Cape Peninsula
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THE EFFECT OF SALINITY AND SUBSTRATES ON THE GROWTH PARAMETERS AND ANTIOXIDANT POTENTIAL OF *TETRAGONIA DECUMBENS* (DUNE SPINACH) FOR HORTICULTURAL APPLICATIONS

by

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DECLARATION

I, Avela Sogoni, declare that the contents of this dissertation/thesis represent my own unaided work and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

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ABSTRACT

Climate change, expanding soil salinization and the developing shortages of freshwater supplies indicate the need for a sustainable and sufficient crop production method. Seawater and salinized lands represent potentially cultivable areas for edible salt tolerate plants. Majority of the accessible fields with these qualities in South Africa are located close to coastal areas and suffer from salinity. Therefore, knowledge on propagation, cultivation, fertigation and salt stress responses of the edible halophyte, *Tetragonia decumbens* (Dune spinach) is needed in developing a viable growth protocol for both home gardeners and commercial farmers for the utilization of saline soils. Hence, this study was conducted to evaluate the effects of salinity, hormone treatment and substrates on the vegetative growth, nutrient uptake and antioxidant potential of *T. decumbens* Mill.

The study consisted of two separate experiments, with the 1st experiment presented in chapters 3 and the 2nd experiment presented in chapters 4 & 5. The 1st experiment investigated the possibility of root initiation of *T. decumbens* nodal stem cuttings as influenced by rooting media and hormone. The cuttings were obtained from a single stock plant growing along the Cape Town coast at Granger bay campus (CPUT). Only cuttings taken using homogeneous methods; i.e stem cuttings with about two-thirds of leaves removed, ± 15 cm long with a stem thickness of approx. 8 mm were used for the experiment. The nodal stem cuttings were treated with different concentrations of rooting hormone (Dynaroot 1, 2 and 3 0- Control) and planted into four different rooting media: Sand (S), Sand: Peat (SP) (1:1), Perlite: Peat (PP) (1:1) and Peat:Perlite: Vermiculite (PPV) (1:1:1) and placed on a specialized propagation bed with heating cables/rod underneath at 26 ºC. A completely randomised design was utilized for the experiment. The total number of cuttings used in the experiment was 528 for 16 treatments in three replicates (11 cuttings per treatment x 16 treatments x 3 replicates). The parameters evaluated were percentage survival, root length, number of roots, number of leaves and height of the cuttings. The data was statistically analysed using Analysis of Variance. The significant differences between treatment means at $p \leq 0.05$ were compared using Fisher's least significant difference. The result indicated significant growth differences in various rooting media, whereas the rooting hormone and its interaction with rooting media were not significant on most assessed variables. Improved rooting was observed from nodal cuttings planted in the sand: peat mixture with or without the use of the rooting hormone. This study showed that dune spinach does not require additional rooting hormones which could be a cost-saving option for potential commercial growers of the species.

The 2nd experiment investigated the effects of salt stress on plant growth, nutrient uptake, chlorophyll content and antioxidant potential of *T. decumens* grown in soilless culture. The

objectives of the study were to measure plant growth, nutrient uptake, SPAD-502 values (chlorophyll content) and metabolite (phenolics) formation in the leaves of dune spinach, grown under various salt concentrations. Rooted cuttings of uniform size were arranged into four treatments each containing fifteen replicates. Salt stress were set up on three treatments by adding increasing NaCl concentrations in the NUTRIFEED™ nutritive solution (50, 100 and 200 mM). A total of 300 mℓ nutrient solutions were prepared for each plant with and/or without NaCl addition, the plants were then watered every three days using a plastic beaker. The control treatment was sustained and irrigated only by the nutritive solution. In all treatments, the pH was maintained at 6.0. SPAD-502 values were measured weekly using a handheld SPAD-502 meter. Determination of nutrient uptake of macronutrients N, K, P, Ca, Mg and Na and micronutrients Cu, Zn, Mn, Al and B were measured from dry plant material at the end of the experiment by Bemlab, 16 Van Der Berg Crescent, Gants Centre, Strand. Plant growth parameters such as shoot length, number of branches, wet and dry weight of shoots, stem and root were measured after harvest. Determination of concentrations of secondary metabolites (phenolic compounds) were assayed and measured spectrophotometrically at the end of the experiment.

