moenga & Achieng Odeny, 2022).

The Gilo group is widespread in humid areas and plants from this group display inedible hairy leaves and edible green or white fruits, which can be round, elongated, ribbed or smooth (Yang & Ojiewo, 2013). They usually have one to three fruits per node and the bushy plants can reach up to 2 m, even though most commercial varieties range from 65 to 110 cm in height (Schippers, 2000). Gilo plants grow well at a temperature between 25 and 35°C during the day and 20 to 27°C at night (Han et al., 2021). Due to Gilo's higher morphological complexity than Shum, it has been suggested that the former has evolved from the latter.

Plants from the Kumba group, most commonly found in arid areas, have hairless leaves and medium to big ribbed fruits, both edible (Yang & Ojiewo, 2013). Plants from this group do not grow very tall, around 40 to 50 cm (Schippers, 2000). Nowadays, Kumba varieties with hairy leaves and only grown for their fruits are preferred in some regions due to their increased tolerance to pests (Schippers, 2000). Kumba plants tolerate high temperatures up to 45°C during the day (Han et al., 2021).

Finally, plants from the Aculeatum group produce inedible fruits and leaves, mostly used as ornamentals, and are the least common group grown in Africa (Yang & Ojiewo, 2013).

Each group is hypervariable with hundreds of local varieties across Africa and sub-groups can be distinguished based on various metrics such as fruit shape or size (Mungai et al., 2016). Traits have evolved through time based on farmers' and consumers' selection, leading to very diverse fruits across the continent. Phenotypic variations can be observed at all developmental stages, offering great breeding potential due to the high genetic pool within the African eggplant species (Kamga et al., 2015).

1.2.2 Cultivation of the African eggplant

Like many IVs, the African eggplant is mostly grown by small-scale farmers (Arumugam et al., 2022). It is a perennial crop but the yield is dramatically reduced after the first season. Most commercial plants will thus be kept alive for one season of about six months before being removed to make space for new seedlings that will be planted for the following growing season. As this crop is mostly rain-fed, it is primarily cultivated during the rainy season but can be grown during the dry season in the case farmers have access to irrigation (Govindasamy et al., 2020). Even though the total production might be lower than in the rainy season, the market price of fruits produced during the dry season will be at its highest and the incidence of diseases and pests will be lowered and is thus often preferred. To promote growth and income stability, the African eggplant, highly shade-tolerant, is often intercropped with other crops such as amaranth or coconut (Moenga & Achieng Odeny, 2022). Fertiliser input is not a common practice in small-scale cropping systems due, in part, to the lack of availability and high price. Instead, manure is commonly used by farmers to

enhance crop vitality (Govindasamy et al., 2020).

Leaf harvest from Shum varieties usually starts two months after sowing and can be repeated up to five times through one season (Schippers, 2000). Flowering, pollination, and fruit formation for fruity varieties generally start a month and a half after transplanting in the field while fruits are typically ready to harvest about one month after fruit set (Lester & Seck, 2004). The earliest varieties belong to the Kumba group and can be harvested around 85 days after sowing while most varieties require 110 to 120 days (Schippers, 2000). During the harvest season, fruits can be harvested twice a week to avoid quick over-ripening and promote plant vigour but are most generally harvested every 5 to 6 days to balance harvested quantity with cost (Horna & Gruère, 2007).

Fruit weight ranges from 25 to 110 g and yield varies significantly between areas, from 0.1 $t.ha^{-1}$ to 7.7 $t.ha^{-1}$, and reaching sometimes 20 $t.ha^{-1}$ under irrigation (Dinssa et al., 2016; Lester & Seck, 2004). Improved cultivars under favourable conditions have shown drastic yield improvement reaching around 60 $t.ha^{-1}$ (Lukumay et al., 2018). Leaf yield averages at 30 $t.ha^{-1}$ despite a potential of 225 $t.ha^{-1}$ under specific farming management (Lester & Seck, 2004).

In addition to being cultivated for its fruits or leaves, the African eggplant has also attracted attention as a potential rootstock for close relatives such as the tomato or brinjal eggplant to enhance plant vigour and/or tolerance against certain soil-born pathogens (Sabatino et al., 2019). Indeed, as interspecies hybridisation can sometimes face challenges such as infertility or incompatibility, the reliance on rootstock in commercial farms is more common to provide resistance against important soil-born pathogens (Boncukçu et al., 2023).

1.2.3 Distribution and use of the African eggplant

The African eggplant is popular in sub-Saharan countries such as Tanzania, Uganda, Benin, Mali, or Ghana, and is also being grown in Brazil under the name 'jilo' (Dinssa et al., 2016; Han et al., 2021). The fruit is consumed in East Africa while leaves are primarily consumed in Uganda and both leaves and fruits are eaten in West Africa (Dinssa et al., 2016). Marketable fruits are creamy to green in colour, while red and ripe fruits are used to collect seeds but generally not eaten (Dinssa et al., 2016). In cuisine, it is used similarly to tomato in stews to accompany local dishes or as soup thickeners, but the fruit can also be eaten raw (Han et al., 2021). Size, colour, taste and shape are the main attributes checked by consumers, with a preference for non-rounded shaped fruits due to their association with a reduced bitterness (Han et al., 2021). The highly bitter fruits of some cultivars, as well as the roots, are sometimes used as medicine to treat colic, high blood pressure or uterine complaints. Consumers are particularly attracted to the nutritional and medicinal value of the fruits and leaves.

A mapping pilot project in Arusha (Tanzania) undertaken as part of this thesis (see Appendix A for methods) highlighted the popularity of the African eggplant in markets across the city, with the crop being present in every market visited (Appendix A, Fig. A.1). Moreover, the African eggplant was in the top five vegetables sold in all of the markets (Fig 1.2). It was mostly sold alongside okra in the markets visited for cooking purposes, highlighting its importance regarding the marketing of other indigenous vegetables (Fig 1.3).

1.2.4 Nutritional properties of the African eggplant

The African eggplant fruits have a high moisture content and very low caloric value and provide several fundamental mineral elements such as calcium, iron,

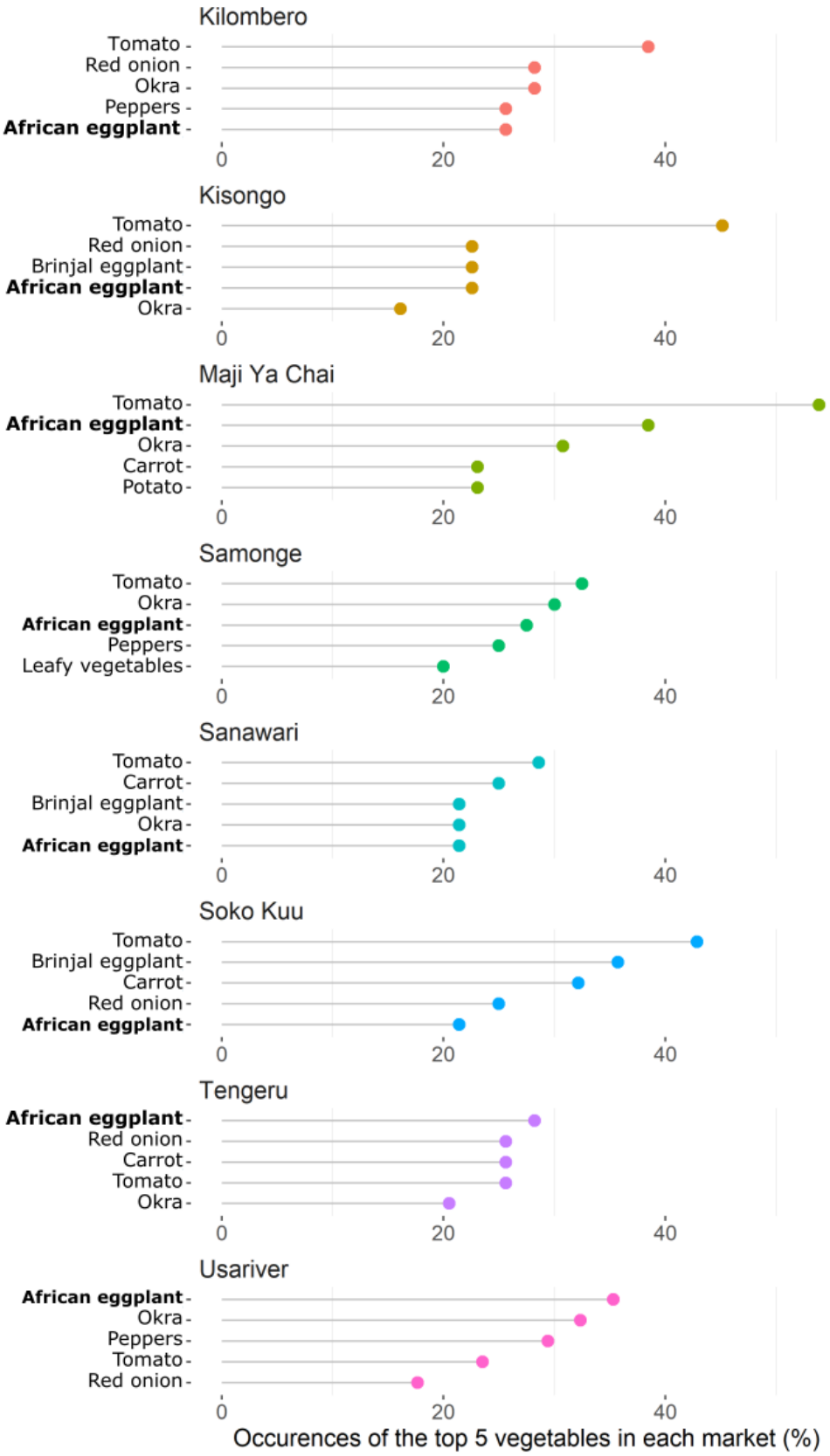


Figure 1.2: Occurrence of the five most common vegetables sold in markets in Arusha among the vendors sampled. Each line represents the percentage of vendors selling a particular vegetable (out of 100%).

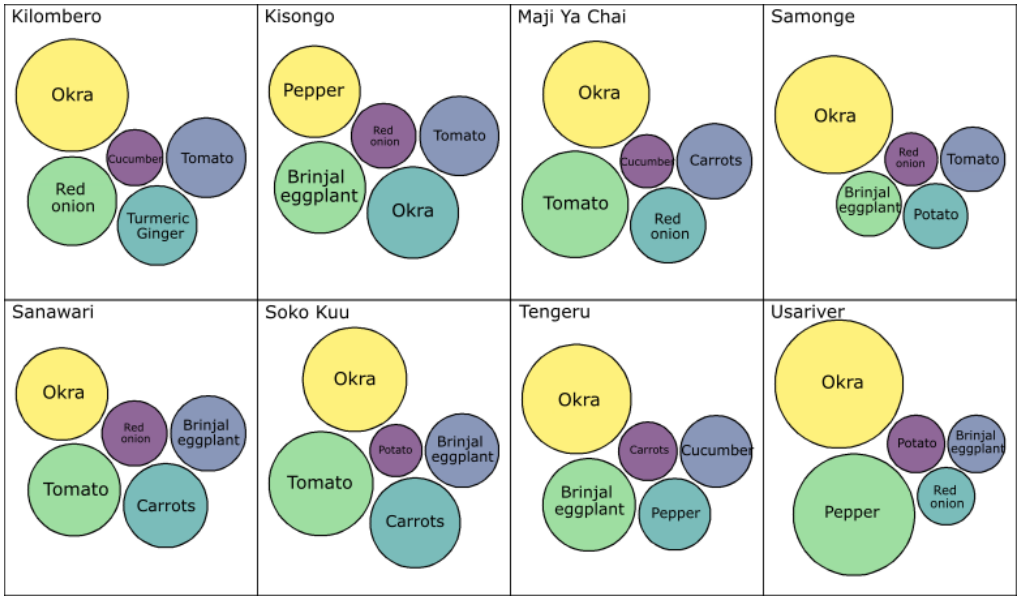


Figure 1.3: Proportions of the vegetables sold alongside the African eggplant in each Arusha market. The colour and the area of each circle correspond to the proportion of vendors selling the vegetables stated along the African eggplant with the area of each circle being proportional.

zinc, and vitamins (Afful et al., 2019). Fruits and leaves contain many phytochemicals, such as phenols, saponins, and flavonoids, which can benefit human health but are also important as secondary metabolites to protect the plant from stress (Han et al., 2021). Kumba plants tend to have the highest level of antioxidant activity while Aculeatum plants have the lowest nutritional value, highlighting a potential selection by growers and consumers for nutritious varieties. Different cultivars, even within the same group, can display high variability in nutritional quality as shown by Nwanna et al. (2019) when assessing fruits from two markets. They recorded large differences in total phenols (253 vs 499 mg gallic acid equivalent 100^{-1}), total flavonoids (1.54 vs 3.92 mg quercetin g^{-1}), and total antioxidants (1.24 vs 3.50 mM trolox equivalent antioxidant capacity) (Nwanna et al., 2019). When assessing fruit nutritional content as part of the pilot exercise in Arusha (see Appendix A for methods), variability across markets was only observed for fruit nitrogen, phosphorus, and copper concentrations (Table 1.1).

Table 1.1: Mean comparisons for dry fruit nutrient concentrations of African eggplants bought in markets in Arusha.

Source of variance	N	P	K	Ca	Mg	S	B	Cu	Mn	Fe	Zn
	g kg^{-1}						mg kg^{-1}				
Variety											
DB3	19.1	4.0	26.5	2.3	2.0	1.7	13.1	6.3	18.1	41.8	14.4
Tengeru White	21.7	4.4	28.9	2.0	2.6	1.8	16.5	6.0	24.3	44.8	15.3
Location											
Kilombero	19.4	4.0	27.3	2.1	2.2	1.6	13.2	5.0	19.1	42.0	13.6
Kisongo	17.9	4.2	27.2	2.2	2.4	1.8	17.2	6.6	18.6	39.7	14.4
Maji Ya Chai	20.1	5.1	26.4	1.9	2.3	2.0	12.0	7.8	17.9	37.0	15.2
Samonge	21.1	4.4	28.7	2.6	2.2	1.8	14.1	6.8	20.2	52.4	15.9
Sanawari	21.0	4.5	28.8	2.2	2.4	1.9	16.2	8.1	23.5	42.4	16.4
Soko Kuu	21.1	4.3	28.4	2.1	2.4	1.7	17.0	5.0	24.0	45.2	14.1
Tengeru White	17.8	3.6	25.7	1.9	2.0	1.6	11.2	4.6	17.2	40.0	13.5
USARiver	22.4	4.0	26.1	2.2	2.0	1.8	12.8	6.5	22.3	39.0	15.1

N = Nitrogen, P = Phosphorus, K = Potassium, Ca= Calcium, Mg = Magnesium, S = Sulphur, B = Boron, Cu = Copper, Mn = Manganese, Fe = Iron, Zn = Zinc.

The African eggplant is used in traditional medicine to treat different conditions such as mental disorders or diabetes (Abubakar et al., 2022). Due to the high antioxidant levels within the plant, they have been suggested to be used as nutraceutical supplements (Faraone et al., 2022). The supplementation of diets with dry or fresh leaves from the African eggplant has indeed been investigated in multiple studies which have seen some beneficial effects on obesity development (Asuquo et al., 2022), diabetes (Singh et al., 2020) and iron intake (Saha et al., 2022) for example. Red fruits displayed higher levels of essential minerals, highlighting a potential to dry these fruits instead of marketable ones to obtain a highly nutritional powder while limiting waste and increasing farmers' income (Michael et al., 2017).

Chapter 2

Literature Review

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2.1 Drought stress in *Solanum* species

Drought takes place when the water available at the roots does not cover the transpirational requirements of the plants (Kapoor et al., 2020). It is one of the most damaging factors in crop production with effects on plants' morphology, physiology, and biochemical processes (Table 2.1).

Table 2.1: Sub-sample of the available studies on the effect of drought on *Solanum* species.

Species							Responses										
S. aethiopicum	S. lycopersicum	S. melongena	S. insanum	S. incanum	S. anguivi	% Pot / field capacity	Shoot biomass	Leaf area	Plant height	Yield	Stomatal conductance	Photosynthesis activity	Chlorophyll	Sugars	Non-enzymatic antioxidans	Enzymatic antioxidants	Reference
						50	=		=	↓	↓		↓				Ghannem, Ben Aissa, & Majdoub (2021)
						Wilting	=							=	↑		Klunklin & Savage (2017)
						50	↓	↓	↓				↓		↑	↑	Kusvuran & Dasgan (2017)
						40		↓	↓	↓	↓		↓	↑			Lagat (2016)
						50				↓	↓	↓	↓				Limbu, Sharma, & Rao (2018)
						40				↓			↓	↑			Mohawesh (2016)
						25	↓			↓							Nakanwagi et al. (2017)
						No water	↓								=	=	Plazas et al. (2022)
						No water	↓								↑	=	
						45					↓	↓	↓	=		↑	Sun et al. (2016)

↑ Increase ↓ Decrease = No changes

The different colours represent different Solanum species. A yellow upward arrow depicts an increase in the measured response, a red downward arrow a decrease, and a purple equal sign represents no signifcant differences. Characteristics with no signs have not been researched in the study associated.

2.1.1 *Solanum aethiopicum* under drought

In a study by Lagat (2016), the African eggplant's morphological and physiological aspects were hindered in every accession tested under reduced field capacity. although at a different rate for each accession. For example, under a 20% irrigation reduction, stomatal conductance decrease ranged from 6% to 40% with an average of 19% (Lagat, 2016). The variability observed, also observed in Shum cultivars in another study by Nakanwagi et al. (2017), highlights the range of tolerance among cultivars despite the absence of absolute tolerance

of morphophysiological metrics. In addition, fruits' sugars, acids, beta-carotene, and vitamin C increased under 60% field capacity, highlighting the activity of the plant's secondary metabolism (Lagat, 2016). These benefits were, however, counter-balanced by a decrease in mineral elements such as magnesium, calcium, iron, and zinc, reducing the overall gain in fruits' nutritional quality (Lagat, 2016).

Another study by Mibei et al. (2017) investigating the drought effects on the African eggplant secondary metabolism also showed strong variability between accessions. Out of the 19 accessions of African eggplant tested, 10 displayed an increase in carotenoids, 5 a decrease, and 4 no change (Mibei et al., 2017). Individual carotenoids were affected differently as well, with an overall decrease in chlorophylls and carotenes and an increase in xanthophyll, suggesting a decrease in leaf pigmentation alongside an increase in other dietary neutraceutical carotenoids (Mibei et al., 2017). Further to their carotenoids study, Mibei et al. (2018) analysed a range of metabolites in leaf extracts, including organic acids, sugars, and amino acids. Similarly, accessions reacted differently even though a general trend of increasing sugars and organic acids appeared (Mibei et al., 2018). The authors shed light on important parts of the oxidative responses to drought in various accessions of the African eggplant and highlighted key metabolites involved in drought stress adaptation.

In field conditions, Mwinuka et al. (2021) demonstrated that watering at 80% of the crop requirement was optimal to balance the cost of irrigation while maintaining the same yield. Similarly, Gilo cultivar Morro Grande had a reduced plant growth and fruit yield at 50% irrigation but not at 75%, highlighting a tolerance threshold situated between these levels (de Castro Seron et al., 2019). In another study, however, a drop from 100% pot capacity to 75% reduced a variety of morphological attributes such as leaf number, area, and plant height, even though a reduction to 50% did not damage these characteristics further (Limbu

et al., 2018). Photosynthesis and fruit yield, in opposition, were maintained at 75% pot capacity (Limbu et al., 2018). The African eggplant pathways to tolerance are thus varied and the conservation of fruit production over vegetative growth seems to be in place under low-intensity drought. Different responses were also noted when drought was applied at different growth stages in another study, with the flowering stage being the most critical stage for watering (Nakanwagi et al., 2020). This knowledge is important for farmers to ensure crop protection at key growing points.

2.1.2 Other *Solanum* species under drought

Morphological defects caused by drought on *Solanum* can be detected at early stages with a reduced germination rate and seedling growth in eggplant under water stress (Steiner & Zuffo, 2019). A reduction of leaf area was noted in later stages in tomato by Kusvuran and Dasgan (2017) with the maintenance of leaves number, suggesting the production of smaller leaves instead. This observation was also made by Zhou, Yu, et al. (2017) in two different tomato cultivars, indicating an adaptive mechanism to limit water loss through leaf thinning. Drought also impacted fruit development in eggplant with a reduction in diameter and length, reducing eventually marketable yield and profitability (Aly et al., 2019). Even if drought impacts morphological characteristics at every growth stage, a study by Ghannem et al. (2021) showed that yield and fruit characteristics of tomato were only impacted when intermittent drought perturbations were applied at the harvesting stage, suggesting the presence of recovery mechanisms earlier.

Root length was reduced in multiple tolerant and susceptible brinjal eggplant cultivars under no irrigation (Plazas et al., 2022). Root dry weight, however, was not reduced for tolerant cultivars in another study, showing diverse responses of

root development by producing either larger roots or a higher number of small roots (Plazas et al., 2022). While drought avoidance mechanisms can lead to an extended root network accessing water more easily, especially in the field, the reduction in root growth is often seen as a tolerance mechanism to maintain resources. Stem development is also hindered by the lack of water with plants producing thinner stems and reducing shoot dry weight overall, as observed in the brinjal eggplant and tomato (Aly et al., 2019; Zhou, Kong, Yu, et al., 2019). These reductions seem to only appear above a certain threshold with a reduction in irrigation by 20% or 25% not impacting significantly the brinjal eggplant development and yield, as seen by Mahmud et al. (2021). Under higher stress intensity, however, the reduction of stem diameter eventually leads to reduced water and nutrient flow within the plant.

Reducing water flow throughout the plant affects plant water status whose maintenance is crucial for many physiological processes. A decrease in leaf relative water content (LRWC) has regularly been reported under drought and is now a common stress marker in multiple species (Çelik et al., 2017). A reduced LRWC was noted in a drought-tolerant tomato cultivar, even though a sharper decrease was observed in a sensitive one (Kusvuran & Dasgan, 2017). This decrease is generally observed even at a low level of drought and at early stages but is quickly recovered after re-wetting the soil, highlighting the dynamic nature of this marker (Parkash & Singh, 2020). The reduction of LRWC enables plants to withstand drought periods better by reducing water loss through leaves but limits cell expansion and functions.

A decrease in water status is a signal for plants to close their stomata to limit further water loss through transpiration (Kapoor et al., 2020). This closure was noted in tomato even at a low-stress level of 15% irrigation reduction and at every growth stage (Çelik et al., 2017; Ghannem et al., 2021; Zhou, Yu, et al., 2017). Stomatal closure has been generally said to be the main driver for de-

creased photosynthesis under drought, eventually leading to reduced fruit production. Other mechanisms can, however, decrease photosynthesis following drought due to the complex photosynthetic mechanisms. For example, drought often leads to a reduction in chlorophyll production, as reported in tomato, eventually reducing the light-harvesting capacity of the plants (Alharby et al., 2020; Sun et al., 2016). In a study by Çelik et al. (2017) on tomato, leaf photosynthetic pigments were reduced two days after the beginning of stress, showing a highly responsive process. A chlorophyll decrease in tomato plants was most prevalent during the vegetative stage, when sufficient photosynthesis activity is crucial to develop resources to start flowering, and at fruit set (Ghannem et al., 2021). Nonetheless, increases in chlorophyll have also been noted under water stress, as seen in the brinjal eggplant in the study by Mahammed et al. (2021). This increase, measured per leaf area, can be due to the reduced leaf size observed under drought, leading to a higher concentration of chlorophyll per area but can also be an intrinsic mechanism to enhance light harvest while limiting water loss. Alongside photosynthesis pigments, membrane stability is crucial to maintain photosynthesis activity (Kapoor et al., 2020). Under drought, membrane stability, measured by the amount of electrolyte leakage, has regularly been reported to decrease proportionally to the level of stress in both the tomato and brinjal eggplant (Cornejo-Ríos et al., 2021; Mahammed et al., 2021). Leakage of electrolytes has often been related to photosynthetic and mitochondrial activity reductions in plants.

Net photosynthesis rate has been observed to decrease sharply under the absence of irrigation in tomato (Zhou, Yu, et al., 2017). Limited irrigation also increased non-photochemical quenching in the same study, showing an adaptive process to limit the creation of reactive oxygen species (ROS) produced due to the imbalance of the energy harvested and its utilisation (Ashraf & Harris, 2013). Reactive oxygen species, while beneficial for plant stress response

at low levels as signalling molecules, can have damaging effects on cells when present at a high concentration and lead to oxidative stress (Das & Roychoudhury, 2014). Malondialdehyde (MDA), a marker of oxidative stress, increased gradually as pot water content decreased in tomato, as did total protein content driven by a sharp increase in antioxidant enzymes (Çelik et al., 2017). Interestingly, levels of MDA were similar between tolerant and susceptible brinjal eggplant cultivars in a study by Plazas et al. (2022), highlighting that MDA levels do not automatically translate to plant tolerance but more likely trigger stronger responses leading to tolerance.

Drought stress increased oxidative stress in tomato alongside an increase in enzymatic and non-enzymatic antioxidant levels as a response (Alharby et al., 2020). Lycopene, a major antioxidant in tomato, increased under stress in three cultivars alongside phenols, flavonoids, and total antioxidants in another study (Klunklin & Savage, 2017). Antioxidants are the main defenders to limit ROS damage and maintain cell processes (Das & Roychoudhury, 2014). When comparing susceptible, intermediate, and tolerant brinjal eggplant cultivars, Plazas et al. (2022) showed that even if all accessions had an increase in phenols and flavonoids, this increase was more important for tolerant cultivars. The same observation was made for the antioxidant enzyme catalase, while only susceptible and intermediate cultivars increased their levels of ascorbate peroxidase, another antioxidant enzyme (Plazas et al., 2022). The antioxidative response is thus tightly controlled under stress with selected enzymes and non-enzymatic antioxidants being activated under certain conditions. Some *Solanum* cultivars seem to rely on their antioxidant activity for stress tolerance, ensuring cell processes are maintained and damage by ROS compounds is limited.

A range of biochemical processes are affected by drought due to the reduction in mineral elements uptake by the roots and the following reduction in the distribution of these elements to different plant organs. Leaf nitrogen, potassium,

phosphorus, and iron decreased in tomato at 15% irrigation reduction, with further decreases noted when irrigation was withdrawn even more (Ragab et al., 2019). In the brinjal eggplant, however, a decrease of 20% irrigation did not impact nitrogen, potassium, or phosphorus, which were only reduced when irrigation was withdrawn by 60% or more (Mohawesh, 2016). *Solanum* nutrient uptake under drought is thus different based on the species, highlighting a potential genetic factor involved. This was observed in tomato cultivars where most cultivars had a reduction in their leaf nitrogen levels under drought except the tolerant ones, supporting their importance in tolerance (Sánchez-Rodríguez et al., 2010). In the same study, potassium, phosphorus, magnesium, and iron were unchanged in the sensitive cultivars but were increased in the most tolerant cultivar, highlighting the importance of enhancing selected mineral elements to maintain plant growth and photosynthetic activity (Sánchez-Rodríguez et al., 2010).

2.2 Heat stress in *Solanum* species

Elevated temperatures and extreme events such as heat waves can hinder plant growth at every development stage depending on the cultivar, intensity, and length of stress. In addition to morphological and physiological changes, antioxidant levels can also be affected by heat stress (Hassan et al., 2021). Short and measured stress can, however, be beneficial if timed right for vegetable quality by improving health-promoting compounds such as antioxidants. Table 2.2 depicts graphically some of the literature available on the effects of high temperatures on *Solanum* species.

Table 2.2: Sub-sample of the available studies on the effect of heat stress on *Solanum* species.

Species			Responses													
S. aethiopicum	S. lycopersicum	S. tubercosum	Heat (°C)	Shoot biomass	Leaf area	Plant height	Yield	Pollen germination	Membrane stability	Stomatal conductance	Photosynthesis activity	Chlorophyll	Sugars	Non-enzymatic antioxidans	Enzymatic antioxidants	Reference
			38	↓						↓	↓	↓			↑	Haque et al. (2021)
			38	↑					↓		↓	↑			↑	Lee et al. (2023)
			30			↓						↓			↑	Mohamed et al. (2016)
			45									↓	↑			Naz et al. (2018)
			40	↑		↑	↓			↑	↓					Nkansah (2001)
			35				↓				↓	↓			↑	Paul, Farooq, & Gogoi (2016)
			38	↓	↓			↓		↑	↓					Poudyal, Rosenqvist, & Ottosen (2018)
			35			↑	↓					↑				Tang et al. (2018)
			36					↓		=	↓	↓	↑	=		Zhou et al. (2015)
			36			=				↑	↓	↓	↑			R. Zhou, Kjaer, et al. (2017)

↑ Increase ↓ Decrease = No changes

The different colours represent different Solanum species. A yellow upward arrow depicts an increase in the measured response, a red downward arrow a decrease, and a purple equal sign represents no signifcant differences. Characteristics with no signs have not been researched in the study associated.

2.2.1 *Solanum aethiopicum* under heat

A Gilo cultivar of the African eggplant has been shown to have an increased photosynthesis activity and stomatal conductance at 35°C when compared to 30°C which eventually were decreased at 40°C, showing a high tolerant threshold (Nkansah, 2001). Conversely, plant height and shoot weight kept increasing under 40°C (Nkansah, 2001). Despite an enhancement of vegetative growth under heat, fruit numbers decreased drastically and the overall yield was reduced, even though the fruits produced were heavier (Nkansah, 2001). This shows a different tolerance threshold for vegetative and reproductive growth which is important to uncover for understanding the potential of the African eggplant in heat-prone fields.

2.2.2 Other *Solanum* species under heat

The vegetative growth of tomato plants was unaffected in a study subjecting them to a 10°C increase, reaching 36°C, as seen by the absence of changes in plant height, leaf number, and stem diameter in both heat-tolerant and heat-sensitive cultivars (Zhou, Kjaer, et al., 2017). When including more cultivars and setting the temperature at 40°C, Sherzod et al. (2020) found an increase in plant height and stem diameter for many cultivars, especially the ones producing large fruits. Blanchard-Gros et al. (2021) also found a cultivar-dependent response of plant height under heat when studying the wild tomato *S. chilense* (Dunal) Reiche with an increase in stem growth under heat for some. The same trend regarding plant height was noted in potato with an increase of 13°C from normal temperature leading to a plant height increase of 47% on average (Tang et al., 2018). The positive effect of heat on plant development seems thus to appear mostly at very high temperatures in *Solanum*. Interestingly, when including a recovery period, Duan et al. (2017) showed that even after no effects on stem growth during the heat period, tomato plants that experienced heat had a slower growth development when placed back under normal temperature than the controls, in contrast to the previous beneficial observations. This suggests long-term damages and a slow recovery regarding vegetative growth in this case.

Leaf production and development are, in general, hindered at high temperatures after a certain threshold as seen in both tolerant and sensitive tomato cultivars and potato (Naz et al., 2018; Poudyal et al., 2018). No change in leaf number was noted in young tomato plants grown at 36°C, however, and leaves produced were the same size in another study growing tomato at 40°C (Sherzod et al., 2020; Zhou, Kjaer, et al., 2017). The different growth stages and stress lengths, with the latter studies focusing on short-term stress at early stages, might explain these opposite effects observed. Under heat stress, smaller leaves are a way to

limit excess transpiration and water loss.

Leaf pigments are also affected by heat with tolerant tomato cultivars displaying higher leaf pigment levels in a study by Zhou et al. (2015). Every potato cultivar investigated by Tang et al. (2018) also displayed higher leaf pigment levels under heat. Chlorophyll b tends to increase to a lesser extent than chlorophyll a due, in part, to its high correlation with light-harvesting proteins (Tanaka & Tanaka, 2011). A high number of light-harvesting proteins can be harmful for plants under heat as the stress limits the amount of light that can be used due to photosynthetic enzyme damage. The excess light can eventually increase the damage to the photosynthesis apparatus and other processes. An increase in chlorophylls is, nonetheless, important to maintain and potentially enhance photosynthesis. In addition, an increase in chlorophyll a and carotenoids can lead to the reduction of photooxidation and photoinhibition by ensuring excess light is dissipated as heat (Ashraf & Harris, 2013). As opposed to the previous observation, a potato cultivar tolerant regarding micro tuber formation showed a slight decrease in leaf pigment, especially chlorophyll b, showing that other tolerance mechanisms are in place to ensure fruit production, in this case (Mohamed et al., 2016). A range of studies supported that observation with a reduction in leaf pigments in potato, tomato, and the brinjal eggplant (Hannachi et al., 2022; Haque et al., 2021; Paul et al., 2016).

Varied tolerance levels regarding gas exchange are also seen in *Solanum*. Tolerant tomato cultivars displayed an increase in stomatal conductance which was not observed in sensitive ones in the study by Zhou et al. (2015). Both sensitive and tolerant tomato cultivars increased their stomatal conductance under heat in another study, however (Poudyal et al., 2018). This adaptive mechanism allows better leaf cooling and the maintenance of enzyme activity, crucial to maintaining photosynthesis. This was not observed by Zhou, Kjaer, et al. (2017) and Duan et al. (2017) who reported a decrease in stomatal conductance, suggest-

ing other pathways are in place to limit the negative effects of increasing leaf temperature while maintaining water loss to a minimum. The photosynthetic apparatus is, in general, highly disturbed by heat through a range of mechanisms including enzyme denaturation and increased transpiration (Hassan et al., 2021). Photosynthetic parameters were reduced in tomato plants subjected to an increase of 15°C or higher in studies by Parrotta et al. (2020) and Raja et al. (2020) but no negative effects were noted at a 6°C increase in another cultivar, showing a certain tolerance of the photosynthetic system (Zhou, Yu, et al., 2017). Maximum photochemical efficiency of photosystem II (*Fv/Fm*) can be a powerful way of identifying heat tolerant plants as its maintenance under heat stress is associated with multiple other tolerance traits (Poudyal et al., 2018; Zhou et al., 2015). As a fast-responding indicator of a plant's photosynthesis efficiency, *Fv/Fm* can help researchers quickly identify damage to the photosynthesis process.

Despite the boost sometimes observed in the vegetative growth of *Solanum* plants, the most drastic effects of heat stress are observed during the reproductive stages, hindering final fruit yield (Alsamir et al., 2021). In tomato, both short and long-lasting heat stress reduced pollen germination and the number of fruits produced (Fragkostefanakis et al., 2016; Sherzod et al., 2020). Pollen germination was decreased in both tolerant and sensitive tomato cultivars even though the effects were most noticeable in the sensitive one (Zhou et al., 2015). Pollen tube length, on the other hand, was not affected by a 10°C increase in tolerant tomato plants but decreased strongly in sensitive ones (Zhou et al., 2015). In one study, the number of flowers increased significantly under heat despite a net reduction in fruit yield, showing that pollen defects are the main reason for a reduced fruit set and yield, alongside defects in photosynthesis (Amuji et al., 2020).

A range of mechanisms is activated under heat stress to help maintain critical

processes running, such as photosynthesis. To counteract oxidative stress induced by heat which damages proteins and DNA, plants, in particular tolerant ones, can enhance their antioxidant metabolism as was seen by an increase in phenols and antioxidant enzymes in a few studies (Dasgan et al., 2021; Haque et al., 2021; Paul et al., 2016). Osmolytes are also often increased to maintain membrane stability and a range of cell processes, as was observed in a study by Dasgan et al. (2021) with an increase in sugars in both tolerant and susceptible tomato cultivars. The maintenance of membrane stability, and cell turgor and expansion is also assured by an increase in proline in a range of plants, an adaptive mechanism to tolerate short-term heat stress (Naz et al., 2018; Raja et al., 2020).

2.3 Salinity stress in *Solanum* species

Saline soils contain excessive soluble salts, mainly sodium chloride (NaCl) and sodium sulphate (Na_2SO_4). They are generally described as having an electrical conductivity higher than 4 dS m^{-1} (FAO & ITPS, 2015). This represented 412 million hectares of soil in 2015, 122.9 of which in Africa (FAO & ITPS, 2015). Different salinity intensities are further defined in Table 2.3. Salinity disturbs every growth stage, with a particularly high impact during seedling development, via osmotic or ion imbalance stresses (Machado & Serralheiro, 2017). The former is due to the lower uptake of water by the plant due to the high salt concentration in soil and has a rapid onset, while the latter is caused by the excessive uptake of sodium and chloride ions over a long exposure period (Altaf et al., 2022). A range of processes is affected by salinity in *Solanum* crops including morphological, physiological, and molecular pathways (Table 2.4).

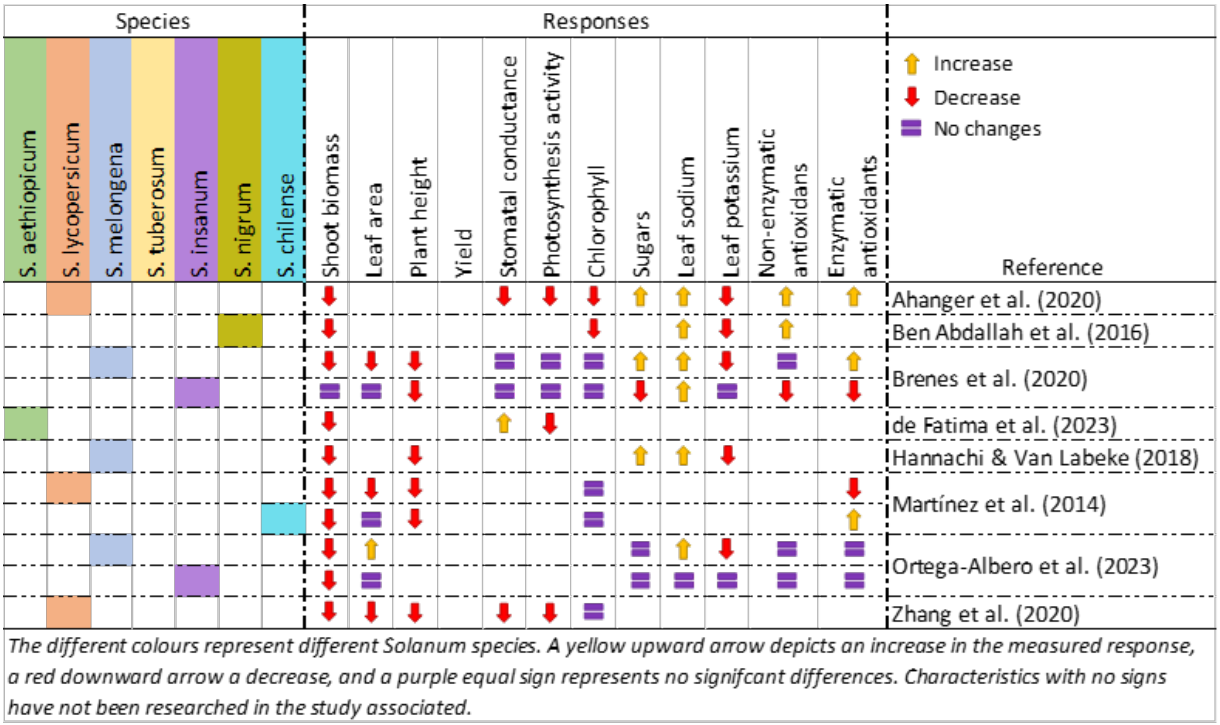
Table 2.3: Soil salinity classes based on the soil electrical conductivity as defined by (Omuto et al., 2020)

Salinity rating	Non-saline	Weakly saline	Moderately saline	Strongly saline	Very strongly saline
ECe (dS m^{-1})	<2	2 - 4	4 - 8	8 - 16	>16

2.3.1 *Solanum aethiopicum* under salinity

Increasing salinity decreased root and shoot biomass production in the African eggplant (de Fatima et al., 2023). Interestingly, stomatal conductance was enhanced by salinity, an increase explained by the energy re-deployment from plant growth to stomatal dynamics (de Fatima et al., 2023). This was not sufficient to maintain CO_2 assimilation rate, however (de Fatima et al., 2023). Overall, reductions regarding plant development and gas exchange of more than 10%

Table 2.4: Sub-sample of the available studies on the effect of salinity on *Solanum* species.



The different colours represent different Solanum species. A yellow upward arrow depicts an increase in the measured response, a red downward arrow a decrease, and a purple equal sign represents no signifcant differences. Characteristics with no signs have not been researched in the study associated.

were observed above 1.37 dS m^{-1}, highlighting its sensitivity to salt accumulation. This sensitivity to saline irrigation was further supported by the study by Mensah et al. (2020) who demonstrated a negative effect of salinity on the African eggplant's development already at low salinity levels. The negative impacts were significant only from the 7^{th} week of application, however, suggesting the importance of stress length in determining the stress tolerance of the plant (Mensah et al., 2020). In contrast to the study by de Fatima et al. (2023), however, Mensah et al. (2020) found a reduction in the stomata area. As the increase in stomatal conductance observed by de Fatima et al. (2023) was highly dependent on the salinity level, it can be hypothesised that the different methods of salt application and timing of measurement might be part of the reasons behind these contrasting results.

2.3.2 Other *Solanum* species under salinity

Salinity stress affects *Solanum* species from the germination stage, with both tomato and its close relative the black nightshade *S. nigrum* L. displaying reduced seed germination under NaCl irrigation, even if *S. nigrum* was less affected (Al Khateeb et al., 2019). Of the germinated seeds, both root and shoot growths were limited by the stress (Al Khateeb et al., 2019). This negative effect on root and shoot was also observed later, during the plants' vegetative stages (Ben Abdallah et al., 2016; Brenes et al., 2020). Interestingly, even high salt levels did not reduce plant growth parameters in the wild eggplant *S. insanum* L., highlighting the tolerance potential of some *Solanum* species (Brenes et al., 2020). *Solanum pennellii*, a salt-tolerant species, formed the central focus of a study by Albaladejo et al. (2017) in which the it maintained leaf thickness under salinity while the sensitive tomato lines studied displayed thinner leaves under stress. Leaf thickness has previously been suggested to be a reliable indicator of

a plant's water status and stress level, with thinner leaves reducing water loss. In the same study, the authors found a slow tolerance mechanism in place for *S. pennellii* (Albaladejo et al., 2017). Shoot and root growth rate was slowed down by salinity in *S. pennellii* to a higher extent than in tomato after seven days of stress, but this was reversed after 14 days (Albaladejo et al., 2017). Due to the two-step nature of salinity toxicity, tolerance can take place at different stages and might only be perceived when a threshold is passed. Leaf development was reduced in the brinjal eggplant at lower salinity levels than stem or root development, showing differences between plant organs as well (Brenes et al., 2020). This decrease in leaf production was also observed in tomato, *S. chilense*, *S. nigrum*, and *S. insanum* (Ben Abdallah et al., 2016; Martínez et al., 2014; Ortega-Albero et al., 2023; Zhang et al., 2020).

Brenes et al. (2020) reported the maintenance of LRWC in *S. insanum* under salinity stress reaching 300 mM NaCl while the brinjal eggplant's LRWC was already reduced at 100 mM NaCl. A reduction in LRWC was also noted in two tomato cultivars (Alsafari et al., 2019), impacting leaf expansion, nutrient transfer, and photosynthesis activity. The water status of tomato, *S. chilense*, and potato was also impacted by a decrease in leaf osmotic pressure under stress (Efimova et al., 2018; Martínez et al., 2014). This reduction in leaf osmotic pressure is a protective mechanism in place to limit ion accumulation due to the adapted osmotic gradient. This only works as a short-term tolerance strategy, though, as a long-term reduction in osmotic pressure can lead to negative effects on various parameters such as cell membrane stability, which was reduced in the brinjal eggplant and every tomato accession tested under salinity (Ahsan et al., 2022; Hegazi et al., 2015). A reduction in leaf osmotic pressure was only observed in the most sensitive brinjal eggplant accessions while tolerant ones maintain it under stress, suggesting other mechanisms in place for long-term tolerance (Hannachi & Van Labeke, 2018).

Variations in leaf morphology and water content can, in turn, affect chlorophyll levels. Under very high salinity levels (above 300 mM NaCl), chlorophyll was noticeably reduced in tomato plants (Tanveer et al., 2020). A range of studies on *Solanum* reported no or little effects on total chlorophyll under more moderate salinity levels (Brenes et al., 2020; Ortega-Albero et al., 2023; Zhang et al., 2020). Carotenoids were, however, regularly reduced, suggesting a potential shift in light-harvesting wavelengths throughout the stress. Photosynthesis activity can eventually be affected due to a combination of affected pathways including light harvesting changes. Liao and Zhang (2021) reported a gradual decrease in assimilation rate, stomatal conductance, and intracellular carbon in *S. nigrum* as the salinity level increased. Similarly, a reduction in net photosynthesis rate and stomatal conductance was observed in tomato, the brinjal eggplant, and *S. nigrum* (Bacha et al., 2017; Brenes et al., 2020; Zhang et al., 2020). In addition, non-photochemical quenching, representing the amount of light dissipated as heat by the plant to avoid photodamage, was increased in *Solanum* (Ahanger et al., 2020). Despite this increase, ROS hydrogen peroxide (H_2O_2) and O_2^- were still recorded at high levels in these crops (Ahanger et al., 2020). The increase of H_2O_2 is not consistent, however, with some reports of no effects of salinity on its levels (Ben Abdallah et al., 2016; Ortega-Albero et al., 2023). When comparing H_2O_2 levels in potato leaf, stem, and root, Jaarsma et al. (2013) showed that leaf levels were unchanged in most accessions but stem and root H_2O_2 were highly increased under salinity, highlighting plant organ differences in stress marker accumulation and explaining in part the variability of the observations previously made.

Ion accumulation within plants under salinity is broadly accepted as a common effect with the increase of sodium and decrease of the potassium over sodium ratio in various plant parts. This disruption causes secondary negative effects including damage to cell membranes and various enzymes. Albaladejo et al.

(2017) noted a clear sodium increase in roots and leaves of *S. pennellii* and tomato under salinity with *S. pennellii*, more tolerant than tomato, showing a less drastic increase in leaf sodium concentration. This clear increase was also noted in the brinjal eggplant and *S. insanum* alongside an increase in chloride ions (Brenes et al., 2020; Hegazi et al., 2015). A range of secondary metabolites is produced in response to this stress including osmolytes such as proline, glycine betaine or sugars, which help to maintain a low osmotic potential, necessary to ensure water flow through the plant (Jogawat, 2019). Fruit sugars increased under salinity in the brinjal eggplant and tomato, but not in *S. chilense*, considered the most tolerant species among those (Hegazi et al., 2015; Martínez et al., 2020). In contrast, *S. insanum* accumulated proline and sugars under stress to a higher level than the brinjal eggplant, more sensitive than *S. insanum* (Brenes et al., 2020). These osmolytes and their localisation seem thus to be important in plants' tolerance to salinity by acting as signalling molecules but also directly protecting cells from the damaging effects of ion accumulation (Jogawat, 2019).

Changes in ROS level under salinity trigger an antioxidant response by crops, as observed by the increase of antioxidant enzymes in potato, tomato, the brinjal eggplant and *S. nigrum* (Ahanger et al., 2020; Efimova et al., 2018; Hegazi et al., 2015; Liao & Zhang, 2021). The antioxidant response can affect greatly crop tolerance as was seen in potato where the most tolerant cultivar showed an increase in shoot ascorbate peroxidase and glutathione reductase that was not observed in the sensitive accession (Aghaei et al., 2009). Ahanger et al. (2020) reported an increase in all antioxidant enzymes measured, total flavonoids, and phenols in tomato, with the latter also increased in the brinjal eggplant fruits and tomato leaves in other studies (Bacha et al., 2017; Hegazi et al., 2015). This antioxidant response seems to be triggered at low salinity levels as shown by Ben Abdallah et al. (2016) who demonstrated that phenols increased already at

50 mM NaCl. Surprisingly, a higher level of salinity removed this effect with no increase or decrease noted at 100 and 150 mM NaCl compared to no salinity (Ben Abdallah et al., 2016). In the same vein, *S. villosum* and *S. insanum* only showed changes in phenols and flavonoids at medium salinity levels while no differences were noted between non-stressed plants and plants watered with a 150 mM NaCl solution (Ben-Abdallah et al., 2019; Brenes et al., 2020). In some studies, no effects were noted at all (Brenes et al., 2020; Martínez et al., 2020). These differences suggest a complex link between stress and antioxidant responses, heavily reliant on the stress intensity with other mechanisms in place at very high salinity levels.

2.4 Stress combination in *Solanum* species

Exploring the effects of stress combination is of utmost importance to understand how crops will react in natural conditions in current and future agricultural settings. Drought is often associated with high temperatures while high temperatures can increase soil salinity by reducing the soil leaching capacity for example (Mahalingam, 2015). While responses to individual stresses have been extensively studied as seen above, especially in model crops, stress combination is only starting to be researched. Crops can respond to stress combinations by showing completely new responses, the addition of individual stress, or the effects of only one of the stress when this stress is predominant (Suzuki et al., 2014).

2.4.1 Heat and drought

In a study by Francesca et al. (2022), the growth of tomato plants under the combination of heat and drought was reduced to the same level as when under

drought alone, despite the enhancement of vegetative growth by heat (31°C). This was also observed by Duan et al. (2017), suggesting that the positive effect of heat does not compensate for the highly damaging effects of drought in tomato. The same predominance trend was observed regarding tomato's stomatal conductance which decreased under the stress combination following the effects of drought despite the increase noted under heat alone (Blanchard-Gros et al., 2021; Duan et al., 2017; Francesca et al., 2022). Chlorophyll pigments displayed a decrease under the stress combination despite an increase under drought, mostly due to the decrease under heat (Blanchard-Gros et al., 2021; Duan et al., 2017). This predominance effect is not always seen, however, with tomato and brinjal eggplant development reaching their lowest level under the stress combination in a range of studies, showing an additive effect in these cases (Duc et al., 2018; Hannachi et al., 2022; Zhou, Kong, Wu, et al., 2019). In addition, when both heat and drought reduced chlorophyll levels individually in the brinjal eggplant, their combination showed an additive effect (Hannachi et al., 2022). The intensity and length of stress seem thus to be major factors determining whether one stress will be predominant or not, as well as the initial response of the crop of interest to the individual stresses.

Overall photosynthesis was generally further decreased by the stress combination in *Solanum*, mostly showing an additive effect of the stresses rather than a predominance effect (Blanchard-Gros et al., 2021; Duc et al., 2018; Hannachi et al., 2022). Tomato plants grown at 45° C without irrigation, for example, had a more severe effect on photosynthesis and biochemical stress markers than the individual temperature increase and irrigation withdrawal (Raja et al., 2020). Under a less intense heat treatment reaching 32°C combined with no irrigation, tomato plants mostly displayed characteristics of drought alone (Zhou, Yu, et al., 2017). These results suggest a complex interaction of the stresses depending on their intensity and length which will determine the mechanisms of the

plants to withstand stress.

Hydrogen peroxide and MDA were increased further by the stress combination than the individual stresses, suggesting a stronger oxidative stress response when both stresses are present in both tomato and the brinjal eggplant (Duc et al., 2018; Hannachi et al., 2022). This was not matched by a higher level of antioxidant enzyme activity in either study, suggesting a plateau already reached under individual stresses or the triggering of other pathways instead to control the higher concentration of damaging compounds (Duc et al., 2018; Hannachi et al., 2022). Similarly, Hannachi et al. (2022) reported a rise in stress hormones under the stress combination reaching the same levels as observed under drought alone in the brinjal eggplant while Francesca et al. (2022) observed this in sugars in tomato. Despite an additive effect in some growth parameters, a range of commonly measured characteristics seems to not be further exacerbated by the stress combination, especially when focusing on secondary metabolites.