The results showed that the use of nutrient solution incorporated with 50 mM and 100 mM of NaCl on plant growth resulted in plants that have higher yields of material with regards to both wet and dry weights of shoots, stem and roots, total weights and number of branches compared to the plants irrigated with nutrient solution only. Moderate (100 mM) to high (200 mM) NaCl increased chlorophyll content in leaves for up to seven weeks, then decreased on week eight and ten. Salt stress also positively affected polyphenolic compounds and antioxidant capacity (FRAP excluding ABTS) in the leaves. Polyphenolic compounds increased with increasing concentration of NaCl with the highest content found in 200 mM (highest NaCl conc.). This was not the case when FRAP capacity was evaluated, where the lower (50 mM) and moderate (100 mM) NaCl concentration proved to strengthen the FRAP capacity more than the highest concentration. As for the ABTS capacity, salt stress did not have any significant effect since the control had a slightly more capacity than all NaCl concentration.

In conclusion, this study found that the use of peat and sand as a rooting media had a positive impact on root initiation and growth of the nodal stem cuttings of dune spinach. On the other hand, salt stress at certain levels positively affected the total wet weight, nutrient uptake and antioxidant activities in the leaves of dune spinach. Based on these results it is evident that there is potential for seawater cultivation of the leafy vegetable *T. decumbens*. This could be a water-saving option in provinces experiencing the adverse effect of drought and salinity, where seawater or underground saline water could be diluted and used as irrigation water in the production of this vegetable.

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DEDICATION

I humbly dedicate this piece of work to my loving parent, Mrs Nomawethu Mxwebisa for her endless guidance and support, to my daughter Siwenzamhle Avuzwa Ntakani, may this be a reminder that “Success is no accident, it is hard work, perseverance, learning, sacrifice and most of all love for whatever you’re doing”-Pele.

STRUCTURE OF THE THESIS

The thesis is drafted differently to the alternative of a traditional format for a thesis. The article-format thesis examples of published, co-published and/or "ready-for-publication" articles were prepared during candidature and applies to the format prescribed by CPUT for 100% master's studies which complies to the following guidelines:

1. The overriding principle of the thesis is that it remains an original contribution to the discipline or field by the candidate.

2. Chapters containing the journal articles form a coherent and integrated body of work, which focused on a single project or set of related questions or propositions. All journal articles form part of the sustained thesis with a coherent theme.

3. The study does not include work published prior to commencement of the candidature.

4. The number of articles included depending on the content and length of each article and take full account of the university's requirements for the degree as well as the one article already published or "ready-for-publication" expected for a master's degree in this discipline.

5. The thesis should be examined in the normal way and according to the normal requirements as set out by the "Guidelines for Examiners of Dissertations and Theses" (using form HDC 1.7).

The thesis consists of the following chapters, which are concisely discussed as:

Chapter One: This chapter provides the significance of the research, its aim and the overall list of specific objectives, which guided the study.

Chapter Two: This chapter provides insight on the potential of *T. decumbens* **(**Dune spinach) as a leafy vegetable. It also highlights its uses, propagation, distribution, the environmental effect on growth as well as potential cultivation methods, which could be adopted for commercial use.

Chapter Three: This chapter evaluated the effect of rooting media and hormone on rooting success of South African dune spinach, *T. decumbens*. The research justification, materials and methods, results and discussions are presented.

Chapter Four: This chapter evaluated the effect of salt stress on plant growth, nutrient uptake and chlorophyll content in *T. decumbens* (Dune spinach). The research justification, materials and methods, results and discussions are presented.

Chapter Five: This chapter evaluated the effect of salt stress on the polyphenolic content and antioxidant capacity of *T. decumbens* (Dune spinach) leaves. The research justification, materials and methods, results and discussions are presented.

Chapter Six: General discussion, conclusions and recommendations.
This chapter deals with the general discussion, which connects the previous chapters and is followed by the conclusions of the study. Recommendations are made for further work; to introduce future research topics.

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GLOSSARY

Terms/Acronyms/Abbreviations	Definition/Explanation
CPUT	Cape Peninsula University of Technology
NaCl	Sodium chloride
CAM	Crassulacean Acid Metabolism
FRAP	Ferric reducing antioxidant power
ORAC	Oxygen Radical Absorbance Capacity
ANOVA	Analysis of Variance

CHAPTER ONE

RESEARCH PROBLEM, AIMS, HYPOTHESES AND OBJECTIVES

1.1 RESEARCH PROBLEM

Climate change, expanding soil salinization and the developing shortages of freshwater have negatively affected crop production in many countries including South Africa. The world development report has estimated that crop production should increase by 70% to 100% by 2050 to meet global food demand. However, increasing crop production has led to a loss of soil fertility and the phenomena of salinization and desertification, which make soils unsuitable for cultivation. This indicates the need for a creative, sustainable, and sufficient crop production method. With this in mind, numerous researchers have pointed out the use of salt-tolerant plant species with possible commercial value as a proposed upfront strategy for salinized land (Debez et al., 2010; Ventura and Sagi, 2013). The use of halophytic plants for food production could be an adaptation strategy for climate change as freshwater continues to become scarce and the rainfall, particularly in Sub-Saharan African become more sporadic (Glenn *et al*., 1999; Bartels *et al.,* 2005). Seawater and salinized lands represent potentially cultivable areas for edible salt tolerate plants. Majority of the accessible fields with these qualities in South Africa are located close to coastal areas and suffer from salinity. Therefore, knowledge on propagation, fertigation, nutrition, and salt stress responses of the unexploited edible halophyte *(Tetragonia decumbens*) could support its cultivation potential as a leafy vegetable. Thus, this study was carried out to investigate the effects of salinity and substrates on the growth parameters and antioxidant potential of *T. decumbens* Mill 'Dune spinach' for horticultural applications.

1.2 AIM

The study aims to investigate the effects of salinity, commercial rooting hormone and substrates on the vegetative growth, nutrient uptake and antioxidant potential of *T. decumbens* Mill 'Dune spinach'. In order to formulate a viable growth protocol for both home gardeners and potential commercial farmers.

1.3 HYPOTHESES

It is hypothesised that moderate salinity stress will increase nutrient uptake and plant growth of *T. decumbens* and have a positive effect on the accumulation of metabolites. Additionally, commercial rooting hormone and a correct rooting media could positively influence root initiation in vegetative propagation of *T. decumbens*.

1.4 OBJECTIVES

1.4.1 Main objective

The purpose of this investigation is to determine the vegetative growth of *T. decumbens* in response to various commercial rooting hormone concentrations, salinity concentrations and growth substrates in order to establish a suitable growth protocol.

1.4.2 Specific objectives

1) To evaluate the rooting percentage, root length, number of roots, and number of leaves and plant height of *T. decumbens* stem cuttings in response to varying hormone treatments and growth substrates.
2) To evaluate the nutrient uptake by *T. decumbens* in response to varying salinity concentrations.
3) To evaluate the chlorophyll content in *T. decumbens* leaves in response to varying salinity concentrations.
4) To evaluate the fresh and dry weight of *T. decumbens* in response to varying salinity concentrations.
5) To evaluate the antioxidant potential of *T. decumbens* in response to varying salinity concentrations.
6) To evaluate optimal responses of *T. decumbens* gathered under the specific objectives 1-5 for a suitable growth protocol.

CHAPTER TWO

DUNE SPINACH: A POTENTIAL CASH CROP FOR SALINE LANDS IN SOUTHERN AFRICA

Dune spinach: a potential cash crop for saline lands in southern Africa

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2.1 Abstract

Climate change, expanding soil salinization and the developing shortages of freshwater have negatively affected crop production in many countries including South Africa. Current attempts to adapt to these conditions include the use of salt-tolerant plant species with potential economic value. The wild edible halophyte commonly known as dune spinach has potential to be used as a leafy vegetable. It is well adapted in saline sand dunes, which are characterised by low soil fertility and require minimal cultivation needs. However, it remains underutilised as a leafy vegetable. This review examined the potential of dune spinach as a leafy vegetable and explores its uses, morphology, distribution, propagation and potential cultivation methods. Furthermore, this appraisal is expected to be useful towards further research and popularization of this unexploited edible halophyte.