2.4.2 Salinity and drought

Tomato plant growth was only affected by the combination of drought and salinity more significantly than each stress individually when the intensity of salinity was elevated, following the same trend observed under heat and drought (Yang et al., 2019). Below that level, drought was predominant over salinity (Yang et al., 2019). In the same study, photosynthetic activity was not further impacted by drought and salinity combination with a decrease to the same extent as the decrease observed under individual stresses (Yang et al., 2019). In another study on tomato when both stresses were more intense, the combination of drought and salinity led to a lower leaf water potential, higher sodium accumulation, and increased proline concentration (Kautz et al., 2014). This was also the case for leaf fluorescence, supporting the importance of stress length

and intensity regarding tolerance to the stress combination (Kautz et al., 2014). A study in tomato plants by Ors et al. (2021) showed that salinity reduced the drought threshold needed before negative effects on CO_2 assimilation and mineral concentrations were observed. Understanding this interaction is thus crucial as monitored water deficit treatment following guidelines based on the application of water stress alone is sometimes used to improve fruit quality (Sarker & Oba, 2018). The presence of another stress during this process might lead to unwanted effects such as reduced growth and final yield depending on the combined intensity.

2.4.3 Salinity and heat

In a study investigating heat and salinity on tomato, sodium transport rate was reduced under the stress combination when compared to salinity alone, potentially limiting the negative effects of ion accumulation observed under salinity (Rivero et al., 2014). The high temperature also had a protective effect on the photosynthetic activity of plants under salinity with a reduction of the defects under the stress combination when compared to salinity on its own (Rivero et al., 2014). This was further observed by Lopez-Delacalle et al. (2021) who showed a recovery of the assimilation rate when heat was present alongside salinity while a significant drop was noticed under salinity alone. These positive interactions are, however, not observed every time. Sousa et al. (2022), using a 7°C higher temperature than the research discussed above on tomato plants, showed an 8% increase in shoot sodium accumulation under heat and salinity when compared to salinity alone. The stress combination also reduced shoot calcium despite an increase under salinity and heat individually and highlighted responses that are neither additive nor predominant but completely new (Sousa et al., 2022). In another study on tomato, the highest decrease in photosynthetic

activity was observed under the combination of stresses (García-Martí et al., 2019). Similarly, heat protection was not observed on tomato yield in a separate study, showing limitations to the positive interactive effect, especially when salinity levels are low and temperatures are high (Botella et al., 2021). The stress combination also impacted plant metabolites differently than individual stresses with sugars and some acids being further increased by their interaction in the study by Botella et al. (2021) and antioxidants being regulated differently in the study of García-Martí et al. (2019). Thus, understanding field-like conditions is important to predict whether a stress interaction will be beneficial or detrimental based on each stress intensity.

2.5 Improving stress tolerance with biostimulants

A range of reviews tackles the long-term crop improvements through breeding (Araus & Kefauver, 2018; Thudi et al., 2021). In this part of the review, the focus will be on biostimulants as a short-term solution for farmers.

To strengthen plant stress response mechanisms, the use of biostimulants has been employed in multiple cases in pot or field conditions. Biostimulants are described as ”substance(s) and/or micro-organisms whose action when applied to plants or the rhizosphere is to stimulate natural processes to enhance/benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality” according to the European Biostimulants Industry Council (EBIC, 2021). They can be categorised as follows (Du Jardin, 2015):

- Humic and fulvic acids (Canellas et al., 2015)
- Protein hydrolysates and N-containing compounds (Colla et al., 2015)
- Seaweed extracts and botanicals (El Boukhari et al., 2020)

- Chitosan and biopolymers (Pichyangkura & Chadchawan, 2015)
- Inorganic compounds (Pilon-Smits et al., 2009)
- Microbial compounds (Afzal & Asad, 2019)

Biostimulants have been shown to have beneficial effects mostly when horticultural crops are under stress (Bulgari et al., 2019). Under optimal conditions, the use of biostimulants does not tend to improve plant growth or nutritional quality, making them redundant. Hernández-Herrera et al. (2014) even showed that some seaweed extracts slowed down germination and limited seedling growth in tomato under non-stressed conditions.

Under drought, the levels of enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) increased in stressed tomato plants treated with a biostimulant, supporting their beneficial effect to limit ROS damage (Behnamnia et al., 2009; Bindu et al., 2018; Shi et al., 2014). Yield parameters, including biomass, fruit production, and fresh and dry weight of the plant, also increased in treated plants under drought due to a combination of beneficial responses boosted by the biostimulants (Goñi et al., 2018; Paul et al., 2019; Peripolli et al., 2021). In tomatoes grown under heat stress, fruit set and development have been improved by different biostimulants, as has the activity of SOD, CAT, and POD, and stomatal conductance and photosynthetic assimilation rate (Ogweno et al., 2008; Sang et al., 2016; Sasaki et al., 2005). Tomatoes grown under salinity reported an increase in leaf area, biomass production, and yield when treated with biostimulants (Cordero et al., 2018; Di Stasio et al., 2018; Miceli et al., 2021). Fruit quality was also enhanced by biostimulants with an increase in antioxidants, total soluble sugars, and minerals (Balliu et al., 2015; Khan, 2016; Xiong et al., 2019). When using seaweed extract on the African eggplant under salinity, de Fatima et al. (2023) found a positive impact on plant morphology and development. However, the positive impact was vari-

able based on the seaweed extract intensities as well as the salinity stress level (de Fatima et al., 2023). The effect of biostimulants can fluctuate highly, with some reports of no or small effects. The success of biostimulants depends on a variety of factors, such as the timing and method of application, stress and cultivar studied, or growth stages (Bulgari et al., 2019). Their combination seems particularly promising (Rouphael & Colla, 2018). The *one-size-fits-all* approach is not appropriate for biostimulant use and research on particular conditions is needed to provide farmers with efficient methods of coping with environmental stresses now.

2.6 Project overview

The constant environmental stresses faced by farmers can negatively impact plant development, yield, and crop nutritional quality. The interplay of stresses, often observed in the field, adds a level of complexity when predicting the effects of these stresses on our food systems. The African eggplant is an indigenous crop with a high potential to ensure the sustainability and resilience of food systems in Africa due, in part, to its high genetic variability and adaptability to a range of environments. Research on its stress tolerance is sparse, however, despite some knowledge based on its evolutionary path. The variability of results previously observed within the *Solanum* kingdom under drought, heat, salinity, and their combination highlights the need to investigate the African eggplant responses to get a better understanding of its role in stressed environments. In addition, quickly available solutions to increase field resilience against various environmental stresses are needed. Biostimulants are promising but their success depends on a variety of factors; the *one-size-fits-all* approach is not appropriate for their use.

Different cultivars of the African eggplant will thus be grown under various combinations of environmental stress and their morphology, physiology, and biochemical content analysed to get a better understanding of the African eggplant's stress responses and help fight food insecurity. Research on the effects of biostimulants on one cultivar of the African eggplant under salinity and drought will be investigated during this project to provide farmers with efficient methods of coping with important environmental stresses.

Chapter 3

Different cultivars of the African eggplant under drought or salinity

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3.1 Introduction

Africa is home to more than 200 indigenous plant species adapted to local environments, but only a few of them are commonly marketed due to consumer acceptability and limited productivity (Kansiime et al., 2018). In contrast, conventional cereals and horticultural crops improved during the Green Revolution are omnipresent but not always adapted to the environmental conditions (Ulian et al., 2020). Recently, indigenous vegetables have received increased interest from researchers, policy-makers, and consumers due to their diversity and adaptability to local, and often harsh, environments. Their use could offer numerous benefits including increased diet diversity, raised field resilience, and improved farmers' income (Akinola et al., 2020).

Agricultural fields worldwide are threatened by environmental stresses, such as soil salinity and drought, set to increase in intensity, length, and frequency due to climate change (Bezner Kerr et al., 2022). Soil salinity problems are due to seawater inundation, poor irrigation and drainage, or atmospheric deposition for example (FAO & ITPS, 2015). The first constraint created by salinity is osmotic stress, triggered by the reduced water uptake by the roots due to the high concentrations of sodium and chloride ions around the roots. To compensate, plants have mechanisms in place to limit water loss through stomatal closure and reduced leaf expansion (Zhao et al., 2020). Secondly, salinity imposes ion imbalance which impairs a range of cellular mechanisms due to the increase of sodium concentration in different plant organs associated with a decrease in potassium, calcium, and nitrogen (Zhao et al., 2020). Sodium toxicity is not fully understood but is believed to be associated with reduced activity of various enzymes involved in photosynthesis activity, chlorophyll production, and sugar metabolism (Zhao et al., 2020). Drought also triggers osmotic stress where plants limit water transpiration to compensate for the low soil water availability

by reducing leaf production and closing their stomata, eventually reducing stomatal conductance (Kapoor et al., 2020). Plant productivity is also impacted by a change in light-harvesting compounds, leading to an increase in photo-oxidation and reactive oxygen species production (Kapoor et al., 2020). Eventually, yield is reduced under both stresses, negatively impacting food security and farmers' livelihoods.

The World Vegetable Center (WVC), working to improve African indigenous vegetables, have developed a list of priority crops for breeding to improve fields' sustainability and resilience based on their nutritional quality, current local importance, income generation, and role in food security (Dinssa et al., 2016). This list includes the African eggplant (*Solanum aethiopicum* L.) which is highly popular in sub-Saharan Africa and has great genetic diversity with local varieties grown across different geographical locations (Han et al., 2021). It is liked for its high nutritional value and is used to treat a range of conditions such as high blood pressure or diabetes (Han et al., 2021). In addition, since the African eggplant grows well in shaded areas and small spaces, it can be promoted in small urban gardens and other modern structures to enhance soil quality and diet diversity (Han et al., 2021). Despite some knowledge about its tolerance to abiotic stress, research is lacking in understanding its tolerance pathways and diversity.

The study by de Fatima et al. (2023) highlighted a low tolerance threshold for salinity on the cultivar tested while Lagat (2016) showed a large diversity in terms of drought tolerance when assessing multiple African eggplant accessions. Previous research on various *Solanum* crops confirmed the large diversity in drought and salinity stress responses, including between varieties, suggesting a genetic basis of tolerance within the genus (Blanchard-Gros et al., 2021; Dasgan et al., 2018). It is thus important to compare different cultivars of the African eggplant to understand if a similar diversity is found in regards to drought and

salinity tolerance and highlight cultivars of interest for future research on this crop. This project aimed to compare four cultivars of the African eggplant under drought and salinity by measuring growth parameters and stomatal conductance. Both experiments are pilot projects before exploring further the different abiotic stress on the African eggplant in the following chapters.

3.2 Materials and Methods

3.2.1 Plant material and growth conditions

Two experiments were established to examine the impact of salinity and drought on different cultivars of the African eggplant. For the salinity experiment (experiment S), repeated once, *Solanum aethiopicum* L. cv., DB3 (Gilo), F1 Djamba (Kumba), Meketan (Kumba), and Kotobi (Kumba) were used. For the drought experiment (experiment D), cultivars Meketan, Kotobi, N'Goyo, and genotype E11 (Shum) were used. A description of the origin, line stability, and market of the lines used is shown in Table 3.1. DB3 seeds were generously provided by the WVC Eastern and Southern Africa (Arusha, Tanzania) and Novagenetic (Technisem, Longué-Jumelles, France) kindly provided us with the seeds of F1 Djamba, Meketan, Kotobi, and N'Goyo. E11 seeds were obtained from Uganda Christian University (Mukono, Uganda). Seeds were sown in modular trays in John Innes No.2 soil compost (7 loam:3 peat:2 sand v/v/v) (J Arthur Bower's, Westland®, Dungannon, Northern Ireland) and grown at 27/21°C for a 12 h photoperiod at 70% relative humidity in an MLR-352 growth cabinet (PHC Holdings Corporation®, Tokyo, Japan). On the 10^{th} day, seedlings were transferred to 7 cm x 7 cm germination pots filled with the same compost and placed under the same growing conditions for four weeks. After the time elapsed and seedlings displayed 4-6 true leaves, six plants per cultivar (24 plants in total)

were transplanted in 3 L pots (1 plant per pot) and placed in three growth cabinets (Plant growth chamber A1000, Conviron®, Winnipeg, Canada) fitted with Valoya LEDs lights (BX NSI spectrum 'white' LED, Valoya®, Helsinki, Finland) providing 350 μmol m^{-2} s^{-1} light intensity. The same growing conditions were maintained (27/21°C for a 12 h photoperiod at 70% relative humidity). Pots were positioned in each cabinet using a randomised complete block design with 3 replicates so that each combination of treatment and cultivar was present in each cabinet.

Name	Group	Edible part	Origin	Line stability	Market
DB3	Gilo	Fruits	The World Vegetable Centre	Fixed	Central and Eastern Africa
F1 Djamba	Kumba	Fruits	Novagenetic	Hybrid	Central and Western Africa
Meketan	Kumba	Fruits	Novagenetic	Fixed	Central and Western Africa
Kotobi	Kumba	Fruits	Novagenetic	Fixed	Central and Western Africa
N'Goyo	Kumba	Fruits	Novagenetic	Fixed	Central and Western Africa
E11	Shum	Leaves	Uganda Christian University	Hybrid	Central and Western Africa

Table 3.1: Description of the African eggplant lines used during the salinity and drought experiments.

3.2.2 Treatment application

Salinity - Experiment S

After a seven-day acclimatisation period in the growth cabinets, the salinity treatment was initiated. Half of the plants were irrigated every day with 100 mM NaCl while the other half were irrigated without NaCl (0 mM NaCl) for 18 days. The soil water content and pore water conductivity were monitored daily using an HH2 moisture meter attached to a WET sensor (Delta-T devices®, Burwell, UK) to decide the amount of irrigation needed. The temperature and humidity throughout the experiment are shown in Appendix B, Figure B.1.

Drought - Experiment D

At transplanting, the air temperature was raised from 27/21°C to 30/24°C for a 12 h photoperiod. After seven days, irrigation was suspended on half of the plants for 12 days. The rest of the plants were irrigated every other day at optimal conditions based on the soil water content monitored daily by an HH2 moisture meter attached to a WET sensor (Delta-T devices®). The temperature and humidity throughout the experiment are shown in Appendix B, Figure B.2.

3.2.3 Growth and physiological measurements

Chlorophyll index and gas exchange

SPAD measurements were made throughout the stress period with a CCM-200 chlorophyll content meter (Opti-Sciences®, Hudson, NH, United States). The SPAD meter measures the transmittance of red and infrared through the leaf and calculates a SPAD value that corresponds to the amount of leaf chlorophyll (Uddling et al., 2007). One measurement was made on two distinct leaves for each plant. The leaves were fully expanded and located at the top of the canopy. Abaxial and adaxial stomatal conductance (gs) were recorded using a leaf porometer (Model SC-1, Decagon Devices, METER Group, Pullman, WA, United States) 5 h after the start of the photoperiod for midday measures. Measurements on days 0, 1, 10, 15, and 18 after the beginning of the salinity stress were taken for experiment S and on days 0, 1, 5, 7, and 9 after irrigation withdrawal in experiment D. Diurnal stomatal conductance was recorded at 1, 3, 5, 8, 10, and 13 hours after the start of the photoperiod on day 9 for experiment D, with the last measurement being done one hour after the end of the photoperiod. Stomatal conductance was recorded on one fully expanded leaf per plant located at the top of the canopy.

Leaf electrolyte leakage

Five discs (10 mm diameter) were cut out from fully expanded leaves and rinsed three times with distilled water at the end of each experiment to measure leaf electrolyte leakage as described by Whitlow et al. (1992). The discs were placed in 20 mL of distilled water and incubated on a shaker plate (200 rpm) at room temperature for 24 h. The electrical conductivity of the solution (EC1) was recorded using a LAQUAtwin EC-33 meter (Horiba®, Kyoto, Japan). A second reading (EC2) was taken after samples were incubated for 60 mins at 95°C in a water bath. The electrolyte leakage (EL), expressed as a percentage, was calculated as:

$$EL = EC1/EC2 * 100\ (\%) \tag{3.1}$$

Soil electrical conductivity 1:5

The soil electrical conductivity 1:5 (EC 1:5) was measured at four time points during experiment S as described by He et al. (2012). A soil sample was extracted from each pot, air-dried for 48 h, and sieved (2 mm). Distilled water was added to the soil following a 1:5 soil:water (w/w) ratio. The solution was placed on a shaker for 24 h and the electrical conductivity of the settled solution was measured using a LAQUAtwin EC-33 meter (Horiba®).

Destructive harvest

At the end of each experiment, the weight of leaves and stems was recorded separately using a two-digit electronic balance. Leaf number, stem diameter, and plant height were recorded. Total leaf area was measured with a Li-3000 area meter (LiCor®, Lincoln, NE, United States) and the shoot biomass was oven-dried at 80°C until it reached a constant weight.

Leaf chlorophyll content

Two leaf discs of 1.54 cm^2 area each from the first two fully expanded leaves were samples from each plant on the last day of experiment S. The discs were immersed in 10 ml 95% (v/v) cold ethanol and kept at 4°C in the dark for 48 h. The absorbance of the supernatant was read at 470, 649, and 665 nm using a UV-VIS spectrophotometer (Ultrospec III, Pharmacia LKB, Stockholm, Sweden). The amount of chlorophyll a (Chl a) and b (Chl b) and carotenoids (Car) per unit area were calculated according to the method described in Wintermans and De Mots (1965) as follows:

$$Chl\,a = (13.95 * A649)/1.54 \tag{3.2}$$

$$Chl\,b = (24.96 * A649 - 7.32 * A665)/1.54 \tag{3.3}$$

$$Car = (10^3 * A470 - 2.05 * Chl\,a - 114.8 * Chl\,b)/1.54 \tag{3.4}$$

3.2.4 Statistical analysis

All statistical analyses were performed using R version 4.1.2 and models are detailed in Appendix C. Pearson's correlation coefficients were computed for each experiment to assess the relationship between some of the measured characteristics and the stress parameter.

The data was assessed for residual normality and variance homogeneity. The Shapiro test and visual evaluation including density plots, Q-Q plots, and histograms were used to determine normality. Variance homogeneity was checked using Levene's test and visual inspection of the residuals. A two-way analysis of variance (ANOVA) was then performed on continuous data using the stress (salinity or irrigation) and cultivars as parameters. Their interaction was also

included as well as the growth cabinet as fixed parameters. Tukey adjustment at a 0.05 significance level was used for pairwise comparison among and across groups when needed. Count data was analysed using a generalised linear model fitted with a Poisson distribution checked for overdispersion.

3.3 Results

3.3.1 Experiment S - Salinity

Correlation

Salinity level was significantly and negatively correlated with leaf area, stem diameter, leaf and stem dry weight, and abaxial gs (Fig. 3.1). Electrolyte leakage, showing the strongest correlation among the parameters analysed, was positively correlated to the salinity level (Fig. 3.1). In contrast, plant height, concentration of chlorophylls a and b, and carotenoids were not significantly correlated with the salinity stress (Fig. 3.1).

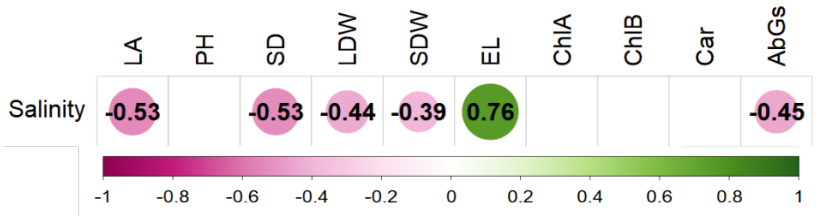


Figure 3.1: Pearson's correlation between salinity and plant characteristics of the African eggplant. Only significant correlations are shown ($P < 0.05$, $n = 6$). The colour scale indicates the direction and intensity of the correlation where dark green denotes a positive correlation and dark pink a negative correlation. Larger circles represent strong correlations and smaller circles represent weaker correlations. Numbers within each circle are Pearson's correlation coefficients. LA = Leaf area, PH = Plant height, SD = Stem diameter, LDW = Leaf dry weight, SDW = Stem dry weight, EL = Electrolyte leakage, ChlA = Chlorophyll a, ChlB = Chlorophyll b, Car = Carotenoids, AbGs = Abaxial stomatal conductance.

Soil salinity

Salinity significantly increased pore water conductivity from the second day after the stress initiation (DASI) (Fig. 3.2a). The increase was even more noticeable as the stress went on, with a 35% increase on average when compared to control conditions at 2 DASI and an increase higher than 100% from 11 DASI onwards. At 11 DASI, the pore water conductivity of DB3 cultivars was significantly lower than that of F1 Djamba and Kotobi (Fig. 3.2a). At 16 DASI, Kotobi's pore water conductivity was lower than that of DB3 and F1 Djamba (Fig. 3.2a). Salinity also significantly raised soil EC 1:5, measured on a soil:water solution, at 10, 14, and 18 DASI (Fig. 3.2b). The soil electrical conductivity 1:5 plateaued from 10 DASI with no significant differences noted between 10, 14, and 18 DASI.

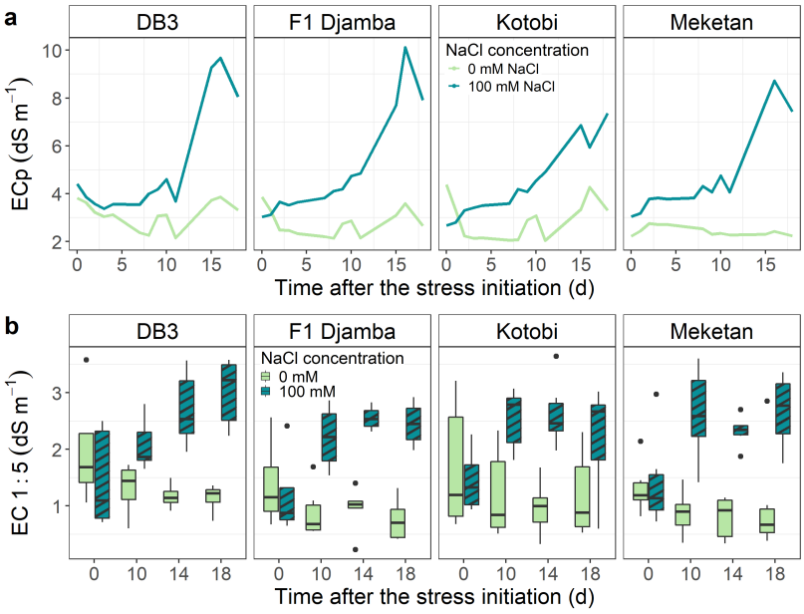


Figure 3.2: Salinity levels as measured by **(a)** the pore water conductivity (ECp) and **(b)** the soil electrical conductivity (EC) 1:5 at four time-points throughout the salinity stress. The mean values of ECp are represented while EC 1:5 is displayed by the lower and upper whiskers, 25% and 75% quartiles, and median values (n = 6). Two ECp measurements per pot were taken daily while one soil sample was extracted at each time-point selected to measure EC 1:5.

Plant growth

Plants' morphology was significantly affected by salinity (Fig. 3.3). Salinity affected leaf and stem development by reducing leaf and stem dry weight, leaf area, and stem diameter (Fig. 3.4a,b,c,e). Leaf area was the most affected characteristic with a reduction of 34% on average under salinity. There was no significant difference in stem dry weight reductions based on cultivar with reductions of 24%, 18%, 43%, and 34% for DB3, F1 Djamba, Kotobi, and Meketan, respectively. In contrast, significant differences were noted between

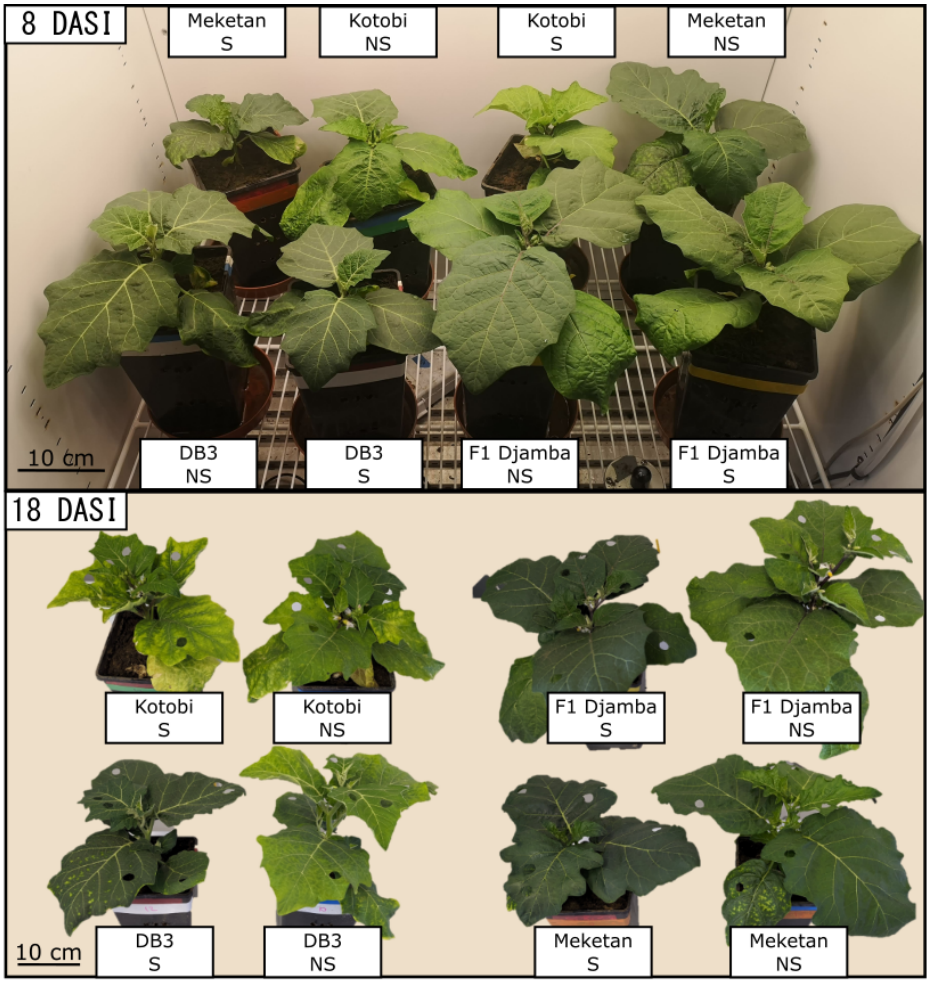


Figure 3.3: Plants at 8 and 18 days after the stress initiation (DASI). The cultivar's name is shown on top and the treatment is at the bottom of each descriptive box. Pictures were not taken with the same focus and a comparison of growth between 8 and 18 DASI is thus invalid. NS = Non saline (0 mM NaCl), S = Saline (100 mM NaCl).

cultivars regarding leaf number with salinity only reducing it for Kotobi (Fig. 3.4d). Salinity increased significantly leaf EL by an average of 64% in all tested cultivars (Fig. 3.4f).

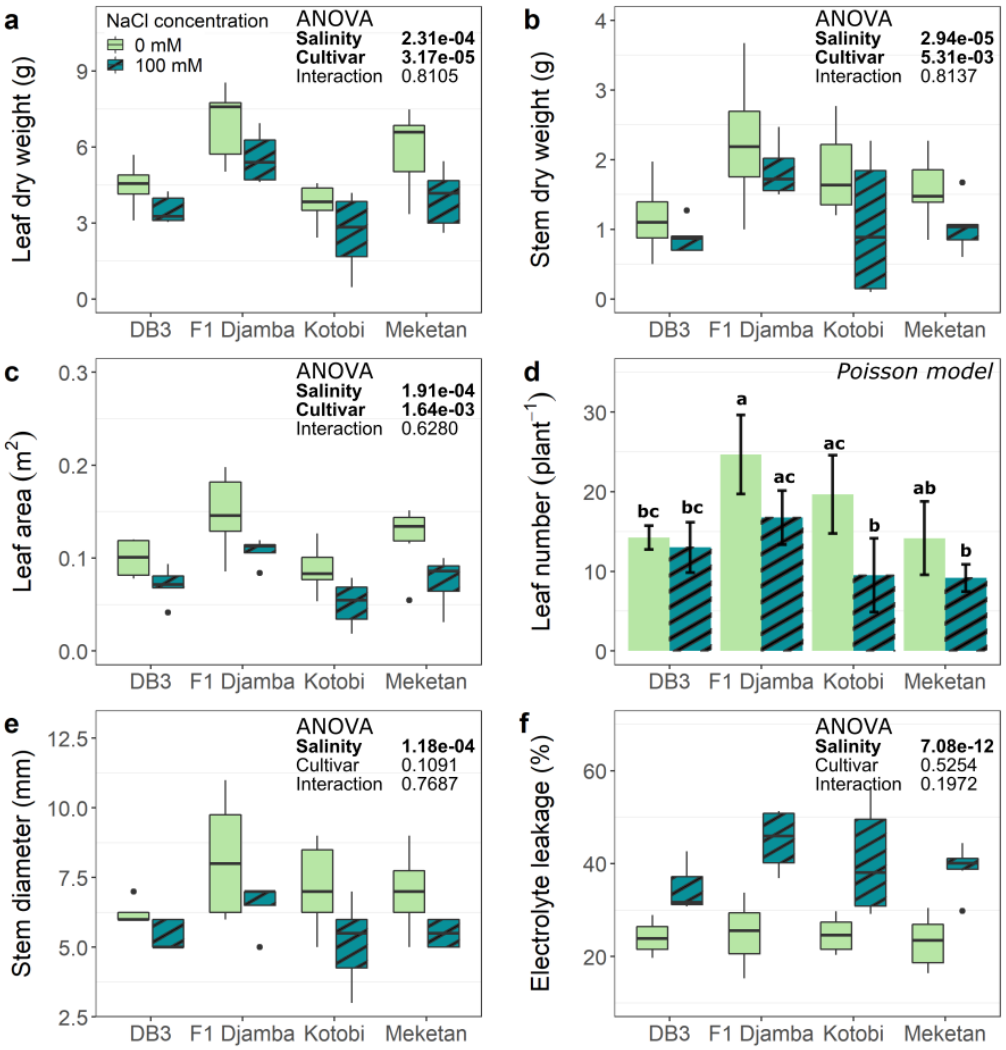


Figure 3.4: Plant morphological characteristics of the African eggplant cultivars at the end of the salinity treatment. Sub-plots represent **(a)** leaf dry weight, **(b)** stem dry weight, **(c)** leaf area, **(d)** leaf number per plant, **(e)** stem diameter, and **(f)** leaf electrolyte leakage. The dataset is represented by the lower and upper whiskers, 25% and 75% quartiles, and median (n = 6). Black dots represent outliers. Leaf number data is presented as a barplot showing the mean value +/- standard deviation. The p-values of the statistical test done are shown for each source of variation. For the leaf number, barplots not sharing any letters are significantly different based on the Tukey test at 0.05% level of significance.

Leaf chlorophyll and stomatal conductance

Salinity increased chlorophyll a concentration significantly only in F1 Djamba cultivar (Fig. 3.5a). Chlorophyll b and carotenoid levels were increased significantly by salinity, in contrast (Fig. 3.5b,c). The ratio of chlorophyll a over b remained unchanged under salinity (Fig. 3.5d).

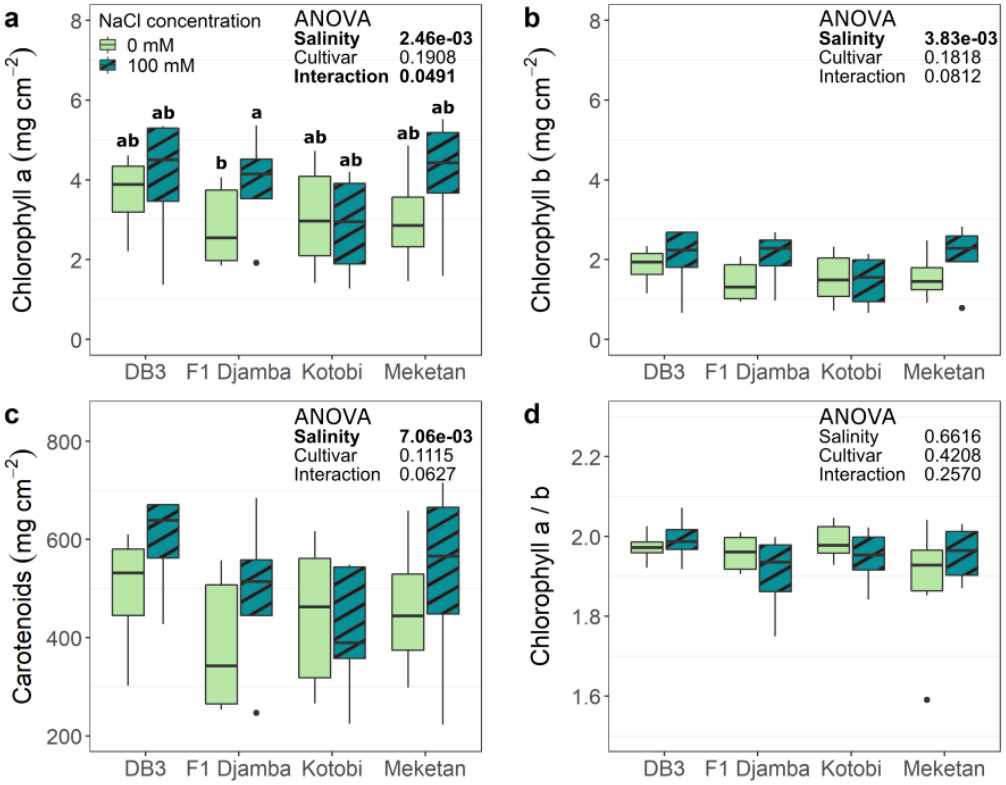


Figure 3.5: Leaf pigments content at the end of the salinity period in the salinity experiment for each cultivar. Sub-plots represent **(a)** chlorophyll a, **(b)** chlorophyll b, **(c)** carotenoids, and **(d)** the ratio of chlorophyll a over chlorophyll b. The dataset is represented by the lower and upper whiskers, 25% and 75% quartiles, and median (n = 6). Black dots represent outliers. The p-values of the statistical test done are shown for each source of variation. In the case when the interaction of the stressors is significant, letters are used to display the significant interactions for clarity. Boxplots not sharing any letters are significantly different based on the Tukey test at 0.05% level of significance.

Midday abaxial gs showed no significant differences between the salinity treatments at 0, 1, and 10 DASI (Fig. 3.6). It was, however, reduced by salinity on the last two measurement dates, 15 and 18 DASI (Fig. 3.6). Initial differences among cultivars were observed on day 0 before the stress began, with Kotobi and

Meketan showing significantly lower midday abaxial gs than E11 and N'Goyo, but no differences were noted later in the stress (Fig. 3.6).

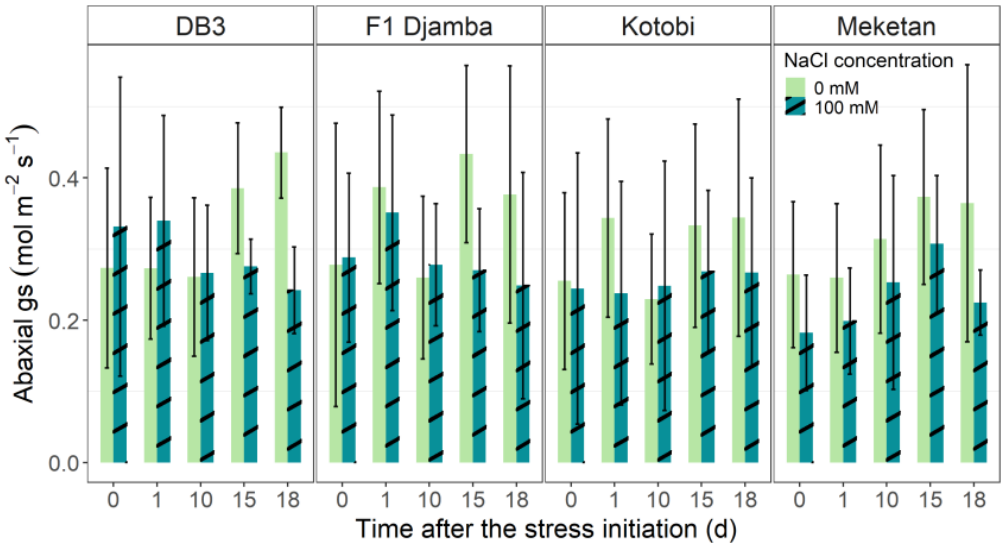


Figure 3.6: Midday abaxial stomatal conductance (gs) of each cultivar throughout the salinity period. The data are represented as mean +/- standard deviation (n = 6). Measurements were taken on one leaf per plant located at the top of the canopy.

SPAD index was increased by salinity at 15 DASI and not significantly affected before (Fig. 3.7). At 18 DASI, the increase was significant only for cultivars F1 Djamba and Meketan (Fig. 3.7).

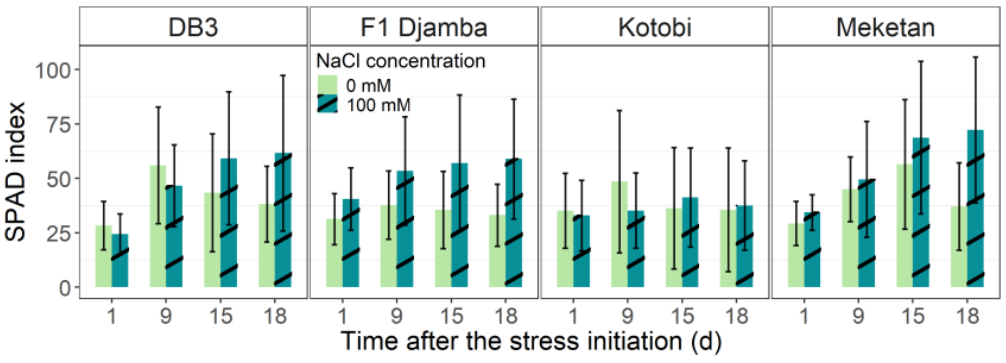


Figure 3.7: SPAD index throughout the salinity treatment in experiment S of each cultivar. The data are represented as mean +/- standard deviation (n = 6). One measurement of the SPAD index was taken on two distinct leaves per plant located at the top of the canopy.

3.3.2 Experiment D - drought

Correlation

The irrigation level was significantly and positively correlated with plant height, stem diameter, leaf and stem dry weight, and adaxial gs but was not significantly correlated to abaxial gs (Fig. 3.8). The strongest correlation with the irrigation level was the stem diameter while the weakest of the significant ones was noted for leaf dry weight.

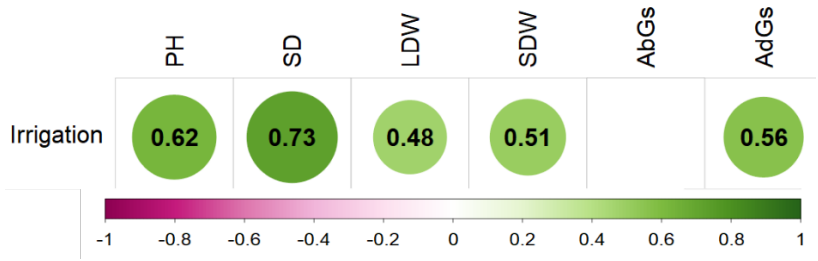


Figure 3.8: Pearson's correlation between the irrigation level and plant characteristics of the African eggplant. Only significant correlations are shown (P <0.05, n = 6). The colour scale indicates the direction and intensity of the correlation where dark green denotes a positive correlation and dark pink a negative correlation. Larger circles represent strong correlations and smaller circles represent weaker correlations. Numbers within each circle are Pearson's correlation coefficients. PH = Plant height, SD = Stem diameter, LDW = Leaf dry weight, SDW = Stem dry weight, AbGs = Abaxial stomatal conductance, AdGs = Adaxial stomatal conductance.

Plant growth

Water stress altered plant morphology (Fig. 3.9), causing reductions in leaf and stem dry weight, plant height, and stem diameter (Fig. 3.10a-d). These reductions were not significantly different between cultivars. Leaf dry weight was reduced by 40%, 34%, 41%, and 23% in E11, Kotobi, Meketan, and N'Goyo, respectively. N'Goyo also had the smallest reduction regarding leaf number (32%), plant height (13%), and stem diameter (19%). The final leaf number was the most impacted characteristic with an average reduction of 43%. The leaf dry weight of both Meketan and Kotobi was greater than that of N'Goyo

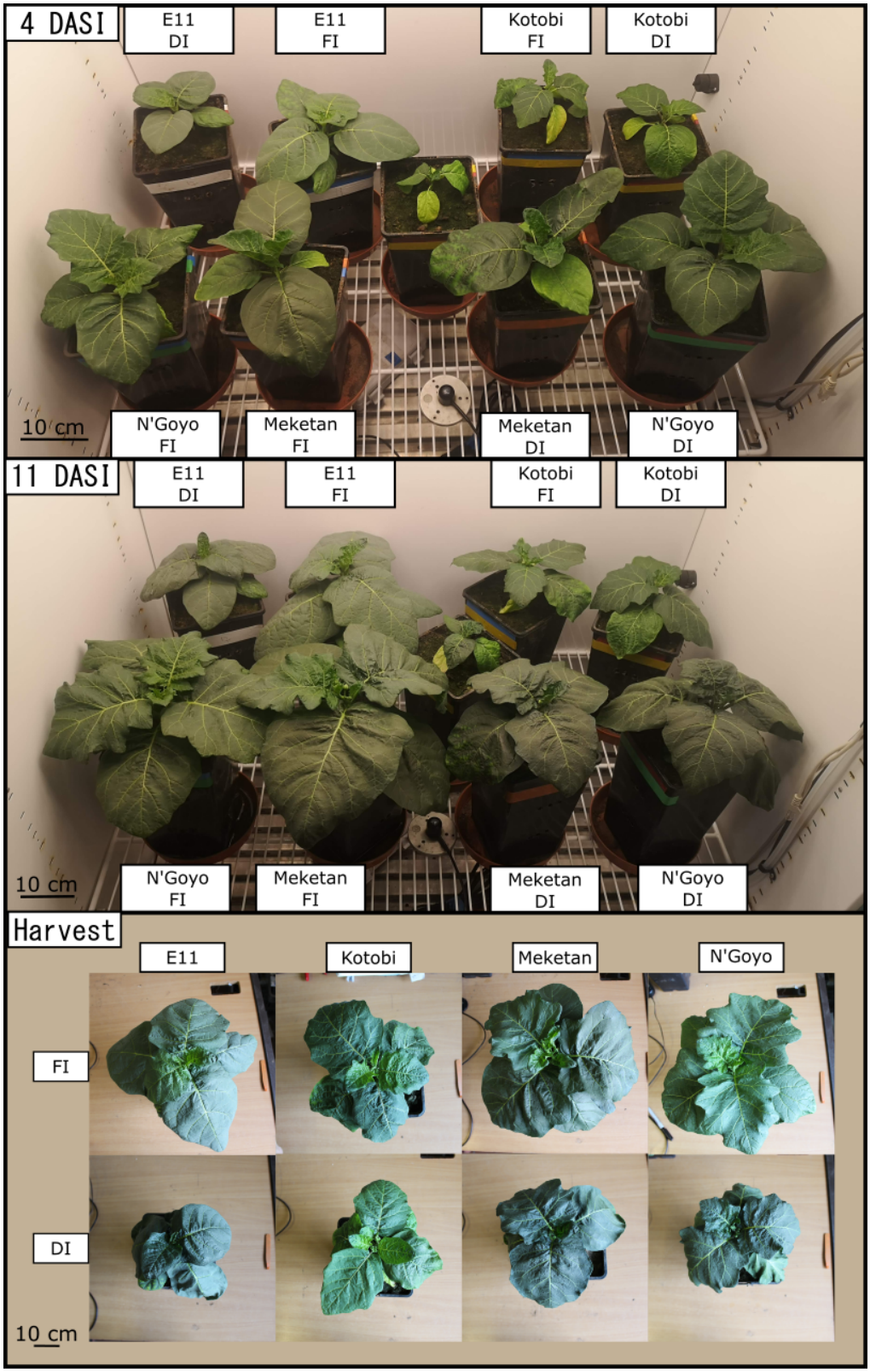


Figure 3.9: Plants at 4 and 11 days after the stress initiation (DASI) and at harvest (12 DASI). The cultivar's name is shown on top and the treatment is at the bottom of each descriptive box. The extra plant in the middle at 4 and 11 DASI was initially used as a backup and eventually used to measure light intensity at the plant level (data not shown). Pictures were not taken with the same focus and a comparison of growth between days is thus invalid. FI = Full irrigation, DI = Deficit irrigation.

(Fig. 3.10a). N'Goyo showed a significantly shorter height than Meketan and a smaller stem diameter than both Meketan and E11 (Fig. 3.10c,d).

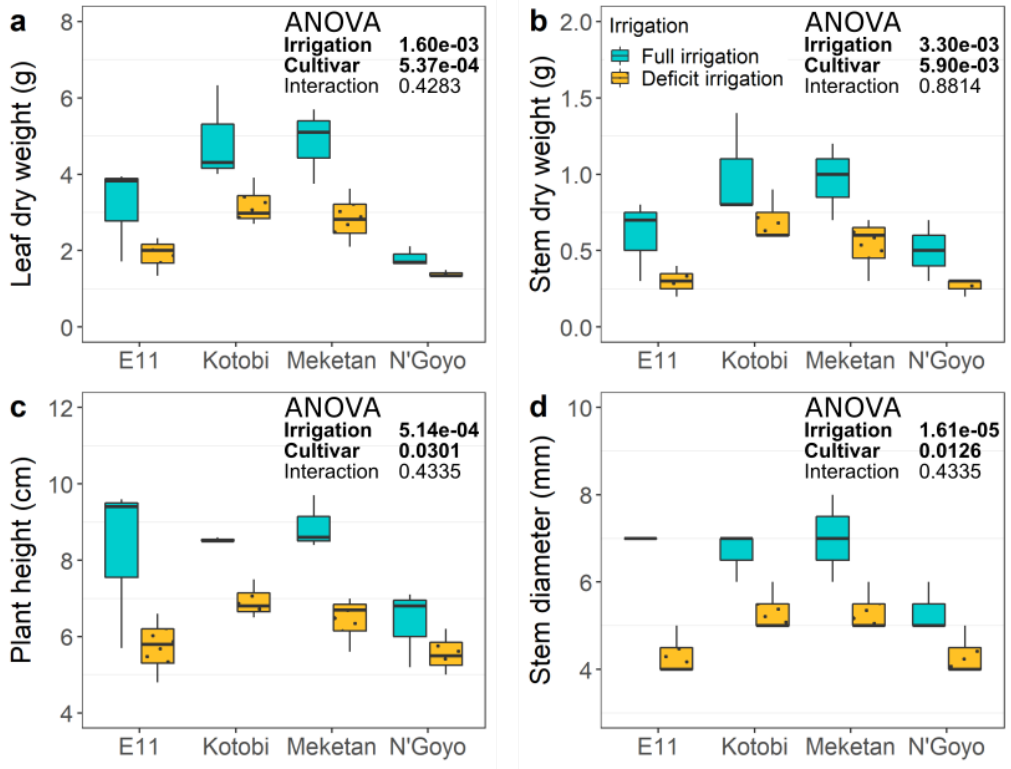


Figure 3.10: Plant morphological characteristics of the African eggplant cultivars at the end of the drought treatment. Sub-plots represent **(a)** leaf dry weight, **(b)** stem dry weight, **(c)** plant height, and **(d)** stem diameter. The dataset is represented by the lower and upper whiskers, 25% and 75% quartiles, and median (n = 3). The p-values of the statistical test done are shown for each source of variation.

Stomatal conductance and chlorophyll index

Water stress did not significantly impact midday abaxial gs over the nine days of measurements despite some non-significant reductions in Meketan and N'Goyo (Fig. 3.11). Midday adaxial gs, on the other hand, was reduced by water stress on days 7 and 9 after irrigation withdrawal (Fig. 3.11).

Diurnal abaxial gs, only recorded nine days after the irrigation withdrawal, showed a significant reduction due to water stress on the first hour of the photoperiod (Fig. 3.12). Although water stress did not significantly impact abaxial

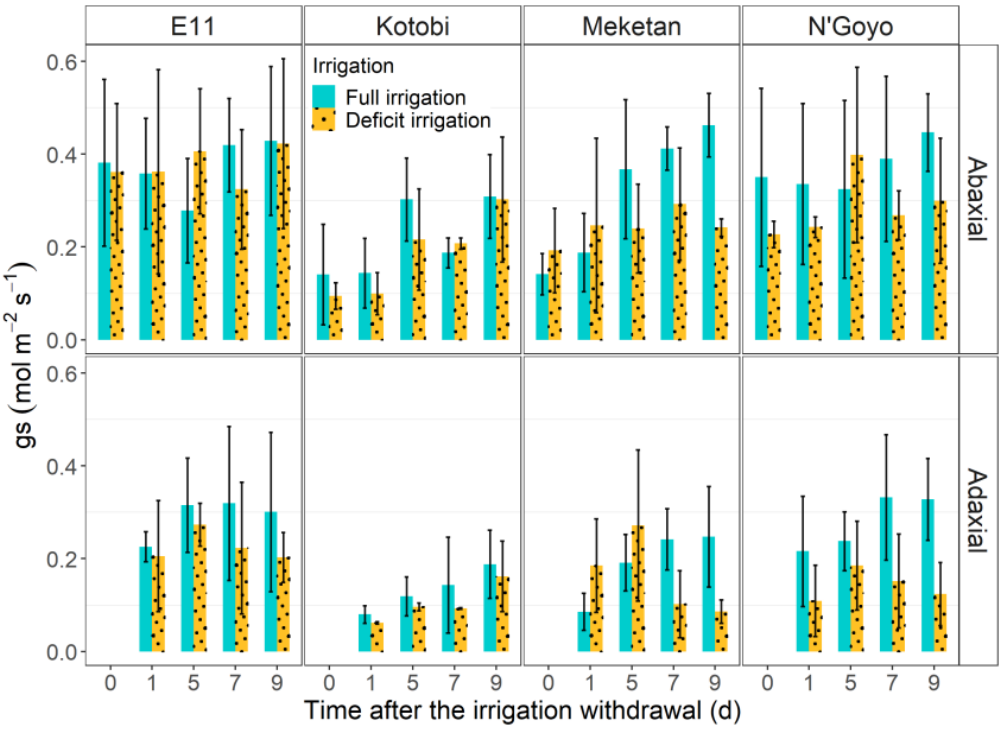


Figure 3.11: Midday abaxial and adaxial stomatal conductance (gs) of each cultivar throughout the drought treatment. The data are represented as mean +/- standard deviation (n = 3). Measurements were taken on one leaf per plant located at the canopy.

gs at three and five hours after the onset of the photoperiod, a decrease was noted again at eight and ten hours (Fig. 3.12). In contrast, adaxial gs showed no significant difference one hour after the start of the photoperiod but decreased at three and five hours under water stress (Fig. 3.12). While the water stress effect was not significant at eight hours, a decrease was observed again at ten hours, significant only for the N'Goyo cultivar. Both abaxial and adaxial gs stabilised at the same level under stressed and non-stressed conditions at the end of the day (Fig. 3.12).

Water stress did not influence the SPAD index before day 9 for any cultivar (Fig.3.13). By day 9, the SPAD index was significantly higher in water-stressed plants and was maintained higher on the last measurements day (Fig.3.13).