Key words: Halophyte; Functional foods; *Tetragonia decumbens;* Salt tolerance

2.2 Introduction

Global agriculture feeds over 7 billion people and alarmingly this number is expected to increase by a further 50% by 2050 (Loconsole *et al.,* 2019). The global need for food production has never been greater, especially in developing nations where an increase of 90% of the population growth anticipates that food insecurity will become a greater problem (Gidamis & Chove, 2009). To meet the additional food demand, the world development report has estimated that crop production should increase between 70% to 100% by 2050 (World Bank, 2007). However, increasing soil salinization and the rising shortages of freshwater have negatively affected the yield of important crops like wheat, maize, rice and barley around the world including South Africa (Acquaah, 2007). Moreover, soil salinity is predicted to be a major problem in many regions in the future due to global climate change (Guo et al., 2018). This indicates the need for a creative, sustainable and sufficient crop production method. With this in mind, numerous researchers have pointed out the use of salt-tolerant plant species with possible commercial value as a proposed upfront strategy (Debez et al., 2010; Ventura and Sagi, 2013). The use of halophytic plants for food production could be an adaptation strategy for climate change as freshwater continues to become scarce and the rainfall, particularly in Sub-Saharan African become more sporadic (Glenn *et al*., 1999; Bartels *et al.,* 2005). Seawater and salinized lands represent potentially cultivable areas for edible salt tolerate plants. In South Africa majority of the fields with these qualities are located close to coastal areas and suffer from salinity. Therefore, saline agriculture with halophytes could enable coastal and saline lands to be productive. Hence, knowledge on the unexploited edible halophyte, *Tetragonia decumbens* is needed to support its cultivation potential on saline lands. This review examined the potential of dune spinach as a leafy vegetable and explored its uses, morphology, distribution, propagation, and potential cultivation methods.

2.3 Introducing the strandveld species *Tetragonia decumbens*

Tetragonia decumbens Mill. also known as Dune Spinach or Duinespinasie (Afrikaans) belongs to the genus *Tetragonia* which is native to Africa, Australia, South America, and Asia (Forrester, 2004). Of the *Tetragonia* genus, about 67 species are endemic in southern Africa. *T. decumbens* is an endemic and sprawling perennial shrub with branches (runners) that can go up to 1 m long. It has papillose-hirsute, oblong, or lance-shaped fleshy leaves that feel hairy to the touch as seen in (Figure 2.3) (Manning, 2013; Snijman, 2013). The glistening leaves have a shiny and warty appearance due to small shiny epidermal bladder cells (Manning, 2013; Snijman, 2013; Van Wyk & Gericke, 2017). This plant usually starts flowering from August to November (summer months) with seeds that vary in size which have a brown hard outer casing with distinct rigid wings when dry (Rusch, 2016; Tembo-Phiri, 2019). This widespread dune plant performs a vital role in stabilizing the sand dunes, acting as a seed trap, and generating organic matter, enabling the dune to become a friendly surrounding for different vegetation (Van Wyk & Gericke, 2017). In southern Africa, this genus is largely distributed along the coast from southern Namibia to the Eastern Cape (Figure 2.1 and Figure 2.2).

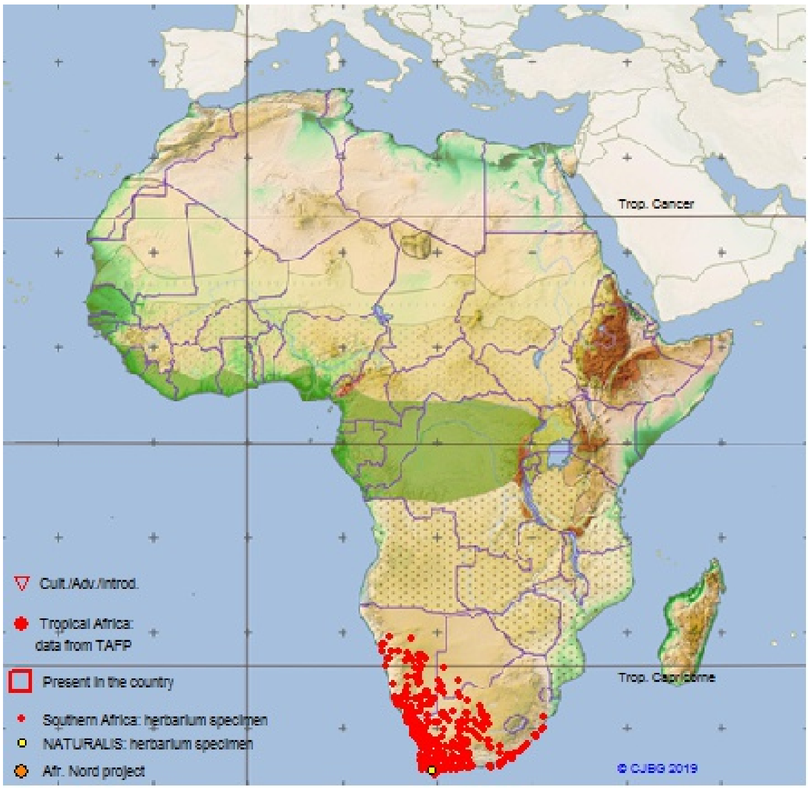

Figure 2.1: Geographical distribution map of *T. decumbens* (Adapted from: http://www.ville-ge.ch/musinfo/bd/cjb/africa/details.php?langue=an&id=48025)

Figure 2.2 *T. decumbens* growing in its natural habitat along the South Western Cape coast of South Africa (Laubscher, 2019).

Figure 2.3: Hairy and glistening edible leaves of dune spinach with flowers appearing in summer (Sogoni, 2019).

2.4 Potential of *Tetragonia decumbens* as leafy vegetable

Culinary edible halophytes are used in many green dishes in restaurants and have gained popularity in some cultures across the world due to their saltiness and crunchiness (Agudelo *et al.,* 2021). The species climatic adaptation to high temperatures compare to conventional leafy vegetable crops makes them an ideal choice in some countries (Ebert, 2014). In Australia and Brazil *Tetragonia expansa* (New Zealand spinach) which is native to New Zealand, Australia, Argentina, and Japan is commercially cultivated as a new choice of nutrient-rich leafy vegetable (Cecilio Filho *et al*., 2017). In South Africa, *T. decumbens* as a leafy green vegetable remains underutilized as commercial farming of this species has never been explored. More recently, the species is piloted as a possible commercial crop at a community garden in Khayelitsha, Cape Town, along with several other potential winter rainfall crops (Tembo-Phiri, 2019). The leaves and soft stems of dune spinach have a salty taste and are used like green spinach, eaten raw in grain salads, or cooked with other vegetables as demonstrated in Figures 2.4 and 2.5. Dune spinach can also be fermented, pickled, used in stews and soups, and particularly tasty in a stir-fry (Rusch, 2016). When boiled it has a granular texture and tastes bland but mixed with *Oxalis pes-caprae* (sorrel/suring) and butter, makes a tasty dish (Tembo-Phiri, 2019; Van Wyk & Gericke, 2017). Even though this plant is edible, its nutritional composition has not been explored, whereas the New Zealand spinach have been reported to have good nutritional value with total amino acid, which also increased after cooking and freezing (Slupski *et al.,* 2010). For this reason, New Zealand spinach was recommended as suitable for processing as a frozen product and convenience food. Additionally, a comparable study conducted by Jaworska and Kmiecik (1999) on the accumulation of selected mineral elements between New Zealand spinach and spinach (*Spinacia oleracea* L.), reported that while New Zealand spinach had good mineral levels, spinach was higher at fresh weight. Based on this result it was concluded that New Zealand spinach is an alternative good source of calcium, magnesium and iron (Jaworska & Kmiecik, 1999). As no data exists on the nutritional importance of *T. decumbens,* further studies are recommended on the nutritional profile of this species to support its consumption as a leafy vegetable.

Figure 2.4: Oep ve Koep's Sandveld restaurant dumplings with dune spinach (Armbruster, 2014).

Figure 2.5: Dune spinach salad served with feta cheese (Angela, 2018).

2.5 Cultivation of *Tetragonia decumbens*

The valuable adaptive nature of dune spinach to withstand biotic and abiotic stresses makes it an ideal plant to cultivate. Wild vegetables have great agronomic potential in regions experiencing the adverse effects of drought, high temperatures and soil salinity and sodicity (Laurie *et al.,* 2017). Naturally, attention needs to be given to the propagation, cultivation and use of wild indigenous vegetables in order to enhance local food systems, which will then upscale to commercial markets.

2.5.1 Growing *Tetragonia decumbens*

A report by Forrester (2004), suggested that dune spinach can be easily propagated by pulling up runners (branches) from the sand and separate pieces with roots appended as cuttings. These cuttings can be planted directly in-situ into well-drained, sandy soil, which must be kept slightly moist until the plants have established. Nevertheless, the potential utilization of this plant by local communities and small-scale farmers in the future could lead to over-harvesting along the coast, thus disturbing its natural use to stabilise the dunes. Therefore, a suitable propagation method should be developed to protect its population from the wild and for use by potential farmers. Hence furthers studies should be conducted on the effect of rooting hormone and media on root initiation of this plant. Dune spinach can also be propagated through seeds, however, Rusch (2016) reported the re-sprouting ability of the species when watered can be utilised for repeated harvest without the need to save seeds and re-sowing every year. Thus, the re-sprouting ability of this plant when continuously harvested should be investigated to measure whether there is a difference in fresh weight, nutrient composition and secondary metabolites produced. In order to formulate and recommend a viable continuous harvesting schedule for potential growers.