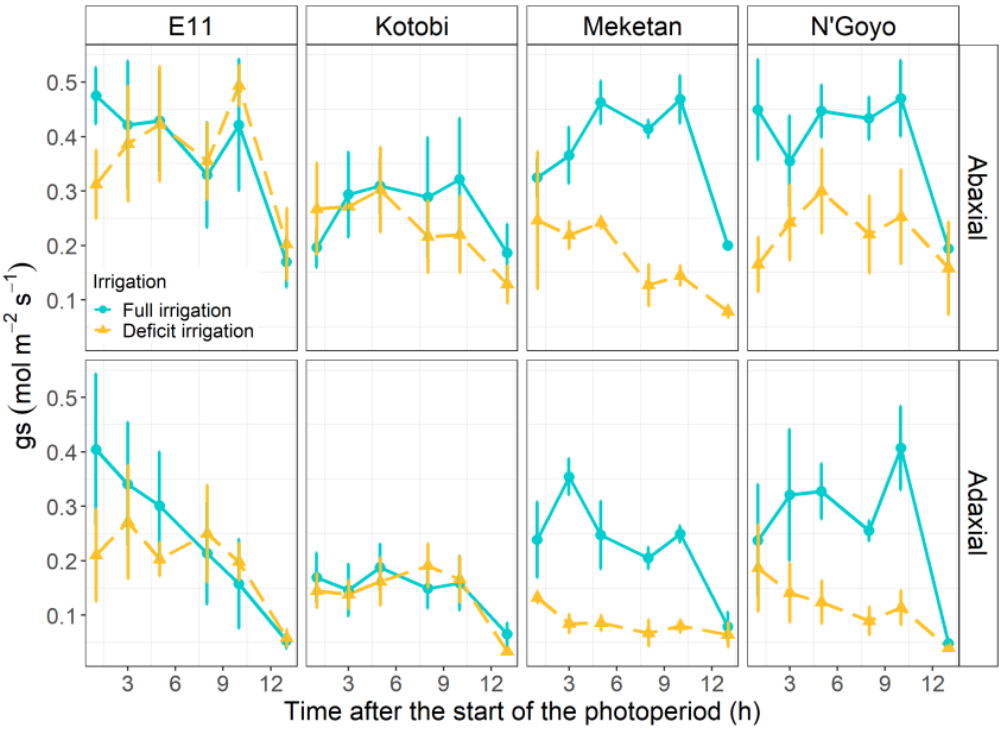


Figure 3.12: Diurnal abaxial and adaxial stomatal conductance (gs) of each cultivar throughout the 9th day of the irrigation withdrawal. The data are represented by the mean +/- standard error (n = 3). Stomatal conductance measurements were taken on one leaf per plant located at the top of the canopy.

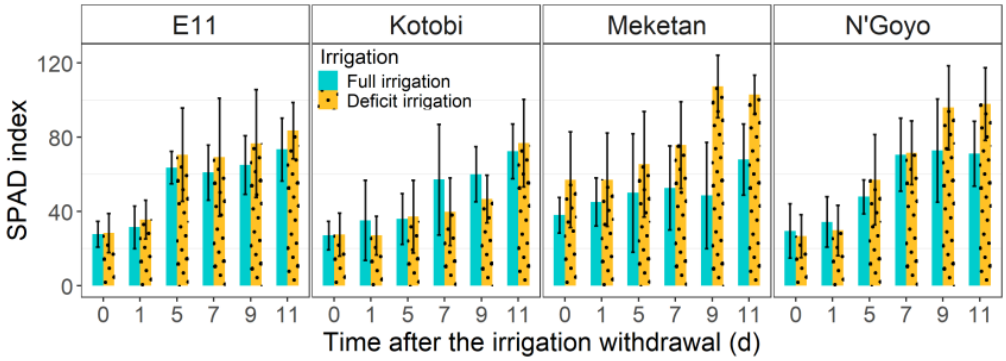


Figure 3.13: SPAD index during the drought treatment in experiment D of each cultivar. The data are represented as mean +/- standard deviation (n = 3). One measurement of the SPAD index was taken on two distinct leaves per plant located at the top of the canopy.

3.4 Discussion

Both salinity and drought are major environmental stresses causing important losses in agricultural fields (Fita et al., 2015). Understanding the stress tolerance of multiple varieties of under-researched crops is thus important to inform small-scale farmers of the best cultivar to use under different conditions and highlight cultivars of interest for breeding programmes. A description of how well different cultivars of the African eggplant perform under these stresses is lacking, limiting their usage. Therefore, the current study described how four African eggplant cultivars respond to water and salinity stress in two separate experiments. The limited number of cultivars tested in the current study is, however, a limiting factor to observe genetic variations. Indeed, the use of a mapping population, or a higher number of replicates, would be more appropriate to effectively highlight tolerance traits within a group. This current research, in opposition, was mostly a pilot study useful to give indications on the cultivars to use for further studies within this thesis.

The cultivars investigated in this study responded similarly to salinity and drought stress with a decrease in dry mass, a common response under these stresses. When comparing wild (*S. cheesmaniae* L.Riley and *S. galapagense* S.C. Darwin & Peralta) and commercial tomatoes (*S. lycopersicum* L.), Pailles et al. (2020) observed a reduction in dry mass under salinity for all the cultivars tested. However, wild tomatoes showed less of a decrease than commercial tomatoes, supporting their higher tolerance despite still being affected by the stress (Pailles et al., 2020). The genetic variability observed in this study, although not significant, might thus be a first step in highlighting the more tolerant cultivars. Growth parameters are negatively correlated to the level of salinity and are powerful estimates of salinity tolerance, especially at the seedling and vegetative stages as it is not possible to measure commercially relevant traits such as fruit

yield (Negrão et al., 2017). Similarly, dry weight production is an important parameter dictating drought tolerance with morphological characteristics being positively correlated to the irrigation amount (Kapoor et al., 2020). Reduced leaf area is, however, positive for drought tolerance by limiting water loss, with plants displaying high drought resistance often producing smaller and thicker leaves (Fang & Xiong, 2015).

Another avoidance mechanism to limit water loss in plants under water or salinity stress is to close their stomata, reducing gs (Fang & Xiong, 2015). Under salinity, midday abaxial gs of the African eggplant tested was decreased over the last few days of the stress as seen in tomato, eggplant (*S. melongena* L.), and *S. nigrum* L. (Leiva-Ampuero et al., 2020; Liao & Zhang, 2021; Wu et al., 2012). A reduced gs was noted in a range of crops independently on their tolerance under drought as well (Hlahla et al., 2022; Khan et al., 2023; Küçükyumuk, 2020). Midday abaxial gs of the African eggplant tested in this current were not significantly affected by water stress, in contrast. However, midday adaxial gs were reduced in the last few days of the stress and were significantly and positively correlated to the irrigation level. This contrasts with previous results by Driesen et al. (2023) in basil (*Ocimum basilicum* L.) where both adaxial and abaxial gs were reduced under drought and by Li et al. (2017) where drought impacted stomata characteristics of wheat (*Triticum aestivum* L.) on both the upper and lower side of the leaves. However, various sources highlighted that upper and lower stomata often respond differently to environmental factors (Aston, 1978; Mott & O'Leary, 1984; Richardson et al., 2017). Driesen et al. (2023) showed that basil's upper stomata were much more sensitive to drought stress than lower ones. As the upper side is exposed to more environmental factors such as light, adaptive mechanisms may be involved in ensuring an early stomata closure on this side to limit water loss and damage to the photosynthetic apparatus, explaining the current observations (Aston, 1978). It has to be noted that the African

eggplants used in the current study displayed a decrease in gs under long-term drought (see Chapter 7). The response of only the upper stomata may thus be mostly due to the length of the stress.

Upper and lower stomata reacted differently to the drought stress throughout the day too. Despite water stress not affecting abaxial gs at midday, a significant drop later during the day was noted, suggesting an earlier closure of stomata under stress. Under drought, high-performing tomato cultivars maintained a high gs early in the day before a noticeable drop in the afternoon (Gosa et al., 2022). Low-performing cultivars, on the other hand, presented a lower gs already in the early hours of the day, suggesting the importance of diurnal dynamics for yield maintenance (Gosa et al., 2022). This early morning peak of gs, sometimes referred to as the ”golden hour”, was observed under drought for a range of tomato lines and linked to better field performance, suggesting the adaptation of the cultivars tested in the current study (Gosa et al., 2022). These diurnal dynamics might thus explain the absence of significant differences under drought when measuring abaxial midday stomatal conductance in this pilot study and support an impact of drought later during the day instead. In addition, abaxial and adaxial gs peaked at different times throughout the day in the study by Gosa et al. (2022) on tomato, supporting the differences observed in the current study with the adaxial side displaying a lower gs than the abaxial side early in the day. A quick adaxial gs closure under stress, while maintaining abaxial gs open for longer, observed in this pilot study, was associated with an improvement in the plant's performance and might thus be an adaptive response of the African eggplant to drought stress (Gosa et al., 2022).

The chlorophyll content is another important parameter under stress due to its role in light absorption and maintenance of photosynthetic activity under stress (Li et al., 2006). Both salinity and drought stress increased the SPAD index in the cultivars tested, an observation made in other crops under drought stress too

(Mahammed et al., 2021). A range of reports observed a decrease of photosynthetic pigments under stress for sensitive cultivars while tolerant crops generally increased their chlorophyll level (Sudhir & Murthy, 2004). The African eggplant cultivars tested might thus be tolerant to the level of salinity and drought used regarding chlorophyll level.

3.5 Conclusion

The cultivars tested under salinity and drought mostly responded similarly to the stresses despite some non-significant variability. The African eggplant reduced its shoot development under both stresses and increased its chlorophyll levels. Salinity significantly decreased abaxial stomatal conductance while only the adaxial stomatal conductance was reduced under drought. Diurnal measurements highlighted how drought impacted the dynamics of adaxial and abaxial stomatal conductance differently throughout the day. The results recorded in this study were used to select the cultivars to be analysed further in each chapter.

The cultivar E11 was used under the combination of heat and drought in Chapter 4. Indeed, E11 was not impacted more negatively by drought than the other cultivars despite being the only Shum cultivar, normally grown in humid and well-watered areas due to their requirements in water. Moreover, the impact of heat on its vegetative development is interesting to explore as some positive effects of heat can be observed in leaf development up to a threshold (Hwang et al., 2018; Sublett et al., 2018). Due to the improvement of chlorophyll a levels in F1 Djamba under salinity, this cultivar was used in Chapter 5 combining heat and salinity. The protective effects of chlorophyll levels on heat stress have been shown before and the current observations might suggest a positive effect

of salinity under heat stress in this cultivar (Jha et al., 2014).

The only Gilo cultivar tested in this pilot study, DB3, was used for further analysis of salinity as long-term stress in Chapter 6. Different salinity levels were tested to understand a potential tolerance threshold and further developmental stages were explored as Gilo cultivars are only cultivated for their fruits. An understanding of the effect of salinity on DB3's fruit yield was thus highly relevant.

Meketan had one of the highest stomatal conductance which was only reduced by salinity and not drought in the measured time-frame. This cultivar was thus used under the combination of salinity and drought in Chapter 7 to understand whether salinity would be predominant in this cultivar or whether new mechanisms would be in place to withstand the combined stress. As biostimulants generally have a stronger impact on the most sensitive cultivars as shown by Zuluaga et al. (2023), Kotobi was selected to be tested in Chapter 7 under the combination of drought and salinity and biostimulants due to its initial lower stomatal conductance and reduced number of leaves, suggesting a potentially high impact of stress above a certain threshold.

Chapter 4

The combination of drought and transient elevated temperatures on the African eggplant Shum

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4.1 Introduction

Climate change increases the likelihood of extreme events such as drought and heat waves worldwide (Mbow et al., 2019). Both of these stresses already significantly limit crop production, especially in Africa (Niang et al., 2014). Drought stress is a consequence of insufficient rainfall or deficit soil moisture and can lead to various morphological, physiological or biochemical defects in plants (Farooq et al., 2009). Heat waves, and heat stress in general, also have deleterious effects on plant growth especially at the reproductive stage (Bita & Gerats, 2013; Zhou et al., 2022). These stresses are often associated in the field, making it necessary to study their combination on plant responses (Lamaoui et al., 2018). Most studies have, however, focused on the responses of various crops to individual stresses to uncover the mechanisms of stress tolerance and sensitivity (Fahad et al., 2017; Suzuki et al., 2014).

A combination of stresses can lead to different, and often more severe, responses than individual ones such as seen in maize (*Zea mays* L.) and citrus plants (Yousaf et al., 2022; Zandalinas et al., 2016). Despite some shared responses between plants under stress, signalling pathways induced by combined stress can sometimes be different or opposite to the ones induced by individual stresses (Suzuki et al., 2014). Chlorophyll, the primary photosynthetic pigment, is, for example, degraded under drought due to the instability of the chlorophyll membrane, increased photo-oxidation, and decreased chlorophyll production (Parkash & Singh, 2020). Under high temperatures, the photosynthetic apparatus might be under-performing due to reduced enzymatic activity (Hassan et al., 2021). The combination of heat and drought might thus reduce photosynthetic activity on multiple fronts, making one cultivar tolerant to one stress not adapted to the stress combination (Fahad et al., 2017). Stress also increases the production of reactive oxygen species which can damage proteins, cells, and

DNA when present at high concentrations and need detoxification by antioxidants (Fahad et al., 2017). The variety of responses observed in crops, such as an increase in total phenols and a decrease in anthocyanins under elevated temperatures and an increase in antioxidant enzymes under drought, might lead to new pathways activated under their combination (Fahad et al., 2017).

To increase field resilience to environmental stress, the use of indigenous crops has recently attracted attention due to their higher tolerance to stress. The African eggplant (*Solanum aethiopicum* L.) Shum is an indigenous vegetable from Africa and a close relative to the common brinjal eggplant (*S. melongena* L.) and tomato (*S. lycopersicum* L.) (Aubriot & Daunay, 2019). It is mostly grown by small-holder farmers in peri-urban areas for its edible leaves (Schippers, 2000). The leaves are rich in nutrients but their market value can be highly affected by limited water supply as leaf quality can deteriorate quickly (Han et al., 2021). Every Shum accession studied by Nakanwagi et al. (2017) had a decrease in leaf and stem growth under drought, however, a high variation was observed with some cultivars recovering much better than others, indicating some genetic basis of tolerance. Drought also affected the secondary metabolism of *S. aethiopicum* such as carotenoids and sugars (Mibei et al., 2017, 2018). The heat tolerance of the African eggplant Shum has not been investigated to our knowledge.

4.2 Materials and Methods

4.2.1 Plant material and growth conditions

A pot experiment was carried out to investigate the responses of the African eggplant Shum (*Solanum aethiopicum* L. genotype E11) to a combination of

heat and drought stress. The Uganda Christian University generously provided us with the seeds. Before use, seeds were disinfected by immersion in 10% (v/v) trisodium phosphate decahydrate solution for three hours and rinsed with distilled water, placed for 1 h in a dilution of 70% water:30% commercial bleach, and rinsed again. Seeds were then left to dry at room temperature for 15 days. At the start of the experiment, seeds were placed in 7 cm x 7 cm germination pots filled with John Innes No.2 compost (7 loam:3 peat:2 sand v/v/v) (J Arthur Bower's, Westland®, Dungannon, Northern Ireland). Pots were placed in an MLR-352 growth cabinet (PHC Holdings Corporation®, Tokyo, Japan) set at 70% relative humidity and 27/21°C for a 12 h photoperiod, and watered from the bottom when needed. Thirty seedlings were transplanted in 3 L pots after four weeks and placed following a split-plot design in three growth cabinets (Plant growth chamber A1000, Conviron®, Winnipeg, Canada). Valoya LEDs lights (BX NSI spectrum 'white' LED, Valoya®, Helsinki, Finland) providing 350 μmol m^{-2} s^{-1} light intensity were fitted in the cabinets and the growing conditions stayed the same (27/21°C for a 12 h photoperiod, 70% relative humidity). Natural predators were applied once, four days after transplanting (Anderline, Starskii, Chrysoline C, Bioline AgroSciences®, Little Clacton, UK). The final harvest of plants took place 36 days after transplanting.

4.2.2 Treatment application

Plants were left to acclimate for seven days in the growth cabinets at 27/21°C for a 12 h photoperiod and were well-watered. Irrigation was withheld for five plants in each cabinet for four days until the soil matric water potential (SWP) reached -100 kPa as monitored by MPS-2 sensors (Decagon devices, Meter Environment®, Pullman, WA, United States) connected to a datalogger EM50g (Meter Environment®) and maintained at this level after that. The other

15 plants (five in each cabinet) were irrigated to maintain an SWP of approximately -10 kPa. This water stress level was selected based on previous research (data not shown) and on the relationship between SWP and water content. A soil matric water potential of -100 kPa represents roughly a 50% decrease in water content from a SWP of - 10 kPa, a level often used for water stress research (Evett, 2007). Soil water content was also monitored daily using an HH2 moisture meter attached to a WET sensor (Delta-T devices®, Burwell, UK). When plants reached the required SWP under deficit irrigation, the heat treatment was initiated gradually to reduce the risk of an osmotic shock and mimic natural conditions. Two sequential heatwave-type stresses were imposed with a maximum of 32/26°C (T32) and 37/31°C (T37) day/night temperature when compared to 27/21°C (T27) for control plants. The heat increased gradually over day one (1 DAHS, days after the start of heat stress) and was maintained for seven days, followed by a six-day recovery period at T27. The temperature rose again after the recovery period (14 DAHS) over eight days, followed by a second recovery period. The harvest took place at 26 DAHS. These elevated temperatures were selected based on temperatures sometimes reached in Central Africa during heat waves with T37 being on the higher end of the average temperature in Central African countries under normal conditions (Worlddata, 2023). The daily schedule for each of the treatments for one heat-wave period is shown in Figure 4.1. The recorded temperatures in each cabinet are shown in Appendix B, Fig. B.3.

4.2.3 Growth and physiological measurements

Leaf expansion rate

Two young leaves (less than 30 mm length-wise) were labelled on each plant at a similar height and developmental stage at 0 DAHS. Their length and maximum width were recorded three times a week. The growth difference between the

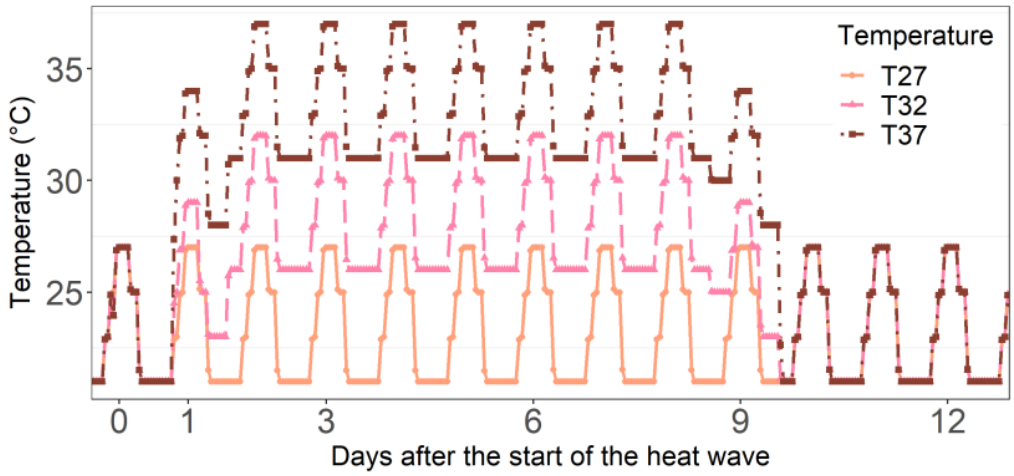


Figure 4.1: Daily air temperature schedule of 27/21°C (T27), 32/26°C (T32), and 37/31°C (T37) treatments throughout one heatwave. This treatment was repeated twice between 1 and 26 days after the start of the heat stress.

measurements was considered to be the daily length and width expansion rates, respectively. On the day before the second heatwave (13 DAHS), new young leaves were labelled to limit the impact of ageing on the results.

SPAD index

The SPAD index, measuring the transmittance of red and infrared through the leaf, was measured three times a week at midday from 1 to 26 DAHS using a MultispeQ V2.0 device with Photosynthesis RIDES 2.0 protocol (PhotosynQ®, East Lansing, MI, United States) (Kuhlgert et al., 2016). One measurement was taken on each plant at the top of the canopy.

Destructive harvest

The leaf area and weight of a sub-sample representing a mix of developing and developed leaves were measured on the last day of the experiment using a two-digit electronic balance and a Li-3000 area meter (LiCor®, Lincoln, NE, United States). This sub-sample was then stored at -80°C for biochemical analysis.

The rest of the leaves were weighed after measuring their total leaf area using the same equipment. Stem weight was recorded before the shoot biomass was oven-dried at 80°C until it reached a constant weight.

Leaf electrolyte leakage

At the end of the second recovery period (26 DAHS), one leaf disc (10 mm diameter) was sampled from five fully expanded leaves to measure leaf electrolyte leakage (Whitlow et al., 1992). Each disc was washed three times with distilled water before being placed in 20 mL of distilled water. After incubating the discs at room temperature on a shaker (200 rpm) for 20 h, the electrical conductivity of the bathing solution (EC1) was recorded (LAQUAtwin EC-33 meter, Horiba®, Kyoto, Japan). A second electrical conductivity measurement (EC2) was taken after incubating the discs in a water bath at 95°C for 60 min. The electrolyte leakage (EL) was expressed as a percentage and calculated as follows:

$$EL = EC1/EC2 * 100\ (\%) \tag{4.1}$$

4.2.4 Biochemical analysis

Leaf chlorophyll content

Wintermans and De Mots (1965) protocol was followed to determine leaf chlorophyll content on the last day of the experiment. Two leaf discs (15 mm diameter) were sampled from the first two fully expanded leaves and placed in 10 mL 95% (v/v) cold ethanol (Fischer Scientific, Hampton, NH, United States). After 48 h in the dark at 4°C, the absorbance of the supernatant was read at 470, 649, and 665 nm (Ultrospec III, Pharmacia LKB, Stockholm, Sweden). The amount of chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoids (Car) per unit area

were calculated as follows:

$$Chl\,a = (13.95 * A649)/1.54 \tag{4.2}$$

$$Chl\,b = (24.96 * A649 - 7.32 * A665)/1.54 \tag{4.3}$$

$$Car = (10^3 * A470 - 2.05 * Chl\,a - 114.8 * Chl\,b)/1.54 \tag{4.4}$$

Total carbohydrates

Total carbohydrates were quantified following the phenol-sulfuric acid colourimetric method described by Dubois et al. (1956) and using glucose (Merck, Darmstadt, Germany) as a standard. Fresh leaf tissue (300 mg) from the subsample obtained on the last day of the experiment was digested in 10 mL of 80% (v/v) cold methanol (Fischer Scientific) and 100 μL of the digested sample was added to 900 μL of distilled water, 2.5 mL sulfuric acid (95% (v/v)) (Fischer Scientific), and 500 μL of a 5% (v/v) phenol (Fischer Scientific) solution. After 20 mins at room temperature, the absorbance of the solution was read at 490 nm using a UV-VIS spectrophotometer (Ultrospec III).

Total soluble protein

The procedure of Bradford (1976) was followed to quantify total proteins on the last day of the experiment. Briefly, 50 mg of dry leaf sample was digested in 3 mL of 100 mM sodium phosphate buffer (pH 7.0 at 20°C) (Fischer Scientific) and 10 μL of the supernatant was pipetted in a 96-well plate (96-Well Microtiter™Microplates, Fischer Scientific). Then, 290 μL Bradford reagent (Merck) was added to each well. The plate was incubated on a shaker for 5 mins at 200 rpm at room temperature. The sample absorbance at 595 nm was

measured using a plate reader (CLARIOstar, BMG Labtech, Ortenberg, Germany) and bovine serum albumin (Merck) was used as a standard.

Total antioxidants

The TEAC (Trolox Equivalent Antioxidant Capacity) method was used to determine the leaf total antioxidants at the end of the stress period with trolox (Merck) used as a standard (Re et al., 1999). A solution of 7 mM ABTS (Merck) and 2.45 mM potassium persulfate (Merck) was made and left in the dark overnight. When ready to use, this solution was diluted with 95% (v/v) ethanol (Fischer Scientific) until it reached an absorbance of 0.9 at 734 nm and warmed on a hotplate at 40°C. Approximately 50 mg of dry leaf sample was digested in 5 mL of 80% (v/v) cold methanol (Fischer Scientific) and 30 μL of the sample supernatant was pipetted in 3 mL of the diluted ABTS working solution and placed in a water bath at 40°C for 15 min. After the time elapsed, the absorbance of the samples was read at 734 nm in a UV-VIS spectrophotometer (Ultrospec III).

Total phenols

The Folin-Ciocalteau method described by Singleton et al. (1999) was used to determine leaf phenols using gallic acid (Merck) as a standard. A fresh leaf sample (300 mg) from the last day of the experiment was digested in 10 mL of 80% (v/v) cold methanol (Fischer Scientific) and 300 μL of the digested sample was added to 600 μL of 10% (v/v) Folin-Ciocalteau reagent (Merck). After 2 mins, 2.5 mL of 700 mM sodium carbonate (Fischer Scientific) was added. The mixture was left to stand at room temperature for 1 h, after which the absorbance of each sample was read at 765 nm using a UV-VIS spectrophotometer (Ultrospec III).

Leaf nutrient concentration

Three hundred milligrams of dried and milled leaf sample from the last day of the experiment was digested in 6 mL of (70% (v/v)) nitric acid and placed in a digestion microwave (Multiwave PRO, Anton Paar, Graz, Austria) reaching 175°C over a 20 mins period and maintained for a further 20 mins followed by a 10 mins cool down at 55°C. The digested sample was diluted using 18.2 milli-Q water to reach 2% (v/v) nitric acid concentration for analysis by ICP-MS (Inductively coupled plasma mass spectrophotometry, Thermo-Fisher Scientific iCAP-Q; Thermo Fisher Scientific, Germany). A multi-element solution (Claritas-PPT grade CLMS-2 from SPEX Certiprep Inc., Metuchen, NJ, United States) and a bespoke external multi-element calibration solution (PlasmaCAL, SCP Science, Quebec, Canada) were used as calibration standards.

Leaf nitrogen concentration was analysed separately using a FlashEA® 1112 elemental analyser (Thermo Fischer Scientific, Waltham, MA, United States). A dried and milled sample (50 mg) was placed in a foil capsule. Elemental nitrogen was detected after the conversion and filtration of the gas mixture generated by combustion, providing a nitrogen percentage.

4.2.5 Statistical analysis.

Statistical studies were performed on R version 4.1.2 and the models used are detailed in Appendix C. The data were assessed for normality and variance homogeneity of residuals. Normality was checked using a combination of the Shapiro test and visual evaluation (density plot, Q-Q plot, and histogram). Variance homogeneity was assessed using Levene's test and visual inspection of residuals. A two-way analysis of variance (ANOVA) was then performed with "Irrigation" and "Temperature" as the main factors and including their interac-

tion. For time series, a repetitive measure model was fitted (`lmer`) with two random effects including the repetitive measures on each plant and the interaction between the individual plants and the measurement dates. Tukey test was used to perform pairwise comparisons among both irrigation and temperature groups at a 0.05 significance level.

4.3 Results

4.3.1 Plant development and characteristics

The evolution of the stresses on plant morphology is shown in Figure 4.2. Water stress reduced the length and width leaf expansion rate (LER) at 3 and 5 DAHS compared to the control (Fig. 4.3). Only length LER was further reduced on day 7, while width LER was maintained at each irrigation level. After that, water stress did not significantly impact either LER (Fig. 4.3). T32 increased LER from 5 to 13 DAHS, corresponding at the end of the first heatwave (Fig. 4.3). During the recovery period, however, the LER of heat-stressed plants slowed down quickly and reached the same level as T27 plants (Fig. 4.3). The leaf expansion rate continued to decrease throughout the recovery, with every temperature treatment following the same trend (Fig. 4.3). The newly labelled leaves at the start of the second heatwave had a noticeably slower growth for every treatment, peaking at the end of the heatwave (Fig. 4.3). The decrease in daily LER noted for every treatment during the first recovery period was not present during the second one with plants maintaining their LER from the last heatwave day (Fig. 4.3).

Despite the maintenance of LER under limited irrigation for most of the treatment period, the final leaf dry weight and total leaf area were significantly re-

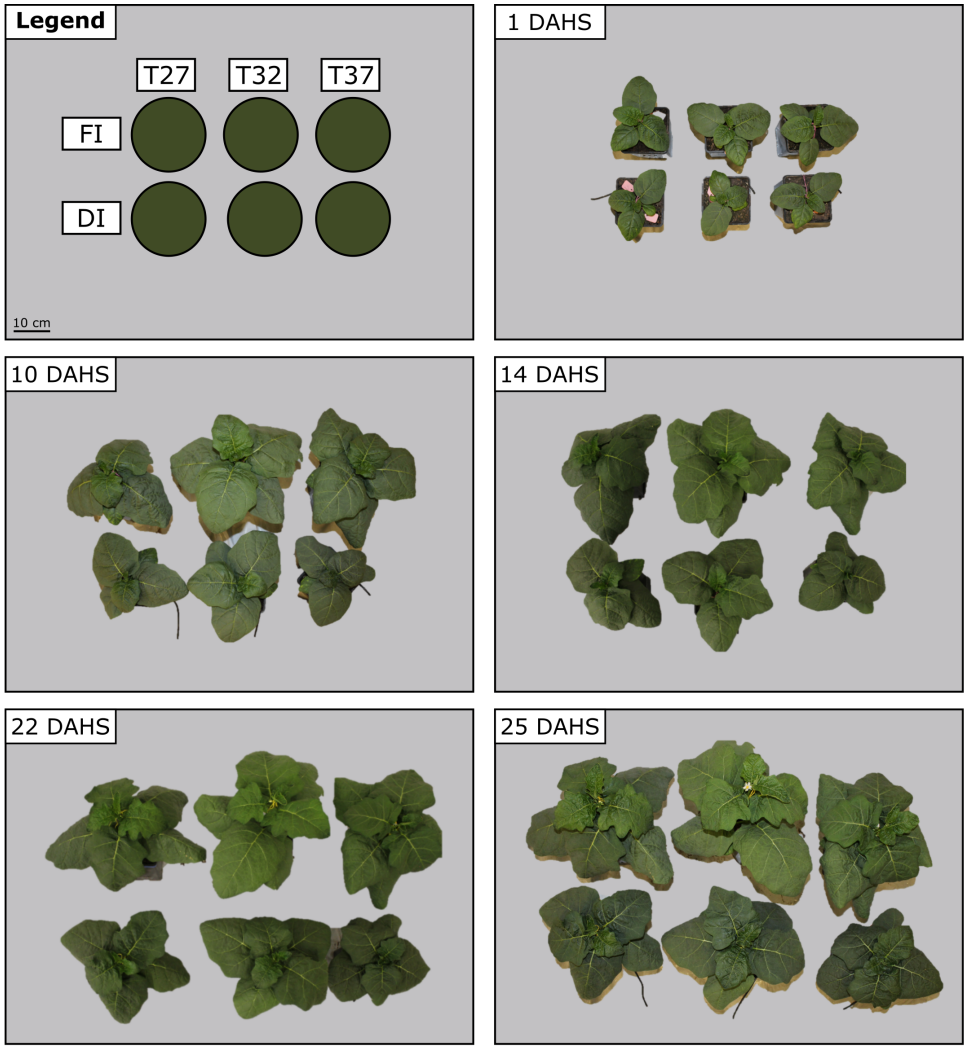


Figure 4.2: Pictures of the E11 Shum plants at 1, 10, 14, 22, and 25 days after the start of heat stress (DAHS). FI = Full irrigation, DI = Deficit irrigation. Temperature treatments as T27 = 27/21°C, T32 = 32/26°C, and T37 = 37/31°C.

duced for plants under water stress (Fig. 4.4a,c). Heat stress, in contrast, did not impact leaf dry weight (Fig. 4.4a). Under full irrigation, plants grown at T32 had a larger leaf area than the ones grown at T27 but this was not seen under deficit irrigation (Fig. 4.4c). Stem dry weight was reduced under deficit irrigation at every temperature (Fig. 4.4b). T32 increased stem dry weight only under full irrigation (Fig. 4.4b). Electrolyte leakage increased significantly under drought (Fig. 4.4d). Under deficit irrigation, EL was reduced under the highest temperature when compared to T27 (Fig. 4.4d).

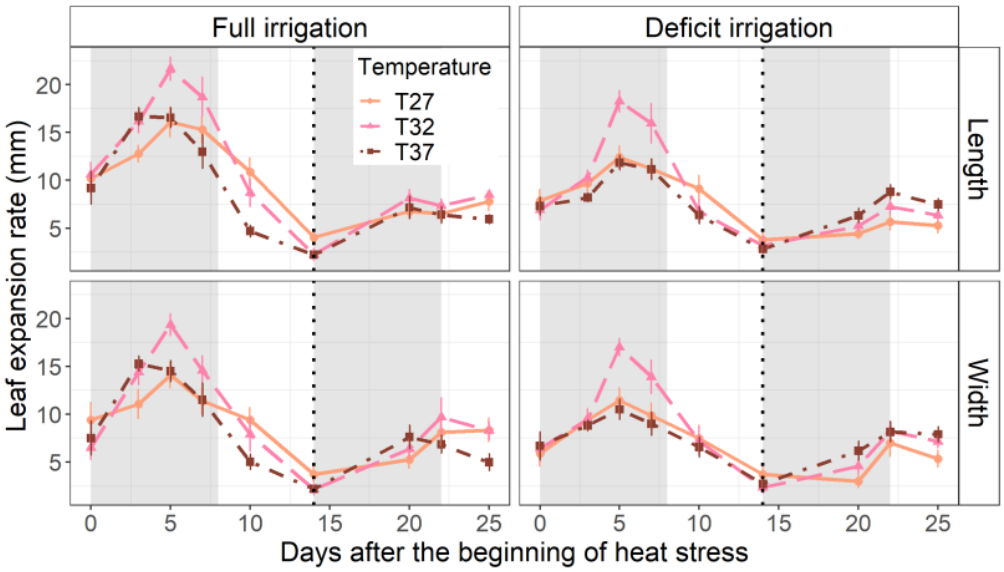


Figure 4.3: Leaf length (top row) and width (bottom row) expansion rate throughout the stress period. The data are represented by the mean +/- standard error (n = 5). The greyed areas delimit the heat wave periods. Measurements were taken on two leaves per plant and the dotted vertical line delimits the newly labelled leaves at day 14. Temperature treatments as T27 = 27/21°C, T32 = 32/26°C, and T37 = 37/31°C.

4.3.2 Leaf chlorophyll content

The SPAD index values were consistently and significantly increased by deficit irrigation from 3 DAHS (Fig. 4.5). During the heatwave, the highest temperature increased SPAD values only for plants under water stress (Fig. 4.5). During the first recovery, however, the SPAD of heat-stressed plants quickly dropped while T27 plants maintained their levels (Fig. 4.5). At the beginning of the second heatwave, there was a rapid increase in chlorophyll levels among T37 plants, especially those subjected to both heat and water stress (Fig. 4.5). This increase was temporary as the levels reverted to those of the T27 plants by 20 DAHS. During the initial heatwave, the T32 SPAD index exhibited a slight increase, aligning with the pattern observed in T27 plants. However, in the subsequent heatwave, when subjected to full irrigation, SPAD declined and only recovered during the recovery period (Fig. 4.5). On the last day of the experiment, only the irrigation treatment had a significant effect, marked by an increase for plants under water stress (Fig. 4.5).

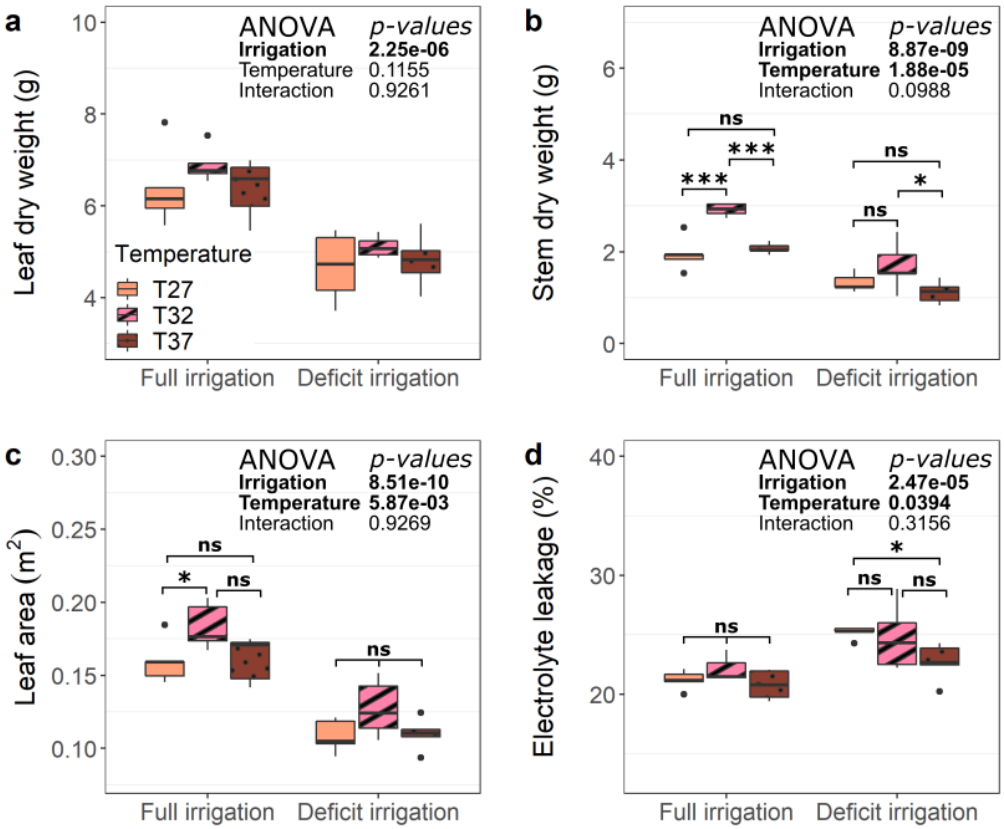


Figure 4.4: Plant morphological characteristics of the African eggplant at the end of the 26 days under increased temperature and/or reduced irrigation treatment. Sub-plots represent **(a)** leaf dry weight, **(b)** stem dry weight, **(c)** leaf area, and **(d)** leaf electrolyte leakage. The dataset is represented by the lower and upper whiskers, 25% and 75% quartiles, and median (n = 5). Black dots represent outliers. Stars denote the significance level of the pairwise comparisons by the Tukey test (* p <.05 and *** p <.001.). ns = Non-significant. Temperature treatments as T27 = 27/21°C, T32 = 32/26°C, and T37 = 37/31°C.

At the end of the experiment, chlorophylls a and b, and carotenoids were increased under deficit irrigation (Fig. 4.6a,b,c). The ratio of chlorophyll a over chlorophyll b was not affected under either stress or their combination (Fig. 4.6d).

4.3.3 Biochemical analysis

No differences were noted in antioxidant concentration between temperature treatments under full irrigation but an increase in T32 plants when compared to

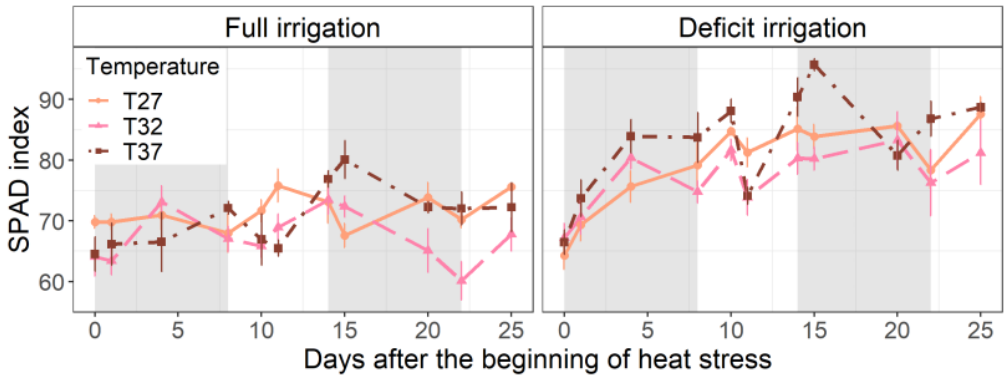


Figure 4.5: SPAD index throughout the stress period. The greyed areas represent the heat wave periods. The data are represented by the mean +/- standard error (n = 5). SPAD measurements were taken on one leaf per plant located at the canopy. Temperature treatments as T27 = 27/21°C, T32 = 32/26°C, and T37 = 37/31°C.

T37 was noted under deficit irrigation (Fig. 4.7a). Neither deficit irrigation nor

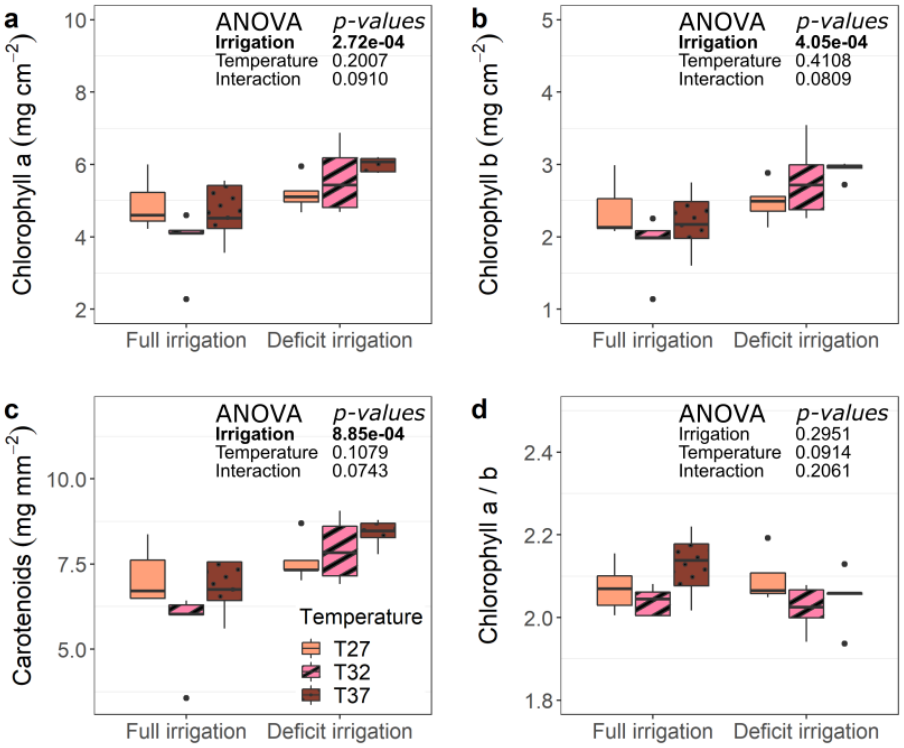


Figure 4.6: Leaf pigment content per unit area of the African eggplant after 26 days under increased temperature and/or reduced irrigation treatment. Sub-plots represent **(a)** chlorophyll a, **(b)** chlorophyll b, **(c)** carotenoids, and **(d)** the ratio of chlorophyll a over b. The dataset is represented by the lower and upper whiskers, 25% and 75% quartiles, and median (n = 5). Black dots represent outliers. The p-values are displayed in bold when significant alongside the statistical test used. Temperature treatments as T27 = 27/21°C, T32 = 32/26°C, and T37 = 37/31°C.

increasing temperature impacted the concentration of leaf phenols (Fig. 4.7b). Under full irrigation, the protein concentration was reduced for T32 plants when compared to T27 plants, which had the highest concentration (Fig. 4.7c). Under deficit irrigation, however, T27 and T32 had a similar concentration of protein while T37 had an increased concentration (Fig. 4.7c). Irrigation only reduced leaf protein concentration at T27 (Fig. 4.7c). Total carbohydrates increased linearly with heat for plants well irrigated but were only significantly higher for plants grown at T37 when compared to T27 (Fig. 4.7d). Carbohydrates were not significantly impacted by the different temperatures under deficit irrigation and the irrigation treatment did not impact their concentrations either (Fig. 4.7d).

4.3.4 Leaf nutrient concentrations

Leaf phosphorus concentration decreased under drought and increased at the highest temperature level (Table 4.1). Potassium leaf concentration was not significantly affected by either drought, heat, or their interaction (Table 4.1). While leaf magnesium and calcium concentrations were not significantly different between heat treatments, deficit irrigation decreased both nutrients (Table 4.1). Leaf boron concentration was significantly reduced under deficit irrigation across all temperature levels and the highest heat treatment resulted in a positive effect on leaf boron concentration (Table 4.1). Leaf sodium concentration was reduced under deficit irrigation and unaffected by the different temperatures (Table 4.1). The T37 treatment increased leaf manganese concentration when compared to T27 and T32 plants under both irrigation levels (Table 4.1). The stress interaction caused a reduction in leaf iron concentration for T32 plants under full irrigation and an increase in plants at T37 under deficit irrigation (Table 4.1). Reduced irrigation increased leaf zinc and selenium concentrations at every temperature while their concentration remained unaffected by the temper-

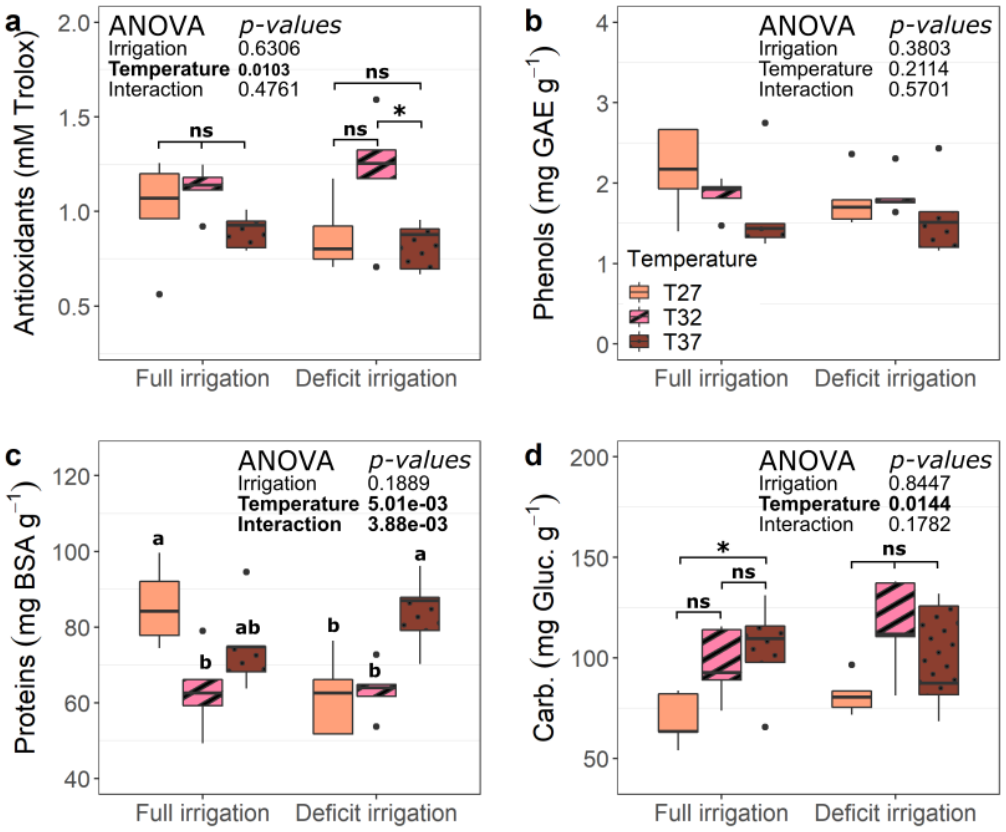


Figure 4.7: Leaf biochemical analysis of the African eggplant at the end of the 26 days under increased temperature and/or reduced irrigation treatment. Sub-plots represent **(a)** total antioxidants in dry leaves, **(b)** total phenols in mg gallic acid equivalent (GAE) per g fresh weight, **(c)** total proteins in mg bovine serum albumin (BSA) per g dry weight, and **(d)** total carbohydrates in mg glucose (Gluc.) per g fresh weight. The dataset is represented by the lower and upper whiskers, 25% and 75% quartiles, and median (n = 5). Black dots represent outliers. The p-values are displayed in bold when significant alongside the statistical test used. Stars denote the significance level of the pairwise comparisons by the Tukey test at 0.05% level of significance with * for p <.05, ** p <.01, and *** p <.001. For measures where the interaction of the parameters was significant after an ANOVA, letters are used to display the difference between treatments for clarity. Boxplots not sharing any letters are significantly different in this case. ns = Non-significant. Temperature treatments as T27 = 27/21°C, T32 = 32/26°C, and T37 = 37/31°C.

ature treatment (Table 4.1).

Table 4.1: Analysis of variance and mean comparisons for dry leaf nutrient concentrations of African eggplants grown under different irrigation and temperature levels (n = 5). Temperature treatments as T27 = 27/21°C, T32 = 32/26°C, and T37 = 37/31°C.

Source of variance	P	K	Mg	Ca	B	Na	Mn	Fe	Zn	Se
	g kg^{-1}				mg kg^{-1}					µg g^{-1}
Irrigation (I)	*	ns	***	**	***	*	***	***	***	*
Temperature (T)	*	ns	ns	ns	**	ns	***	**	ns	ns
I x T	ns	ns	ns	ns	ns	ns	*	**	ns	ns
Irrigation										
Full irrigation	4.44^{a}	37.87	6.59^{a}	30.27^{a}	40.83^{a}	56.79^{a}	118.0	80.64	14.40^{b}	8.11^{b}
Deficit irrigation	3.80^{b}	39.41	5.49^{b}	24.65^{b}	23.59^{b}	37.26^{b}	97.47	108.9	19.56^{a}	9.16^{a}
Temperature										
T27	3.81^{b}	39.43	6.49	28.30	28.75^{b}	38.14	73.76	96.89	16.02	8.54
T32	3.76^{b}	36.94	5.73	26.89	31.75^{ab}	45.17	90.93	85.76	16.51	9.02
T37	4.78^{a}	39.56	5.90	27.20	36.13^{a}	57.76	158.5	101.7	18.62	8.35
I x T										
Full irrigation + T27	4.38	41.05	6.97	28.61	37.72	36.79	77.15^{c}	93.14^{bc}	14.03	8.31
Full irrigation + T32	3.92	34.68	6.33	31.00	39.21	58.06	97.04^{c}	68.78^{d}	13.76	8.57
Full irrigation + T37	5.02	37.87	6.48	31.20	45.56	75.51	179.9^{a}	79.99^{cd}	15.28	7.45
Deficit irrigation + T27	3.25	37.81	6.02	27.99	19.77	39.49	70.36^{c}	100.6^{b}	18.01	8.77
Deficit irrigation + T32	3.59	39.19	5.12	22.77	24.30	32.28	84.83^{c}	102.7^{b}	18.71	9.48
Deficit irrigation + T37	4.55	41.24	5.33	23.20	26.71	40.02	137.2^{b}	123.3^{a}	21.95	9.24

*P = Phosphorus, K = Potassium, Mg = Magnesium, Ca = Calcium, B = Boron, Na = Sodium, Mn = Manganese, Fe = Iron, Zn = Zinc, Se = Selenium. NS, *, **, *** Non-significant or significant at P ≤ 0.05, 0.01, 0.001, respectively. Means not sharing any letters within each column are statistically different according to Tukey's test (P = 0.05).*

4.4 Discussion

While a few groups have researched the effects of drought on the African eggplant, the effects of drought in combination with heat have not been studied despite the combination of these stresses commonly observed in the field (Nakanwagi et al., 2020; Sseremba et al., 2018; Suzuki et al., 2014). In some cases, the stress combination does not increase plant damage further than the single stresses when one of the stresses is predominant, as was the case in this study for leaf development and chlorophyll content, and was previously observed in tomato (Nankishore & Farrell, 2016; Zhou, Yu, et al., 2017). For other mea-

sures, the stress combination can lead to new responses as reported by Duan et al. (2017) and observed in the current study for leaf protein and some mineral elements.