2.5.2 Pests and diseases

The leaves of dune spinach have been reported to be rarely infected or eaten by insects and are more likely to be damaged to a very small extent due to the plant antimicrobial activity, which has been reported as one of the reasons this plant hardly show pest, disease and fungal infestation (Słupski *et al.,* 2010). However, changing environmental conditions have contributed to the increasing prevalence of diseases in plants as they interfere with the important physiological and biochemical processes (van Puijenbroek *et al.,* (2017). Studying the biochemical responses of this species to salinity, light and drought may be vital in enhancing its overall secondary metabolites, which are beneficial in plant protection and human health through antioxidants.

2.6 Influence of environmental factors on plant growth

2.6.1 Effect of salinity on plant growth

Salt stress is considered as the most serious problem, which restricts plant growth and hampers production in the world (Jouyban, 2012). Salinity in agricultural soil refers to the presence of excessive accumulation of soluble salts in the soil moisture of the root zone. The accumulation of these soluble salts affects important physiological and biochemical processes in plants, which later cause a reduction in plant growth and development (Munns, 2002; Tester & Davenport, 2003). These adverse effects restrict the flow of water and nutrients in plants parts or by direct injury to plant cells through the accumulation of toxic ions. According to Munns *et al.* (2006), plant responses to salt stress occur in two stages, the first and second stage. In the first stage, growth reduction occurs very rapidly due to the development of a water deficit. The second stage is caused by the accumulation of salts in the shoots at toxic levels results in a slow growth process due to the build-up of Na+ inside the cells of the plants. Additionally, the uptake of high amounts of salt by the plant roots leads to the increase of osmotic pressure, which is the main contributor in growth reduction of new leaves and lateral buds' development in the initial stages of plant growth (Munns & Tester, 2008). Osmotic stress also has a negative impact on photosynthesis by reducing CO_2 availability as a result of diffusion limitations (Flexas *et al.,* 2007; Machado & Serralheiro, 2017) and a reduction of the contents of photosynthetic pigments (Ashraf & Harris, 2013; Delfine *et al.,* 1999). In spinach, salt accumulation inhibits photosynthesis (Di Martino *et al.,* 1999; Emanui *et al.,* 2020), primarily by decreasing stomatal and mesophyll conductance's to

CO_2 (Delfine *et al.,* 1998) and reducing chlorophyll content, which can affect light absorbance.

The ion toxicity occurs when certain ionic species disturbs cell ion homeostasis by making both the inhibition in the uptake of essential elements such as K+, Ca 2+, and NO_3 − and the accumulation of Na+ and Cl¯. Specific ion toxicities cause the accumulation of sodium, chloride, and/or boron in the tissue of transpiring leaves to damaging levels (Benito *et al.*, 2014). However, several authors have reported the positive effect of NaCl in prompting changes in qualitative and quantitative compositions of metabolites biosynthesis to a greater extent (Salema *et al.,* 2014). Khan *et al.* (2011) stated that plants exposed to salt stress could result in enhanced production of metabolites that have an economic value such as isoflavones, isoprenoids, phenols or alkaloids and phytosterols. Therefore, further studies are recommended on salt responses of wild coastal edible plants such as *T. decumbens* to evaluate its commercial cultivation potential and to ensure that species are not exploited in their natural habitat.

2.6.2 Salt stress and antioxidant system in plants

Salinity and drought stress are well-known factors to induce oxidative stress in plants through the production of superoxide radicals by the process of Mehler reaction (García-Caparrós *et al.,* 2020). These free radicals initiate the chain of reactions that produce more harmful oxygen radicals (Chao *et al.,* 2009). These Reactive Oxygen Species (ROS) are continuously generated during normal metabolic processes in mitochondria, peroxisomes and cytoplasm which disturb normal metabolism through oxidative damage of lipids, proteins, and nucleic acids when produced in excess (Hernandez *et al.,* 2001; Ahmad *et al.,* 2010). Plants throughout their lives are prone to oxidative damage caused by environmental factors due to their sessile nature (Hippeli & Elstner, 1996). There is a constant need for efficient mechanisms to compensate for possible oxidative damage to cellular components. Plants have evolved efficient systems for ROS removal, which include specific ROS-scavenging anti-oxidative enzymes and small non-enzymatic molecules that act as ROS scavengers such as ascorbate, glutathione, α tocopherol, flavonoids, anthocyanines, polyphenolic compounds and carotenoids.