Under heat alone, an increase in SPAD, directly proportional to the absolute chlorophyll content (Jiang et al., 2017), was observed in tomato (Bhattarai et al., 2021; Ro et al., 2021). This was in line with the observations made in the current study during the heatwave period, especially for plants under deficit irrigation. This can be an acclimation response to heat where plants promote chlorophyll production and/or thermostability to compensate for the chlorophyll degradation pathway initially triggered under heat (Havaux, 1993; Wang et al., 2018). Chlorophyll content might also have increased per unit area due to smaller leaves being produced, without an increase in production or stability. However, it has to be noted that SPAD measurements can be affected by leaf water status, which was affected in this study and might have thus interfered with the accurate chlorophyll content (Martínez & Guiamet, 2004; Schlemmer et al., 2005). The final chlorophyll content, measured following a destructive method after the recovery period, was unaffected by heat. A recovery period after heat stress in tomato also led to the absence of effect at the end of the experiment despite an increase during the heat stress period (Lee et al., 2023). This may be due to a fast response of chlorophylls and carotenoids to outside temperatures to limit photodamage under lower temperatures. Chlorophylls a and b and carotenoids were, however, still increased by the drought stress, as observed throughout the stress with the SPAD measurements, suggesting an accurate chlorophyll content measurement. Despite an increase under drought alone, chlorophylls a and b in *S. chilense* (Dunal) Reich and *S. lycopersicum* decreased under the combination of heat and drought, following the same trend as under heat alone, thus highlighting the predominant effect of heat, in contrast to observations made in the current study (Blanchard-Gros et al., 2021). The

predominance of one of the stresses might be related to the adaptation of the crop of interest with drought being predominant in the current study due to the long-term adaptation of the African eggplant to high temperatures.

Deficit irrigation had previously been reported to reduce leaf area, number, and dry weight in the African eggplant as observed in this study (Gaveh et al., 2011; Limbu et al., 2018; Nakanwagi et al., 2017). Reducing leaf area is part of avoidance mechanisms aiming at reducing water loss through evapotranspiration (De Micco & Aronne, 2012). Heat stress can, in contrast, promote leaf production as seen in lettuce (*Lactuca sativa* L.) and amaranth (*Amaranthus tricolor* L.) and observed in this study in T32 (Hwang et al., 2018; Sublett et al., 2018). The increase in leaf area was not noted at the highest temperatures tested, suggesting that larger leaves are not beneficial under high temperatures as they reduce the plant's ability to regulate leaf temperature and sustain cellular processes (Hassan et al., 2021).

The reduced growth is often due to damage to the cell membranes and other compounds by reactive oxygen species produced under stress. Electrolyte leakage has been used routinely as an indicator of membrane damage and is a useful parameter to identify stress-tolerant genotypes as seen in wheat (*Triticum durum* Desf.) and tomato for example (Aleem et al., 2020; Alsadon et al., 2006; Bajji et al., 2002). While the increase observed in EL under deficit irrigation in the current study is consistent with previous observations in eggplant and tomato, the reduction of EL following moderate heat stress and recovery under deficit irrigation has not been reported previously (Cornejo-Ríos et al., 2021; Kirnak et al., 2001, 2002). The highest temperature might have played a protective role against cell membrane damage caused by drought through other mechanisms in place above a certain temperature. A lower EL was associated with beneficial traits under heat such as maintained pod set or grain yield in cowpea (*Vigna unguiculata* (L.) Walp.) (Thiaw & Hall, 2004). After a recovery period, EL

was quick to recover in wheat and tomato despite a marked increase during the stress period, hinting at a very dynamic process (Grigorova et al., 2011; Lee et al., 2023). The decrease of EL under the highest temperature observed in this study might thus be explained by the sampling timing after the recovery period.

Reactive oxygen species formation under stress is balanced by the presence of antioxidant compounds which are essential for their removal (Fahad et al., 2017). Total antioxidants and phenols were not increased under stress in the current study. A study by Hwang et al. (2018) showed no differences in antioxidant capacity under drought in amaranth while only one tolerant tomato cultivar had an increase in phenols under heat in a study by Zhou et al. (2015). Under the combination of heat and drought, enzymatic antioxidants were either reduced or unchanged in tomato after long-term stress, supporting results observed in the current study (Zhou, Kong, Yu, et al., 2019). Independently of the drought tolerance of the cultivars used, an increase in leaf and fruit antioxidant compounds was, however, previously noted in tomato, eggplant, and spinach under heat (Khosa et al., 2022; Klunklin & Savage, 2017; Plazas et al., 2022). These observations highlight the variability of responses to detoxify cells depending on growth stage, stress intensity, and plant organ. Another important stress tolerance mechanism is the increase in soluble sugars due to their role in leaf turgor maintenance (Lamaoui et al., 2018). Total carbohydrates were increased linearly under heat stress in this study as reported previously in potato (Naz et al., 2018). Sugar concentration increased under mild water stress in tomato but decreased under more severe stress, showing a dynamic response to the stress intensity (Sun et al., 2016). Stress combination affected sugar concentration differently in the study by Zhou, Yu, et al. (2017), highlighting an interplay of heat and drought in carbohydrate metabolism as seen in the current study with an increase driven by heat only under full irrigation.

The effect of abiotic stress on leaf nutrient concentration tends to be nutrient-

specific as well as highly dependent on the stress intensity (Soares et al., 2019). Interestingly, leaf calcium concentration decreased in the current study despite a range of studies previously reporting an increase in other *Solanum* crops (Matías et al., 2021; Pimenta et al., 2022; Sarker & Oba, 2018). While an increase in leaf calcium concentration in certain plant parts is important for the regulation of stress responses, a reduced soil water potential can reduce calcium uptake (Ahanger et al., 2016). Surprisingly, leaf potassium and phosphorus concentrations, whose uptake is also reduced under low soil water potential, were not affected by drought in this case, in contrast to previous observations in eggplant and tomato (Ali et al., 2021; Kirnak et al., 2001). In field conditions under a more moderate temperature increase, however, leaf phosphorus concentration increased under heat in quinoa (*Chenopodium quinoa* Willd.) (Matías et al., 2021). In contrast, leaf potassium concentration was not affected, similar to the current observations and suggesting different mechanisms acting depending on the stress intensity and other environmental conditions (Matías et al., 2021).

The maintenance of leaf magnesium concentration under different temperature treatments in this study is consistent with the observation made in quinoa under heat (Matías et al., 2021). The decrease noted under water deficit at high temperatures was, however, in contrast with previous results in tomato where no effect was recorded (Pimenta et al., 2022). Adequate leaf magnesium concentration plays an important role in chlorophyll production, and plant growth maintenance, and has been previously shown to be highly beneficial in heat stress tolerance by promoting a range of secondary metabolism pathways (Siddiqui et al., 2018). The pathways triggered under heat by the genotype used in the current study seem, however, to interact with magnesium uptake and/or transport under water stress, leading to reduced leaf magnesium concentration at high temperatures under drought.

Micronutrients can have a beneficial effect on stress responses, for example,

iron can reduce oxidative stress (Kumari et al., 2022). Under heat stress, a decrease in leaf iron concentration was noted, however, when water stress was also present, the opposite was true. Under well-watered conditions, iron was not affected by heat in red lettuce, pak choi (*Brassica chinensis* L.), amaranth, and tomato (Hwang et al., 2018; Pimenta et al., 2022; Sublett et al., 2018). The response observed in the current study seems thus not to be a common stress response. As iron uptake is negatively impacted by an elevated concentration of phosphorus, manganese, and zinc, the dynamics of these minerals under stress will influence iron concentration, adding complexity when trying to understand its dynamics (Ahanger et al., 2016). The leaf manganese concentration increase in T37 under both full and deficit irrigation shows a sustained heat response mechanism. Manganese has previously been suggested to help with photosynthesis, respiration, and nitrogen assimilation (Kumari et al., 2022). In the same experiment, leaf manganese concentration was also noted to increase in Amaranth under heat, but not Pak Choi, highlighting species differences (Hwang et al., 2018).

4.5 Conclusion

This study aimed to describe the combined effects of drought and high temperature with a recovery period on the African eggplant Shum. Drought stress negatively impacted leaf and stem production, while moderate heat promoted plant growth. Chlorophylls were increased under water stress while they were affected by heat only during the heatwave period and were back to non-heated levels after a recovery period. The simultaneous application of drought and high temperature led to a similar effect to drought applied alone in plant growth and chlorophylls, showing its predominant effects on major plant characteristics. Water stress mostly affected the morphology and physiology of the plant but

some mineral elements were also impacted by this stress. High temperatures affected the plant's primary and secondary metabolisms to different extents, with an increase in carbohydrates and boron only for well-watered plants under heat stress. The African eggplant does not seem to activate its non-enzymatic antioxidative response pathways under drought and heat.

Chapter 5

Heat and salinity stress on a Kumba cultivar of the African eggplant

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5.1 Introduction

The last decade has been marked by increasing temperatures and longer, more intense, and more frequent heatwaves around the world, in particular in Africa (Bezner Kerr et al., 2022). High temperatures threaten food security as most crops grown worldwide are heat-sensitive (Hassan et al., 2021). Yield reductions are due to various mechanisms triggered by heat including the reduction of leaf expansion and gas exchange to limit water loss alongside the damages to enzymes and other key parts of the photosynthetic system due to the high leaf temperature (Hassan et al., 2021). In addition, soil salinisation is increasing worldwide due to a combination of natural and man-made processes such as rising sea levels, poor irrigation practices, and atmospheric deposition (Bezner Kerr et al., 2022; Ondrasek et al., 2022). Soil salinity also negatively impacts crop growth, leading to stunted growth and poor yields due to limited water uptake and ion imbalance (Ondrasek et al., 2022). Under field conditions, heat and salinity often occur concurrently, especially in coastal areas. It is known that the stress combination often leads to responses not observed under individual stresses, thus requiring additional research to understand the mechanisms in play (Nadeem et al., 2023).

The introduction of tolerant and diverse crops in food production systems is crucial to enhancing food security and consumers' nutrition. Indigenous vegetables offer great opportunities to improve field resilience due to their tolerance to abiotic and biotic stress. They are generally more tolerant to stress than their exotic counterparts due to their selection in harsh environments and their large genetic assortment (Akinola et al., 2020). Even though they have been poorly studied in the past, they have strong local importance and include all types of crops including legumes, cereals, and vegetables (Akinola et al., 2020). *Solanum aethiopicum* L., the African eggplant, is indigenous to Africa and present in

multiple forms across sub-Saharan Africa, displaying a great genetic diversity and nutrition (Han et al., 2021). It is liked for its nutritional quality and is an important income stream for small-scale farmers across the African continent (Han et al., 2021). The African eggplant has been selected by the World Vegetable Center as a priority crop for breeding strategies to improve fields' resilience and diversity but was previously reported to have a low tolerance to salinity (de Fatima et al., 2023; Dinssa et al., 2016). New varieties are being developed by breeding companies and researchers but lack research on their abiotic stress tolerance to ensure their relevance in the field in stress-prone areas. This is the case of the cultivar F1 Djamba, for example, developed by Novagenetic (Technisem, Longué-Jumelles, France) and promoted for its high yield. Technisem market includes tropical areas, in particular Northern and Western Africa, which are highly likely to experience a high heat increase due to climate change alongside a constant increase in soil salinity (World Meteorological Organization, 2022). Understanding how F1 Djamba responds to the combination of stresses is thus crucial to ensure its use in every possible area.

This study aimed to understand how soil salinity and heat affect the African eggplant cv. F1 Djamba when present individually and in combination. F1 Djamba was used due to preliminary results obtained in Chapter 3 where chlorophyll a concentration was increased under salinity only in F1 Djamba. Due to the protective effects of chlorophyll concentration on heat stress, salinity was combined with elevated temperatures to learn more about how F1 Djamba, marketed in coastal areas and in West Africa where temperatures are particularly high, responds to this stress combination and highlights potential protective pathways. Leaf biomass, photosynthetic parameters, and leaf biochemical analysis including protein, antioxidants, and nutrient concentrations were assessed to clarify some stress-related mechanisms used by the African eggplant. It was hypothesised that the combination of heat and salinity would lead to specific responses

not observed when the stresses were present individually. This research will be useful to understand how this species could be deployed in future climates.

5.2 Materials and Methods

5.2.1 Plant material and growth conditions

The combination of salinity stress and high temperatures on the African eggplant Kumba (*Solanum aethiopicum* L. cv., F1 Djamba) was investigated. Novagenetic (Technisem, Longué-Jumelles, France) generously provided the seeds which were sown in modular trays in John Innes No.2 soil compost (7 loam:3 peat:2 sand v/v/v) (J Arthur Bower's, Westland®, Dungannon, Northern Ireland) and placed in an MLR-352 growth cabinet (PHC Holdings Corporation®, Tokyo, Japan). The environmental conditions were set at 70% relative humidity and 27/21°C for a 12 h photoperiod. After four weeks, 24 homogenous seedlings were transplanted in 3 L pots in John Innes No.2 soil and placed following a split-plot design in three growth cabinets (Plant growth chamber A1000, Conviron®, Winnipeg, Canada) fitted with Valoya LEDs lights (BX NSI spectrum 'white' LED, Valoya®, Helsinki, Finland) providing 350 μmol m^{-2} s^{-1} light intensity. The growing conditions stayed at 27/21°C for a 12 h photoperiod at 70% relative humidity.

5.2.2 Treatment application

After 11 days of acclimation at 27/21°C for a 12 h photoperiod at 70% relative humidity and well-watered, salinity stress was applied to four plants per cabinet by watering them using 100 mM NaCl solution while the other four plants, used as the control, received only irrigation water for 12 days. This sodium chloride

concentration corresponds to a soil electrical conductivity of around 9.8 dS m^{-1}, considered highly saline (West, 1975). As the stress was applied as irrigation for only 12 days, rather than an initial mix of the salt into the soil, this high concentration was selected to ensure a significant increase in soil salinity while being relevant for field conditions. At the same time, considered to be 1 DASH (days after salinity and heat), heat stress was applied in two of the cabinets with a maximum of 37/31°C (T37) and 42/36°C (T42) day/night temperature when compared to 27/21°C (T27) for the control plants, a treatment also maintained for 12 days. These increased temperatures were selected based on previous research on the African eggplant (see Chapter 4) and on temperatures sometimes reached in Northern and Western Africa during heat waves, with T42 also being higher than the average monthly temperature of multiple countries in Africa (Worlddata, 2023). The daily schedules of each treatment are shown in Fig.5.1. The recorded temperatures in each cabinet are shown in Appendix B, Fig. B.4.

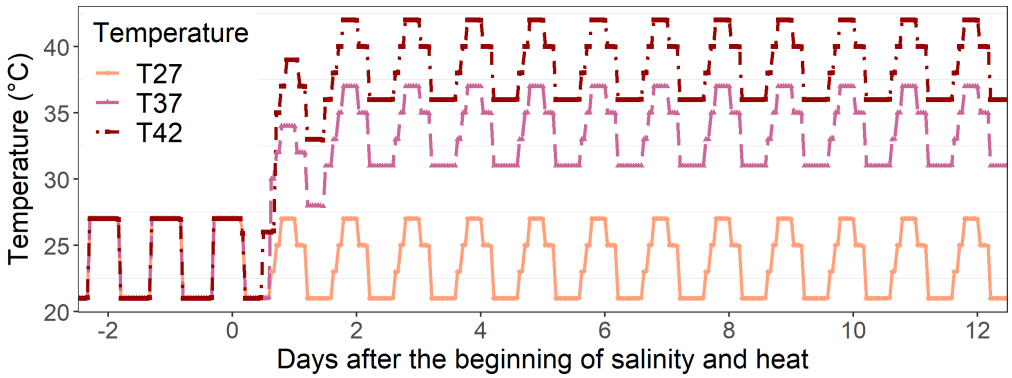


Figure 5.1: Daily air temperature schedule of 27/21°C (T27), 37/31°C (T37), and 42/36°C (T42) treatments throughout the experimental period (from -2 DASH to 12 DASH).

5.2.3 Growth and physiological measurements

Leaf expansion rate

Three times a week, the length and maximum width of two labelled leaves were recorded. These leaves were originally labelled at a similar height and developmental stage and were less than 30 mm in length. The length and width leaf expansion rates (LER) were considered to be the growth difference between the repeated measurements.

Chlorophyll fluorescence and gas exchange

Leaf fluorescence parameter SPAD, measuring the transmittance of red and infrared through the leaf to quantify chlorophyll, and photosynthetic parameters photosystem II quantum yield ($\phi 2$) and quantum yields of non-photochemical exciton quenching (ϕNPQ) were measured three times a week 5 h after the start of the photoperiod from 0 to 12 DASH using a MultispeQ V2.0 device with Photosynthesis RIDES 2.0 protocol (PhotosynQ®, East Lansing, MI, United States) (Kuhlgert et al., 2016). Measurements were taken on one leaf per plant at the top of the canopy. Leaf chlorophyll, flavonoids, and nitrogen balance were measured three times a week at the same time from 0 to 12 DASH using a Dualex® device (ForceA, Montpellier, France). Measurements were taken on two leaves per plant at the top of the canopy. Stomatal conductance was recorded 4 h after the start of the photoperiod at 0, 1, 7, and 12 DASH using a leaf porometer (Model SC-1, Decagon Devices, METER group, Pullman, WA, United States) on one leaf per plant at the top of the canopy. At 12 DASH, diurnal measurements of stomatal conductance were taken at 0, 4, 8, and 12 h after the start of the photoperiod.

Destructive harvest

On the last day of the experiment (12 DASH) a leaf sub-sample representing a mix of developing and developed leaves was harvested, placed in liquid nitrogen straight after recording its combined weight using a two-digit electronic scale, and stored at -80°C for biochemical analysis. Leaves and stems were weighed separately, after measuring leaf number, stem diameter and plant height, and oven-dried at 80°C until reaching a constant weight.

Leaf electrolyte leakage

One leaf disc (10 mm diameter) was cut out from five fully expanded leaves on each plant at the end of the treatment (12 DASH). The discs were washed three times with distilled water to remove surface contamination and placed in 20 mL of distilled water. The discs were incubated at room temperature on a shaker at 200 rpm for 24 h and the electrical conductivity of the bathing solution (EC1) was eventually recorded using a LAQUAtwin EC-33 meter (Horiba®, Kyoto, Japan). The discs were then placed in a water bath at 95°C for 60 mins and a second electrical conductivity reading (EC2) was taken after cooling the solution to room temperature. The electrolyte leakage (EL), expressed as a percentage and calculated following the protocol by Whitlow et al. (1992), was calculated as follows:

$$EL = EC1/EC2 * 100\ (\%) \tag{5.1}$$

5.2.4 Soil electrical conductivity

On the last day of the experiment, one soil sample from each pot was extracted and left to air-dry until reaching constant weight. Five grams of the dry soil was then added to 25 mL of distilled water, shaken, and left to settle for 24 h as described in He et al. (2012). The electrical conductivity of the supernatant (EC 1:5) was then measured using a LAQUAtwin EC-33 meter (Horiba®).

5.2.5 Biochemical analysis

Leaf chlorophyll content

Leaf chlorophyll content was determined following the method described in Wintermans and De Mots (1965). At 12 DASH, two leaf discs (1.54 cm^2 each in area) from the first two fully expanded leaves were immersed in 10 mL 95% (v/v) cold ethanol (Fischer Scientific, Hampton, NH, United States) and kept at 4°C in the dark for 48 h. The absorbance of the supernatant was read at 470, 649, and 665 nm using a UV-VIS spectrophotometer (Ultrospec III, Pharmacia LKB, Stockholm, Sweden). The amount of chlorophyll a (Chl a) and b (Chl b) and carotenoids (Car) per unit area were calculated as follows:

$$Chl\,a = (13.95 * A649)/1.54 \tag{5.2}$$

$$Chl\,b = (24.96 * A649 - 7.32 * A665)/1.54 \tag{5.3}$$

$$Car = (10^3 * A470 - 2.05 * Chl\,a - 114.8 * Chl\,b)/1.54 \tag{5.4}$$

Total carbohydrates

The amount of soluble sugars in plants at 12 DASH was quantified using the phenol-sulfuric acid colourimetric method (Dubois et al., 1956). A dry leaf sample (50 mg) was dissolved in 5 mL of 80% (v/v) cold methanol (Fischer Scientific). Subsequently, 100 μL of the resulting solution was combined with 900 μL of distilled water, 2.5 mL of sulfuric acid (95% (v/v)) (Fischer Scientific), and 500 μL of a 5% (v/v) phenol (Fischer Scientific) solution. After incubating for 20 mins at room temperature, the absorbance was measured at 490 nm using a UV-VIS spectrophotometer (Ultrospec III). Glucose (Merck, Darmstadt, Germany) served as the standard.

Total antioxidants

The TEAC (Trolox Equivalent Antioxidant Capacity) method was used to determine total antioxidants (Re et al., 1999). A solution of 7 mM ABTS (Merck) with 2.45 mM potassium persulfate (Merck) was made and left in darkness overnight. This solution was then diluted with 95% (v/v) ethanol (Fischer Scientific) until reaching an absorbance of 0.9 at 734 nm and warmed on a hotplate at 40°C. Fifty milligrams of dry leaf sample (extracted at 12 DASH) was diluted in 5 mL of 80% (v/v) cold methanol (Fischer Scientific) and 30 μL of the sample supernatant was pipetted in 3 mL of the diluted working solution. After 15 mins in a 40°C water bath, the absorbance of the samples was read at 734 nm in a UV-VIS spectrophotometer (Ultrospec III).

Total phenols

The Folin-Ciocalteau method described by Singleton et al. (1999) was used to measure leaf phenol content on the final day of the experiment (12 DASH). Cold

methanol (80% (v/v), Fischer Scientific) was used to digest 50 mg of dry leaf sample and 300 μL of the digested sample was added to 600 μL of 10% (v/v) Folin-Ciocalteau reagent (Merck) and left to stand for 2 min. Then, 2.5 mL of 700 mM sodium carbonate (Fischer Scientific) was added. The mixture was left to stand at room temperature for 1 h, after which the absorbance of each sample was read at 765 nm in a UV-VIS spectrophotometer (Ultrospec III). Gallic acid (Merck) was used as a standard.

Leaf nutrient concentrations

The dried and milled leaf samples (300 mg), extracted at 12 DASH, were digested in 6 mL of 70% (v/v) nitric acid and placed in a digestion microwave (Multiwave PRO, Anton Paar, Graz, Austria) reaching 175°C over a 20 mins period and maintained for a further 20 mins followed by a 10 mins cool down at 55°C. This digested extract was diluted by adding 18.2 milliQ water to reach a 2% (v/v) nitric acid concentration for multi-element analysis by ICP-MS (Inductively Couple Plasma Mass Spectrophotometry, Thermo-Fisher Scientific iCAP-Q; Thermo Fisher Scientific, Germany). Calibration standards included a multi-element solution (Claritas-PPT grade CLMS-2 from SPEX Certiprep Inc., Metuchen, NJ, United States) and a bespoke external multi-element calibration solution (PlasmaCAL, SCP Science, Quebec, Canada).

A FlashEA®1112 elemental analyser (Thermo Fischer Scientific, Waltham, MA, United States) was used to determine leaf nitrogen concentration by burning 50 mg of dried and milled sample placed in a foil capsule. The gas mixture generated by combustion was filtered and elemental nitrogen was detected by conversion, providing a nitrogen percentage.

5.2.6 Statistical analysis

Statistical studies were performed on R version 4.1.2 and the models used are detailed in Appendix C. The data was assessed for normality and variance homogeneity of residuals. Normality was checked using a combination of the Shapiro test and visual evaluation (density plot, Q-Q plot, and histogram). Variance homogeneity was assessed using Levene's test and visual inspection of the residuals. When the requirements were met, a two-way analysis of variance (ANOVA) test was performed on continuous data using "Salinity" and "Temperature" as the main factors and including their interaction. Tukey adjustment at a 0.05 significance level was used to test for pairwise comparison among and across groups. Whenever the interaction of stresses was significant, the interaction parameter was used for posthoc tests and letters were used to show the significance throughout the manuscript for clarity. A linear mixed-effects model was also used to analyse time series of continuous data including the interaction of the parameters of interest with time and the repetitive measurement of each plant as a random factor. For count data, a generalised linear model fitted with a Poisson distribution was checked for overdispersion and used for analysis with "Salinity" and "Temperature" as interacting factors.

The log values of leaf sodium concentration were used for statistical analysis to improve residuals' normality and homogeneity due to the extreme treatment effect observed on the original dataset.

5.3 Results

The salinity treatment significantly increased soil EC 1:5 at every temperature (Fig. 5.2). In addition, T37 and T42 had a significantly higher soil EC 1:5 than T27 under salinity (Fig. 5.2).

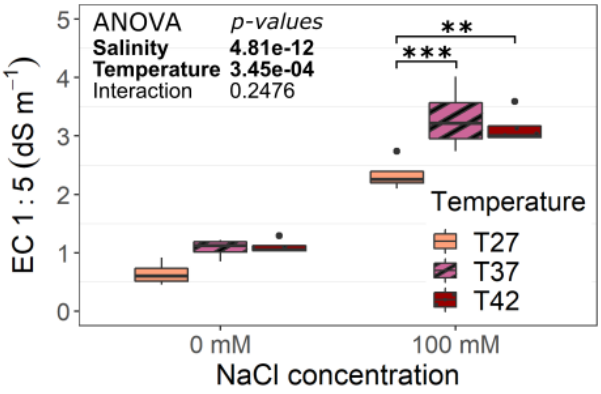


Figure 5.2: Soil electrical conductivity (EC) 1:5 at the end of the salinity treatment. The dataset is represented by the lower and upper whiskers, 25% and 75% quartiles, and median (n = 4). Stars denote the significance level based on the Tukey pairwise comparison at 95% confidence level with * for p <.05, ** p <.01, and *** p <.001. Non-significant interactions are not shown for clarity. Temperature treatments as T27 = 27/21°C, T37 = 37/31°C, and T42 = 42/36°C.

5.3.1 Plant development

A linear decrease of leaf dry weight was observed under increasing temperature as well as under salinity (Fig. 5.3a). Stem dry weight was maintained at T37 and reduced at T42 under 0 mM NaCl but was reduced for both T37 and T42 under 100 mM NaCl when compared to T27 (Fig. 5.3b). Salinity did not impact stem dry weight. Plant height was only reduced by T42 under 0 mM NaCl conditions while plants under salinity were not impacted when compared to the control (Fig. 5.3c). Salinity also impacted shoot development by reducing stem diameter while only the highest temperature reduced it in both 0 and 100 mM NaCl treatments (Fig. 5.3d). Leaf number followed a similar pattern and was only reduced by T42 under 0 and 100 mM NaCl conditions (Fig. 5.3e).

Electrolyte leakage was increased by salinity but not significantly impacted by the temperatures at 0 mM NaCl and reduced by T37 when compared to T27 under 100 mM NaCl (Fig. 5.3f).

While an increase in both length and width LER was observed from the 1st day of the treatment to the 3rd, only T27 plants continued to increase up to

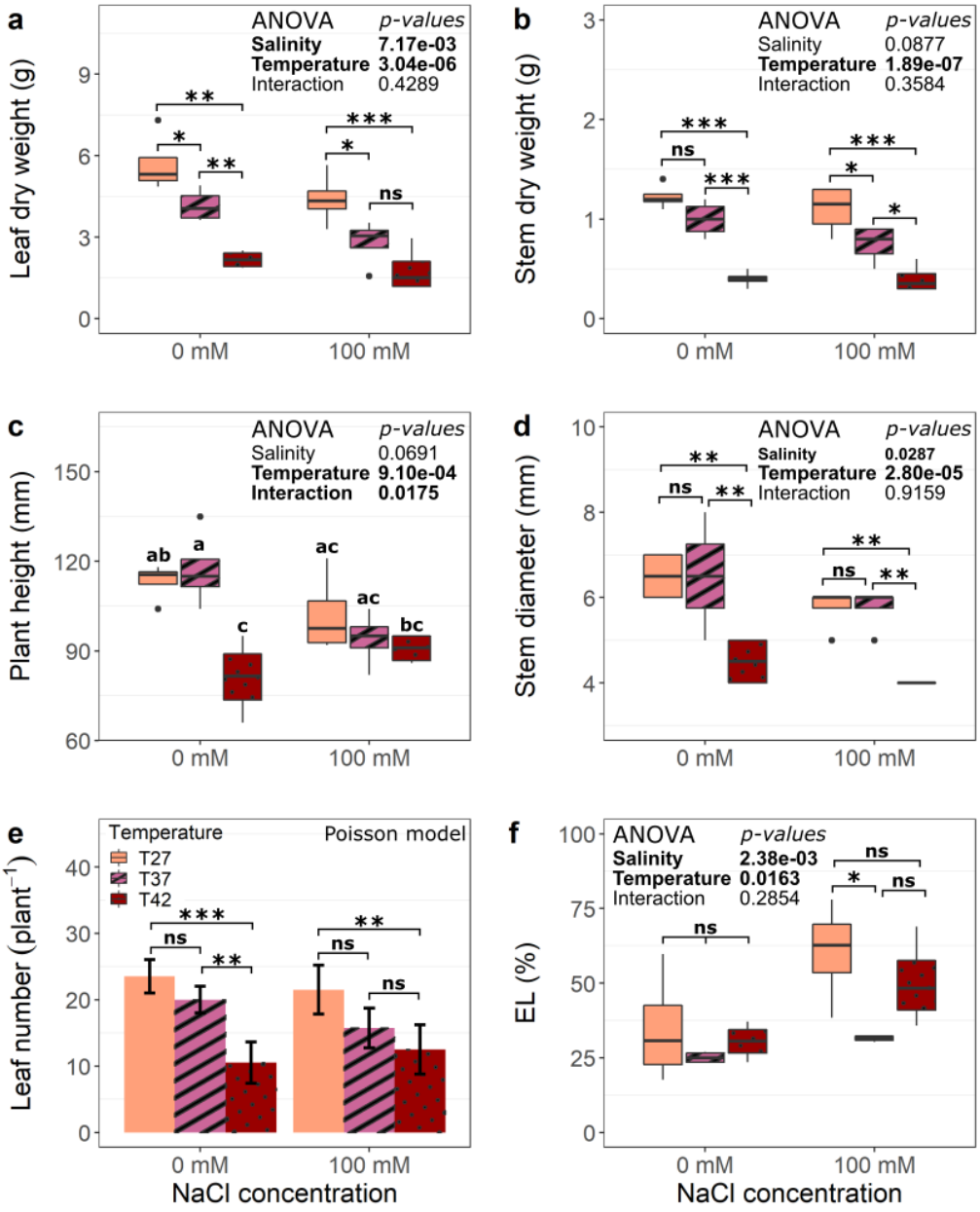


Figure 5.3: Morphological characteristics of the African eggplant at the end of the 12 days under salinity and increased temperature. Sub-plots represent **(a)** leaf dry weight, **(b)** stem dry weight, **(c)** plant height, **(d)** stem diameter, **(e)** number of leaves per plant and **(f)** leaf electrolyte leakage (EL). The continuous dataset is represented by the lower and upper whiskers, 25% and 75% quartiles, and median (n = 4). Black dots represent outliers. The count dataset is represented by the mean +/- standard deviation. Stars denote the significance level based on the Tukey test at 95% confidence level with * for p $<.05$, ** p $<.01$, and *** p $<.001$. For measures where the interaction of the parameters was significant after the ANOVA, letters are used to display the difference between treatments for clarity. Boxplots not sharing any letters are significantly different in these cases. Temperature treatments as T27 = 27/21°C, T37 = 37/31°C, and T42 = 42/36°C.

day 6 with T37 and T42 LER reducing slightly (Fig. 5.4). From the 6th day, T42 treatment reduced length LER significantly when compared to T27 in both salinity treatments but only reduced width LER of plants under 0 mM NaCl on day 6 and 8 (Fig. 5.4). Salinity reduced length LER on day 6 at every temperature (Fig. 5.4). On day 8, length and width LER were only reduced for T27 and T37 plants by salinity and no further impact was noted for the rest of the experiment due to salinity (Fig. 5.4). Both plants under saline and non-saline conditions had the same pattern of initial LER increase before slowing down and stabilising.

5.3.2 Leaf chlorophyll and polyphenols

Salinity increased chlorophyll index throughout the stress (Fig. 5.5a). Under 100 mM NaCl, the chlorophyll index increased more quickly at T37 and T42 than at T27 until 4 DASH and stabilised thereafter. The chlorophyll index of T27 plants, however, continued to increase until the end of the experiment and surpassed T37 and T42 levels at 8 DASH (Fig. 5.5a). Although at non-saline

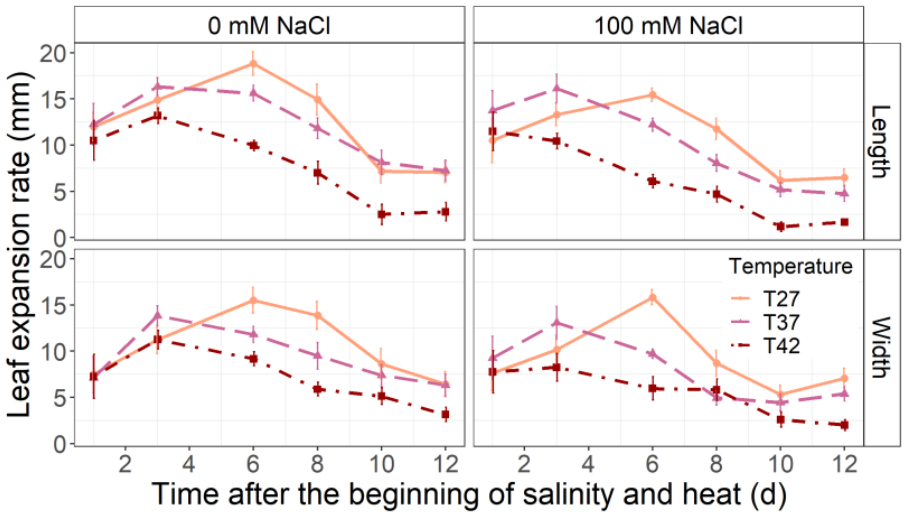


Figure 5.4: Leaf length (top row) and width (bottom row) expansion rate throughout the salinity and increased temperature treatment. The data are represented by the mean +/- standard error (n = 4). Two leaves per plant were measured to create this data. Temperature treatments as T27 = 27/21°C, T37 = 37/31°C, and T42 = 42/36°C.

conditions the chlorophyll index was not statistically different throughout the stress period between the temperature treatments, T42 had lower levels than the other two temperatures at 12 DASH (Fig. 5.5a). Salinity increased the SPAD index halfway through the experiment with significant differences appearing from 6 until 10 DASH (Fig. 5.5b). The temperature stress also affected SPAD under 0 mM NaCl, with a decrease from day 8 on plants grown at T42 (Fig. 5.5b). Under 100 mM NaCl, T42 only significantly reduced SPAD on day 10 and day 12 (Fig. 5.5b). T42 reached its peak SPAD earlier than the other temperatures at 100 mM NaCl (Fig. 5.5b).

Neither flavonoids nor nitrogen balance index were impacted by salinity, but they decreased and increased, respectively, under T37 and T42 from the 3rd day of stress (Fig. 5.5c,d). Temperature decreased flavonoids from day 3 to 8, after which the T37 and T42 levels started to increase without fully recovering (Fig. 5.5c). While the nitrogen balance index at T27 remained stable throughout

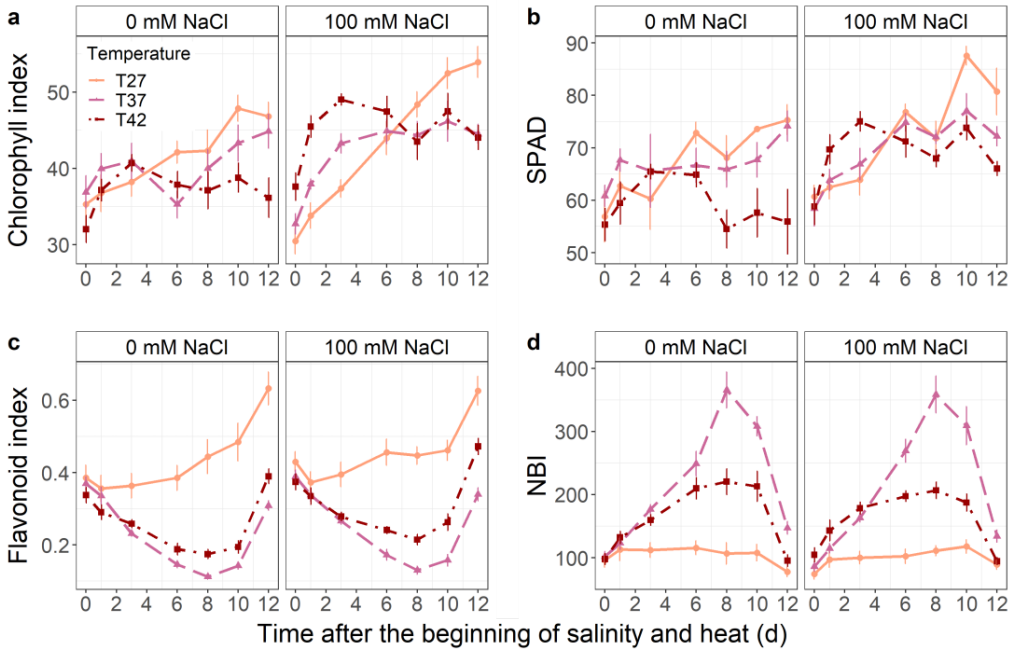


Figure 5.5: Leaf chlorophyll and polyphenols throughout the stress period. Sub-plots represent **(a)** chlorophyll index, **(b)** SPAD, **(c)** flavonoid index and **(d)** nitrogen balance index (NBI). The data are represented by the mean +/- standard error (n = 4). Two measurements per plant were taken on two distinct fully expanded leaves on top of the canopy.

the experiment, both T37 and T42 increased from 3 to 8 DASH (Fig. 5.5d). From day 8, the nitrogen balance index of T37 and T42 started to decrease and eventually reached the same level as T27 at the end of the experiment (Fig. 5.5d). The highest nitrogen balance index was reached under the T37 treatment on day 8 while the increase seen under the T42 treatment was more moderate (Fig. 5.5d).

Salinity did not impact final chlorophyll content (Fig. 5.6). The highest temperature, on the other hand, decreased chlorophylls a and b, and carotenoids (Fig. 5.6a,b,c). The ratio of chlorophyll a over b was increased for T42 plants compared to T37 but not T27 (Fig. 5.6d).

5.3.3 Stomatal conductance and leaf fluorescence

Midday stomatal conductance increased significantly only for T37 at 100 mM NaCl when compared to the control at 1 DASH (Fig. 5.7a). At 7 DASH, however, stomatal conductance at T42 was significantly higher than the control but not T37 (Fig. 5.7a). The temperature treatment had the biggest effect on midday stomatal conductance on day 7 with both saline and non-saline T37 and T42 plants reaching their highest level then (Fig. 5.7a). No differences between treatments were recorded on the last day of the experiment. Stomatal conductance of T42 plants dropped significantly on that day and stayed stable for T27 and T37 (Fig. 5.7a).

When looking at diurnal stomatal conductance throughout the last treatment day (12 DASH), the strongest difference was noted at the beginning of the day with a low stomatal conductance under T42 at 0 mM NaCl and under both T37 and T42 at 100 mM within the first hour of light (Fig. 5.7b). As the day progressed, stomatal conductance increased quickly in plants under the T42 treatment while

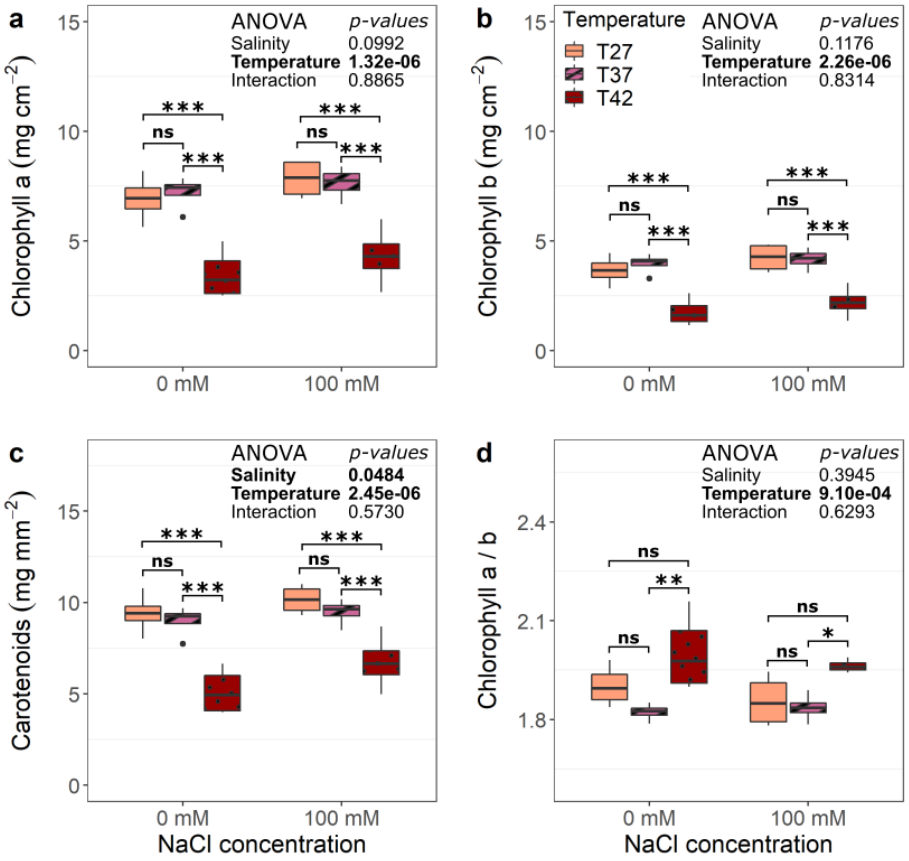


Figure 5.6: Leaf pigment content per unit area of the African eggplant after 12 days under salinity and increased temperature. Sub-plots represent **(a)** chlorophyll a, **(b)** chlorophyll b, **(c)** carotenoids, and **(d)** the ratio of chlorophyll a over chlorophyll b. The dataset is represented by the lower and upper whiskers, 25% and 75% quartiles, and median (n = 4). Black dots represent outliers. Stars denote the significance level based on the Tukey test at 95% confidence level with * for p <.05, ** p <.01, and *** p <.001. Temperature treatments as T27 = 27/21°C, T37 = 37/31°C, and T42 = 42/36°C.

plateaued, or even reduced, in the other temperatures (Fig. 5.7b). The T42 treatment thus reached the same stomatal conductance as T27 and T37 eight hours after the start of the photoperiod at 0 mM NaCl (Fig. 5.7b). T42 plants reached their peak stomatal conductance eight hours after the beginning of the photoperiod at 100 mM NaCl (Fig. 5.7b). At 0 mM NaCl, T37 stomatal conductance also peaked 8 h after the beginning of the photoperiod and was significantly higher than T27 (Fig. 5.7b). The T27 plants reached their peak stomatal conductance earlier, at 4 hours after the beginning of the photoperiod. Salinity mostly affected T37 plants, which had the lowest stomatal conductance at every time point, even though only significant at the beginning and the end of the

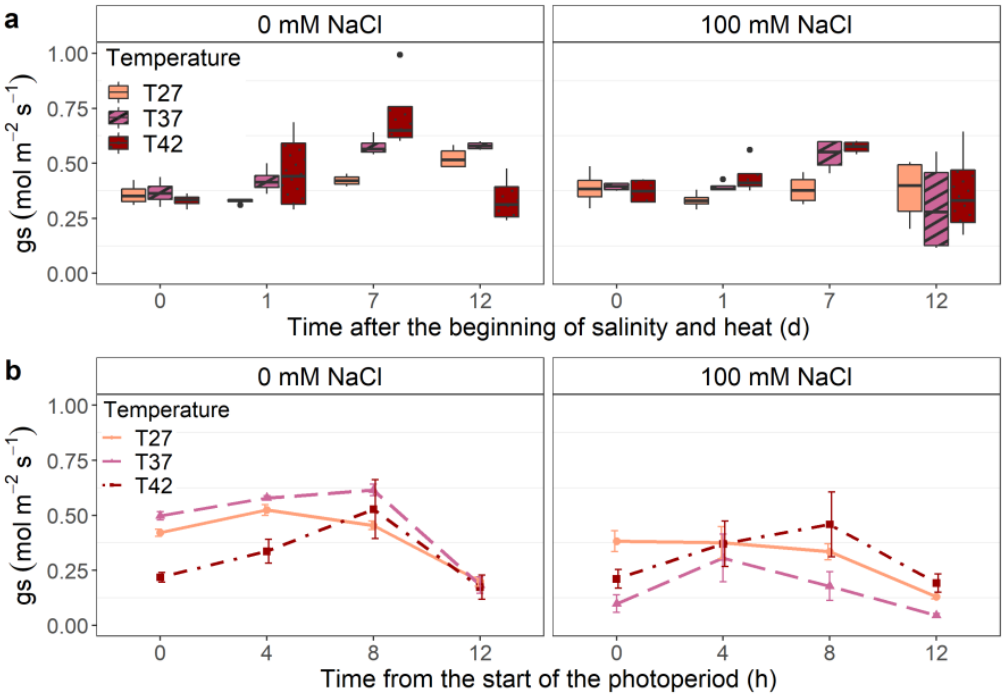


Figure 5.7: Stomatal conductance (gs) of the African eggplant. Sub-plots represent **(a)** midday stomatal conductance throughout the experiment and **(b)** diurnal stomatal conductance throughout the last day of the experiment (12 DASH). The dataset in **(a)** is represented by the lower and upper whiskers, 25% and 75% quartiles, and median (n = 4). The dataset in **(b)** is represented by the mean +/- standard error. Black dots represent outliers. One measurement per plant was taken on a leaf at the top of the canopy.

photoperiod (Fig. 5.7b).

The T42 treatment decreased the quantum yield of photosystem II ($\phi 2$) at 1, 6, 8, and 12 DASH under both salinity treatments (Fig. 5.8a). This was exacerbated by salinity towards the end of the stress period (Fig. 5.8a). The ratio of incoming light going through non-photochemical quenching (ϕNPQ) was only increased by the combination of salinity and the highest temperature at the end of the experiment at 10 and 12 DASH (Fig. 5.8b). The ϕNPQ and $\phi 2$ remained constant throughout the stress period for T27 and T37 at both salinity levels (Fig. 5.8).

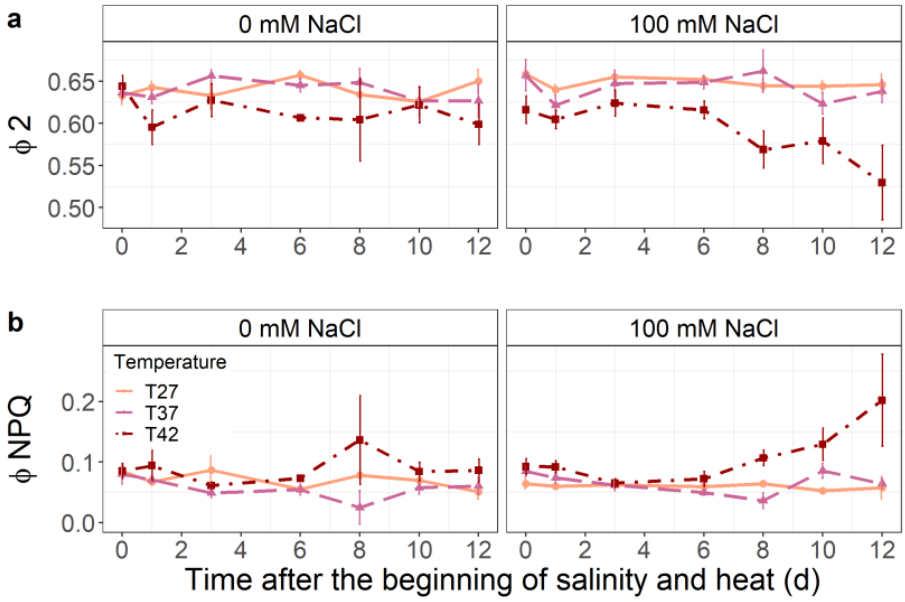


Figure 5.8: Leaf fluorescence parameters throughout the salinity and increased temperature. Sub-plots represent **(a)** quantum yield of photosystem II ($\phi 2$) and **(b)** ratio of incoming light going through non-photochemical quenching (ϕNPQ). The data are represented by the mean +/- standard error (n = 4). One measurement per plant was taken on a fully expanded leaf at the top of the canopy. Temperature treatments as T27 = 27/21°C, T37 = 37/31°C, and T42 = 42/36°C.

5.3.4 Biochemical analysis

Total carbohydrates increased under T37 and T42 at 0 mM NaCl conditions when compared to T27 plants, an observation not made at 100 mM NaCl (Fig. 5.9a). Total phenols were impacted by the increasing temperatures with a decrease as temperature increased in both saline and non-saline treatments while salinity also decreased its concentration (Fig. 5.9b). The decrease in phenol content was noted for both T37 and T42 under 100 mM NaCl but was only significant for T42 under 0 mM NaCl treatment(Fig. 5.9b). Total antioxidants were unchanged by either stress or their interaction (Fig. 5.9c).

Leaf sodium concentration increased significantly for plants grown under 100 mM NaCl (Table 5.1). Both T37 and T42 increased the leaf sodium concentration when compared to T27 while T37 and T42 concentrations were not statistically different (Table 5.1). Leaf nitrogen concentration was reduced as tempera-

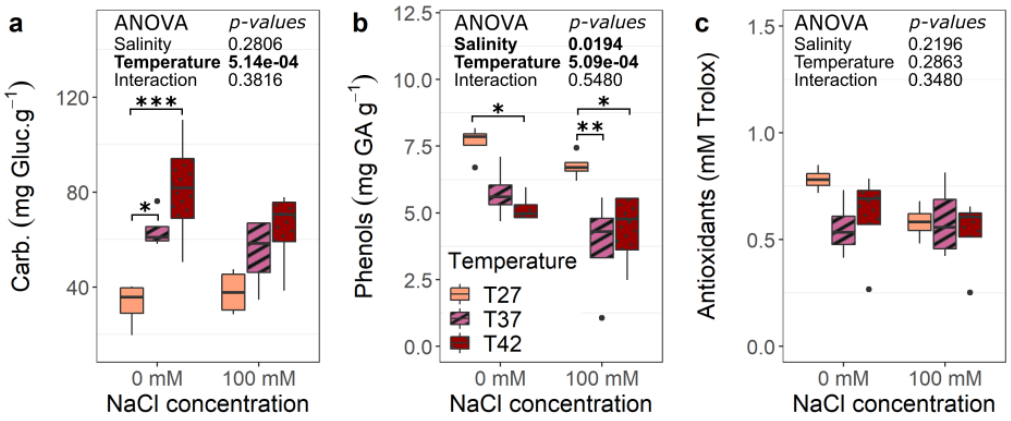


Figure 5.9: Biochemical analysis of the African eggplant dry leaves at the end of the 26 days under salinity increased temperature. Sub-plots represent **(a)** total carbohydrates, **(b)** phenols, and **(c)** total antioxidants activity. The dataset is represented by the lower and upper whiskers, 25% and 75% quartiles, and median (n = 4). Stars denote the significance level based on the Tukey test at 95% confidence level with * for p <.05, ** p <.01, and *** p <.001. Non-significant interactions are not shown for clarity. Carbs = Carbohydrates, Gluc = Glucose, GA = Gallic Acid. Temperature treatments as T27 = 27/21°C, T37 = 37/31°C, and T42 = 42/36°C.

ture increased and when under salinity (Table 5.1). Leaf phosphorus concentration was unaffected by the salinity treatment but was increased by T37 and T42 when compared to T27 (Table 5.1). Leaf potassium concentration was reduced by salinity at both T27 and T37 but not at T42 (Table 5.1). Under 100 mM, T42 plants displayed the highest leaf potassium concentration while, under 0 mM NaCl, leaf potassium concentration was the highest in the T37 treatment (Table 5.1). Leaf calcium concentration was only affected by T42 with a significant increase when compared to T27 but was not significantly changed by salinity (Table 5.1). The treatments and their interaction did not affect leaf magnesium concentration (Table 5.1).

Leaf manganese concentration was increased by T37 and T42 under both salinity concentrations (Table 5.1). Leaf copper concentration was increased by salinity and by T37 significantly but not by T42 when compared to T27 (Table 5.1). T37 was also the only treatment to increase leaf zinc concentration while T42 and salinity did not impact it significantly. (Table 5.1) Under 0 mM NaCl, leaf iron concentration was initially increased by T37 from T27 but decreased

significantly at T42 (Table 5.1). The initial increase was not observed under 100 mM NaCl but the decrease at T42 was also present (Table 5.1). Salinity also decreased leaf iron concentration for T37 plants (Table 5.1).

Table 5.1: Analysis of variance and mean comparisons for dry leaf nutrient concentrations of the African eggplants grown under different salinity and temperature levels (n = 4). Temperature treatments as T27 = 27/21°C, T37 = 37/31°C, and T42 = 42/36°C.

Source of variance	Na	N	P	K	Ca	Mg	Mn	Cu	Zn	Fe
	g kg^{-1}						mg kg^{-1}			
Salinity (S)	***	**	ns	***	ns	ns	ns	**	ns	***
Temperature (T)	***	***	***	ns	**	ns	***	**	**	***
S x T	ns	ns	ns	*	ns	ns	**	ns	ns	*
Salinity										
0 mM NaCl	0.27^{b}	60.20^{a}	7.55	43.70	25.77	6.43	110.1	2.26^{b}	25.45	96.52
100 mM NaCl	12.27^{a}	55.08^{b}	7.43	31.70	29.40	7.17	108.3	3.01^{a}	30.02	82.35
Temperature										
T27	2.45^{b}	63.14^{a}	6.67^{b}	36.14	23.20^{b}	7.00	68.08	2.04^{b}	22.48^{b}	98.47
T37	7.31^{a}	57.72^{b}	8.03^{a}	39.42	25.24^{ab}	6.54	123.1	3.18^{a}	34.20^{a}	103.9
T42	9.04^{a}	51.81^{c}	7.78^{a}	37.55	34.31^{a}	6.87	136.4	2.69^{ab}	26.53^{b}	65.91
S x T										
0 mM + T27	0.045	66.94	6.44	41.75^{ab}	18.89	6.01	58.24^{b}	1.63	20.75	101.7^{ab}
0 mM + T37	0.23	60.80	8.26	48.39^{a}	25.27	6.68	123.2^{a}	2.76	31.35	117.9^{a}
0 mM + T42	0.55	54.84	7.94	40.94^{bc}	33.14	6.60	148.8^{a}	2.40	24.26	69.91^{c}
100 mM + T27	4.86	60.28	6.89	30.52^{d}	27.51	7.98	77.92^{b}	2.45	24.22	95.24^{b}
100 mM + T37	14.40	56.18	7.79	30.44^{d}	25.22	6.40	122.9^{a}	3.61	37.05	89.91^{b}
100 mM + T42	17.54	48.78	7.62	34.16^{cd}	35.48	7.13	124.1^{a}	2.99	28.79	61.90^{c}

*Na = Sodium, N = Nitrogen, P = Phosphorus, K = Potassium, Ca = Calcium, Mg = Magnesium, Mn = Manganese, Cu = Copper, Zn = Zinc, Fe = Iron. NS, *, **, *** Non-significant or significant at P ≤ 0.05, 0.01, 0.001, respectively. Means not sharing any letters within each column are statistically different according to Tukey's test (P = 0.05).*

5.4 Discussion

The harmful or beneficial effects of salinity and heat have been reported for a range of crops but their combination is still only sparsely studied (Suzuki et al., 2014). In this study, while some plant parameters were affected by the individual stresses and not further impacted by the stress combination, such as chlorophyll and shoot dry weight, other traits responded differently to the combination of

heat and salinity, in particular leaf fluorescence parameters.

Despite a slight decline in leaf expansion rate, salinity did not impact the final leaf production in the current study, which contrasts with what was previously observed in other *Solanum* species (Alsafari et al., 2019; Efimova et al., 2018; Ortega-Albero et al., 2023). Bacha et al. (2017) observed a decrease in tomato (*S. lycopersicum* L.) leaf area after 14 days of stress while no differences were noted after 7 days, highlighting the role of salinity build-up and tolerance variation through time which could explain the current results. In the current study, both high temperatures reduced leaf dry weight but leaf expansion rate and leaf number were only reduced at 42°C, suggesting another explanation necessary for the reduction observed at 37°C. A similar pattern was noted in a tomato cultivar with no reduction in leaf area and leaf number but a reduction in final shoot dry weight under heat (Zhou, Yu, et al., 2017). The production of thinner leaves at high temperatures to reduce leaf temperature, despite its impact on plant water status and membrane stability, might explain the reduction in leaf dry weight without impacting leaf number (Schiattone et al., 2017).

Salinity decreased membrane stability in the current study as in Ben Abdallah et al. (2016) study on *S. nigrum* L.. Shunkao et al. (2022) reported a further decrease in membrane stability in wheat (*Triticum aestivum* L.) when the crop was exposed to both heat and salinity, similar to what was observed in this study at T42. However, T37 had a mitigating effect with a non-significant decrease in membrane stability which might be due to a level of protection offered by a moderate increase in temperature. Despite studies having previously shown that heat can have a protective effect against salinity on photosynthesis or sodium transport rate, similar observations in membrane stability have yet to be made (Lopez-Delacalle et al., 2021; Rivero et al., 2014). The protection effect observed in the current study might be linked to the increased leaf zinc concentration under moderate heat, a mineral element previously linked to improving

membrane stability under stress in rice and other crops (Hassan et al., 2020; Tufail et al., 2018). In accordance with the protective effects of temperature on membrane stability only appearing at T37, the increased leaf zinc concentration was not noted at T42, at which point membrane stability was reduced. The absence of an impact of temperature alone on membrane stability was surprising in the current study as the negative impact of T42 on other measured characteristics was significant. Electrolyte leakage is positively correlated to the reactive oxygen species (ROS) accumulation and lipid peroxidation, not measured in the current study, and its maintenance is generally a sign of tolerance (Demidchik et al., 2014). The negative effects of T42 observed in the current study on parameters depending on membrane stability such as chlorophylls are, however, a sign of its irrelevance regarding the overall tolerance of the African eggplant. These negative impacts might be due to other damaging processes under heat such as enzyme inhibition or transcriptional changes (Zhao et al., 2020). It has to be noted, however, that midday stomatal conductance was maintained, and even increased, under the T42 treatment. As F1 Djamba is marketed as a particularly high-yielding cultivar, mechanisms might be in place to maintain gas exchange and carbon assimilation even under stress, thus requiring sustained membrane stability. The maintenance of membrane stability might thus have been important for characteristics not measured in this study and thus require further research.

Midday stomatal conductance was increased by the T42 treatment seven days after the beginning of the stress but not by the T37 treatment, suggesting different mechanisms in place to cope with high temperatures depending on their intensities. The increase in stomatal conductance, despite increasing water loss, helps regulate leaf temperature to maintain the activity of photosynthetic enzymes (Poudyal et al., 2018). This increase was noted in different tomato cultivars at lower temperatures, highlighting the high tolerance threshold of the African

eggplant (Amuji et al., 2020; Poudyal et al., 2018). The increased stomatal conductance promoted by high temperatures was, however, reduced under salinity. This was previously observed in tomato plants where the lowest stomatal conductance was reached under the stress combination due to the osmotic stress created by the salinity stress, leading to mechanisms in place to limit water loss such as stomatal closure (García-Martí et al., 2019).

Plants grown under higher temperatures kept their stomata closed for longer at the start of the day in the current study. Similarly, the largest difference between drought-stressed and non-stressed *Carapa guianensis* Aubl. was observed early in the day (Carvalho et al., 2013). Stomatal conductance of sorghum (*Sorghum bicolor* L.) was reduced early as well but did not return to control levels during the day, unlike the observations made in the current study (Sonobe et al., 2009). The current observations may be due to adaptive mechanisms under which the plants only fully open their stomata after reaching an increase in light intensity to balance water loss with carbon gain. However, it has to be noted that the temperature started to reduce eight hours after the start of the photoperiod as per the gradual increase and decrease temperature set-up. While still the highest temperature, T42 treatment was at 40°C at that time, reduced from 42°C which only lasted from the 2^{nd} to the 6^{th} hour after the start of the photoperiod. A reduction in temperature is linked to a reduction in vapour pressure deficit (VPD), allowing better gas exchange (Merilo et al., 2018). In the current study, the 2°C reduction from 42°C might be enough for the plants to recover stomatal conductance. Even if the VPD of the other treatments also decreased at this time, the change might not have improved stomatal conductance as the VPD was already within optimal levels, leading to T42 plants recovering to the levels of the other treatments.

In addition to stomatal conductance changes, the stresses in the current study impacted the photosynthetic apparatus through chlorophylls and leaf fluores-

cence modifications. While SPAD and final chlorophylls and carotenoids were reduced significantly by T42, leaf fluorescence parameters were not drastically reduced by this temperature. This observation might be due to the relationship between chlorophyll content per area and leaf thickness (Jinwen et al., 2009). Leaf thickness is positively correlated with SPAD index but negatively correlated with $\phi 2$ due to loss of transmittance at high leaf thickness which limits the optimal utilisation of each chlorophyll molecule (Jinwen et al., 2009; Knapp & Carter, 1998). In the current study, plants with a lower SPAD (under the T42 treatment) may have had a higher light use efficiency, leading to the mitigated impact of the stresses on $\phi 2$ and ϕNPQ despite the noticeable loss of chlorophyll molecules.

In terms of biochemical changes, the increase in total carbohydrates under heat was also observed by Zhou, Yu, et al. (2017) in tomato. In contrast, no changes in glucose and fructose were noted under heat by Botella et al. (2021) in tomato but salinity increased these compounds significantly, while the stress combination further increased them. The increase noted by other studies was attributed to the enhanced enzyme activity within the sugar biosynthesis pathways, the insufficient sink activity limiting the export of the produced sugars from leaves, and the role of sugars as osmoprotectants (Botella et al., 2021; Zhou, Yu, et al., 2017). In the current study, heat might have activated one or a combination of the stated accumulating mechanisms to withstand stress but not salinity. Salinity also did not trigger a non-enzymatic antioxidative response with the absence of changes in antioxidants and a reduction in total phenols. Phenols and antioxidants are important to detoxify ROS created under stress and are sometimes a key part of the tolerance mechanisms (Das & Roychoudhury, 2014). The observations made in the current study were in line with the results of Martínez et al. (2020) who reported no changes in antioxidants in tomato and *S. chilense* L. under salinity. Phenols were increased by salinity in tomato (Bacha et al.,

2017) but not in brinjal eggplant (*S. melongena* L.) and only at 50 mM NaCl in *S. nigrum* (Ben Abdallah et al., 2016; Ortega-Albero et al., 2023), showing the importance of the cultivar of interest and the level of stress.

Sodium accumulation under salinity, a quick and common observation, has major negative impacts on plant development (Sousa et al., 2022). In the current study, leaf sodium concentration was highly increased under salinity, an increase further exacerbated by heat as seen previously in tomato plants (Sousa et al., 2022). The antagonist effect of plant's sodium concentration on calcium uptake was not seen in the current study despite previous reports of calcium reduction in *Solanum* under salinity (Ben Abdallah et al., 2016; Ben-Abdallah et al., 2019; Sousa et al., 2022). The decrease in leaf calcium concentration in *S. villosum* Mill. reported by Ben-Abdallah et al. (2019) was, however, only seen under 150 mM NaCl and not at 50 or 100 mM NaCl, suggesting a threshold under which its uptake is not hindered in leaves. Tomato leaf potassium and phosphorus concentrations were reduced under heat in the study by Ali et al. (2021), which was not observed in the current study with leaf phosphorus and potassium concentrations increased and unchanged, respectively. This corroborates findings in the brinjal eggplant and potato (*S. tuberosum* L.) (Efimova et al., 2018; Ortega-Albero et al., 2023). The maintenance of these primary nutrients under stress is important for enzyme activity, cell membrane stability, and ROS detoxification among others and the variability of responses under stress highlights the difference in mechanisms triggered for each stress (Kumari et al., 2022). Leaf nitrogen concentration, another primary nutrient, was reduced under salinity only at T27 and T42 but not at T37. Nitrogen has key roles in various tolerance plant mechanisms and moderate temperature stress might improve its uptake or transport to enhance tolerance through improved membrane stability or photosynthetic activity (Kumari et al., 2022).

Despite the role of magnesium in enzyme activity maintenance, the abiotic

stresses did not affect leaf magnesium concentration in this study as was reported previously, suggesting its non-primary role in *Solanum* stress response (Ben-Abdallah et al., 2019). Leaf zinc and copper concentrations were only increased at T37 when compared to T27, while T42 concentrations were similar to the T27 ones. The increase seen at T37 might be due to the increased nutrient uptake of selected important nutrients for tolerance observed under a certain threshold in different species due to the maintained ATPase activity and increased water uptake to compensate for the higher transpiration (Dias & Lidon, 2009; Klock et al., 1996). Interestingly, the temperature experienced by the roots was not significantly different between the T37 and T42 treatments, both much higher than the T27 treatment (data not shown). The reduction in leaf zinc and copper at T42 when compared to T37 was thus not due to an increase in heat damage on the roots which commonly reduces nutrient uptake (Bravo-F & Uribe, 1981; Mishra et al., 2023). This observation might instead be due to a translocation mechanism not impacted at 37°C but hindered at 42°C. Indeed, the translocation of zinc and copper from roots to shoot and the different plant organs relies on a range of proteins which might be denatured under high temperatures (Gupta et al., 2016; Mishra et al., 2023). The promotion of nutrient uptake alongside denatured proteins might thus have led to the same leaf zinc concentration observed between T27 and T42. It has to be noted that copper, zinc, and manganese play an important role in the activity of the antioxidant enzyme superoxide dismutase (Tyagi et al., 2019). The increase observed at T37 might be part of some tolerance mechanisms increasing the concentrations of important nutrients to detoxify ROS created during the stress with the increase in non-photochemical quenching and other damage. Further investigation is required to better understand the role of zinc, copper and manganese in the African eggplant under heat stress and their potential impact on antioxidant enzymes.

5.5 Conclusion

The current study aimed to describe the effects of the stress combination on an African eggplant Kumba cultivar and showed that despite some unique responses under the combination of heat and salinity, several plant attributes followed the same trend as the individual stresses. Elevated temperatures significantly impacted leaf weight, polyphenols, and chlorophyll concentrations, salinity mostly had an effect on electrolyte leakage, stomatal conductance, and sodium uptake, while the stress combination impacted $\phi 2$, ϕNPQ, and potassium. The level of heat stress was important in dictating whether the stress combination had a stronger negative effect than the individual stresses, highlighting the importance of stress intensity when multiple stresses are present. Moderate heat offered some protection against salinity stress on the African eggplant regarding cell membrane stability, leaf fluorescence, and zinc and potassium concentrations, highlighting some positive effects of stress combination under certain conditions. The African eggplant did not rely on non-enzymatic antioxidant mechanisms to maintain photosynthetic activity, leaf expansion or chlorophylls, suggesting other mechanisms triggered under stress to maintain these processes.

Chapter 6

The African eggplant DB3 maintained fruit quality under salinity but not plant growth

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6.1 Introduction

Salinity is a major environmental stress affecting more than 424 million hectares of topsoil globally according to the FAO, an area that is steadily increasing, especially in irrigated land (FAO, 2021). Soil salinity results from a combination of human and natural processes such as seawater inundation, atmospheric deposition, water evaporation, and inadequate irrigation and drainage systems (FAO & ITPS, 2015). Climate change exacerbates this issue through rising sea levels, more persistent drought, and irregular rainfall (Corwin, 2021). Furthermore, most of our global food supply depends on a limited variety of crops, many of which are highly sensitive to salinity (known as glycophytes, such as *S. lycopersicum* or *S. tuberosum*) as they have been domesticated on arable land (Zörb et al., 2019).

In glycophyte crops, high salt concentrations lead to the onset of osmotic stress by limiting water uptake and impact the plant's ion homeostasis due to the accumulation and antagonist effects of sodium (Altaf et al., 2022). The salt-induced osmotic stress hampers nutrient uptake and damages a range of cellular processes, ultimately restricting growth and marketable yield (Ahmad et al., 2023). The ionic stress adds to these damages and further limits plant physiology by disrupting enzyme activity (Altaf et al., 2022).

Worldwide, various solutions are being employed to combat salinity, including the installation of drainage systems, to prevent salt build-up, and the use of organic amendment and land reclamation to remove sodium ions from the soil (Department for International Development, 2004; Wang et al., 2014). Another strategy is to rely on salt-tolerant crops, known as halophytes, to increase field resilience and crop diversity. While certain under-researched crops show promise for thriving in saline soil, it is essential to quantify their tolerance and identify potential cultivars of interest. Some wild *Solanum* species, for exam-

ple, have shown high tolerance to salinity, suggesting a genetic basis of salinity tolerance in this genus (Pailles et al., 2020). The African eggplant, *Solanum aethiopicum* L., is highly popular in sub-Saharan Africa due to its high nutritional value and yield (Moenga & Achieng Odeny, 2022). Due to its high shade tolerance and quick fruit production, coconut farmers are showing a growing interest in this crop as an extra income through intercropping. It is little researched regarding its salinity tolerance and responses to stress, however, limiting its wider use in salt-affected soils, common near the seafront. Studies by de Fatima et al. (2023) and Mensah et al. (2020) highlighted the sensitivity of the cultivars tested to salinity but also noted differences in their response pathways. Both studies showed different salinity tolerance thresholds and further research is thus needed to understand whether these results are similar between other cultivars.

The objective of the present study was to compare the responses of the African eggplant DB3 to four concentrations of soil salinity. Cultivar DB3 was selected for this experiment due to its popularity amongst farmers and its importance in coastal areas as an intercrop of coconut, where salinity can be an issue. In addition, as cultivar DB3 is part of the Gilo group of the African eggplant, it is only grown for its fruits so a long-term experiment including yield data is highly relevant. Plant morphology, physiology, and yield were assessed alongside the fruit and leaf nutrient concentrations. It was hypothesised that salinity would reduce photosynthetic parameters and yield once a tolerance threshold is reached, and nutrient uptake is expected to be reduced above the same threshold.

6.2 Materials and Methods

6.2.1 Plant material and growth conditions

The responses of the African eggplant Gilo (*Solanum aethiopicum* L. cv., DB3) to four soil salinity concentrations were assessed in a pot experiment. Seeds were generously provided by The World Vegetable Center Eastern and Southern Africa (Arusha, Tanzania). Seeds were disinfected by immersion in 10% (v/v) trisodium phosphate decahydrate solution for three hours and rinsed with distilled water. Seeds were then placed for one hour in a solution of water and commercial bleach (70:30 v/v), rinsed again, and left to dry for 10 days at room temperature. Germination pots (7 cm x 7 cm) were filled with John Innes No. 2 compost (7 loam:3 peat:2 sand v/v/v) (J Arthur Bower's, Westland®, Dungannon, Northern Ireland) and one seed was placed in the centre of each pot. Seedlings were grown at 27/21°C and 70% relative humidity over a 12 h photoperiod in an MLR-352 growth cabinet (PHC Holdings Corporation®, Tokyo, Japan).

6.2.2 Treatment application

Saline water was prepared to salinise the soil before transplanting based on the ASTM Synthetic seawater standard (ASTM D1141-98) by diluting in distilled water (g L^{-1}): NaCl 24.53, $MgCl_2$ 5.20, Na_2SO_4 4.09, $CaCl_2$ 1.16, KCl 0.695, $NaHCO_3$ 0.201, KBr 0.101, and H_3BO_3 0.027. The saline water was then applied to John Innes No.2 soil at the following rate:

- No salinity (NS): 0 L of saline water for 70 L of soil
- Low salinity (LS): 3 L of saline water for 70 L of soil

- Medium salinity (MS): 9 L of saline water for 70 L of soil
- High salinity (HS): 18 L of saline water for 70 L of soil

The soil was left to air-dry for five days. Seedlings of equal size were transplanted into 10 L pots filled with saline soil nine weeks after sowing (one seedling per pot, 28 in total). They were placed in a glasshouse heated at 27°C when needed between 7 am and 7 pm and at 21°C between 7 pm and 7 am. Additional lighting was used between 7 am and 7 pm and a cooling down system with fans was relied upon when temperatures inside the glasshouse reached 31°C (detailed environmental data in Appendix B, Fig. B.5). A randomised complete block design with seven replicates for each of the four salinity concentrations was used. This represented the first day of stress (1 DASS, days after salinity stress) and the final harvest of plants took place at 91 DASS.

Automatic irrigation was set up using fertigation with Krystalon Brown (NPK 3-11-38, YaraTera™, Yara, Oslo, Norway) following the manufacturer's guidelines (1 kg of fertiliser dissolved in 10 L of water). Soil moisture was recorded throughout the experiment with SM150 theta probe soil moisture sensors connected to a GP2 Delta-T data logger (Delta-T Devices®, Burwell, UK). Sensors were placed in three pots of each salinity treatment (12 pots in total) across the glasshouse. Natural predators were applied throughout the experiment (Anderline, Starskii, Chrysoline C, Bioline AgroSciences®, Little Clacton, UK). Air temperature and relative humidity within the glasshouse were recorded by a weather station (RHT2nl, Delta-T devices®) while light intensity was measured by an SQ-110 quantum sensor (Apogee Instruments Inc., Logan, UT, United States), both connected to a GP2 Delta-T data logger.

6.2.3 Growth and physiological measurements

Chlorophyll fluorescence and gas exchange

The SPAD index was measured from 7 DASS at midday three times a week with a CCM-200 chlorophyll content meter (Opti-Sciences®, Hudson, NH, United States) on three leaves per plant. The SPAD meter measures the transmittance of red and infrared through the leaf and calculates a SPAD value corresponding to the amount of leaf chlorophyll (Uddling et al., 2007). After the start of fruiting (around 45 DASS), the CO_2 uptake and stomatal conductance were recorded on one fully expanded leaf at the top of the canopy using the LI-6800 portable photosynthesis system (LiCor Biosciences®, Lincoln, NE, United States) three times a week. Measures were only taken from 45 DASS due to the equipment availability.

Flowering and fruiting

When plants started flowering, manual observations of the total number of flowers were made three times a week to record the total number of flowers alive and dead each day. To measure the impact of salinity on flower abortion, the percentage of dead flowers over the total number of flowers was used for each day. Dead flowers were left to fall on their own, at which point they were not counted anymore. Fruits were labelled as they developed. When they reached the marketable stage based on their colour, their weight, height, and maximum width were recorded. Fruits were then sliced in half, placed in liquid nitrogen quickly, and stored at -80°C. Before nutrient analysis, fruits were freeze-dried (Alpha 1-4 LSCplus, Martin Christ, Osterode am Harz, Germany).

Destructive harvest

On the last day of the experiment (91 DASS), around 100 g of fresh leaves (a representative mix of developed and developing) were removed from the plant, weighed, placed in liquid nitrogen, and freeze-dried (Alpha 1-4 LSCplus) for nutrient analysis. The rest of the leaves and stems were weighed separately on a two-digit electronic balance, after measuring stem diameter and plant height, and oven-dried at 80°C until reaching constant weight.

Leaf electrolyte leakage

A leaf disc (10 mm diameter) was taken from five fully expanded leaves at 28 and 91 DASS to measure leaf electrolyte leakage as described by Whitlow et al. (1992). The discs were placed in 20 mL distilled water and incubated at room temperature on a shaker (200 rpm) for 20 h after being washed three times with distilled water. The electrical conductivity of the bathing solution (EC1) was recorded using a LAQUAtwin EC-33 meter (Horiba®, Kyoto, Japan) and a second reading (EC2) was taken after placing the discs in a water bath at 95°C for 60 mins and cooling them down. The electrolyte leakage (EL), expressed as a percentage, was calculated as:

$$EL = EC1/EC2 * 100\ (\%) \tag{6.1}$$

Pore water conductivity and soil electrical conductivity

Soil water content and pore water conductivity at the top, middle, and bottom of each pot were monitored regularly using an HH2 moisture meter attached to a WET sensor (Delta-T devices®). One soil sample was taken from each pot at

28 and 91 DASS and air-dried for 48 h. The dried soil was then sieved (2 mm) and 5 g was added to 25 mL of distilled water. The samples were placed on a shaker for 24 h and the electrical conductivity of the settled solution (EC 1:5) was measured using LAQUAtwin EC-33 meter (Horiba®) as detailed by He et al. (2012).

6.2.4 Leaf and fruit nutrient concentrations

The dried and milled leaf and fruit samples (300 mg) sampled on the last day of the experiment were digested in 6 mL of 70% (v/v) nitric acid and placed in a digestion microwave (Multiwave PRO, Anton Paar, Graz, Austria) reaching 175°C over a 20 mins period and maintained for a further 20 mins followed by a 10 mins cool down at 55°C. These digested samples were diluted with 18.2 milliQ water to reach 2% nitric acid concentration for multi-element analysis by ICP-MS (Inductively Couple Plasma Mass Spectrophotometry, Thermo-Fisher Scientific iCAP-Q; Thermo Fisher Scientific, Germany). Calibration standards included a multi-element solution (Claritas-PPT grade CLMS-2 from SPEX Certiprep Inc., Metuchen, NJ, United States) and a bespoke external multi-element calibration solution (PlasmaCAL, SCP Science, Quebec, Canada).

6.2.5 Statistical analysis

All statistical studies were performed using R version 4.1.2 and the models used are described in Appendix C. The normality of residuals and homogeneity of variance were checked using both visual and statistical tests. Shapiro-Wilk test was used to test for normality alongside density plots and Q-Q plots. Levene's test and visual analysis of the residuals were used to confirm variance homogeneity. A one-way analysis of variance (ANOVA) test with Tukey's honest

significance test post hoc was applied at a 0.05 confidence level on data meeting both the normality and homogeneity assumptions. The logarithmic values of electrolyte leakage and leaf and fruit sodium concentrations were used for analysis to improve the residual's normality and homogeneity due to the extreme treatment effect observed on the original dataset.

Count data was analysed using the Poisson distribution and checked for overdispersion. When analysing the data for the number of dead flowers, the number of total flowers was used as an offset to be able to compare the abortion rate rather than the absolute number of dead flowers.

Finally, time series were analysed using a linear mixed-effects model (`lmer`) to include time as a factor and the random effect of the repetitive measures on each plant. The interaction between the salinity treatment and time was included.

6.3 Results

6.3.1 Substrate electrical conductivity

The pore water conductivity of plants under salinity was higher than the controls throughout the treatment (Fig. 6.1a). Both the medium (MS) and high (HS) salinity treatments had a very high pore water conductivity at the beginning of the treatment before decreasing from 10 DASS and stabilising for the rest of the experiment. The MS treatment reached the same level as LS at four time points between 35 and 70 DASS while the HS treatment was also reduced to the MS level between 40 and 50 DASS before slightly increasing again. The soil electrical conductivity 1:5 was significantly increased for the MS and HS treatment at both 28 and 91 DASS when compared to the no salinity (NS) control (Fig. 6.1b).

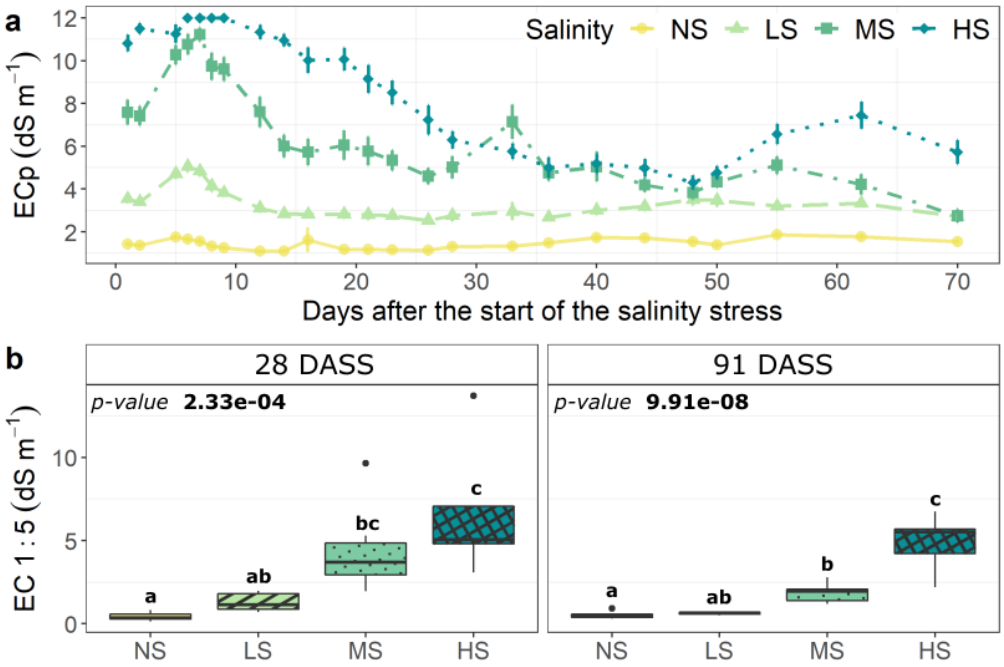


Figure 6.1: Soil salinity levels throughout the experiment as measured by **(a)** pore water conductivity (ECp) and **(b)** substrate electrical conductivity (EC) 1:5 of the soil at 28 and 91 days after the start of the salinity stress (DASS). ECp is represented by the mean +/- standard error. The substrate EC 1:5 is represented by the lower and upper whiskers, 25% and 75% quartiles, and median (n = 7). Black dots represent outliers. Boxplots not sharing any letters are significantly different based on the Tukey test at 0.05% level of significance. NS = No Salinity, LS = Low Salinity, MS = Medium Salinity, HS = High Salinity. Three ECp measurements per pot were taken daily while one soil sample was extracted at each time-point selected to measure EC 1:5.

6.3.2 Plant growth

Salinity affected plant growth throughout the stress (Fig. 6.2). Salinity decreased significantly leaf dry weight only at the two highest concentrations (Fig. 6.3a). Stem dry weight, on the other hand, followed a linear decrease as the salinity concentration increased, significantly from the lowest concentration (Fig. 6.3b). Only the HS concentration reduced significantly final plant height and both MS and HS concentrations led to a smaller stem diameter when compared with the NS concentration (Fig. 6.3c,d).

Leaf electrolyte leakage was only significantly increased under the MS and HS treatments at both 28 and 91 DASS (Fig. 6.4). The electrolyte leakage of plants

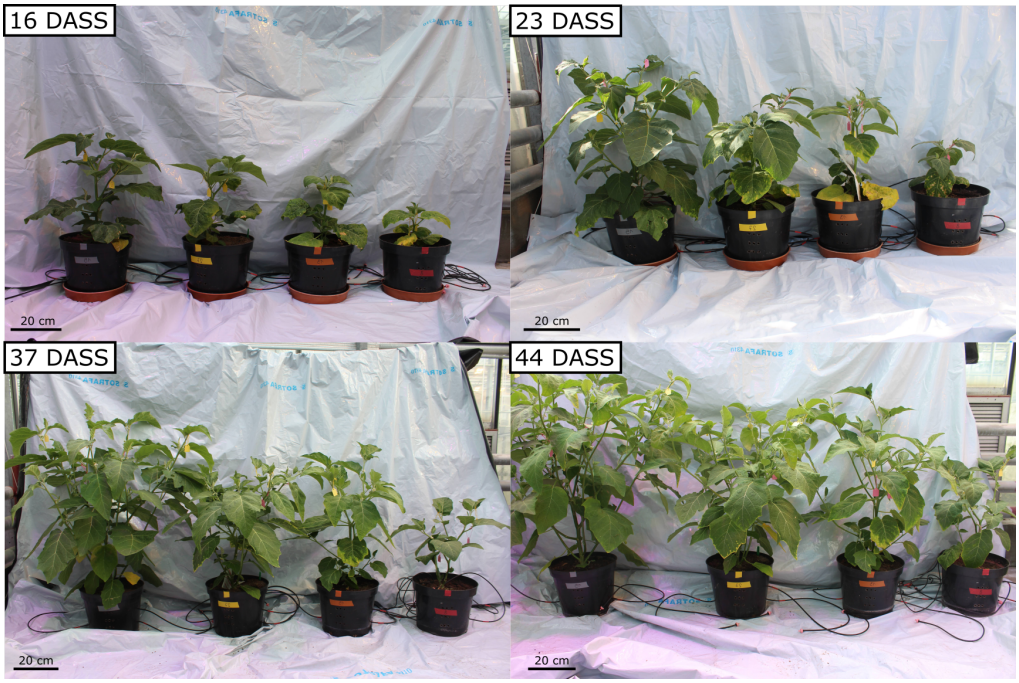


Figure 6.2: Plants at 16, 23, 37, and 44 days after the start of salinity stress (DASS). For each picture, from left to right no salinity (NS), low salinity (LS), medium salinity (MS), and high salinity (HS) treatments.

at NS, LS, and MS remained unchanged between 28 and 91 DASS (Fig. 6.4). For the HS treatment, leaf EL was significantly reduced by 31% between 28 and 91 DASS (Fig. 6.4).

6.3.3 Photosynthesis dynamics

An initial decrease in SPAD was observed in all treatments from 9 to 16 DASS, followed by an increase until 28 DASS (Fig. 6.5). Between 14 and 16 DASS, the HS treatment reduced SPAD significantly when compared to NS (Fig. 6.5). After 28 DASS, SPAD decreased in the NS and LS quicker than the decrease observed for the MS and HS treatments (Fig. 6.5). From 48 DASS until the end of the experiment, MS and HS plants had a significantly higher SPAD index than the control (Fig. 6.5). On the last measurement day, the MS and HS SPAD indexes were significantly higher than the index of the LS plants too.

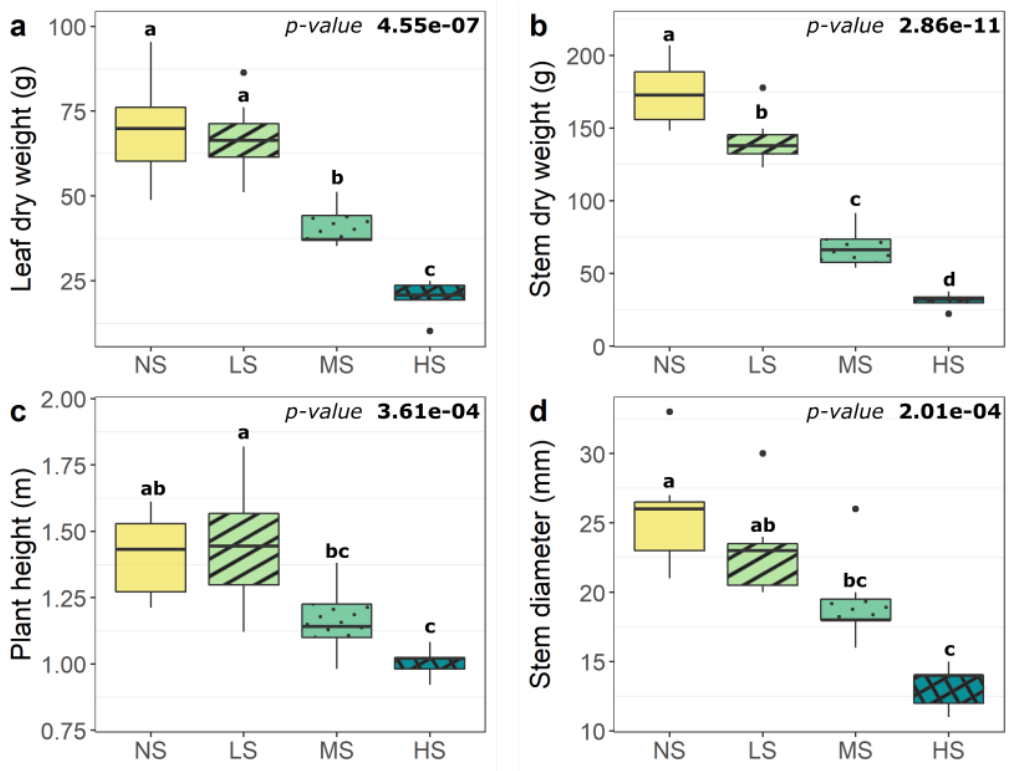


Figure 6.3: Morphological characteristics of the African eggplant at the end of the salinity stress. Sub-plots represent **(a)** leaf dry weight, **(b)** stem dry weight, **(c)** plant height, and **(d)** stem diameter. The dataset is represented by the lower and upper whiskers, 25% and 75% quartiles, and median (n = 7). Black dots represent outliers. Boxplots not sharing any letters are significantly different based on the Tukey test at 0.05% level of significance. NS = No Salinity, LS = Low Salinity, MS = Medium Salinity, HS = High Salinity.

Throughout the measurement period between 45 and 91 DASS, no significant differences of CO_2 assimilation rate were noted between NS and the other salinity treatments (Fig. 6.6a). Stomatal conductance (gs) was reduced by the LS treatment only at 50 DASS (Fig. 6.6b). The MS and HS treatments, in contrast, reduced gs from 45 DASS to 58 DASS (Fig. 6.6b). For the remaining growing period, there were no significant differences between treatments except at 70 DASS where the MS treatment significantly decreased the gs compared to NS (Fig. 6.6b).

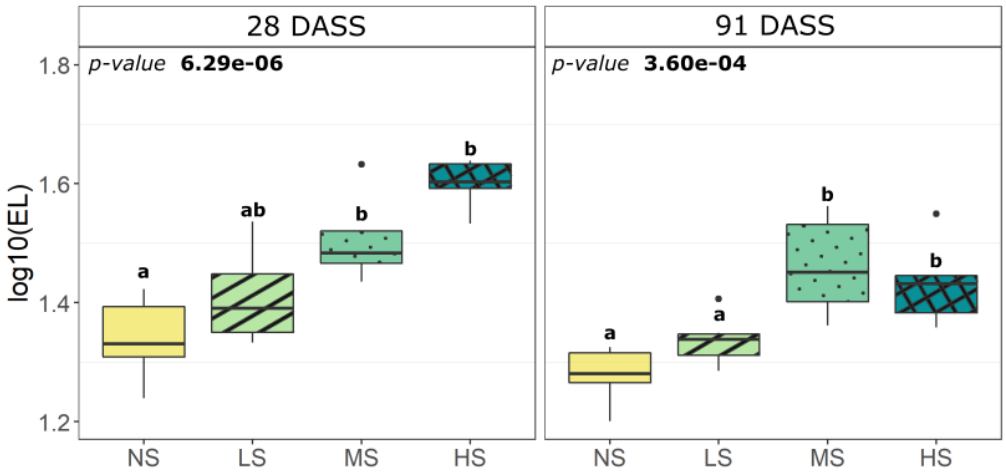


Figure 6.4: Logarithmic values of the leaf electrolyte leakage (EL) at 28 and 91 days after the beginning of salinity stress (DASS). The dataset is represented by the lower and upper whiskers, 25% and 75% quartiles, and median (n = 7). Black dots represent outliers. Boxplots not sharing any letters are significantly different based on the Tukey test at 0.05% level of significance. NS = No Salinity, LS = Low Salinity, MS = Medium Salinity, HS = High Salinity.

6.3.4 Fruit characteristics

The daily number of flowers alive was significantly reduced by the HS treatment compared to all the other salinity levels from 14 DASS until the end of the treatment (Fig. 6.7). The LS and MS levels reduced flowering from 26 DASS until the end of the treatment, with the effect being more substantial for the

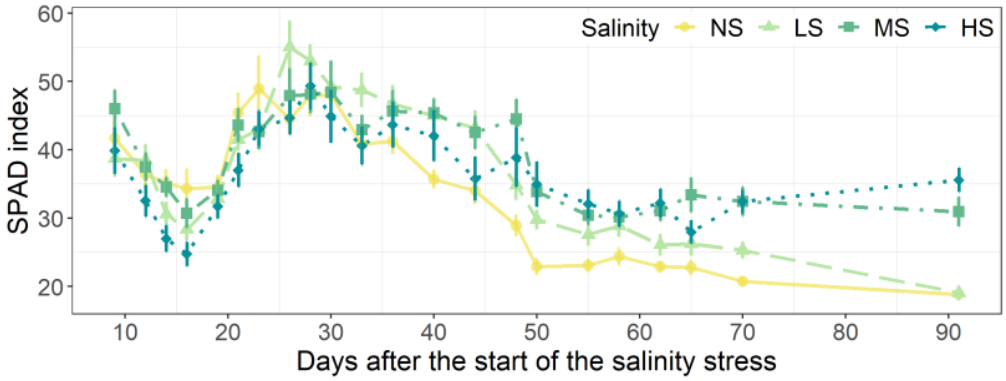


Figure 6.5: Leaf SPAD index of the African eggplant throughout the treatment period. The data are represented by the mean +/- standard error (n = 7). SPAD measurements were taken on three fully expanded leaves on each plant. Leaves at different growth stages were selected across the plants. NS = No Salinity, LS = Low Salinity, MS = Medium Salinity, HS = High Salinity.

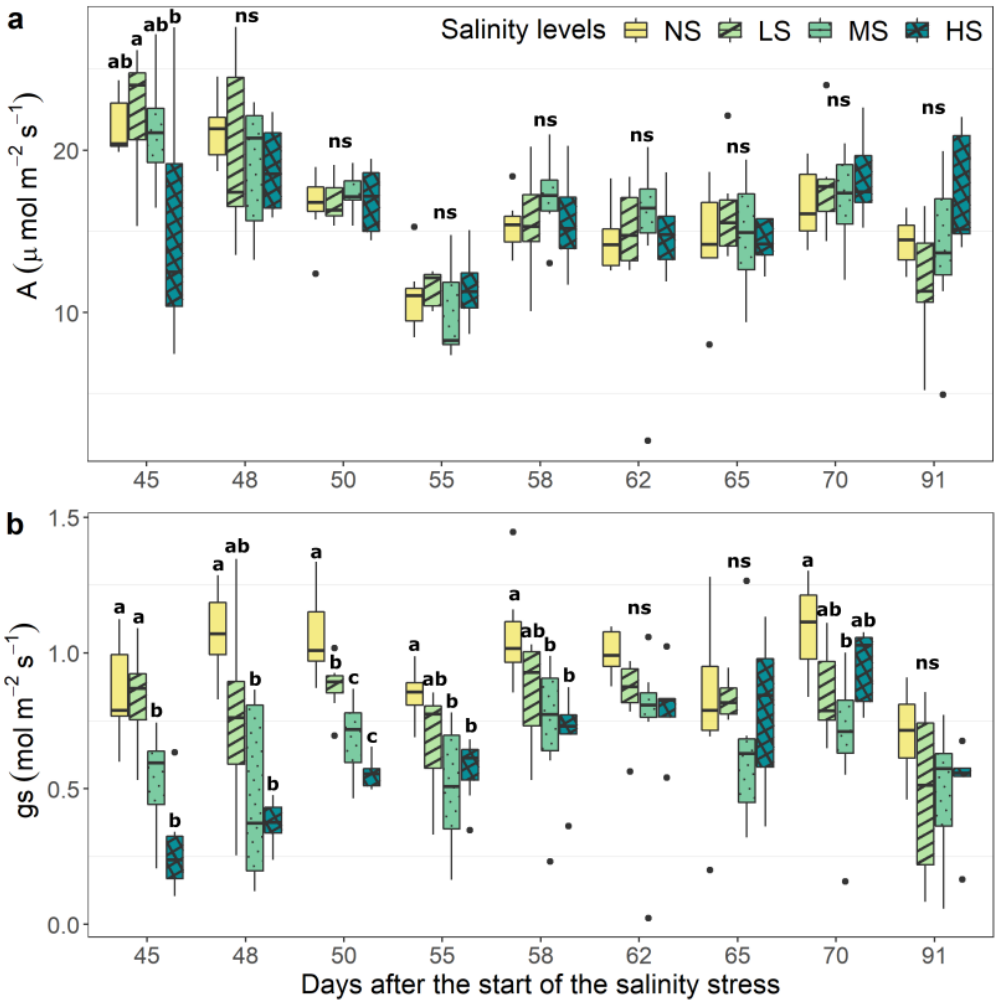


Figure 6.6: Photosynthesis parameters of the African eggplant from 45 to 91 DASS. Sub-plots represent **(a)** CO_2 assimilation rate (A) and **(b)** stomatal conductance (gs). The dataset is represented by the lower and upper whiskers, 25% and 75% quartiles, and median (n = 7). Black dots represent outliers. Boxplots not sharing any letters are significantly different at each DASS based on the Tukey test at 0.05% level of significance and ns = Non-significant. The measurements were taken on one fully expanded leaf per plant at the top of the canopy. NS = No Salinity, LS = Low Salinity, MS = Medium Salinity, HS = High Salinity.

MS treatment (Fig. 6.7). The LS treatment increased the percentage of dead flowers only at 26 DASS while the MS treatment increased it at 30, 36, and 58 DASS (Fig. 6.7). The highest salinity treatment, on the other hand, increased the percentage of dead flowers on most days (28, 30, 33, 40, and 58 DASS) (Fig. 6.7).

As only one fruit was produced by the plants under the HS treatment, only the results of the effects of NS, LS, and MS treatments on fruit characteristics were

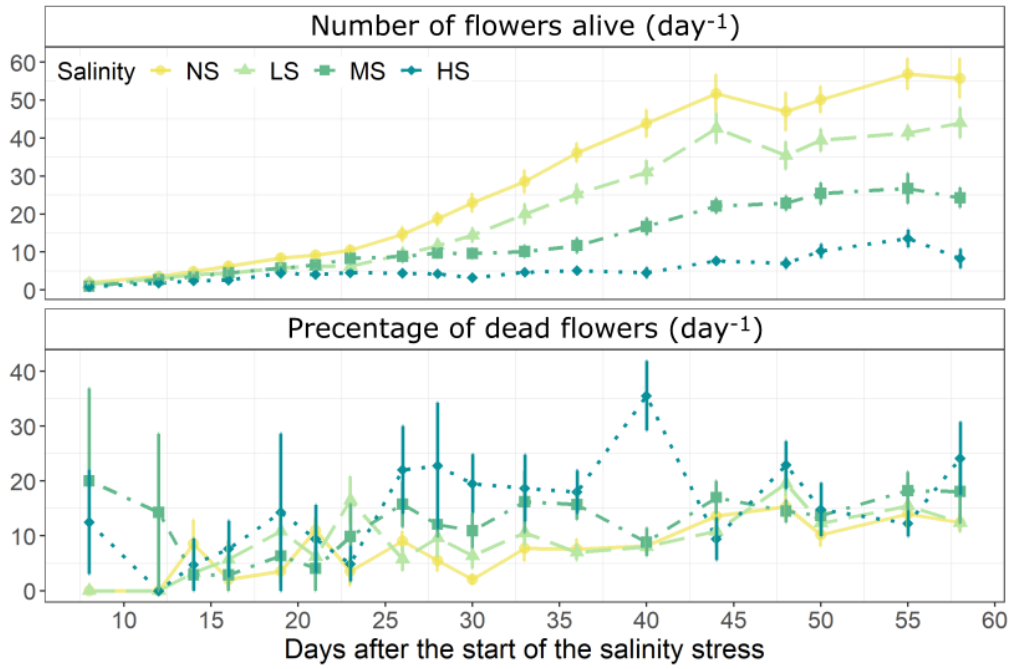


Figure 6.7: Number of flowers alive and percentage of dead flowers per day during the salinity stress period. The data are represented by the mean +/- standard error (n = 7). The number of flowers alive and dead were counted on each plant on the dates shown and the ratio of dead flowers over alive flowers was used for the percentage of dead flowers. NS = No Salinity, LS = Low Salinity, MS = Medium Salinity, HS = High Salinity.

compared. Pictures of fruits produced for each salt concentration are shown in Figure 6.8. A reduction in fresh yield per plant was significant only at the MS treatment (Fig. 6.9a). The fruits produced under the different treatments were of the same weight and dimensions, however (Fig. 6.9b–d). The salinity treatments also did not impact the number of days between fruit set and ripening to a marketable stage (Fig. 6.9e). The total number of fruits produced per plant was only lowered by the MS treatment and not by LS (Fig. 6.9f).

6.3.5 Leaf and fruit nutrient concentrations

Leaf and fruit sodium concentrations increased substantially under the MS treatment, and under HS for leaf concentration (Table 6.1). Fruit phosphorus, potassium, calcium, sulphur, magnesium, boron, and manganese concentrations were

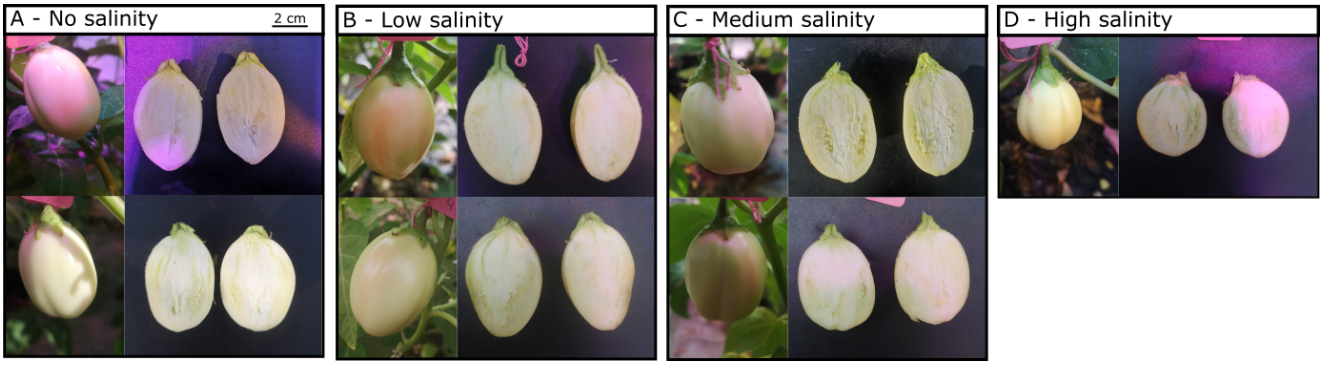


Figure 6.8: Example of fruits harvested during the experiment at each salinity level; **(A)** No salinity, **(B)** low salinity, **(C)** medium salinity, and **(D)** high salinity. The whole fruit is shown next to the open fruit for each sample.

not significantly affected by any salinity level (Table 6.1). In contrast, leaf nutrient concentrations were changed by the salinity treatment. Leaf phosphorus and potassium concentrations were increased by the highest salinity level only (Table 6.1). Leaf calcium concentration was reduced by the MS and HS treatments while the LS level increased leaf sulphur concentration only compared to MS (Table 6.1). Leaf magnesium concentration also increased in LS plants but only significantly compared to HS (Table 6.1). Leaf boron concentration decreased as the salinity levels increased with a significant difference noted at MS and HS levels and, in opposition, leaf manganese concentration was increased by the salinity treatment at every level, following a linear relationship with salinity (Table 6.1). Iron and zinc concentrations were not significantly affected by salinity in either the fruits or the leaves (Table 6.1). Leaf selenium concentration decreased significantly only for the highest salinity level compared to the control after a non-significant increase at the lowest level, leading to LS plants having the highest leaf selenium concentration (Table 6.1). Fruit selenium of LS plants was significantly higher than MS plants even though the concentration in both treatments was not statistically different from the control (Table 6.1).