To overcome salt-mediated oxidative stress, plants detoxify ROS by up-regulating antioxidative enzymes which includes the superoxide dismutase (SOD) found in various cell compartments (Rasheed *et al.,* 2020). This enzyme catalyses a conversion from two O_2 radicals to H_2O_2 and O_2 (Scandalios, 1993). In alternative ways, several antioxidant enzymes can also eliminate the H_2O_2 such as catalases (CAT) (Scandalios, 1993) and peroxidases (POX) by converting it to water (De Gara *et al.,* 2003). During this process, the antioxidant capacity of some species increases when exposed to salinity stress to eliminate or reduce the ROS. However, this process varies among species. For example, sugar beet is known to be salt-tolerant at later stages of growth whilst it is sensitive at germination phases. Some wild greens such as *Salicornia*, *Sarcocornia* and *Cichorium* tolerate salt stress throughout their growing process without significant effects on plant growth and yield (Petropoulos *et al.,* 2017). These wild greens are used in human diets in many other countries and several studies confirm their nutritional value and antioxidant activity (Morales *et al.,* 2012). These antioxidants are very useful against free radicals that predispose humans to sickness and diseases. Thus, research concerning the impact caused by salinity on secondary metabolism of the underutilised edible halophyte, *T. decumbens* is needed, to further promote large-scale cultivation of wild coastal vegetables.

2.6.3 Effect of moisture stress on plant growth

Water stress is considered as the most critical environmental factor causing reduction in plant growth and development (Farooq *et al.,* 2009; Ali, 2010; Zhao et al., 2020). Water stress is mainly caused by too low water content in the soil or by the excessive accumulation of soluble salts in the root zone, which reduce the plant's ability to absorb water from the soil in order to compensate for the water loss through transpiration as water stress conditions develop (Ali, 2010). Plants suffer from water stress in two ways at cellular level and at plant growth level. In spinach, moderate water stress inhibits photosynthesis, primarily by decreasing stomatal and mesophyll conductance of CO_2 (Machado & Serralheiro, 2017). This reduction in stomatal conductance adversely affects the exchange of water and oxygen leading to reduction in photosynthesis and other important metabolic processes in plants, which will cause dehydration and disorganization of the protoplasm with a resultant cause of death.

Most sub-Saharan African countries including South Africa face severe challenges of water scarcity and potable drinking water. Furthermore, a large increase in the vegetable crop production and consumption of edible portions of herbaceous species such as roots, tubers, shoots, stems, leaves, fruits, and flowers, have cause further critical pressure on clean irrigation water in South Africa. The water use efficiency and carbon fixing capacity of edible wild plants such as dune spinach, uses Crassulacean Acid Metabolism (CAM) to be more adaptable to climate, which makes it the ideal climate-conscious vegetable to adopt (Tembo-Phiri, 2019). The innate ability to transition into CAM allows these wild vegetables to store large quantities of carbon dioxide during the night-time, thus increasing photosynthetic rate during the daytime (He *et al.,* 2017). This phenomenon proves to be essential for sustainable production of food plants to address the challenges of poor soils and scarce water conditions. The use of hydroponics and soilless growing media with high water holding capacity could also play an important role to curb water waste in vegetable production. Hence, further studies should be conducted on edible halophytes in hydroponics.

2.7 Potential cultivation method of *Tetragonia decumbens as* a green leafy vegetable

2.7.1 Hydroculture cultivation

Hydroculture, also known as soilless culture or hydroponics, is the term used when plants grow in a nutrient solution without the use of soil (Anderson *et al.,* 1989). Soilless media provide plant support depending on the system and the plant grown (Montanari *et al.,* 2008). Dr. W.F Gericke coined the term hydroponics in 1936, who conducted several studies on cultivating vegetables using water (Abdullah, 2001).