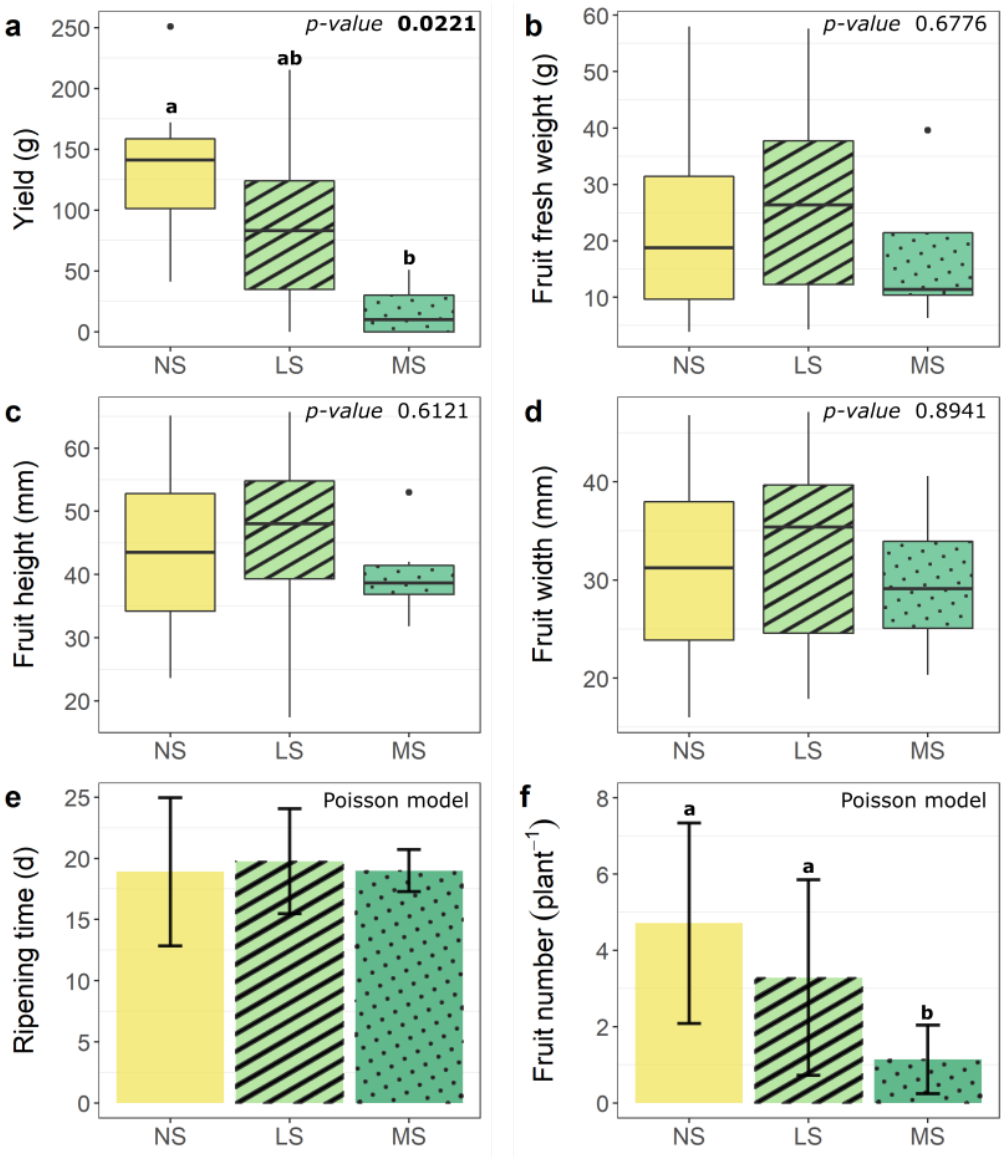


Figure 6.9: Yield and morphological characteristics of marketable fruits. Sub-plots represent fruit **(a)** yield, **(b)** fresh weight, **(c)** height, **(d)** maximum width, **(e)** days from fruit set to ripening, and **(f)** number of fruits produced per plant. The continuous dataset is represented by the lower and upper whiskers, 25% and 75% quartiles, and median (n = 7). Black dots represent outliers. Barplots show the mean value +/- standard deviation. Boxplots and barplots not sharing any letters are significantly different based on the Tukey test at 0.05% level of significance. The p-values are only shown for the data analysed with an ANOVA. NS = No Salinity, LS = Low Salinity, MS = Medium Salinity.

6.4 Discussion

Soil is considered saline above 4 dS m^{-1} and slightly saline between 2 and 4 dS m^{-1} in the field (Omuto et al., 2020). A direct comparison can not be done

Table 6.1: Analysis of variance and mean comparisons for dry leaf and fruit nutrient concentrations of African eggplants grown under different salinity levels (n = 7).

Source of variance	Na	P	K	Ca	S	Mg	B	Mn	Fe	Zn	Se
	g kg^{-1}						mg kg^{-1}				µg g^{-1}
Leaf											
Salinity (S)	***	**	***	***	*	*	***	***	ns	ns	***
Salinity											
None (NS)	0.027^{c}	3.99^{b}	22.97^{b}	25.28^{a}	4.62^{ab}	4.40^{ab}	32.83^{a}	74.98^{c}	234.6	120.0	42.46^{ab}
Low (LS)	0.028^{c}	4.26^{b}	23.10^{b}	22.44^{a}	4.81^{a}	4.98^{a}	30.50^{a}	96.94^{b}	189.7	119.4	48.57^{a}
Medium (MS)	1.14^{b}	4.86^{ab}	29.52^{ab}	15.59^{b}	3.83^{b}	4.40^{ab}	23.34^{b}	143.0^{ab}	196.4	146.6	28.23^{bc}
High (HS)	4.04^{a}	5.80^{a}	33.99^{a}	12.02^{b}	4.00^{ab}	4.01^{b}	16.70^{c}	214.1^{a}	214.1	172.1	18.46^{c}
Fruit											
Salinity (S)	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	*
Salinity											
None (NS)	0.047^{b}	3.44	25.89	2.79	1.72	1.75	12.77	16.19	38.03	20.37	12.50^{ab}
Low (LS)	0.23^{b}	3.12	25.34	2.45	1.64	1.77	12.13	22.07	38.37	16.32	14.97^{a}
Medium (MS)	2.11^{a}	3.40	24.36	2.31	1.54	1.86	11.11	19.23	39.25	14.11	5.35^{b}

*Na = Sodium, P = Phosphorus, K = Potassium, Ca = Calcium, S = Sulphur, Mg = Magnesium, B = Boron, Mn = Manganese, Fe = Iron, Zn = Zinc, Se = Selenium. NS, *, **, *** Non-significant or significant at P ≤ 0.05, 0.01, 0.001, respectively. Means not sharing any letters within each column are statistically different according to Tukey's test (P = 0.05).*

with our results as we measured the substrate EC as a soil/water 1:5 suspension rather than saturated soil paste. Nevertheless, the average pore water conductivity throughout the experiment was 1.43 dS m^{-1} for NS, 3.33 dS m^{-1} for LS, 6.42 dS m^{-1} for MS, and 8.54 dS m^{-1} for HS, showing a clear and significant increase in soil salinity. The pore water conductivity is highly correlated to soil salinity as determined traditionally and used to define the different categories of soil salinity (ECe) (Kumar et al., 2008). It is worth noting that ECp changed significantly throughout the stress, especially for the MS and HS treatment. While the stress seems to have been higher at the beginning, the salinity dropped later on, which might have impacted the results and limited a clear determination of a salinity threshold.

Salinity's negative impact on *Solanum* membrane stability has been reported by multiple groups (Ahanger et al., 2020; Ahsan et al., 2022; Ben Abdallah et al., 2016). The recovery observed here for the HS level between 28 and 91 DASS might be due to the reduction in ECp, hence reducing the stress intensity, or to an

adaptation of the plant to salinity only at the highest level. Adequate membrane stability is key for several processes, including photosynthesis activity which is often highly impacted under stress (Sharma et al., 2020). No significant differences were found between the control and the salinity treatments regarding the CO_2 uptake during the fruiting stage in the current study, despite a reduction in stomatal conductance. Martínez-Cuenca et al. (2020) also observed a reduction in stomatal conductance and no effects on the assimilation rate of the cherry tomato (*S. lycopersicum* L. var. *cerasiforme*) and *S. pimpinellifolium* L. 75 days after the beginning of salinity stress. Prolonged salinity stress (120 days) reduced both parameters, however, showing that stomatal and non-stomatal limitations eventually negatively impacted photosynthesis (Martínez-Cuenca et al., 2020). Similarly, a range of studies on different *Solanum* species reported a decrease in CO_2 assimilation rate under salinity, as opposed to the current results (Bacha et al., 2017; Brenes et al., 2020; Leiva-Ampuero et al., 2020). The maintenance of this parameter was sparsely reported in common crops and mostly observed under low salinity as seen in olive trees (*Olea europaea* L. (Chartzoulakis et al., 2002). Manganese was highly increased in the current study, in contrast to a range of reports showing a reduced uptake under stress, and might have played a part in the maintenance of the assimilation rate due to its key role as an enzyme cofactor in the photosynthesis mechanism (Alejandro et al., 2020). The improved fruit production of chilli pepper under salinity and manganese supplementation was associated with the beneficial effect of manganese on photosynthesis, supporting its key role under stress (Ye et al., 2022).

Salinity impacts a range of processes involved in photosynthesis including stomatal density, light transport, and pigments, which can eventually affect the CO_2 assimilation rate (Huang et al., 2019). In the current study, chlorophylls increased under the highest salinity levels, as observed in *S. nigrum* L. (Liao & Zhang, 2021). Chlorophyll pigments are important to harvest light and have

been positively correlated with the assimilation rate, suggesting a potential role in maintaining the assimilation rate under stress in the current study (James et al., 2002; Sim et al., 2015).

It is important to consider the reduction in ECp towards the end of the stress when the photosynthesis measurements have been made. Photosynthesis is a dynamic process shown to peak at the vegetative stage and slow down during fruiting in tomato (Wang et al., 2019). Hence, it could be hypothesised that earlier changes might have happened, explaining in part the reduced yield, and that the assimilation rate then recovered under the slightly lower salinity level, when the demand for photosynthesis was also lowered. This was observed in castor bean (*Ricinus communis* L.) and barley (*Hordeum vulgare* L.) where an initial decrease in assimilation rate was noted under salinity stress followed by stabilisation at the same level as the control in later developmental stages (Pandya et al., 2005; Pinheiro et al., 2008). In contrast, in this study, stomatal conductance does not seem to have recovered as early as the assimilation rate and was still significantly impacted by the salinity levels for another 13 days. Differences in recovery rate of assimilation rate and stomatal conductance were also observed in kidney beans (*Phaseolus vulgaris* L.) under drought with stomatal conductance taking longer to recover, supporting the current hypothesis (Miyashita et al., 2005).

The maintenance of the assimilation rate during the fruiting stage was not sufficient to maintain fruit yield in the current study. A reduction in final yield under salinity is highly common in *Solanum* as seen in tomato and the brinjal eggplant (*S. melongena* L.) (Carbajal-Vázquez et al., 2023; Salim et al., 2019). Despite fewer fruits being produced under salinity compared to the control, their size and weight were unchanged as noted in a tomato cultivar by Shumaila et al. (2023). In contrast to these observations, salinity reduced the fruit weight of the brinjal eggplant and other tomato varieties due to the reduction in fruit

osmotic potential, causing less water translocation to the fruit, as well as early ripening (Costan et al., 2020; Martínez et al., 2020; Salim et al., 2019). A reduction in fruit water content has been suggested to be the main reason for a reduction in fruit weight under salinity due to the relationship between salinity and the plant's water relations, which might have a lesser impact in the African eggplant cultivar tested in the current study (Chrétien et al., 2000). In addition, this reduction is more notable in larger fruits while the fruits produced by the African eggplant cultivar used in this study are still smaller than the ones produced by highly domesticated plants (Diouf et al., 2018).

The reduced fruit number follows the production of fewer flowers under salinity stress. A reduction in the number of flowers has been reported in a range of crops under salinity mostly due to an impaired carbohydrate metabolism (Martínez-Cuenca et al., 2020; Rahman et al., 2018; Salim et al., 2019). In a study on tomato plants, Ghanem et al. (2009) showed that a low number of flowers reached full development due to a high abortion rate after an accumulation of sodium under salinity. The rise in the dead flowers percentage as salinity increased in this study also suggests an increase in flower abortion. Interestingly, despite a reduction in flower production without an increase in flower death at the low salinity level in this study, the final number of fruits per plant was not significantly reduced. This might be due to an increase in fruit set as observed by Martínez-Cuenca et al. (2020) in one *S. pimpinellifolium* cultivar under salinity stress, suggesting mechanisms to maintain fruit production up to a threshold due to, in part, a maintained carbon assimilation. A note of caution is due here to explain the link between yield, flowering, and salinity. Indeed, a low yield was observed despite a large number of flowers produced even for the control plants. This observation might be due to the absence of pollination during the study as plants were grown in a glasshouse without additional pollinators or manual pollination. As plants under salinity produced fewer flowers, polli-

nation might have been even more restricted, eventually increasing the number of dead flowers and reducing the number of fruits produced due to pollination issues rather than impaired mechanisms or flower abortion.

While the salinity treatment impacted highly the concentration of mineral elements in leaves, their concentrations in fruits were unchanged except for sodium and selenium. This was also reported in the brinjal eggplant due to translocation control (Salim et al., 2019). Sodium accumulation is one of the first markers of salinity stress (Assaha et al., 2017). The degree of leaf sodium accumulation, however, is not a clear marker of salinity tolerance as its transport and compartmentalisation can also affect its toxicity (Tester & Davenport, 2003). Aside from its toxic impact on cell membranes and processes, sodium accumulation can also have adverse interactions with calcium uptake, and consequently, its deficiency can severely impair stress signalling and plant growth (Zhao et al., 2021). The rise in leaf sodium concentration coincided with a reduction in calcium content in the current study, also observed in *S. villosum* Mill. and tomato (Ahsan et al., 2022; Ben-Abdallah et al., 2019). Calcium is an important signalling molecule regulating a range of hormones and has a key role in the interaction of the plant with abiotic stress factors (Ahanger et al., 2016). Its reduction is thus linked to various negative effects also observed here such as stunted growth and lowered membrane stability. Supplementing plants with calcium led to improvements in tomato salinity tolerance by increasing leaf area and germination in the study by Tanveer et al. (2020) and its application has been suggested by a few groups to be beneficial to improve salinity tolerance by promoting plant growth and uptake of potassium and magnesium, for example (Abeed et al., 2023; Elkelish et al., 2019; Islam et al., 2023).

Potassium uptake is also affected by an increasing sodium concentration in the soil due to uptake competition (Zhao et al., 2021). Leaf potassium concentration was reduced in tomato plants under salinity in previous studies, as opposed to

the increase noted in the current study and in *S. insanum* L. (Ahsan et al., 2022; Ortega-Albero et al., 2023; Zhang et al., 2020). Potassium has a key role in a range of metabolic events and the unstressed ratio of sodium over potassium is optimal for many cellular processes (Assaha et al., 2017). Foliar and solution application of potassium increased the salinity tolerance of tomato after its concentration dropped under salinity by maintaining growth and reducing the concentration of reactive oxygen species, supporting its beneficial effects even under salinity and potential role in the maintenance of a few characteristics in the current study, especially at low salinity (Amjad et al., 2016). Similar to the benefits provided by potassium, selenium's role in alleviating salinity-induced damage has been reported by multiple research groups (Nazir et al., 2023). In opposition to the observations made for potassium, however, and despite selenium being at its highest at the LS stress, a sharp reduction in higher salinity levels was noted in both fruits and leaves in this study. This is also linked to the maintenance of leaf production and other characteristics at the LS stress not observed at higher levels, suggesting a potential role only at low-stress levels.

6.5 Conclusion

The African eggplant tolerated low levels of salinity. Its growth and yield were reduced under moderate and high salt concentrations, which could limit its cultivation in highly salt-affected areas. The maintenance of the assimilation rate at the fruiting stage at high salt concentrations was insufficient to maintain fruit production, nevertheless, the fruits produced maintained their quality. Unlike previous reports of reduction of phosphorus and potassium uptake at high concentrations of sodium in other crops, their concentrations increased in the African eggplant, suggesting protective mechanisms in place to ensure their uptake and the protection of mechanisms relying on these nutrients.

Chapter 7

The combination of salinity and drought with biostimulants

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7.1 Introduction

Drought and soil salinity are major abiotic stresses impacting agricultural land worldwide (Fita et al., 2015). Furthermore, drought periods are set to increase in intensity, length, and frequency due to climate change (Bezner Kerr et al., 2022). To overcome this issue and enhance yield, irrigation is often used in arid areas, leading to salt accumulation due to prolonged irrigation and poor drainage (Fita et al., 2015; Shahid et al., 2018). Soil salinisation is due to both human and natural mechanisms, including poor irrigation management, seawater inundation, and lack of drainage (FAO & ITPS, 2015). Both drought and salinity are thus becoming more prevalent and threaten food security by reducing yields (Bezner Kerr et al., 2022).

Water stress occurs when the amount of transpired water is higher than the water available at the roots and impacts the morphological, physiological, and biochemical aspects of crops. Photosynthesis is particularly impacted through reduced leaf area and stomatal closure, eventually reducing plant's yield (Kapoor et al., 2020). A high concentration of salts and ions in the soil also causes osmotic stress due to reduced water uptake (Rahnama et al., 2010). Under the combination of drought and salinity, it is unclear whether photosynthesis and other processes will be damaged by one of the stresses predominantly or if an additive effect will be observed. Additionally, salinity leads to ionic imbalance due to the prevalence of sodium and chloride ions in the soil which impacts the uptake of other essential ions such as potassium, calcium, and magnesium (Altaf et al., 2022). It is not clear, however, how drought impacts the salinity-induced ion imbalance (Angon et al., 2022). The combination of drought and salinity cannot be predicted by the effect of individual stress as shown in multiple studies (Angon et al., 2022). In some cases, the stress combination causes an additive effect on some plant characteristics as seen in tomato (*S. lycopersicum*

L.), but one stress can also be predominant depending on their intensities (Yang et al., 2019; Zhou, Yu, et al., 2017). Understanding how plants react to the stress combination is key to predicting the resilience of fields now and in the future and finding appropriate solutions to drought and soil salinity.

To improve growth and yield under environmental stress, biostimulants have gained interest as they modulate plant physiology and biochemistry, enhancing tolerance through secondary metabolism activation and leaf chlorophyll fluorescence manipulation, for example (Malik et al., 2020). The European Biostimulants Industry Council defines them as "substance(s) and/or micro-organisms whose action when applied to plants or the rhizosphere is to stimulate natural processes to enhance/benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality" (EBIC, 2021). Biostimulants comprise a range of active substances, including microbial organisms, plant hydrolysates (PHs), and seaweed products, which can all have a different mode of action (Paradjiković et al., 2019). Under salinity, PHs improve tomato and lettuce (*Lactuca sativa* L.) tolerance by improving root development, reducing leaf sodium over potassium ratio, and promoting antioxidant enzymes (Zuluaga et al., 2023). Positive effects were also noted when using microorganisms under drought in tomato plants, with an improvement of the plant's water status (Duc et al., 2018). Their positive effects are, however, not always certain and are highly dependent on the stress intensity and length, timing of application, soil structure, and species of interest. Combining biostimulants with different modes of action has thus been suggested to be more efficient than using them individually, especially under stress combination (Rouphael & Colla, 2018).

Indigenous crops offer great potential to increase field diversity and tolerance due to their large gene pool and adaptation to a range of environments (Akinola et al., 2020). The African eggplant (*Solanum aethiopicum* L.) is a highly diverse and nutrient-rich vegetable grown in sub-Saharan Africa (Moenga &

Achieng Odeny, 2022). It is grown near the seafront by farmers cultivating tall fruit crops, such as coconut, thanks to its shade tolerance, offering extra income. According to the studies of de Fatima et al. (2023) and Mensah et al. (2020), its tolerance to salinity is low, however. Variability regarding its tolerance to drought was reported previously by different research groups (Lagat, 2016; Nakanwagi et al., 2017). The large variability observed between cultivars and only sparse reports of its responses under stress highlights the lack of knowledge about its potential in stress-prone areas. Furthermore, no studies on its responses to the combination of salinity and drought have been made. Similarly, biostimulants' impact on its development has not been investigated before.

This project aimed at understanding how the growth, yield, physiology, and nutrient uptake of the African eggplant Meketan were affected by the combination of salinity and drought. In addition, biostimulants were used in a second experiment on the cultivar Kotobi, subjected to the same stresses, to understand their potential as tolerance enhancers on the African eggplant. Meketan cultivar was selected for experiment 1 due to the preliminary results observed in Chapter 3 where Meketan had the highest stomatal conductance when compared to other African eggplant cultivars and landraces which was only reduced by salinity and not drought. Kotobi was used for experiment 2 also based on observations made in Chapter 3 with this cultivar displaying the lowest stomatal conductance and number of leaves. Biostimulants were thus used on Kotobi as they tend to be more efficient on lower-performing crops (Zuluaga et al., 2023). The results will help fill the current knowledge gap on this promising indigenous crop.

7.2 Materials and Methods

7.2.1 Plant material and growth conditions

The effects of drought, salinity, and selected biostimulants on the African eggplant were evaluated across two pot experiments. *Solanum aethiopicum* L. cv., Meketan (experiment 1) and cv., Kotobi (experiment 2) seeds were generously provided by Novagenetic (Technisem, Longué-Jumelles, France). Seeds were sown in modular trays in John Innes No.2 compost (7 loam:3 peat:2 sand v/v/v) (J Arthur Bower's, Westland®, Dungannon, Northern Ireland). Trays were placed in an MLR-352 growth cabinet (PHC Holdings Corporation®, Tokyo, Japan) set at 70% relative humidity and 27/21°C for a 12 h photoperiod, and watered from the bottom when needed. After four weeks, seedlings were transferred to 7 cm x 7 cm pots filled with the same compost and placed under the same growing conditions for 14 days. For each experiment, seedlings of similar size were then transplanted in 10 L (Experiment 1, 28 plants) and 3 L (Experiment 2, 32 plants) pots and placed in a glasshouse following a randomised complete block design. The glasshouse was heated at 27°C when needed between 7 am and 7 pm and at 21°C between 7 pm and 7 am. Additional lighting was used between 7 am and 7 pm and a cooling down system with fans was relied upon when temperatures inside the glasshouse reached 31°C (detailed environmental data are shown in Appendix B, Fig. B.6 and B.7). Automatic irrigation was set up with ML3 thetaprobe soil moisture sensor connected to a GP2 Delta-T data logger (Delta-T Devices®, Burwell, UK) to maintain soil moisture at around 40%. In experiment 1, the sensors were placed in two plants under full irrigation and four plants under deficit irrigation in each salinity treatment. In experiment 2, sensors were placed in five plants in each treatment combination. Fertigation was used with Kristalon Brown (NPK 3-11-38, YaraTera, Yara, Oslo, Norway) following the manufacturer's instructions (1 kg of fertiliser in 10 L of irrigation

water). Natural predators were applied throughout the experiment (Anderline, Starskii, Chrysoline C, Bioline AgroSciences®, Little Clacton, UK). The final harvest of plants took place 73 days after transplanting (DAT) for experiment 1 and 48 DAT for experiment 2.

7.2.2 Treatment application

Saline water was used to salinise the soil before transplanting. For experiment 1, the ASTM Synthetic seawater standard protocol (ASTM D1141-98) was used by diluting (g L^{-1}): NaCl 24.53, $MgCl_2$ 5.20, Na_2SO_4 4.09, $CaCl_2$ 1.16, KCl 0.695, $NaHCO_3$ 0.201, KBr 0.101, and H_3BO_3 0.027 in distilled water. When ready, the saline water was applied to the dry soil (80 mL L^{-1} of soil). For experiment 2, a saline solution of 24.53 NaCl g L^{-1} concentration was applied at a rate of 40 mL to 1 L of John Innes No.2 soil based on preliminary results observed using cultivar Kotobi. Non-saline water was also applied as a control. The soil was mixed and left to air-dry for 48 h before transplanting.

In both experiments, soil moisture content was monitored via soil matric water potential recorded by MPS-2 sensors (Decagon devices, Meter Environment®, METER Group, Pullman, WA, United States) connected to a datalogger EM50g (Meter Environment®). For experiment 1, sensors were placed in two plants in each treatment (8 sensors in total). For experiment 2, sensors were placed in 5 plants in each treatment (20 sensors in total). An HH2 moisture meter attached to a WET sensor (Delta-T Devices®) was also used regularly to manually monitor soil water content and pore water conductivity at the top, middle, and bottom of the pots.

Experiment 1

Two weeks after transplanting in the saline and non-saline soil, the drought treatment was initiated on seven of the plants in saline soil and seven plants in non-saline soil by reducing soil moisture content from around 40% to 20% as measured by the ML3 theta probes (Delta-T Devices®). Air temperature and relative humidity within the glasshouse were recorded by a weather station (RHT2nl, Delta-T devices®) connected to a GP2 Delta-T data logger.

Experiment 2

At transplanting, biostimulants were applied to half of the plants in each of the salinity treatments. The arbuscular mycorrhizal fungi (AMF) were bought from PlantWorks Ltd (Sittingbourne, UK). The rest of the biostimulants (Sion, TTL, Maxicrop, Zonda) were provided by Agrii (Masstock Arable Ltd, Cheltenham, UK). A combination of these products was used due to their different mode of action and based on initial results obtained (not shown) at the following rate:

- Arbuscular mycorrhizal fungi: 4 g per 3 L pot
- Sion: 1 mL per 3 L pot
- TTL: 6 mL per 3 L pot
- Maxicrop: 5 mL per 3 L pot
- Zonda: 2 mL per 3 L pot

Arbuscular mycorrhizal fungi were applied under the roots of the transplanted plants. Sion and maxicrop were applied through a foliar spray while TTL and Zonda were applied directly to the base of the plants. After four weeks, drought condition was initiated by gradually reducing the irrigation from 35% soil moisture to 15% as measured by the ML3 theta probes (Delta-T Devices®). Air temperature and relative humidity within the glasshouse were recorded by a weather

station (RHT2nl, Delta-T devices®) connected to a GP2 Delta-T data logger.

7.2.3 Growth and physiological measurements

Photosynthesis dynamics

The midday CO_2 assimilation rate and stomatal conductance during experiment 1 were recorded at 32 and 60 DAT using a portable photosynthesis system LI-6800 (LiCor Biosciences®, Lincoln, NE, United States). For experiment 2, midday and diurnal stomatal conductance were recorded using a leaf porometer (Model SC-1, METER group) at 26, 31, 33, 35, 39, 41, and 46 DAT. Diurnal stomatal conductance was recorded every three hours from 7 a.m. to 4 p.m. In both experiments, the measurements were taken on one fully expanded leaf per plant at the top of the canopy.

Fruiting

Fruits were labelled as they developed during experiment 1. When fruits reached marketable maturity based on their colour and size (See Fig.7.1), their weight, height, and maximum width were recorded. They were then sliced and placed in liquid nitrogen. Fruits were eventually freeze-dried (Alpha 1-4 LSCplus, Martin Christ, Osterode am Harz, Germany) for laboratory analysis.

Destructive harvest

A leaf sub-sample representing a mix of developing and developed leaves was removed from the plants on the last day of each experiment and weighed using a two-digit electronic scale. The samples were then placed in liquid nitrogen and stored at -80°C for biochemical analysis. Shoot growth was separated between



Figure 7.1: Picture of a marketable Meketan fruit.

leaves and stems and weighed. Stem diameter and plant height were recorded, and leaf area was measured using a Li-3000 area meter (LiCor®) were measured as well. The plant aboveground biomass was then oven-dried at 80°C until it reached a constant weight.

Soil electrical conductivity

The soil electrical conductivity was measured on a 1:5 soil:water (w/w) solution as described by He et al. (2012). A cork borer was used to extract one soil sample from each pot. After leaving the soil to air-dry for 48 h, the substrate was sieved (2 mm) and weighed (5 g). Twenty-five grams of distilled water was added to the weighed soil and the solution was placed on a shaker plate for 24 h. The electrical conductivity of the settled solution (EC 1:5) was measured using a LAQUAtwin EC-33 meter (Horiba®, Kyoto, Japan).

7.2.4 Biochemical analysis

Leaf chlorophyll content

Chlorophyll content was measured using the method outlined in Wintermans and De Mots (1965). Two leaf discs of an area of 1.54 cm^2 each were taken from the first two fully grown leaves, placed in 10 mL of 95% (v/v) cold ethanol, and stored at 4°C in darkness for 48 h. The absorbance of the supernatant was then measured at 470, 649, and 665 nm using a UV-VIS spectrophotometer (Ultrospec III, Pharmacia LKB, Stockholm, Sweden). Chlorophyll a (Chl a) and b (Chl b), and carotenoids (Car) per unit area were calculated as follows:

$$Chl\,a = (13.95 * A649)/1.54 \tag{7.1}$$

$$Chl\,b = (24.96 * A649 - 7.32 * A665)/1.54 \tag{7.2}$$

$$Car = (10^3 * A470 - 2.05 * Chl\,a - 114.8 * Chl\,b)/1.54 \tag{7.3}$$

Total soluble protein

The Bradford (1976) method was followed for total protein quantification on leaves from the last day of the experiments and marketable fruits. For experiment 1, a dry leaf sample (30 mg) and a dry fruit sample (25 mg) were homogenised in 1 mL of 100 mM sodium phosphate buffer (pH 7.0 at 20°C) (Fischer Scientific, Hampton, NH, United States). For experiment 2, a fresh leaf sample (250 mg) was digested in 1 mL of the same buffer. Next, 10 μl of the supernatant from the extracted samples was transferred to a 96-well plate (96-Well Microtiter™Microplates, Fischer Scientific) and 290 μl Bradford reagent (Merck, Darmstadt, Germany) was added to each well. The plate was placed on a shaker for 5 mins at 200 rpm at room temperature. The sample absorbance at

595 nm was then measured using a plate reader (CLARIOstar, BMG Labtech, Ortenberg, Germany), with bovine serum albumin (Merck) used as a standard.

Total antioxidants

The TEAC (Trolox Equivalent Antioxidant Capacity) method detailed by Re et al. (1999) was used to determine the total antioxidants on leaves sampled on the last day of the experiments and marketable fruits. An ABTS (7 mM, Merck) and potassium persulfate (2.45 mM, Merk) solution was made up and left in darkness overnight. This solution was then diluted until it reached an absorbance of 0.9 at 734 nm with 95% (v/v) ethanol (Fischer Scientific) and warmed on a hotplate at 40°C. Thirty microliters of the digested dry leaf sample (10 mg in 1 mL of 80% (v/v) cold methanol) or fruit sample (30 mg in 1 mL of 80% (v/v) cold methanol) were pipetted in 3 mL of the diluted working ABTS solution. After incubating for 15 mins in a water bath at 40°C, the absorbance of the samples was read at 734 nm using a UV-VIS spectrophotometer (Ultrospec III).

Leaf and fruit nutrient concentrations

The dried and milled leaf and fruit samples (300 mg) were digested in 6 mL of 70% (v/v) nitric acid and placed in a digestion microwave (Multiwave PRO, Anton Paar, Graz, Austria) reaching 175°C over a 20 mins period and maintained for a further 20 mins followed by a 10 mins cool down at 55°C. The digested samples were diluted with 18.2 milliQ water to reach 2% nitric acid concentration for multi-element analysis by ICP-MS (Inductively Couple Plasma Mass Spectrophotometry, Thermo-Fisher Scientific iCAP-Q; Thermo Fisher Scientific, Germany). Calibration standards included a multi-element solution (Claritas-PPT grade CLMS-2 from SPEX Certiprep Inc., Metuchen, NJ, United States)

and a bespoke external multi-element calibration solution (PlasmaCAL, SCP Science, Quebec, Canada). Nitrogen concentration was analysed individually using a FlashEA®1112 elemental analyser (Thermo Fischer Scientific, Waltham, MA, United States). Elemental nitrogen was detected after the conversion and filtration of the gas mixture generated by the combustion of 50 mg of dried and milled leaf sample in a foil capsule, providing a nitrogen percentage.

7.2.5 Statistical analysis.

All statistical studies were performed using R version 4.1.2 and the models used are detailed in Appendix C. After checking continuous data for normality and variance homogeneity through statistical and visual tests, a two-way analysis of variance (ANOVA) was performed with Tukey's honest significance test post hoc at a 0.05 confidence level. Count data was analysed using a generalised linear model fitted with the Poisson distribution or the inverse binomial in case of overdispersion. Pearson's correlation coefficients were computed within each treatment sub-group to assess the relationship between some of the measured characteristics.

7.3 Results

7.3.1 Experiment 1 - Salinity and drought

Soil salinity

The pore water conductivity (ECp) was increased by salinity from 6 days after transplanting (DAT) (Fig. 7.2a). From 19 to 29 DAT and again from 38 to 40 DAT, only the ECp of well-watered plants was significantly increased by salinity

(Fig. 7.2a). For the rest of the experiment, salinity significantly increased ECp for both treatments with plants under deficit irrigation displaying a lower ECp than well-watered plants under salinity (Fig. 7.2a). Soil conductivity 1:5 on the last day of the experiment was significantly increased by salinity while the irrigation levels did not impact it (Fig. 7.2b).

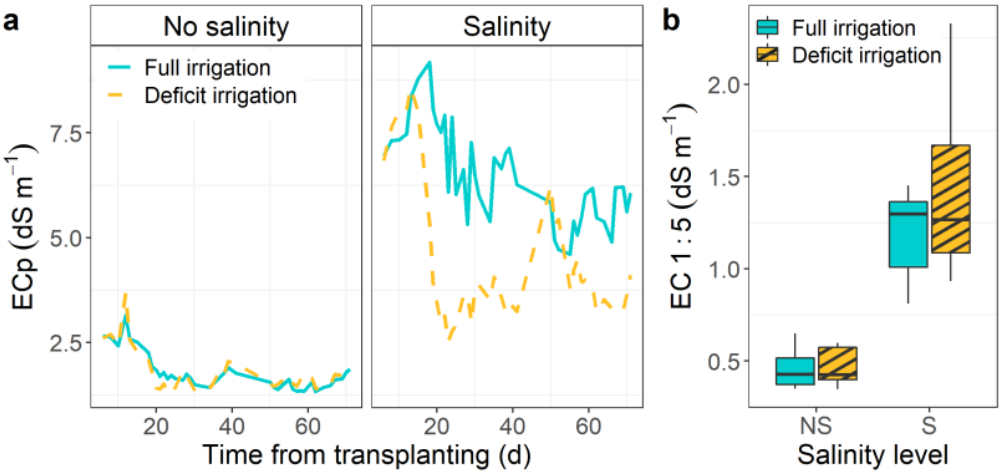


Figure 7.2: Soil salinity levels as measured by **(a)** pore water conductivity (ECp) throughout the stress period and **(b)** soil electrical conductivity (EC) 1:5 at the end of the experiment. The pore water conductivity is represented by the mean of the data and EC 1:5 is represented by the lower and upper whiskers, 25% and 75% quartiles, and median (n = 7). Three ECp measurements per pot were taken daily while one soil sample was extracted at the end of the experiment to measure EC 1:5. NS = No salinity, S = Salinity.

Plant growth parameters

Drought and salinity decreased leaf and stem dry weight, plant height, and stem diameter (Fig. 7.3a–d). The interaction of the stresses was not significant for these parameters. The combination of drought and salinity caused the most significant reduction in leaf area, while salinity and water stress individually did not reduce it compared to the control (Fig. 7.3e). Both the combination of stress and water stress alone led to a decrease in the total number of leaves (Fig. 7.3f). Pictures of the plants grown under the different treatment are shown in Figure 7.4,

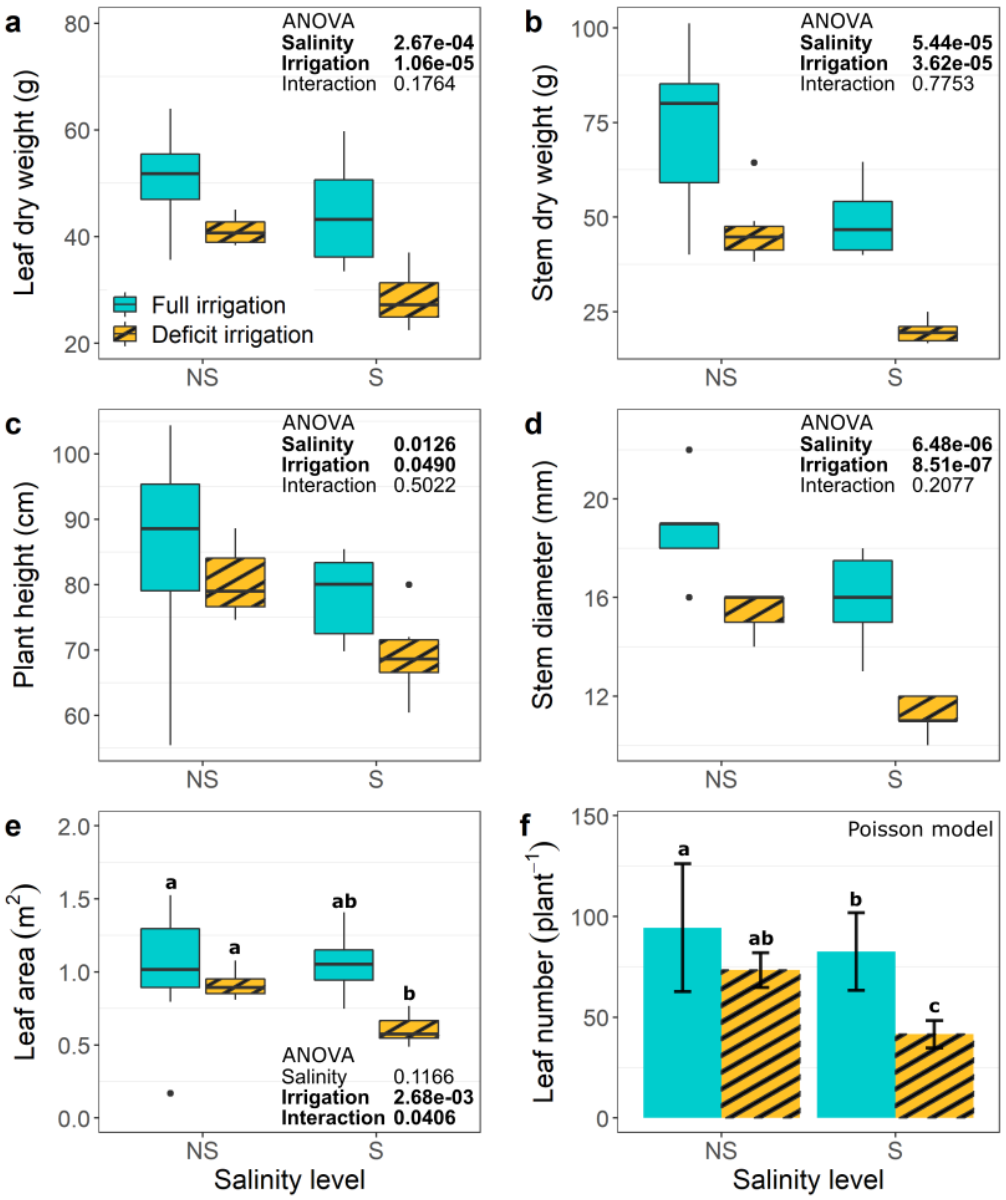


Figure 7.3: Plant growth characteristics of the African eggplant at the end of the salinity and drought treatment. Sub-plots represent **(a)** leaf dry weight, **(b)** stem dry weight, **(c)** plant height, **(d)** stem diameter, **(e)** leaf area, and **(f)** total number of leaves per plant. The continuous dataset is represented by the lower and upper whiskers, 25% and 75% quartiles, and median (n = 7). Black dots represent outliers. The p-values of the ANOVAs are shown for each source of variation. When the interaction of the stresses is significant, letters are used to show the pairwise comparison. Boxplots not sharing any letters are significantly different based on the Tukey test at 0.05% level of significance. The count dataset is presented as a barplot showing the mean value +/- standard deviation. NS = No salinity, S = Salinity.

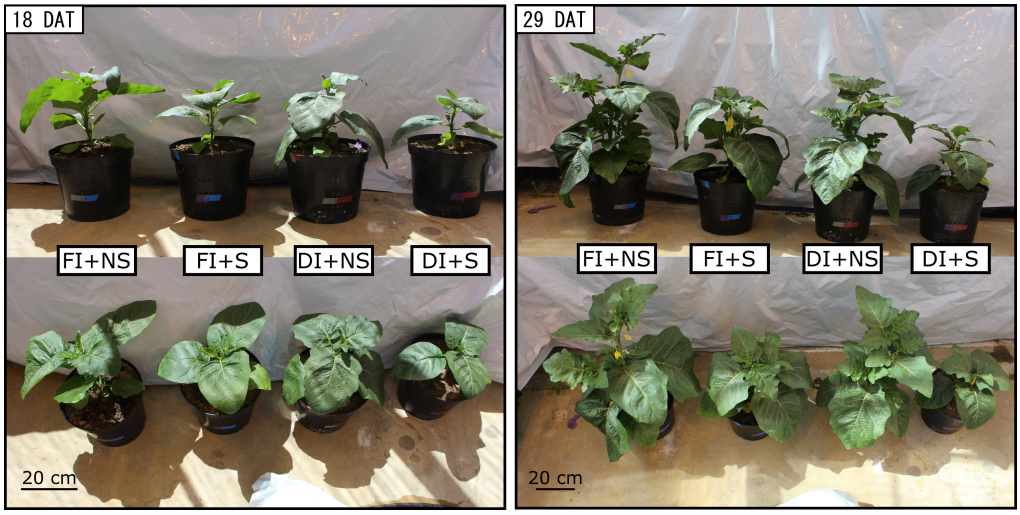


Figure 7.4: Plants at 18 and 29 days after transplanting (DAT). Two different scales are used between 18 and 29 DAT and a comparison between these dates is thus invalid. FI = Full irrigation, DI = Deficit irrigation, NS = No salinity, S = Salinity.

Leaf gas exchange and chlorophyll levels

The CO_2 assimilation rate was not significantly impacted by drought and increased by salinity at 32 DAT (Fig. 7.5a). At 60 DAT, however, both salinity and drought significantly decreased it (Fig. 7.5a). Midday stomatal conductance exhibited a similar trend, initially increasing due to salinity at 32 DAT and decreasing under both stressors later in the stress period (60 DAT) (Fig. 7.5b). The stress interaction did not significantly impact these measures.

Neither drought, salinity, nor their combination impacted significantly on the concentrations of chlorophylls a and b, and carotenoids (Fig. 7.6). The ratios of chlorophyll a over b and of total chlorophyll over carotenoids were also not significantly affected by either stresses or their combination (Fig. 7.6).

Fruit production

Salinity and drought reduced fruit yield significantly with no significant interaction between these factors (Fig. 7.7a). The combination of stress did not lead

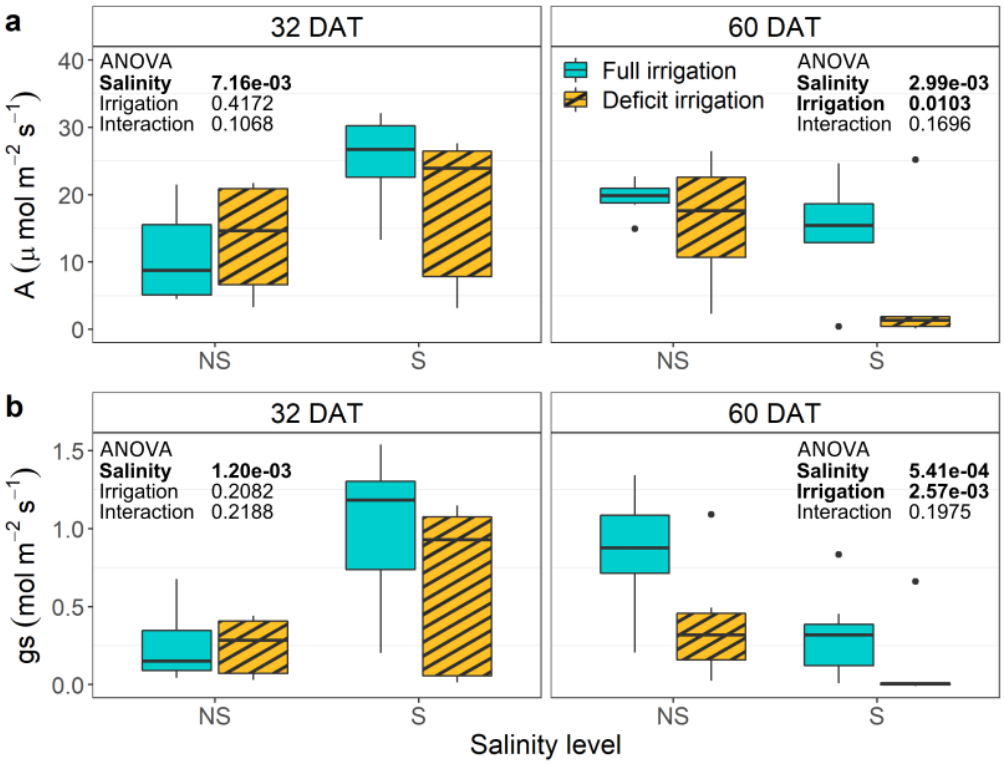


Figure 7.5: Midday **(a)** assimilation rate (A) and **(b)** stomatal conductance (gs) of the African eggplant under drought and salinity at 32 and 60 days after transplanting (DAT). The dataset is represented by the lower and upper whiskers, 25% and 75% quartiles, and median (n = 7) and was recorded on one leaf per plant at the top of the canopy. Black dots represent outliers. The p-values of the ANOVAs are shown for each source of variation. NS = No salinity, S = Salinity.

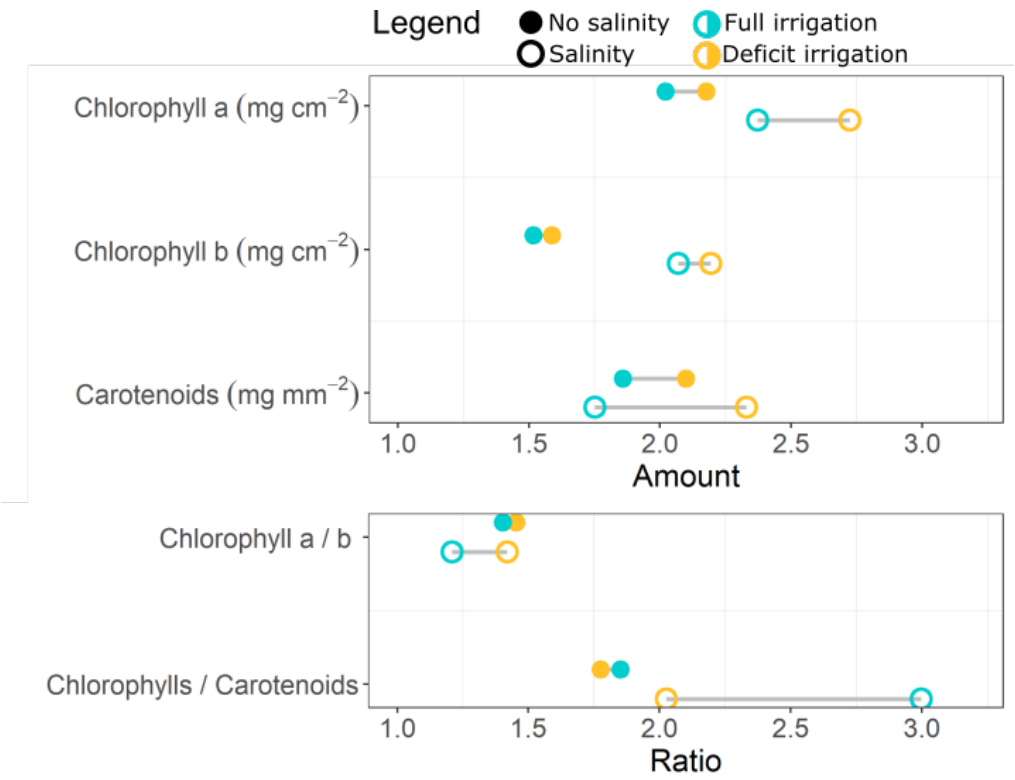


Figure 7.6: Levels of chlorophyll and carotenoid at the end of the salinity and drought treatment. The unit of each measure is shown on the y-axis while the x-axis represents the amount recorded of each measure depicted on the y-axis. For each measure, two segments are used with the filled circles representing the data from plants grown under no soil salinity while the empty circles depict the data from plants grown in the saline soil. Turquoise coloured dots represent the level of each measure of the plants under full irrigation and orange dots show the plants under deficit irrigation (n = 7).

to a further reduction of fruit weight, height, and maximum width, with plants under drought, salinity, and their combination producing fruits of similar characteristics, significantly reduced from the control plants (Fig. 7.7b–d, Fig. 7.8). Salinity reduced the total number of fruits produced only under deficit irrigation (Fig. 7.7 e). Only salinity slowed down significantly the time it took for the fruits to ripen to a marketable stage (Fig. 7.7f).

Biochemical analysis

Total antioxidant concentration was reduced by salinity and drought only in leaves while no changes were noted in fruits (Fig. 7.9 a,b). Salinity increased the concentration of proteins in both fruits and leaves, whereas drought significantly increased it only in leaves (Fig. 7.9 c,d). The interaction of these stresses did not significantly impact antioxidants and protein concentrations.

The salinity treatment increased sodium concentration in both leaves and fruits (Table 7.1). Leaf nitrogen concentration rose under salinity while concentrations of phosphorus and potassium in both leaves and fruits remained unchanged (Table 7.1). Salinity decreased leaf and fruit calcium concentrations but drought had a significant impact only on leaf calcium (Table 7.1). Magnesium and manganese concentrations were increased by salinity in the leaves but not in the fruits (Table 7.1). Neither zinc nor iron concentrations in fruits and leaves were affected by the stresses (Table 7.1). Leaf and fruit selenium were the only concentrations to be affected significantly by the interaction of drought and salinity. Drought increased leaf selenium concentration under no salinity treatment but decreased it under salinity in both leaves and fruits (Table 7.1). The salinity treatment only reduced leaf and fruit selenium concentrations for plants grown under drought (Table 7.1).

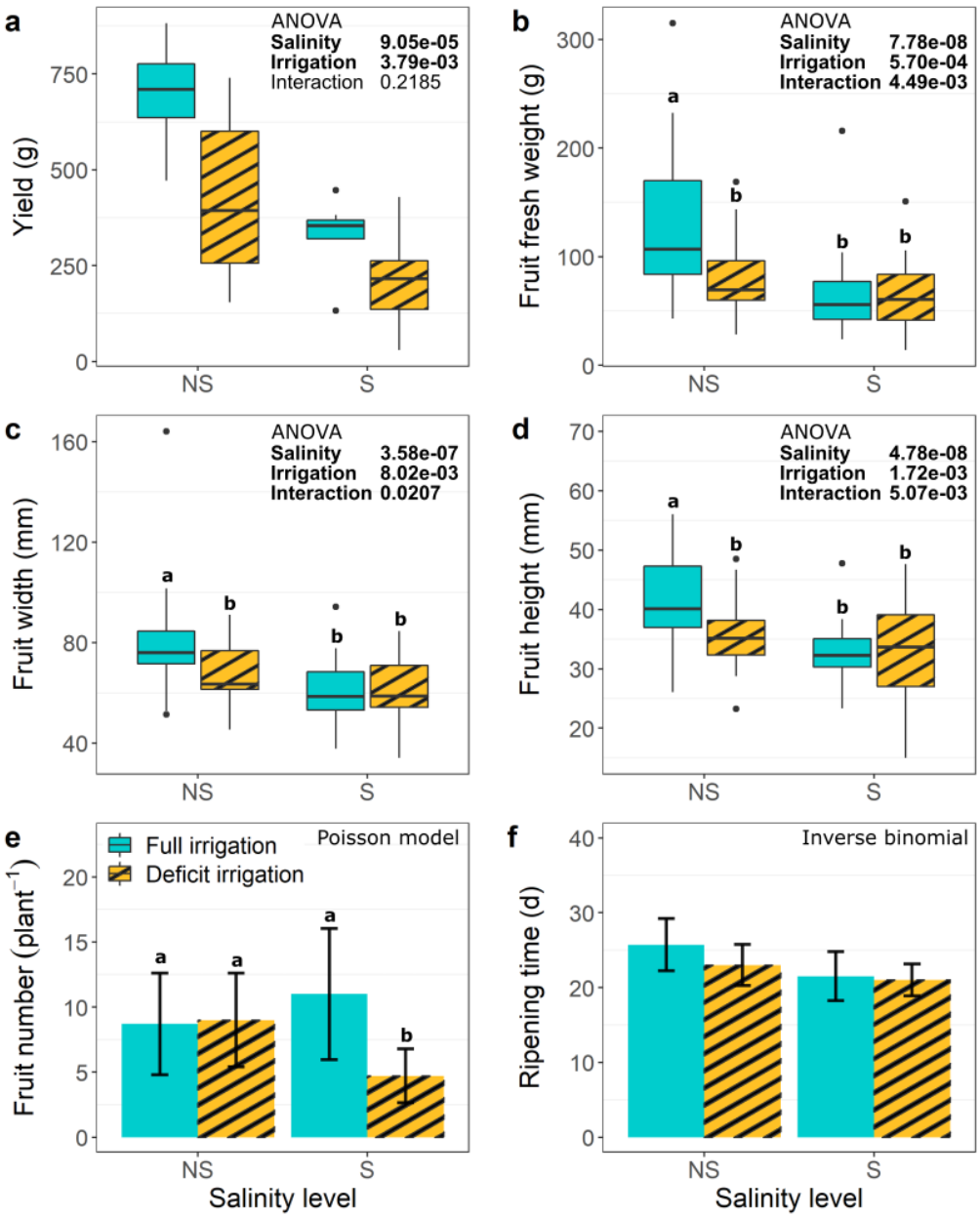


Figure 7.7: Marketable fruit characteristics under the drought and salinity treatment. Sub-plots represent **(a)** total yield, **(b)** individual fruit fresh weight, **(c)** fruit maximum width, **(d)** fruit height, **(e)** number of fruits produced per plant, and **(f)** number of days from fruit set to ripening. The continuous dataset is represented by the lower and upper whiskers, 25% and 75% quartiles, and median (n = 7). Black dots represent outliers. The count dataset is presented as barplots showing the mean value +/- standard deviation. The p-values of the ANOVAs are shown for each source of variation. When the interaction of the stresses is significant, letters are used to show the pairwise comparison. Boxplots not sharing any letters are significantly different based on the Tukey test at 0.05% level of significance. NS = No salinity, S = Salinity.

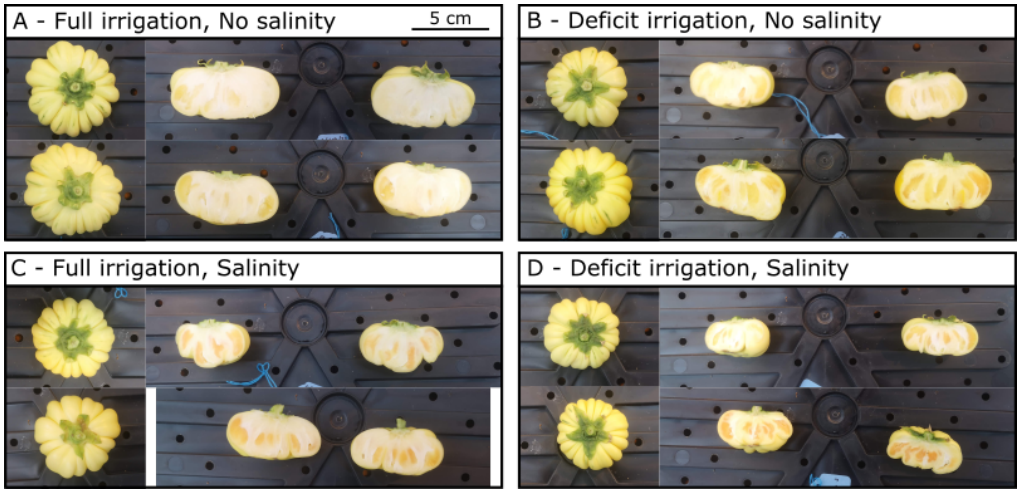


Figure 7.8: Example of fruits harvested for each treatment; **(A)** Full irrigation, no salinity, **(B)** deficit irrigation, no salinity, **(C)** full irrigation, salinity, and **(D)** deficit irrigation, salinity. The whole fruit is presented next to the cut fruit for each sample.

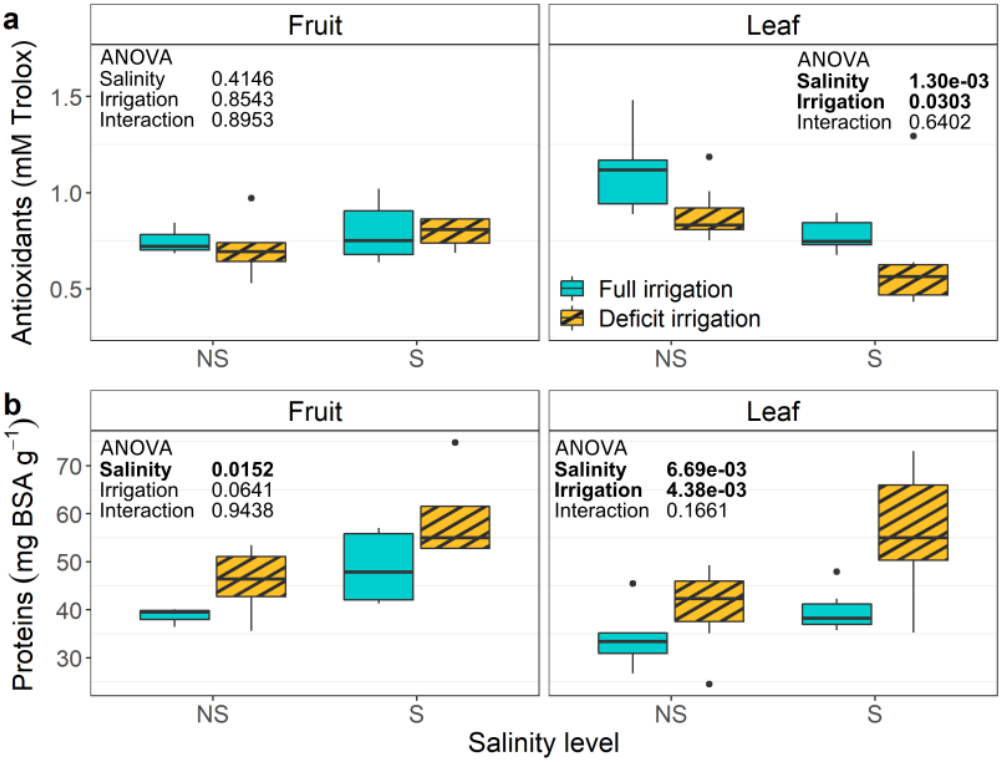


Figure 7.9: Leaf and fruit biochemical analysis of the African eggplant at the end of the salinity and drought treatment. Sub-plots represent dry fruit and leaf **(a)** antioxidant concentration and **(b)** protein concentration. The dataset is represented by the lower and upper whiskers, 25% and 75% quartiles, and median (n = 7). Black dots represent outliers. The p-values of the ANOVAs are shown for each source of variation. NS = No salinity, S = Salinity, BSA = Bovine Serum Albumin.

Table 7.1: Analysis of variance and mean comparisons for dry leaf and fruit mineral element concentrations of African eggplants grown under different salinity and irrigation levels (n = 7).

Source of variance	Na	N	P	K	Ca	Mg	Mn	Fe	Zn	Se
	mg kg^{-1}	g kg^{-1}					mg kg^{-1}			µg g^{-1}
Leaf										
Salinity (S)	**	**	ns	ns	**	***	*	ns	ns	**
Irrigation (I)	ns	***	ns	ns	***	ns	***	ns	ns	ns
S x I	ns	ns	ns	ns	ns	ns	ns	ns	ns	***
Salinity										
None	17.05^{b}	25.86^{b}	2.57	22.28	42.29^{a}	6.33^{b}	90.01^{b}	97.65	14.74	41.92
Saline	25.53^{a}	32.17^{a}	2.89	22.01	33.83^{b}	8.09^{a}	125.6^{a}	104.1	16.85	36.18
Irrigation										
Full irrigation	23.66	26.04^{b}	2.73	21.44	40.88^{a}	7.33	114.4^{a}	95.82	14.70	39.65
Deficit irrigation	17.81	32.00^{a}	2.72	22.86	35.24^{b}	7.09	101.2^{b}	106.0	16.89	38.45
S x I										
None + Full irrigation	18.58	23.83	2.77	22.36	43.30	6.44	96.49	94.82	14.17	37.71^{bc}
None + Deficit irrigation	15.52	27.89	2.38	22.20	41.28	6.23	83.52	100.5	15.31	46.14^{a}
Saline + Full irrigation	28.75	28.25	2.70	20.52	38.46	8.23	132.2	96.81	15.23	41.58^{ac}
Saline + Deficit irrigation	21.03	36.10	3.07	23.51	29.20	7.95	118.9	111.5	18.47	30.77^{b}
Fruit										
Salinity (S)	***		ns	ns	**	ns	ns	ns	ns	**
Irrigation (I)	ns		ns	ns	ns	ns	ns	ns	ns	ns
S x I	ns		ns	ns	ns	*	ns	ns	ns	*
Salinity										
None	33.93^{b}		2.42	24.25	1.56^{a}	1.45	13.11	37.65	9.72	9.44
Saline	244.4^{a}		2.37	22.17	0.94^{b}	1.35	12.93	35.05	9.95	7.25
Irrigation										
Full irrigation	144.0		2.40	23.08	1.22	1.40	12.60	34.64	9.45	8.44
Deficit irrigation	124.3		2.39	23.42	1.32	1.41	13.41	38.02	10.17	8.36
S x I										
None + Full irrigation	40.68		2.38	24.34	1.34	1.34^{ab}	12.16	38.15	9.93	8.83^{ab}
None + Deficit irrigation	29.44		2.46	24.18	1.71	1.53^{a}	13.73	37.31	9.58	9.84^{a}
Saline + Full irrigation	226.7		2.44	22.08	1.11	1.45^{ab}	12.94	31.83	9.07	8.13^{ab}
Saline + Deficit irrigation	266.6		2.28	22.28	0.73	1.23^{b}	12.91	39.07	11.05	6.14^{b}

*Na = Sodium, N = Nitrogen P = Phosphorus, K = Potassium, Ca = Calcium, Mg = Magnesium, Mn = Manganese, Fe = Iron, Zn = Zn, Se = Selenium. NS, *, **, *** Non-significant or significant at P ≤ 0.05, 0.01, 0.001, respectively. Means not sharing any letters within each column are statistically different according to Tukey's test (P = 0.05).*

Pearson's correlation

The treatments and their combination led to different plant characteristics being significantly correlated with the highest number of significant correlations be-

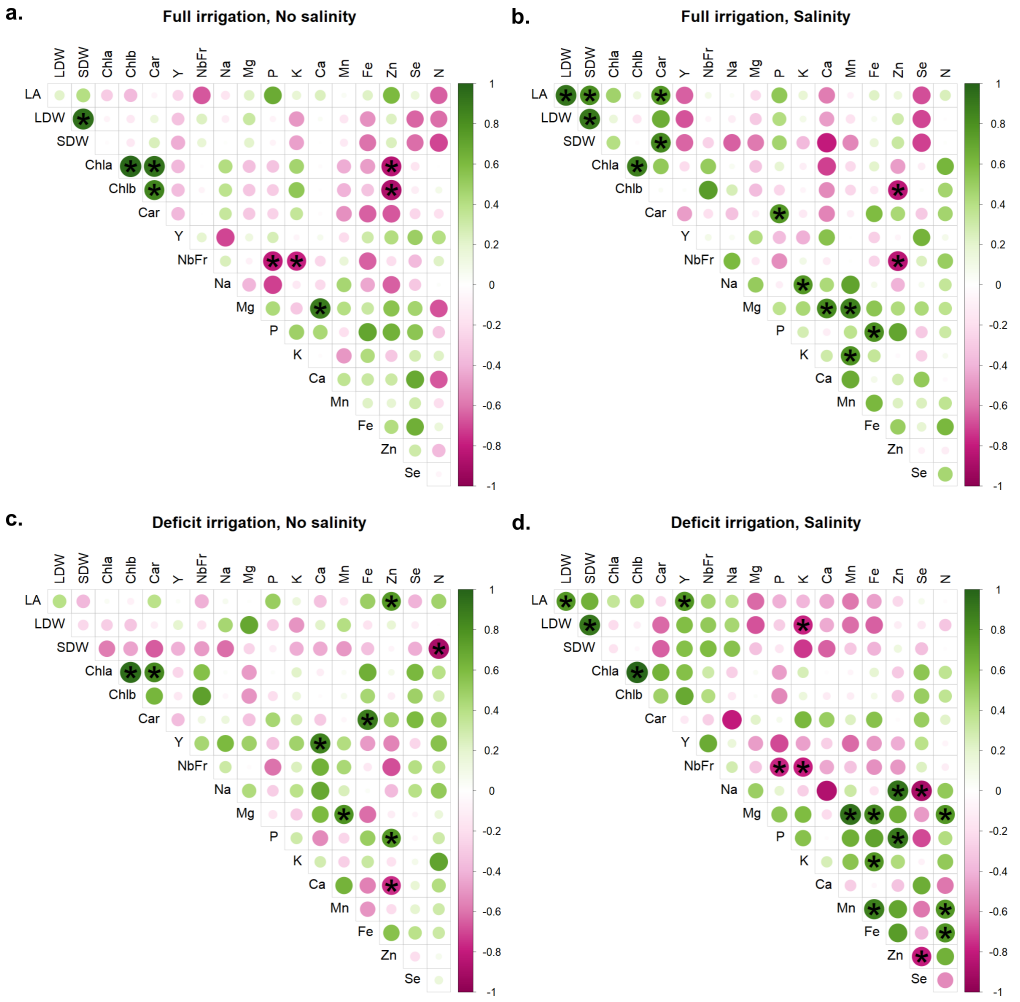


Figure 7.10: Pearson's correlation between plant morphology, yield, and leaf nutrient concentrations. Sub-plots represent the Pearson's correlation of sub-groups **(a)** full irrigation, no salinity (control), **(b)** full irrigation, salinity (salt stress), **(c)** deficit irrigation, no salinity (drought stress), and **(d)** deficit irrigation, salinity (combined stress). Stars denote the statistically significant correlations at a 95% confidence interval (n = 7). The colour represents the level and direction of correlation with dark pink representing negative correlation, white no correlation, and dark green positive correlation. Only leaf nutrient concentrations are used. LDW = Leaf Dry Weight, SDW = Stem Dry Weight, Chla = Chlorophyll a, Chlb = chlorophyll b, Car = Carotenoids, Y = Yield, NbFr = Number of fruits produced, Na = Sodium, Mg = Magnesium, P = Phosphorus, K = Potassium, Ca = Calcium, Mn = Manganese, Fe = Iron, Zn = Zinc, Se = Selenium, N = Nitrogen.

tween the compared parameters noted for plants under the stress combination (Fig. 7.10). The number of fruits produced was negatively correlated with leaf potassium and phosphorus concentrations (Fig. 7.10a). This was also observed under the combination of deficit irrigation and salinity but not under each individual stress (Fig. 7.10b–d). Similarly, differences in correlation between leaf

nutrient concentrations under the treatments were noted, with, for example, positive correlations between leaf magnesium and manganese concentrations only seen under salinity (Fig. 7.10). In contrast, leaf phosphorus concentration was positively correlated to leaf zinc concentration only for plants under deficit irrigation (Fig. 7.10). Chlorophylls a and b were negatively correlated with leaf zinc concentration in the control plants while only chlorophyll b was negatively correlated to leaf zinc under salinity and no correlation between these parameters was noted under drought or the stress combination (Fig. 7.10).

7.3.2 Experiment 2 - Salinity, drought, and biostimulants

Soil salinity

Until 24 DAT, salinity or the biostimulants did not significantly change ECp (Fig. 7.11a). It was then significantly increased until the end of the experiment by salinity while the use of biostimulants did not impact it (Fig. 7.11a). The EC 1:5 at the end of the experiment was, however, not significantly increased by salinity nor the biostimulants (Fig. 7.11b).

Plant growth parameters

Salinity reduced leaf and stem dry weights significantly and the use of biostimulants did not alter these outcomes, whether in stressed and non-stressed conditions (Fig. 7.12a,b). Plant height was not significantly influenced by salinity nor the application of biostimulants (Fig. 7.12c). Stem diameter was reduced by salinity and remained unaffected by the use of biostimulants (Fig. 7.12c).

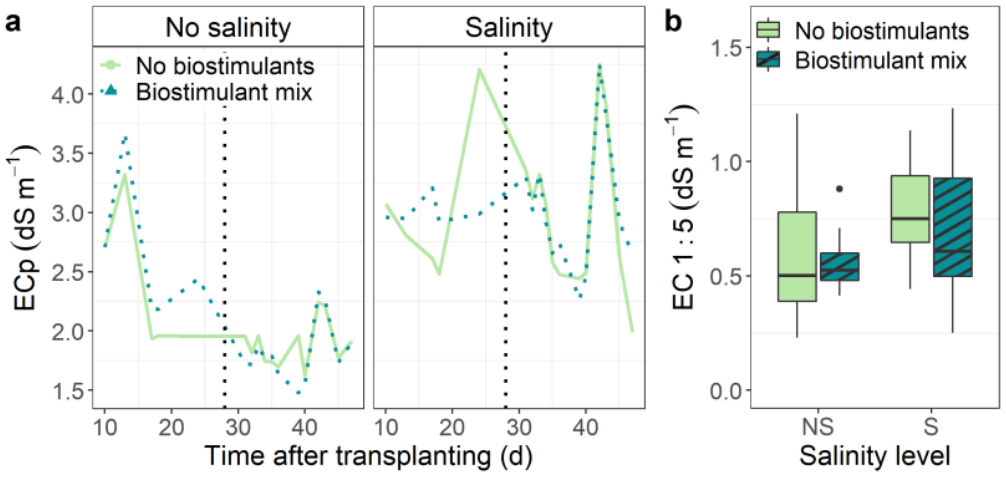


Figure 7.11: Soil salinity levels as measured by **(a)** pore water conductivity (ECp) throughout the stress period and **(b)** soil electrical conductivity (EC) 1:5 at the end of the experiment. The dotted line represents the beginning of the drought stress. The pore water conductivity is represented by the mean and EC 1:5 is represented by the lower and upper whiskers, 25% and 75% quartiles, and median (n = 8). Black dots represent outliers. Three ECp measurements per pot were taken daily while one soil sample was extracted at the end of the experiment to measure EC 1:5. NS = No salinity, S = Salinity.

Leaf chlorophylls

Neither salinity nor biostimulants impacted significantly the concentration of chlorophylls a and b but the ratio of chlorophyll a over chlorophyll b was significantly reduced by salinity (Fig. 7.13). Salinity caused a decrease in carotenoids, increasing the ratio of total chlorophylls over carotenoids (Fig. 7.13).

Stomatal conductance

Salinity reduced midday stomatal conductance at 35, 41, and 46 DAT while the use of biostimulants did not show significant effects at any time point (Fig. 7.14).

Following these results, no significant differences in stomatal conductance due to either salinity or biostimulants were noted throughout the day at 26 DAT (Fig. 7.15a). At 35 DAT, salinity significantly reduced stomatal conductance at 3, 6

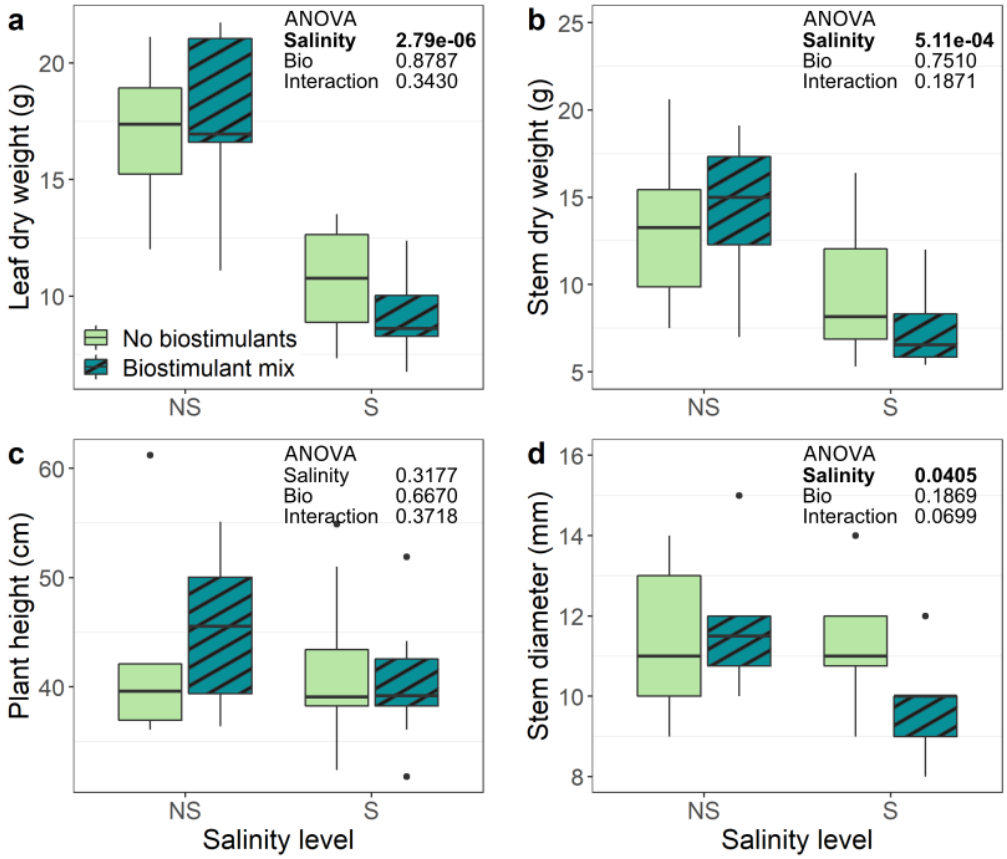


Figure 7.12: Plant morphological characteristics of the African eggplant at the end of the salinity, drought, and biostimulant treatment. Sub-plots represent **(a)** leaf dry weight, **(b)** stem dry weight, **(c)** plant height, and **(d)** stem diameter. The dataset is represented by the lower and upper whiskers, 25% and 75% quartiles, and median (n = 8). Black dots represent outliers. The p-values of the ANOVAs are shown for each source of variation. NS = No salinity, S = Salinity, Bio = Biostimulants.

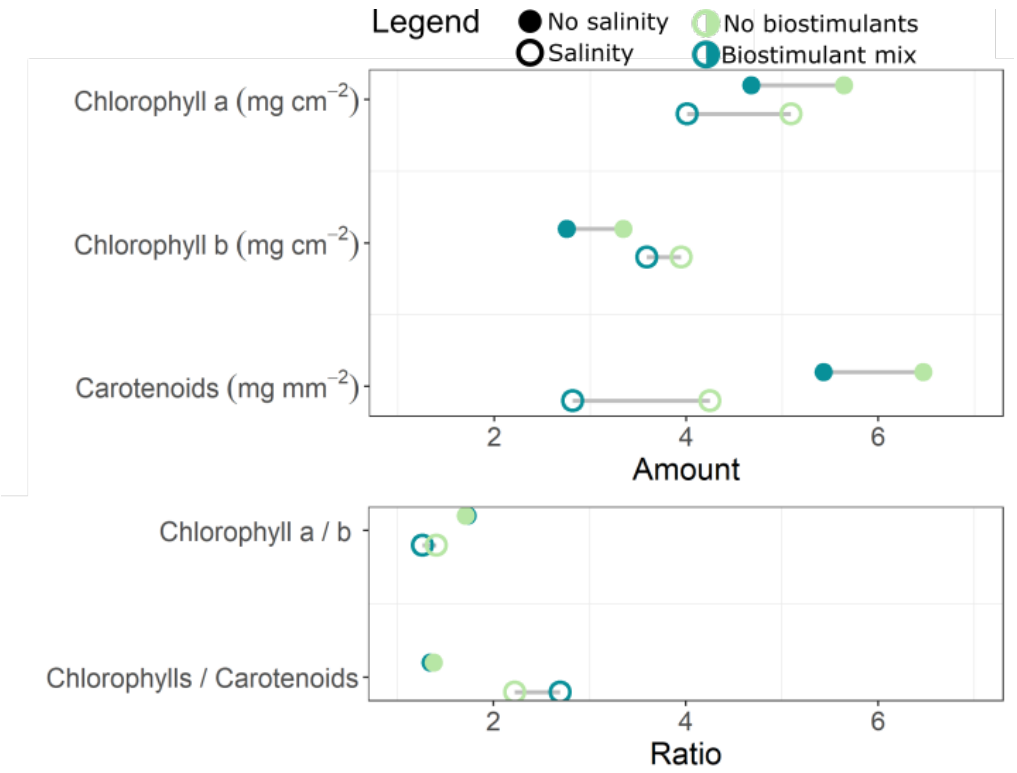


Figure 7.13: Levels of chlorophyll and carotenoid at the end of the salinity, drought, and biostimulant treatment. The unit of each measure is shown on the y-axis while the x-axis represents the amount recorded of each measure depicted on the y-axis. For each measure, two segments are used with the filled circles representing the data from plants grown under no soil salinity while the empty circles depict the data from plants grown in the saline soil. Blue coloured dots represent the level of each measure of the plants under no biostimulants and green dots show the plants grown with a mix of biostimulants (n = 8).

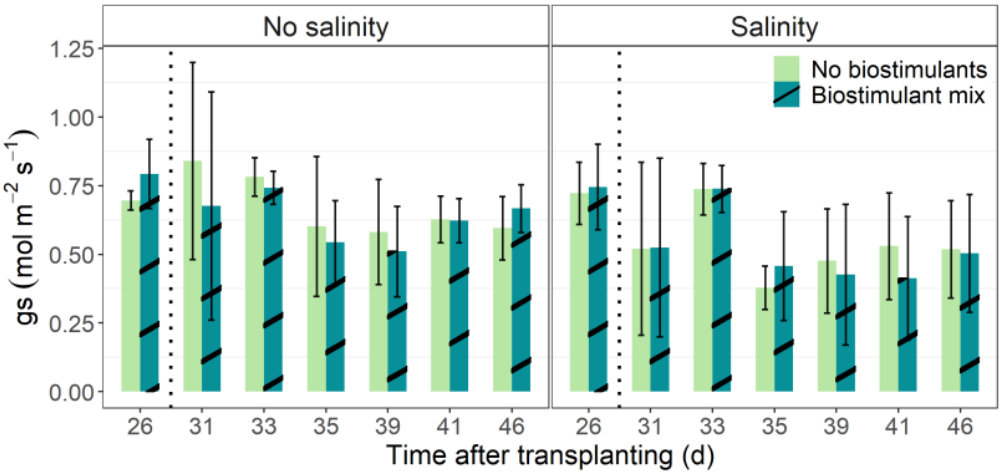


Figure 7.14: Midday stomatal conductance of the African eggplant throughout the salinity, drought, and biostimulant treatment. The dotted line represents the beginning of the drought stress. The data is represented by the mean +/- standard error (n = 8) and was collected on one leaf per plant at the top of the canopy.

(midday), and 9 hours after the start of the photoperiod (Fig. 7.15b). At 41 DAT, a reduction was only observed at 6 and 9 hours while, at 46 DAT, the reduction occurred earlier at 0, 3, and 6 hours after the start of the photoperiod (Fig. 7.15 c,d). In all cases, biostimulants did not have a significant impact on diurnal stomatal conductance (Fig. 7.15).

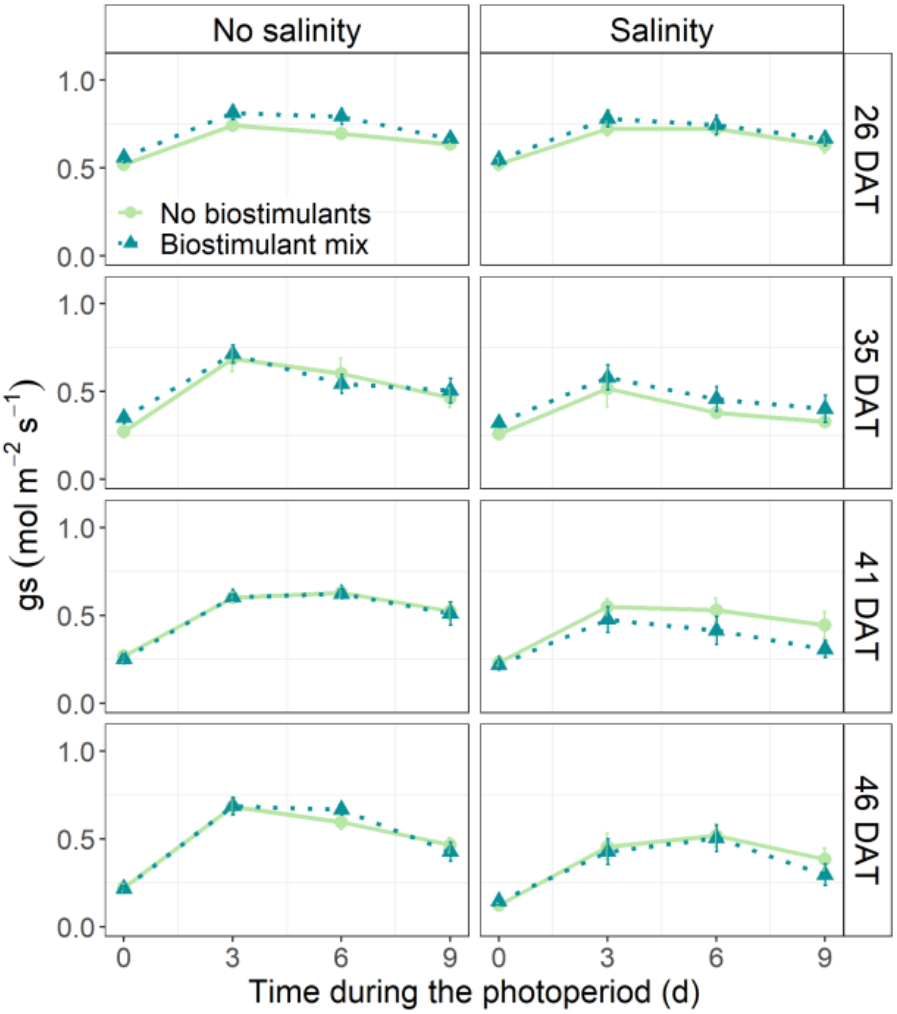


Figure 7.15: Diurnal stomatal conductance of the African eggplant throughout the salinity, drought, and biostimulant treatment at 0, 3, 6, and 9 hours after the start of the photoperiod. Sub-plots represent diurnal stomatal conductance on different days: 26, 35, 41, and 46 days after transplanting (DAT). The data is represented by the mean +/- standard deviation (n = 8) and was taken on one leaf per plant at the top of the canopy.

Biochemical analysis

Salinity decreased leaf protein and antioxidants and the application of biostimulants had no effects on these compounds (Fig. 7.16).

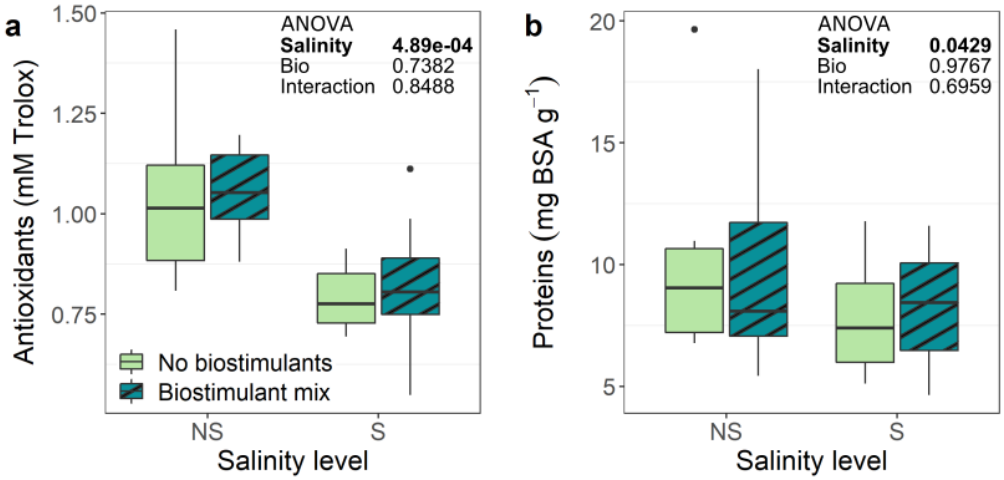


Figure 7.16: Biochemical analysis of **(a)** dry leaf antioxidant concentration and **(b)** fresh leaf protein concentration at the end of the salinity, drought, and biostimulant treatment. The dataset is represented by the lower and upper whiskers, 25% and 75% quartiles, and median (n = 8). Black dots represent outliers. The p-values of the ANOVAs are shown for each source of variation. NS = No salinity, S = Salinity, BSA = Bovine Serum Albumin, Bio = Biostimulants.

Leaf sodium concentration increased under salinity (Table 7.2). In contrast, salinity decreased leaf nitrogen and phosphorus concentrations (Table 7.2). Leaf potassium concentration was reduced by salinity only in the absence of biostimulants (Table 7.2). Under no salinity, however, the presence of biostimulants reduced leaf potassium concentration significantly (Table 7.2). Salinity increased leaf calcium, magnesium, and manganese concentrations and did not significantly impact leaf selenium, iron, and zinc concentrations (Table 7.2). Among those nutrients, biostimulants only impacted leaf iron concentration with a decrease noted under both saline and non-saline conditions when biostimulants were present (Table 7.2).

Table 7.2: Analysis of variance and mean comparisons for dry leaf mineral element concentrations of African eggplants grown under drought and different salinity and biostimulant treatments (n = 8).

Source of variance	Na	N	P	K	Ca	Mg	Mn	Fe	Zn	Se
	mg kg^{-1}			g kg^{-1}				mg kg^{-1}		µg g^{-1}
Salinity (S)	***	*	**	ns	***	***	***	ns	ns	ns
Biostimulants (B)	ns	ns	ns	ns	ns	ns	ns	*	ns	ns
S x B	ns	ns	ns	**	ns	ns	ns	ns	ns	ns
Salinity										
None	7.36^{a}	46.71^{a}	3.93^{a}	22.63	16.56^{b}	5.84^{b}	55.74^{b}	105.2	20.02	39.12
Saline	90.63^{b}	41.44^{b}	3.14^{b}	22.25	25.17^{a}	7.03^{a}	157.1^{a}	105.0	21.24	39.47
Biostimulant										
No application	22.72	46.30	3.69	22.60	19.69	6.50	107.8	112.2^{a}	21.78	38.80
Combination	68.22	41.84	3.38	22.29	22.04	6.36	105.0	97.99^{b}	19.47	39.80
S x B										
None + No application	6.80	50.84	4.23	24.71^{a}	15.94	6.14	56.44	113.6	20.42	38.84
None + Combination	7.93	42.57	3.63	20.54^{b}	17.18	5.54	55.05	96.66	19.61	39.40
Saline + No application	40.92	41.77	3.14	20.48^{b}	23.44	6.86	159.2	110.7	23.14	38.79
Saline + Combination	148.6	41.11	3.13	24.03^{ab}	26.90	7.19	155.0	99.32	19.34	40.15

*Na = Sodium, N = Nitrogen P = Phosphorus, K = Potassium, Ca = Calcium, Mg = Magnesium, Mn = Manganese, Fe = Iron, Zn = Zn, Se = Selenium. NS, *, **, *** Non-significant or significant at P ≤ 0.05, 0.01, 0.001, respectively. Means not sharing any letters within each column are statistically different according to Tukey's test (P = 0.05).*

Pearson's correlation

Varied significant correlations were observed between the treatment (Fig. 7.17). While leaf magnesium and nitrogen concentrations were negatively correlated to both leaf and stem dry weight without the use of biostimulants, this correlation was not observed when biostimulants were used (Fig. 7.17). In fact, under no salinity, the use of biostimulants led to a positive correlation between leaf dry weight and leaf magnesium concentration (Fig. 7.17c). The most noticeable effect of the combination of salinity and biostimulants was seen in the correlations between chlorophylls and leaf nutrients. Chlorophylls were only significantly correlated to leaf magnesium, phosphorus, potassium, calcium, zinc, and nitrogen concentrations under salinity and biostimulants (Fig. 7.17). In contrast, chlorophylls were not significantly correlated to any leaf nutrient under no salinity, while only with leaf iron concentration under salinity alone (Fig.

7.17). The use of biostimulants also impacted the correlations between leaf nutrient concentrations. Leaf zinc concentration was positively correlated with leaf potassium concentration only when biostimulants were used (Fig. 7.17). Under control conditions, leaf zinc and potassium were not correlated with any other leaf nutrient recorded (Fig. 7.17a).

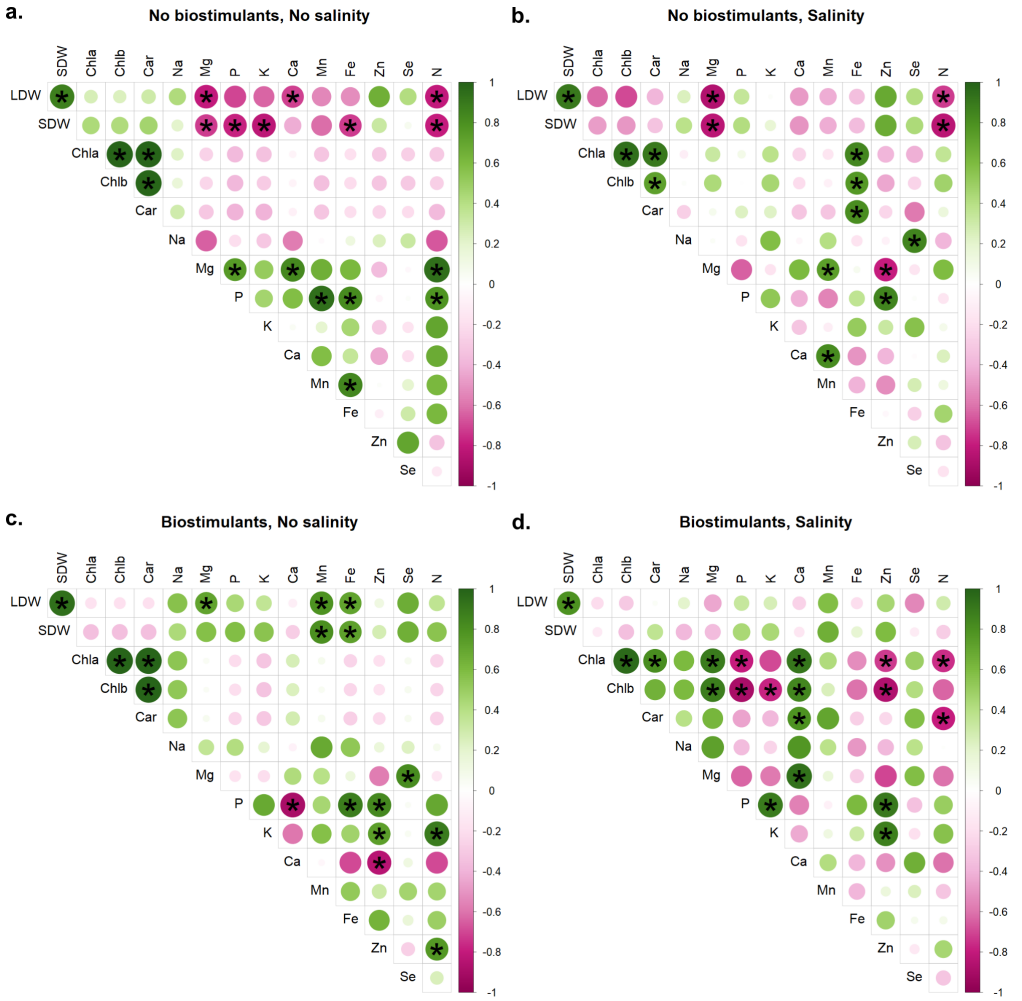


Figure 7.17: Pearson's correlation between plant morphology and leaf nutrient concentrations. Sub-plots represent the Pearson's correlation of sub-groups **(a)** no biostimulants, no salinity (control), **(b)** no biostimulants, salinity (salt stress), **(c)** biostimulants, no salinity (biostimulant effect), and **(d)** biostimulants, salinity (combination). Stars denote the statistically significant correlations at 95% confidence interval (n = 8). The colour represents the level and direction of correlation with dark pink representing negative correlation, white no correlation, and dark green positive correlation. LDW = Leaf Dry Weight, SDW = Stem Dry Weight, Chla = Chlorophyll a, Chlb = chlorophyll b, Car = Carotenoids, Na = Sodium, Mg = Magnesium, P = Phosphorus, K = Potassium, Ca = Calcium, Mn = Manganese, Fe = Iron, Zn = Zinc, Se = Selenium, N = Nitrogen.

7.4 Discussion

When drought and salinity co-occur, they often exacerbate the negative impacts of either stress alone due to the drought-like effect of salinity, as observed in tomato and eggplant (*S. melongena* L.) (Hannachi et al., 2023; Ors et al., 2021). Combining various biostimulants with distinct modes of action might hold the potential to enhance plant resilience to these stresses (Butkevičienė et al., 2022; Rouphael & Colla, 2018). The effectiveness of biostimulants depends, however, on several factors such as the stress intensity and crop involved (Malik et al., 2020).

In the current study, soil salinity was increased by the addition of salts directly on the soil before transplanting. That resulted in an increased pore water conductivity early on in the first experiment. The differences observed between the irrigation treatments might be due to the limited effectiveness of the WET sensor in detecting pore water conductivity at low moisture content as this measure relied on the electrical conductivity of the water available to the plants. The results reported in this current study might thus not be representative of the ECp level experienced by the plants under deficit irrigation. A similar consideration needs to be taken for ECp measurements during experiment 2 as drought treatment was initiated on the 28^{th} day after transplanting. The end-of-experiment measure of soil conductivity confirmed the significant increase of soil salinity during experiment 1 with no effect of the irrigation level, but a similar increase was not seen in experiment 2.

The application of biostimulants did not result in shoot development improvement in this current study, contrary to previous findings in lettuce (*Lactuca sativa* L.) where combining microbial compounds and PHs improved shoot weight under salinity and non-stressed conditions (Rouphael et al., 2017). In maize (*Zea mays* L.), the combination of microbial compounds with PHs led to

comparable improvements in plant growth as using each biostimulant individually, highlighting that there are limitations to biostimulant synergy (Rouphael et al., 2020). In the case of tomatoes, research by Duc et al. (2018) revealed that only one of the two arbuscular mycorrhizal fungi tested provided benefits under heat, drought, and their combination. These gains, although significant, were not enough for the plants to make a full recovery (Duc et al., 2018). The use of these microbial compounds was therefore not sustainable on an industrial scale due to their cost not being covered by the gains. In another study by Zuluaga et al. (2023) examining different PHs on tomato and lettuce, the origin of the compounds affected highly their effectiveness under salt stress. Additionally, PHs showed their greatest effectiveness in the most sensitive vegetable tested, having only a negligible positive effect on the least sensitive crop (Zuluaga et al., 2023). Biostimulants appear thus to be most effective in specific conditions that may not have been met in this study. Compounds with different modes of action than those used in this current study or under a different stress level could potentially impact the African eggplant tolerance by interacting with different mechanisms (Malik et al., 2020).

The inefficiency of the biostimulants used was also noted in the gas exchange dynamics. Both salinity and drought stress decreased midday stomatal conductance, which is consistent with prior observations across various cultivars and crops (Ors et al., 2021; Ors & Suarez, 2017; Yang et al., 2019). It is worth noting that the initial increase in stomatal conductance and CO_2 uptake observed in the current study in Meketan due to salt stress is less commonly reported. This boost might be attributed to the increased leaf magnesium concentration under saline conditions, which is positively correlated to CO_2 assimilation rate (Hauer-Jákli & Tränkner, 2019). As the stress progressed, however, the increased magnesium concentration might not have been sufficient to sustain photosynthesis activity due to the increased damage to various components. As salinity triggers a two-

fold stress in plants, different tolerance mechanisms are often initiated throughout the stress which might also explain the opposite response reported in this current study (Machado & Serralheiro, 2017). While the initial responses might have aimed at promoting the assimilation rate and stomatal conductance to withstand the stress, these parameters were eventually reduced due to the stress intensity, a trend also observed in beet (*Beta vulgaris* L.) (de Melo Filho et al., 2020). An alternative explanation for these results could be related to changes in diurnal photosynthesis dynamics. In the current study, salinity reduced stomatal conductance toward the end of the day during the early stages of the stress while a reduction during the early hours of the day was observed later during the stress. Although most studies have reported reductions in stomatal conductance throughout the day under stress, as Vongcharoen et al. (2019) in cassava (*Manihot esculenta* Crantz) and Ashwani et al. (2019) in wheat (*Triticum aestivum*), alterations in the diurnal pattern under stress have also been observed. In the study by Venkat et al. (2023), for instance, salinity increased stomatal conductance of spinach (*Spinacia oleracea* L.) at 10:00 and 18:00 but not at 13:00 during the early stages of the stress. Stress might thus impact the time stomatal conductance peaks, leading to an increase noted at one time point but not at others. The initial increase recorded in the Meketan cultivar must be interpreted with caution, however, as no such increase in stomatal conductance was observed in the other cultivar tested, Kotobi. This might be due to cultivar differences but also to the different stress intensity, with Kotobi experiencing a lesser salinity stress.

The combination of reduced stomatal conductance, lower assimilation rate, and decreased growth ultimately resulted in a diminished fruit yield in Meketan, a common response to salinity and drought stress in tomato (Dou et al., 2023; Yang et al., 2019). Surprisingly, while smaller and lighter fruits were produced under individual salinity and drought stress, their combined impact did not lead

to an additive effect. The further reduction in yield under the stress combination was primarily due to a reduction in the number of fruits produced per plant instead. This contradicts previous observations in the brinjal eggplant where the stress combination reduced yield due to a reduction in both fruit number and weight (Hannachi et al., 2023). The effects of stress combination on tomato fruit production were significantly stronger than individual stresses when both salinity and drought were present at a high level in a study by Zhang et al. (2022). The levels of stress applied in this study may partially explain the non-additive nature of the results in fruit morphology, being too low for the African eggplant. Additionally, inherent tolerance mechanisms may also explain the current observation where resources might have been used to produce fruits reaching full maturity rather than maintaining the number of fruits produced. One interesting finding is that the number of fruits produced was negatively correlated to leaf phosphorus and potassium concentrations both under control conditions and the stress combination, but not under individual stresses. As plants enter the fruiting stage, leaf potassium and phosphorus concentrations tend to decrease due to their high mobility, relocating to other parts of the plant such as fruits (Vilhena et al., 2022). This explains the negative relationship between the number of fruits produced and the concentration of phosphorus and potassium in leaves. Under individual stresses, however, other processes might be triggered to maintain fruit number and photosynthesis, limiting the clear correlation (Ma et al., 2020). The stress combination increases the overall stress intensity, which was observed to impact the transport dynamics of potassium and phosphorus differently when compared to mild stress in previous studies, potentially explaining the negative correlation observed again under the combination of salinity and drought (Mohawesh, 2016).

Fruits grown under stress showed similar nutritional value to their counterparts regarding antioxidants, magnesium, zinc, and iron concentrations. These results

differ from previous studies in *Solanum* which suggested a nutritional improvement under stress due to the activation of the secondary metabolism (Dere et al., 2022; Dou et al., 2023; Klunklin & Savage, 2017). A decrease in total antioxidants in leaves as observed in this current study is, however, often recorded as in *S. villosum* Mill. and *S. tuberosum* L. (Alhoshan et al., 2019; Ben-Abdallah et al., 2019). A difference between metabolite concentration in different plant organs, as seen in this current study for leaf and fruit antioxidants and magnesium, is also regularly reported and suggests specific effects of stress on metabolite transport and not only production, as seen in tomato (Babu et al., 2012). Unlike the increase in both leaf and fruit proteins observed in this current study due to salinity, previous studies in tomato reported a decrease in leaf proteins while fruit protein concentration was either unchanged or increased (González-García et al., 2022; Pérez-Labrada et al., 2019). In a study by Aazami et al. (2021), salinity initially increased the leaf protein content of a tomato cultivar before decreasing it above 75 mM NaCl, suggesting the importance of stress intensity regarding protein dynamics. An increase in proteins under stress helps to sustain photosynthesis, detoxify reactive oxygen species, and activate stress tolerance pathways (Ma et al., 2020). Maintaining potassium concentration under stress in the current study may have supported nitrogen uptake, ultimately promoting protein production (Mengel, 2015). In Kotobi, salinity and drought reduced leaf potassium concentrations, alongside leaf nitrogen and protein concentration. This was also observed in potato plants in the study by Kafi et al. (2021) in which they also used biostimulants which enhanced tolerance in part due to their effect on increasing potassium uptake. Therefore, the maintenance of potassium uptake and mobility is key in salinity tolerance to limit the increase in sodium over potassium ratio and preserve a range of metabolisms (Ahmad et al., 2023; Rasel et al., 2021).

7.5 Conclusion

The combination of salinity and drought reduced significantly the African eggplant's growth and photosynthesis but the concentration of chlorophylls were maintained in both cultivars tested. While the final yield was further reduced by the stress combination, the fruits produced were of similar quality to the ones produced under each stress. Maintaining the production of marketable fruits under stress is key to ensuring farmers' income and food security, but might not be sustainable using the cultivar tested here due to the slight decrease noted in fruit morphological characteristics. The uptake of potassium, phosphorus, and magnesium were not negatively affected by salinity surprisingly, suggesting some tolerance mechanisms in place to maintain a certain level of ion homeostasis. The biostimulants used in this experiment did not positively impact the African eggplant cv. Kotobi. The reasons behind this inefficiency are unclear, however, and further research is needed to understand the impact of different biostimulants on different cultivars.

Chapter 8

Key results and discussion

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8.1 Introduction

Despite worldwide efforts to mitigate the negative impacts of climate change, farmers find themselves with lower yields and increased insecurity due to environmental stresses (Madegwa et al., 2016). In addition, a loss of crop diversity and the focus on production without considering the crop's nutritional quality in the breeding process have negative impacts on consumers' health with hidden hunger, representing a deficiency in minerals and vitamins, present in almost every continent (Gödecke et al., 2018; Gómez et al., 2013). Food security is key to achieving peace and prosperity and is one of the 17 sustainable development goals (SDGs) set out by the United Nations in 2015 to 'end food, achieve food security, and improve nutrition' (United Nations, 2015). Due in part to the COVID pandemic, this goal is not yet achievable by 2030 as initially set. Further work and research in the area of sustainable and resilient agriculture is needed to improve food security worldwide, especially in Africa.

Part of the solution to improve food security is to rely on indigenous vegetables (IVs). Indeed, IVs are diverse, offer tolerance to local environments, and require fewer inputs in terms of water and fertilisers than conventional crops (Akinola et al., 2020). They are, however, not broadly used due to a range of factors including irregularities in supply, lack of world markets, and lack of information on their use. During a priority-setting exercise by stakeholders, the African eggplant, an important African IV, has been selected as a major strategic crop for breeding and ensuring food security in Africa by the World Vegetable Center (Dinssa et al., 2016). Its importance in current food systems was confirmed by the pilot study conducted in Arusha (Appendix A) where the African eggplant was shown to be highly popular in the markets mapped. As Africa is at high risk of increased environmental stress, it is important to understand the abiotic stress tolerance of this crop and, if any, whether it is of economic value or only

a survival mechanism for its use in the future.

The main aim of this thesis was to understand the potential of the African eggplant under stressed environments by defining its response to heat, drought, and soil salinity. The African eggplant's morphology, physiology, and biochemistry under these stresses individually or combined were analysed to explore its tolerance thresholds and mechanisms. This will help to inform farmers, climate change strategies relying on indigenous vegetables, and breeding programmes interested in stress tolerance.

8.2 The African eggplant & environmental stresses

The African eggplant cultivars tested in Chapter 3 are commercialised for different purposes and grown in different regions. Only one cultivar from the Gilo and Shum groups was tested for each, DB3 and E11, respectively, while the rest of the cultivars belonged to the Kumba group and were produced by Novagenetic. The market of Novagenetic is principally in West Africa, DB3 is mostly distributed in Eastern and Southern Africa and E11, a leafy genotype, is grown principally in Central and Western Africa (Stoilova et al., 2019). Different parts of Africa are subject to different environmental conditions and abiotic stresses and this was considered in the selection of the studied cultivars. Northern and Western Africa are likely to experience the highest heat increase, for example (World Meteorological Organization, 2022). Sea level increase is prevalent all around the continent, but rainfall patterns are changing, with Southern and Eastern Africa receiving abnormally low precipitation (World Meteorological Organization, 2022). Popular varieties in each region need thus to be mostly tolerant to the main stress or stresses experienced. Heat stress was thus studied on one Kumba cultivar and the Shum cultivar. DB3 was used for previous research on

drought tolerance as part of the larger project on the African eggplant (project reference BB/R020655/1) and was thus studied here under salinity due to these stresses being often present together. Issues were faced when growing the cultivars DB3 and E11, however, with poor germination and homogeneity. Cultivars from the Kumba group were therefore used for the rest of the experiments due to their higher germination rate and better growth under glasshouse in the UK. Still, these cultivars are also likely to be used in regions prone to drought and salinity and research on their tolerance is thus relevant.

Within the Kumba cultivars tested, Kotobi and F1 Djamba produce oval and almost smooth fruits and Meketan and N'Goyo cultivars produce flat and ribbed fruits, which are larger and have a longer maturity (Technisem, 2023). These morphological differences explain partially the differences observed regarding their growth and gas exchange dynamics. However, they responded similarly to drought or salinity stress. This suggests similar responses among the cultivars tested at the vegetative stage. Interestingly, F1 Djamba, which has a particularly high fruit production when compared to the other Kumba cultivars developed by Novagenetic, was the only cultivar to increase significantly the chlorophyll a level under salinity stress, the only parameter which was impacted differently among cultivars under stress. Despite the reasons behind this increase still being unclear, chlorophyll content has been positively linked to the assimilation rate and might thus be promoted in F1 Djamba due to its inherent high yield potential (James et al., 2002; Sim et al., 2015). Although not statically significant, some other differences were noted with the reduction in stomatal conductance in Kotobi being smaller than the one observed in the Meketan cultivar. This observation might be important in the field with a more conservative stomatal conductance potentially providing better yield protection under drought (Nemeskéri & Helyes, 2019). Meketan had a higher stomatal conductance than Kotobi under control levels, a characteristic positively correlated with fruit yield, but the better

maintenance of stomatal conductance by Kotobi might provide stability under stress necessary for maintaining production (Faralli et al., 2019; Nemeskéri & Helyes, 2019).

Chapter 4 highlighted that heat and drought generally led to a predominant effect of drought on plant growth and chlorophyll content in the Shum cultivar E11. The predominance of one stress over the other in studies combining stresses is mostly due to the individual stress intensity and crop tolerance (Nadeem et al., 2023). Leaf production was not hindered under a 10°C increase from the optimal temperature tested in this study, suggesting its growth potential even under heat waves. When studying the combination of heat and salinity on F1 Djamba cultivar in Chapter 5, however, a 10°C increase reduced leaf production, highlighting some differences in responses under heat. While the E11 genotype is produced for its leaves, F1 Djamba is cultivated for its fruits. Their development and selection might thus have been based on different criteria, leading to genetic differences at the basis of the differences observed here. Under heat, leaf production of leafy vegetables is sometimes higher than that of fruity vegetables under the same temperature level, as seen in lettuce and tomato (Rajametov et al., 2021; Sublett et al., 2018). This observation, however, is highly dependent on the cultivars tested and a range of leafy vegetables show deleterious effects of heat quickly, highlighting the relevance of cultivar E11 in heat-prone areas (Chaudhary et al., 2022). It has to be noted that leaf fluorescence parameters $\phi 2$ and ϕNPQ were not affected in the study detailed in Chapter 5 under the 10°C increase, suggesting processes in place to maintain photosynthetic activity rather than leaf production in the fruiting crop and supporting its tolerance to heat for some important characteristics. In addition, while the experiment of Chapter 4 was set up as two transient elevated temperature periods lasting 8 days each, the heat was applied as one block of 12 days in the experiment of Chapter 5. A longer duration of heat stress damaged further different charac-

teristics of a calendula (*Calendula officinalis* L.) cultivar in a study by Nazdar et al. (2019), suggesting a potential impact of the different lengths used here as a reason behind the contrasting effects observed. These methodologies were used to answer different research questions. In Chapter 4, the focus was on the effects of a recovery period and re-occurring heat stress while in Chapter 5, the focus was on the heat stress itself. The recovery periods after the heat waves in Chapter 4 are important to take into consideration when doing comparisons as some developmental parameters, such as membrane stability, chlorophyll content, and osmotic stress markers are highly dynamic and recover quickly after the stress as seen in tomato (Lee et al., 2023).

After studying the vegetative growth of the African eggplant under different stress combinations, the flowering and fruiting stages of this vegetable under stress were investigated. Only soil salinity and drought in these stages were tested due to the space required for the plants to reach the fruiting stage. As the increase in temperature could only be accurately controlled in growth cabinets where space is limited, heat stress was not tested on the fruiting stage.

In Chapter 6, the pore water conductivity was significantly increased throughout the stress period at every salinity level tested despite the final EC 1:5 not significantly increased for the lowest salinity level. The pore water conductivity measures the electrical conductivity of the water available to the plant while EC 1:5 is measured on a soil:water solution. This difference can lead to some discrepancies in measurements as observed in this research, despite the strong positive correlation between the two values (Kumar et al., 2008). Interestingly, soil salinity reduced fruit production but the fruits were of similar quality in terms of size and weight as described in Chapter 6. This is an important observation as maintaining the production of marketable fruits under stress is necessary to ensure economic value. However, there is also a limit to the number of fruits being produced to maintain economic value. Soil salinity also reduced stomatal con-

ductance but not the CO_2 assimilation rate during the fruiting stage, highlighting the non-limiting effect of stomatal conductance on downstream photosynthesis activity and the maintenance or recovery of the assimilation rate thanks to other protective mechanisms. Chapter 6 aimed to determine a certain salinity tolerance threshold of the African eggplant but also to highlight potential tolerance mechanisms. A clear salinity tolerance threshold was not determined due to the variability of soil salinity level throughout the experiment but the changes recorded in leaf nutrient concentration are still valuable insights into some tolerance mechanisms of the African eggplant due to their various role in both the primary and secondary plant metabolisms (Ahanger et al., 2016; Fageria, 2001). The mechanistic analysis of tolerance is limited in this current chapter, however, due to the absence of measurements of other compounds such as sugars, proteins, or antioxidants.

Salinity's harmful effects were further exacerbated when drought was present as explored in Chapter 7 on cultivar Meketan. These stresses often occur concurrently due to the link between long-term irrigation, needed in drought-prone regions, and salinity build-up (Shahid et al., 2018). An additive effect was observed in most of the measured developmental characteristics, resulting in highly damaged plants. The leaf area was, for example, further decreased by the stress combination which, alongside a clear reduction of stomatal conductance, reduced eventually overall photosynthesis activity and fruit production. These effects have been reported for a range of crops due to the adaptation to reduce water loss under the osmotic stress caused by both stresses (Angon et al., 2022). Of note, fruits of similar quality were produced under the stress combination and individual stresses. Unlike in the cultivar DB3 grown under salinity alone in Chapter 6 where the fruit characteristics were maintained but the number of fruits dropped, fruits produced under salinity in Chapter 7 were smaller and lighter but the number of fruits produced per plant was maintained. A similar

observation was made for plants grown under drought, suggesting comparable responses to these stresses. The combination of salinity and drought, however, did not further reduce the morphology of the fruits but reduced the number of fruits produced instead. This interesting observation suggests that the African eggplant changes its tolerance mechanisms after a certain threshold, with a focus on maintaining fruit quality over fruit number at higher stress levels. In tomato, fruit diameter was also reduced at a lower salinity level than fruit number (Ahmed et al., 2017). Fruit diameter was not further reduced at the highest salinity level, however, whereas fruit number was, suggesting a similar maintenance of fruit quality over fruit number at high-stress levels (Ahmed et al., 2017). Whether this threshold was attained due to the combination of the two stresses in the current thesis or would also happen if one stress alone was high enough is unknown, though.

When introducing biostimulants to Kotobi plants under the combined drought and salinity stress in Chapter 7, no improvements were observed, in contrast to previous claims about their benefits in stressed conditions (Malik et al., 2020). It has to be noted that the biostimulants used during this study were all made and commercially available in the United Kingdom. The origin, as well as the composition of the biostimulants and soil quality, has previously been demonstrated to impact their beneficial effects (Li et al., 2022). Biostimulants developed in the regions where the African eggplant originates might thus be proven more successful, as seen in West Africa on different crops when using two organic amendments (Eldon et al., 2020). In addition, developing biostimulants in areas where the African eggplant is grown could be a new income stream for farmers and workers, thus increasing the region's economic stability (Eldon et al., 2020). Other reasons might be behind the absence of benefits observed in this research, such as the timing of their application or the stress intensity (Rouphael & Colla, 2020).

Despite measuring leaf biochemical contents under the combination of salinity and drought in Chapter 7, no clear tolerance or sensitivity mechanism was highlighted with an increase and decrease in leaf protein in Meketan and Kotobi, respectively. Response pathways of different African eggplants might not be common, underlying the need to quantify these responses in different cultivars before developing new biostimulants to ensure their positive impact.

8.3 Implication for stakeholders

Despite the African eggplant being under-researched and lacking world markets, it is grown by a large proportion of small-scale farmers in sub-Saharan Africa and the focus of multiple breeding programmes such as the ones run by the WVC, Novagenetic and the Uganda Christian University, leading to a range of stakeholders interesting in this vegetable (Han et al., 2021). While the cultivars used in this thesis were developed mostly for yield gain and/or biotic resistance, this research focused on their tolerance to abiotic stress and nutritional quality. Both factors are essential to consider for ensuring farmers have resilient fields and a sustained income while increasing the local population's health by establishing a high standard for food nutritional quality even under environmental stress. Farmers are already adopting strategies to cope with the effects of climate change and research is important to offer them the best solutions possible (Shisanya & Mafongoya, 2016).

When comparing different cultivars under stress at the vegetative stage, none stood out in terms of their tolerance but differences in responses were observed in later stages for selected cultivars. These observations are important for farmers to ensure they can adapt their cultivation methods and cultivar selection based on the prevalent stress they are facing. No cultivar was highlighted to

show particularly high tolerance to either salinity or drought with the absence of clear differences when directly comparing cultivars. Nevertheless, different mechanisms for tolerance were highlighted with changes in gas exchange, fruit production, or nutrient dynamics based on the cultivar tested. These observations might benefit researchers as exploring different tolerance mechanisms is valuable in the development of tolerant varieties to find the best solution for each scenario (Kamenya et al., 2021).

Since gene transfer between eggplant species is possible, the African eggplant is acknowledged as a source of variations in brinjal eggplant breeding (Daunay et al., 2019). This was shown early on by multiple studies which successfully introduced wilt resistance in the brinjal eggplant from the African eggplant (Ano et al., 1991; Toppino et al., 2008). The tolerance to salinity and heat highlighted in this thesis could thus be used to improve the tolerance of related *Solanum* which might have a lower threshold. Since it is currently used as a rootstock for close relatives, the description of its tolerance mechanisms might be interesting for researchers and farmers of other *Solanum* who need to increase the tolerance of their crops more quickly than through breeding (Sabatino et al., 2019).

The choice of microbial and/or non-microbial biostimulant for achieving a specific objective (e.g. increase the crop resistance to environmental stress factors, enhance the nutrient use efficiency, improve crop quality traits) requires a proven knowledge of the biological activity of the product. The lack of efficiency of the mix of biostimulants tested in this thesis is thus relevant for manufacturers as it is important to gain knowledge about the limitations of marketed products. The observations made in this research show potential avenues to be explored in the development of products improving African eggplant tolerance. The yield was, for example, positively correlated to leaf calcium concentration under drought in Meketan (see Chapter 7), thus biostimulants improving calcium uptake and use might lead to positive effects.

8.4 Limitations, workarounds, and future work

Limitations regarding the equipment used have been encountered throughout this thesis. When measuring chlorophyll fluorescence, the SPAD index can be affected by water status and light levels, thus requiring careful analysis (Martínez & Guiamet, 2004). Destructive measurements on the last day of the experiments following the protocol of Wintermans and De Mots (1965) were thus made to confirm the results of the non-destructive SPAD measurements taken throughout the stress. Light intensity was fixed in our case, and extra light was provided in the glasshouse experiment, but this parameter can be highly variable in the field, especially in shaded conditions, limiting the generalisation of the current results to field conditions. Another equipment limitation appeared for the stomatal conductance measurements using the leaf porometer (Model SC-1, Decagon Devices, METER group, United States). These measurements were slow (around 1:30 min per plant), leading to a large time gap between the first and last measurements. To overcome this issue, plants were separated into blocks which included one repetition of each treatment and blocks were done one at a time. This was especially important when different growth cabinets with different conditions were used (see Chapter 4 and 5). In addition, the LiCor LI-6800 instrument, performing quick measurements of photosynthetic gas exchange and chlorophyll a fluorescence, was used when available.

Throughout the thesis, the plants were grown in pots. Poorter et al. (2012) showed a clear link between pot size and dry mass accumulation, with a pot too small or too large potentially impacting the results in an unwanted manner. While care was taken to choose the correct pot size during these experiments, both time and space were limiting factors. The comparison between treatments can still be accurate as the pot size of all the plants from one experiment was the same, but extrapolating these results to ones obtained in different growing

conditions might be limited. In addition, results obtained in controlled environments (glasshouse or growth cabinet) can differ highly from field ones as seen in tomato (Ro et al., 2021). Only a few trials have tested tolerance genes identified in the lab in field conditions and were generally unsuccessful, indicating that indoor effects might not be repeatable in outdoor conditions (Mittler, 2006). In addition, root growth is an important tolerance parameter that will differ between lab and outdoor conditions (Poorter et al., 2016). Growing plants in glasshouses also limited pollination during flowering as no pollinators were used. The meagre yield observed in Chapter 6 might have thus been due to a lack of pollination and be improved in field conditions. A significantly higher yield was obtained in Chapter 7, however, suggesting genetic variability in addition to pollination issues. In either case, the fruit yield recorded might change significantly when grown in the field.

Although the biostimulants tested in the current study have not shown a beneficial effect, further work is needed to explore their full potential under field conditions. A scientific approach needs to be followed to understand whether some biostimulants impact the factors limiting crop productivity. The growing conditions, application strategies, and biostimulant type are all essential characteristics to take into account to identify the best-performing products. Monitoring morphophysiological and productivity traits as was done in this research would be useful for verifying the biostimulant activity throughout the plant's growth, while laboratory analysis could highlight the biochemical effects of biostimulants. A cost-benefit analysis also needs to be performed to ensure the economic viability of biostimulant use on an industrial scale.

This work focused on understanding the morphophysiological and biochemical changes, and detailed gene work, not in focus in this work, would help inform breeding programmes, especially since the draft genome of the African eggplant has been published (Song et al., 2019). This is important to speed up cultivar

development through targeted genetic modification and for the development of molecular markers that could be used as a diagnostic tool at the seedling stage (Kamenya et al., 2021). In addition, a higher number of cultivars could be used in further studies to understand potential genetic variability by including cultivars grown by farmers in addition to ones that have been recently developed. This was done by Mibei et al. (2017) for drought stress screening, for example, and could highlight a range of tolerance, necessary for breeding applications. The work performed during this thesis could be useful to set up stress experiments on the African eggplant and to highlight the most interesting parameters to record such as stomatal dynamics and key nutrient concentrations.

The thesis presented here is laying down the basis for further research on the African eggplant. On 'Web of Science', when searching for the African eggplant and its derivatives (*Solanum aethiopicum*, *Solanum gilo*, scarlet eggplant), 245 results are found. When adding 'stress' as a search keyword, this falls to 35, highlighting the lack of research on the African eggplant, especially when it comes to stress, either biotic or abiotic. The different methods used throughout this thesis explore some of the ways to assess the abiotic stress tolerance of the African eggplant in a scientific setup. For example, leaf fluorescence parameters were important to quantify only at very high temperatures as they were unaffected by the other stresses. Diurnal stomatal conductance measurements were also important characteristics to record and could be used alongside yield quantification. Most of the laboratory analyses done throughout this work have not been previously reported in African eggplant samples, giving baseline values and method confirmation.

The African eggplant has great potential to improve field diversity and resilience but still requires investment to improve aspects of the cultivars liked by farmers and consumers, such as productivity, simplified harvest, post-harvest handling, and wilt tolerance (Adeniji & Aloyce, 2012).

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Appendices

Appendix A

Pilot study in Arusha, Tanzania

Introduction

Thanks to extra funding obtained from the GCRI Trust and the David Miller award, I was able to organise a three-week trip to Tanzania to meet some of the partners working with the African eggplant. My first stop was in TARI Mikocheni, located in Dar Es Salaam. TARI Mikocheni is working with farmers near the coast and is looking at the African eggplant for intercropping purposes. They were particularly interested in the work about drought, salinity, and wilt tolerance (the latter not done as part of this thesis but as part of the overall project, reference BB/R020655/1). The second visit took place at the World Vegetable Center for Southern and Eastern Africa in Arusha. They provided the seeds throughout the project and gave helpful advice.

The first aim of this pilot project was to exchange knowledge with the partners by sharing some results of my PhD and detailing some techniques used in the laboratory. The second aim was to map the presence of the African eggplant in markets around the cities to understand its popularity and marketability.

Methods

A first trial was performed in markets across Dar Es Salaam to test and develop further the mapping protocol. In Arusha, eight local markets were selected based on their location, opening times, and size (Fig.A.1).

Kisongo, Tengeru, and Usariver are only open once a week on different days while Kilombero, Soko Kuu, Sanawari, and Samonge markets are daily markets, open every day.

At each market, a random number between 3 and 8 was generated and used

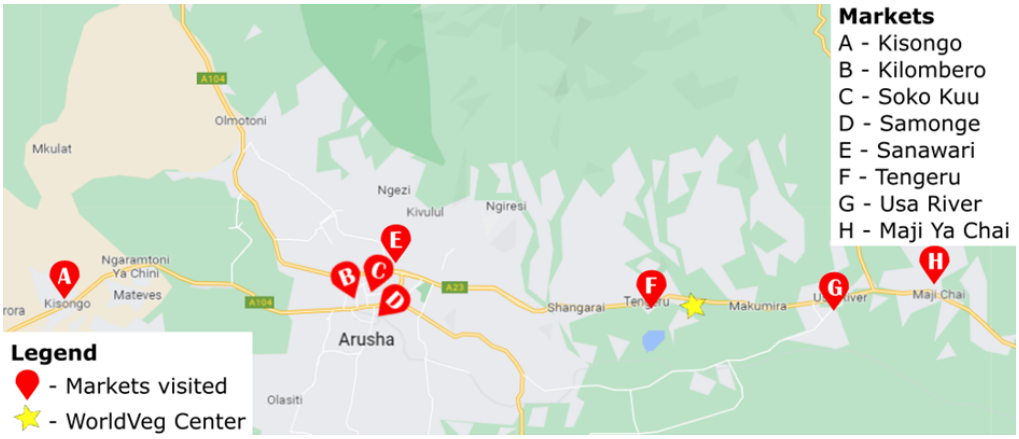


Figure A.1: Location and names of the markets visited as part of the pilot experiment in Arusha.

to sample vendors following a systemic sampling method with up to 40 vendors mapped. The vegetables sold by each vendor were recorded as well as the number of phenotypes of the African eggplant sold. In addition, three random numbers between 1 and 40 were generated to sample the African eggplant from three vendors throughout the market. The fruits' weight, size, and colour (as an indicator of freshness, Fig. A.2) were recorded as soon as possible after collection. The collected fruits were then cut and dried in a fruit dehydrator until they lost all their moisture. They were then packed and sent to NIAB, East Malling, UK (Plant health authorisation no 6996/198773-4 from the Department for Environment, Food, and Rural Affairs and Phytosanitary passport 00173215 from the Ministry of Agriculture, The United Republic of Tanzania) for nutrient analysis. Fruit nutrient concentrations were measured on the dry material by Yara UK (Pocklington, UK).



Figure A.2: Colour-scale used for assessing the collected fruits at the markets. Both varieties are depicted with DB3 at the top and Tengeru White (TW) at the bottom.

Additional figures

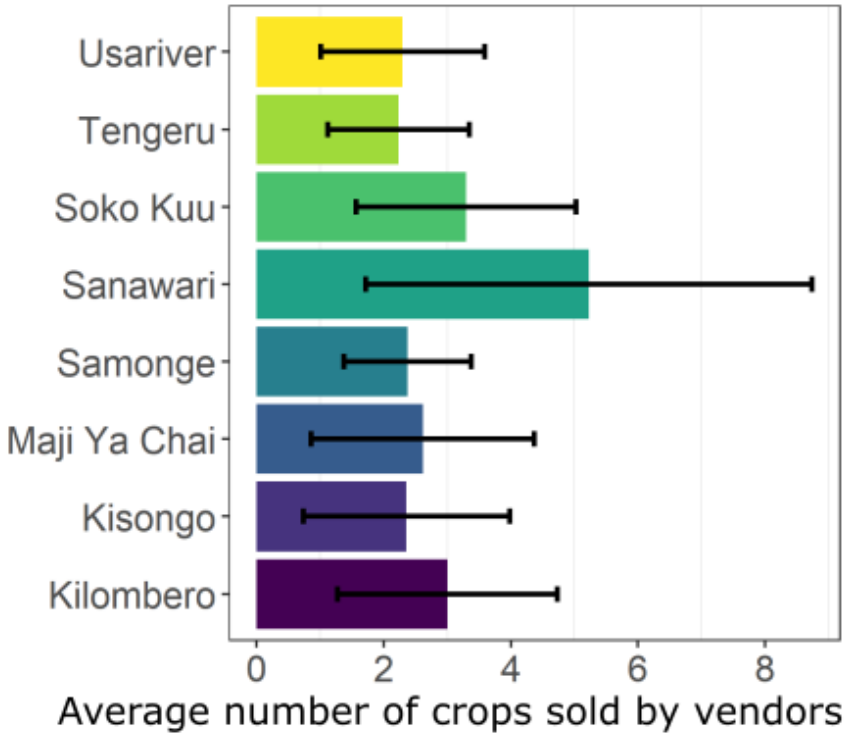


Figure A.3: Average number of fresh vegetables sold by vendors in each market in Arusha. The data is shown as mean +/- standard deviation.

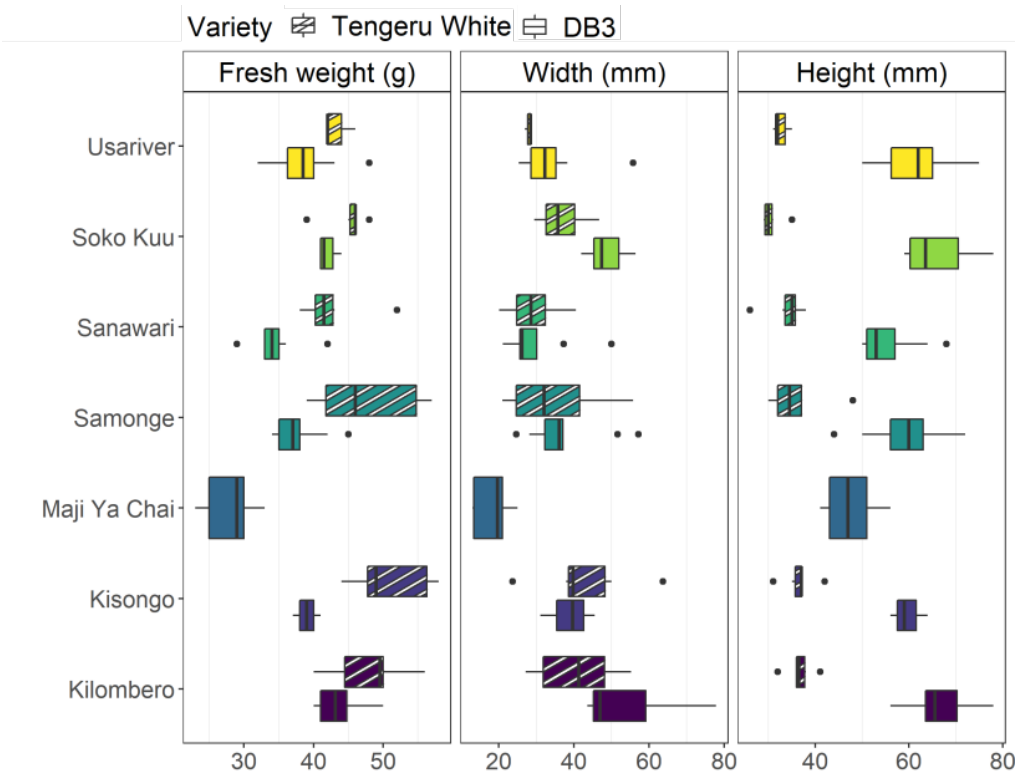


Figure A.4: Morphological characteristics of the fruits bought in markets around Arusha. For each market, both Tengeru White (stripped boxplots) and DB3 (full boxplots) varieties are shown.

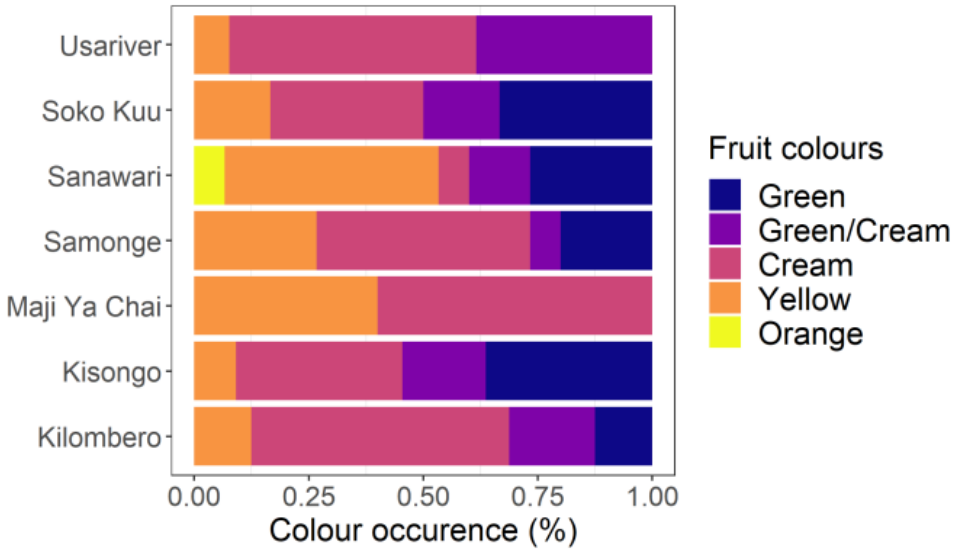


Figure A.5: Occurrence of the six colour categories of the fruits bought in each market.

Appendix B

Environmental data

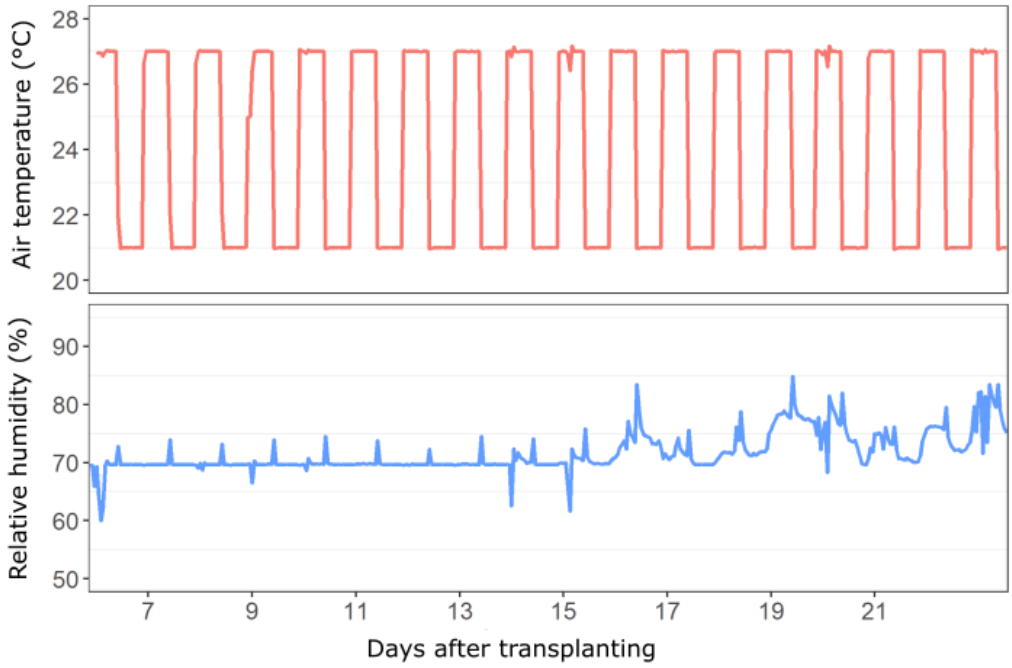


Figure B.1: Temperature and humidity of the three growth cabinets averaged in Chapter 3, Experiment S.

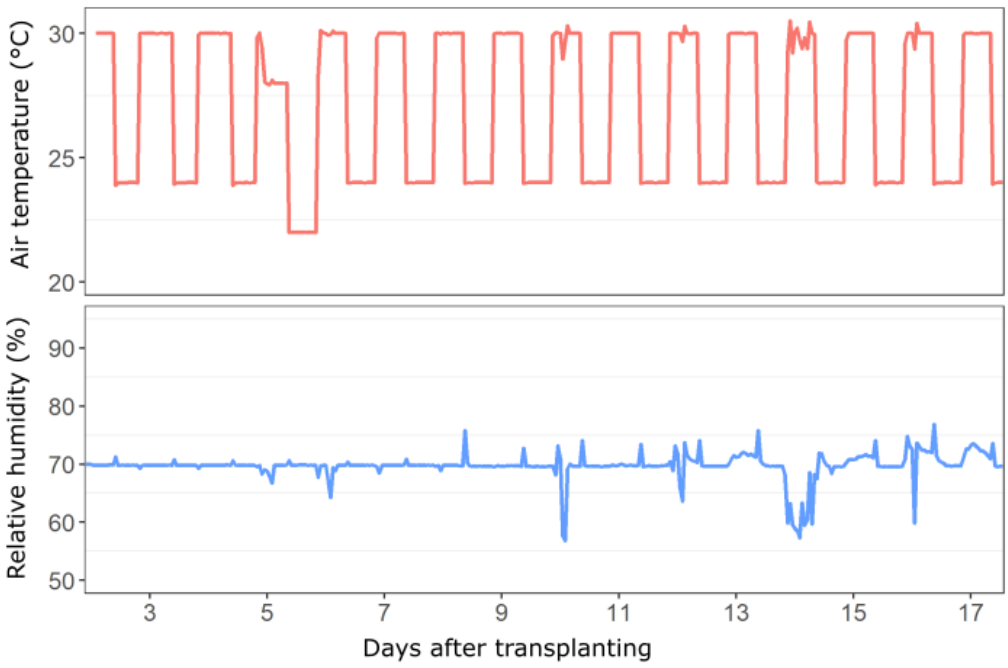


Figure B.2: Temperature and humidity of the three growth cabinets averaged in Chapter 3, Experiment D.

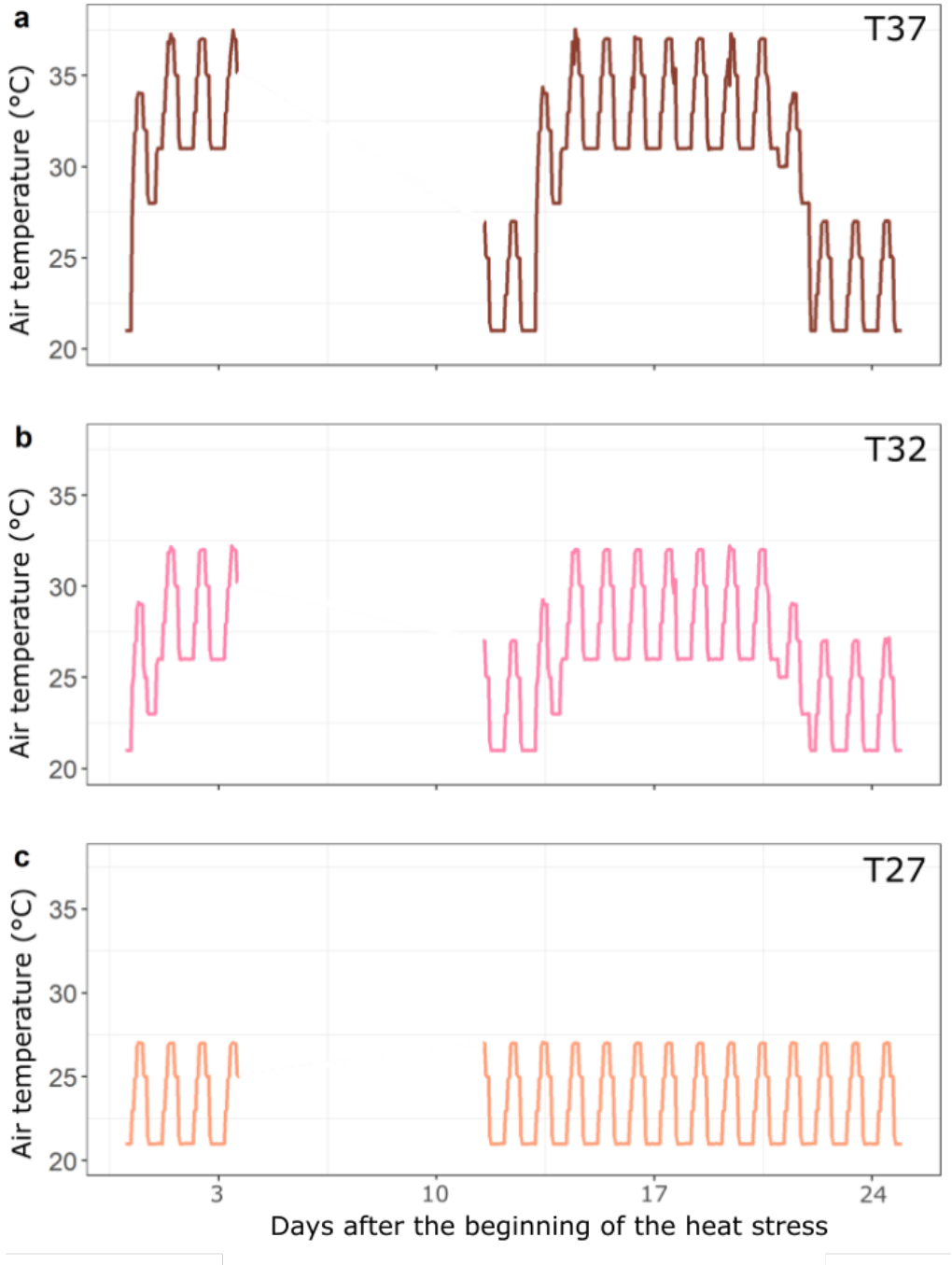


Figure B.3: Environmental data of the three growth cabinets in Chapter 4. Sub-plots represent **(a)** T37 treatment, **(b)** T32 treatment, and **(c)** T27 treatment as recorded by the growth cabinet. The missing data is due to recording failures but the same conditions were set up between 1 and 13 days after the heat treatment (DAHS) than between 14 and 25 DAHS.

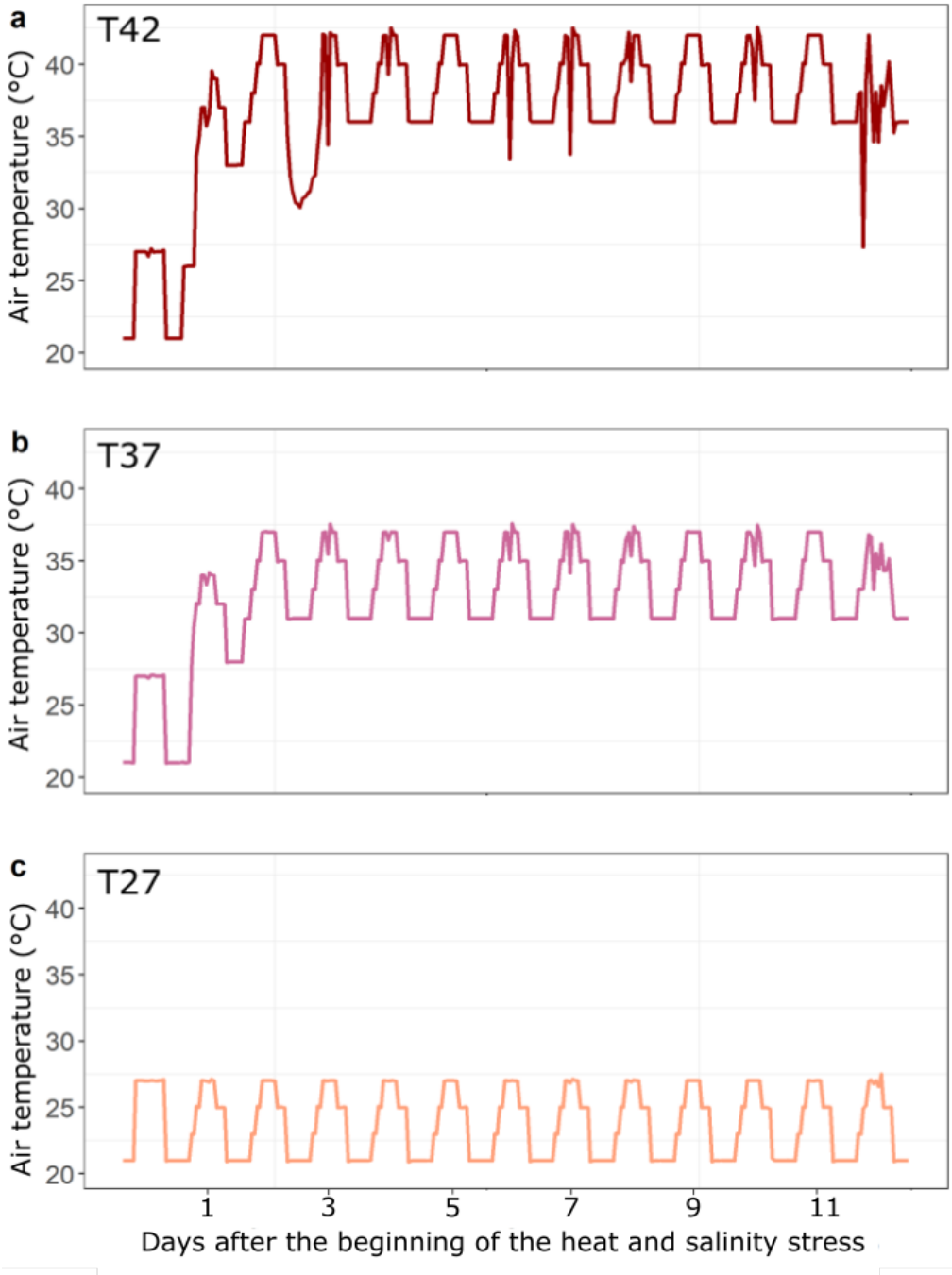


Figure B.4: Environmental data of the three growth cabinets in Chapter 5. Sub-plots represent **(a)** T42 treatment, **(b)** T37 treatment, and **(c)** T27 treatment as recorded by the growth cabinet.

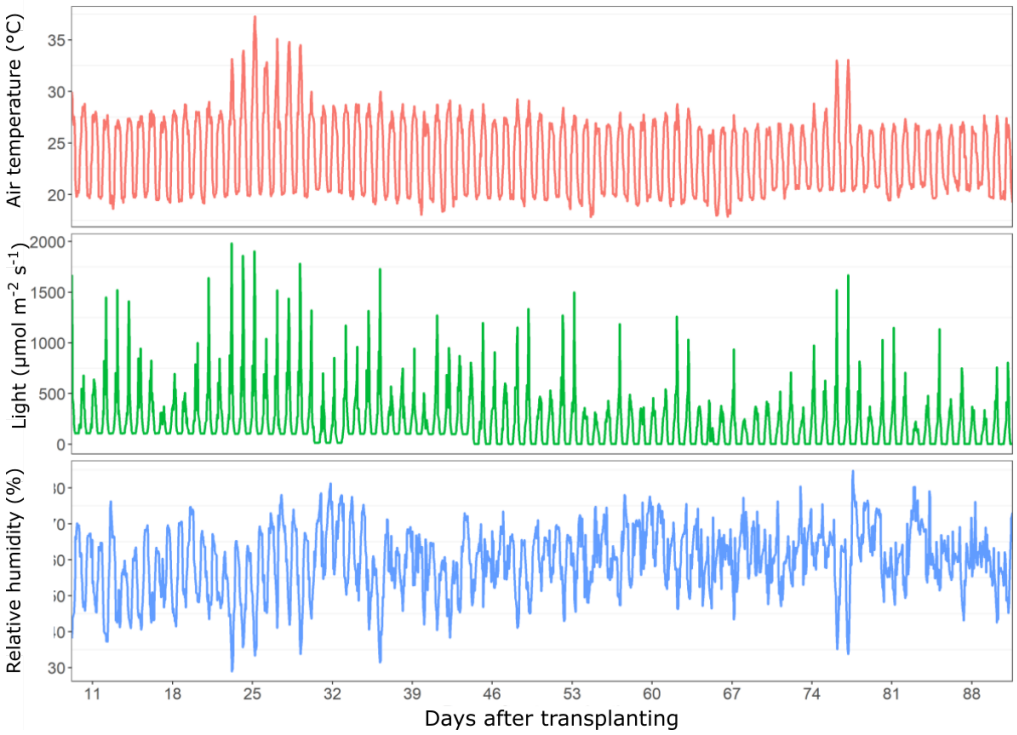


Figure B.5: Environmental data of Chapter 6. From top to bottom: air temperature, light intensity, and relative humidity in the glasshouse throughout the experiment as recorded by data loggers.

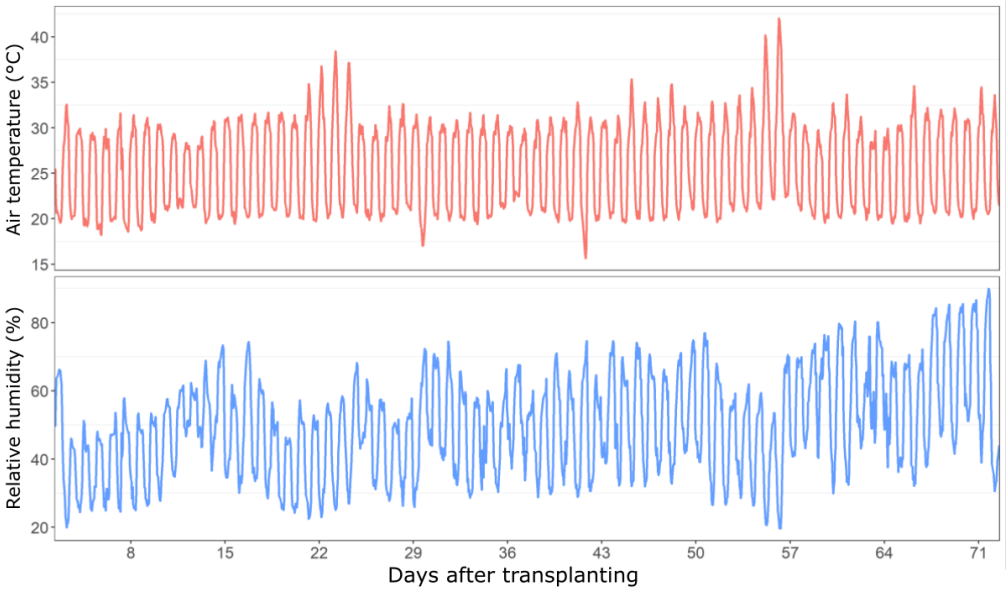


Figure B.6: Environmental data of the salinity and drought experiment in Chapter 7, Experiment 1. From top to bottom: air temperature and relative humidity in the glasshouse throughout the experiment as recorded by data loggers.

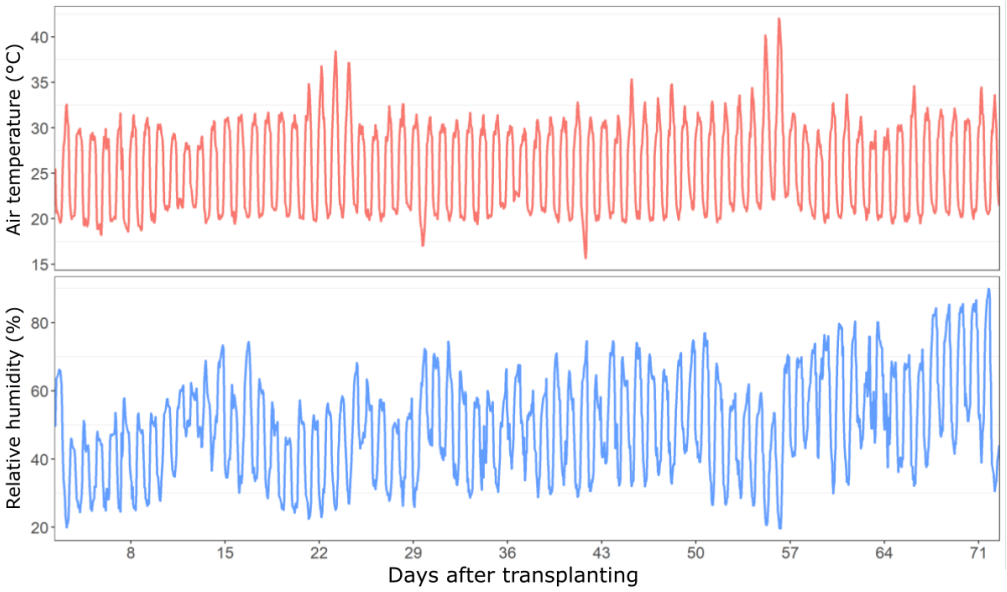


Figure B.7: Environmental data of the drought, salinity, and biostimulant experiment in Chapter 7, Experiment 2. From top to bottom: air temperature and relative humidity in the glasshouse throughout the experiment as recorded by data loggers.

Appendix C

R code and statistical outputs

Models used

The formulas used for each experiment are described in the following sections.

```
#Linear model for continuous data
model <- lm(formula, data = my_data)
anova(model)

#Pairwise comparison based on the ANOVA results with
    Tukey test
emmeans(model, pairwise ~ Parameter1|Parameter2)
emmeans(model, pairwise ~ Parameter2|Parameter1)
emmeans(model, pairwise ~ Parameter1:Parameter2)

#General linear model for count data with the
   Poisson distribution, tested for overdispersion
model <- glm(formula, data = my_data, family =
   poison)
summary(model)

#Linear mixed-effect model for time series of
   continuous data.
model<- lmerTest::lmer(formula_long, data = my_data)

#Generalised linear mixed-effect model for time
   series of count data.
glmer(formula_long, family = poisson, data = my_data
   )

#Pearson's correlation coefficients
coeff <- rcorr(as.matrix(my_data))
```

R code for normality and variance homogeneity test

Each test was performed for each dataset

```
shapiro.test(residuals(model))
ggdensity(residuals(model))
ggqqplot(residuals(model))
ggplot(data = my_data, aes(x = model$residuals)) +
   geom_histogram()
leveneTest(residuals(model) ~ Stress*Cultivar*
   Repetition, data = my_data)
plot(model, 1)
```

Formula for Chapter 3

```
formula <- Measure ~ Cabinet + Repetition + Stress*
   Cultivar
```

Formulas for Chapter 4

```
formula <- Measure ~ Irrigation*Temperature

formula_long <- Measure ~ (1|Plant) + (1|Plant/Leaf)
    + Irrigation*Temperature*TimePoint

#(1|Pot/Leaf) parameter used only for leaf expansion
    rate
```

Formulas for Chapter 5

```
formula <- Measure ~ Salinity*Temperature

formula_long <- Measure ~ (1|Pot) + (1|Pot/Leaf) +
   Salinity*Temperature*TimePoint

#(1|Pot/Leaf) parameter used only for leaf expansion
    rate
```

Formulas for Chapter 6

```
formula <- Measure ~ Block + Salinity
```

```
#Formula used for the dead flowers proportion
   analysis

formula <- dead_flowers ~ Block + Salinity + offset(
   log(TotalFlowers+1)

formula_long <- Measure ~ (1|Pot) + Block + Salinity
   *TimePoint
```

Formulas for Chapter 7

Experiment 1 - Salinity and drought

```
formula <- Measure ~ Block + Salinity*Irrigation
```

Experiment 2 - Drought, salinity, and biostimulants

```
formula <- Measure ~ Block + Salinity*Biostimulant

formula_long <- Measure ~ Block + (1|Pot) + Salinity
   *Biostimulant*TimePoint
```

Appendix D

PIPS reflective statement

Note to examiners:

This statement is included as an appendix to the thesis so that the thesis accurately captures the PhD training experienced by the candidate as a BBSRC Doctoral Training Partnership student (DTP).

The Professional Internship for PhD Students is a compulsory 3-month placement which must be undertaken by DTP students. It is usually centred on a specific project and must not be related to the PhD project. This reflective statement is designed to capture the skills development which has taken place during the student's placement and the impact on their career plans it has had.

Reflective statement:

I completed my Professional Internship for PhD Students (PIPS) at Rothamsted Research (RRes) working under Nicola Yates supervision. Whilst on the placement, I completed two main projects. The first one was about mapping international partners with whom RRes had collaborated since 2015 and creating a new database to easily identify RRes international partners. The second project was in collaboration with the Department of International Trade and aimed at developing the agri-tech UK website to include research institutes as only traders were included at the time.

Different sources provided me with funding lists since 2015 to complete the first project. After multiple steps of filtering, contacting Principal investigators (PIs), and a thorough search of the 'gateway to research' portal, 237 projects were included in an interactive Excel spreadsheet. This spreadsheet included details about the PIs and departments involved at RRes and information about the partners, countries, and publications for each project. This spreadsheet is easily searchable and will be updated every year to include any new collaborations. Multiple descriptive maps were produced as part of the project and used during different presentations by RRes staff to highlight the evolution of international collaborations and the success of each collaboration as measured by

publications.

Alongside the aforementioned data analysis exercise, regular meetings with Elizabeth Warham from the Department of International Trade took place to develop a search engine for their agri-tech website for research (Agritech UK, 2022). I was in charge of creating the research categories under which research institutes and universities would assign themselves based on their expertise. After contacting multiple research institutes to get the best description for each sector (including horticulture, fisheries, livestock, and forestry for example) and doing online research, I came up with categories that were approved by all parties involved. Whilst this part of the website is still under development and waiting for more research institutes/universities to sign up, the research categories are ready for new sign-ups.

During the mapping of international partners, I developed skills in data curing and analysis. I familiarised myself with different data analysis tools and learned to clean up and use large datasets in an efficient manner. I also developed communication skills by interacting with a range of actors throughout my PIPS as I had to contact PIs, communication managers at research institutes, and members of the government. Flexibility was also needed as plans for the website development changed relatively often based on the dynamic needs of the Department for International Trade. This placement also reinforced my teamwork skills as I was involved with different teams for each project and communicated on a daily basis with colleagues to ensure deadlines were met. Working on a different project than my PhD was a great exercise and pushed me to think differently to solve problems due to the very different nature of the data I was working with.

This placement broadened my horizons regarding career choice as I came across a range of stakeholders involved in plant and crop research. This helped me make the decision to pursue a career in policy-making to ensure research is communicated in the best way possible and used to its fullest.