With an increase in population sizes each year agricultural production on soil has been affected by many factors such as limited availability of irrigation water, high infestation of plant pathogens and growing consumer demand on quality and the nutritional value of products (Carruthers, 2002; Correa *et al.,* 2012). With these complex challenges, alternative sustainable, efficient and controllable cultivation methods can change cultivation methods for future vegetable growing. Plants grown on hydroponic systems only require one-tenth of the amount of water used compared to plants grown in soil because in traditional farming, high amounts of water leaches very quickly through the root layer (Ortiz *et al.,* 2009). Savvas, (2003) stated that the switching from soil-based cultivation to hydroponic techniques has led to a decrease in the application of pesticides and other toxic agrochemicals often used in soil-grown crops to disinfest the soil and to control soil-borne pathogens. Anderson *et al.* (1989), later supported by Savvas recognized that soilless culture is the most sustainable, efficient and effective cultivation method in the agricultural industry around the world. Due to the drought periods experienced in the Western Cape Province of South Africa, cultivating *T. decumbens* in hydroponics using dilutions of seawater could provide sustainable agricultural production in coastal areas. Further studies could develop suitable growing protocols for cultivating high-quality edible plants, possibly with antioxidant properties and readily marketed commercially.

2.7.2 Substrates used in soilless culture

Soils of inorganic and or organic origin used to cultivate crops for many decades could harbour soil-borne diseases, unwanted microbial activities, changing acidity levels and high salinity levels. Additionally, poor drainage and poor nutrient soils have caused farmers all over the world large amounts of money to improve and to manage their soils. Hence soil has been replaced by many organic and inorganic substrates since they are disease and pest free inert material capable of holding required sufficient moisture and can be reused year after year (Asaduzzaman *et al.,* 2015). Growing media used in soilless culture serves mainly four functions in providing water to the plant roots, supplies nutrients, allows for gas exchange to and from the roots, and to provide physical support as an anchor for the plant (Fonteno, 1996). According to Hassain *et al*. (2014), the application of a soilless culture system using artificial substrates could result in efficient and effective use of water and fertiliser, thus reducing the use of chemicals applied to treat plant diseases in the process. Ghehsareh *et al.* (2012) further stated that different substrates have several materials (chemical and physical properties) which could have direct or indirect effects on plant growth and development (Table 2.1).

Table 2.1 Physical and chemical properties of organic and non-organic media used in soilless culture. Adapted from Wilkinson *et al.* (2014).

Substrate	**Bulk density**	**Water holding capacity**	**Porosity**	**pH**	**Cation Exchange Capacity**
Coco-peat	Low	High	High	6-7	Low
Perlite	Low	Media	High	6-8	Very low
Vermiculite	Low	High	High	3-6	High
Silica sand	High	Low	High	6-7	Low

2.7.2.1 Coconut coir (Coco-peat)

Coconut coir also more commonly known as coco peat or coco fibre is derived from the mesocarp tissue or husk of the coconut fruit (*Cocos nucifera*) (Raviv *et al.,* 2002). Commercial coconuts are mainly produced in Sri Lanka, the Philippines, Indonesia, southern India and Latin America. Coconut coir is an inert media, which means optimal nutrients levels is more easily controlled and maintained (Kokemuller, 2014). Additionally, coconut coir is a more advantageous over other growing substrates at promoting beneficial bacterial growth and protecting root zone from heat stress (Withers, 2014). According to Mahmood (2004) the coconut coir contains natural anti-fungal properties that offer plants protection against many root diseases. Coconut coir is normally used in most hydroponics systems and also mixed with other growth substrates like clay pellets (Withers, 2014). Many growers have used a 50/50 mix of coconut coir and clay pebbles and proved to be an effective formula, providing high moisture retention, optimum nutrient control, and plenty of oxygenation around the root zone for increased uptake and healthier plant development (Withers, 2014).

2.7.2.2 Perlite

Perlite is composed mainly of minerals and is an expanded amorphous volcanic glass. The expansion process gives perlite a very porous structure and fantastic aeration properties. It also has a neutral pH and is highly absorbent and in retaining water and nutrients, without shrinking or getting soggy (Withers, 2014). Perlite holds three to four times its weight of water. It has a pH of 6.0 to 8.0 with no buffering capacity. Unlike vermiculite, it has no cation exchange capacity and contains no mineral nutrients (Hartmann & Kester, 1983). Expanded perlite is very light with a particle and bulk density of 0.9 and 0.1 g cm ^{3}, respectively. It is provided in various grades, the most common being 0-2 and 1.5-3.0 mm in diameter (Raviv *et al.,* 2002). According to Hall (2008), horticultural perlite has a long and enviable record of performance as a propagating and growing media throughout the world. It has been successfully used in virtually all horticultural applications including hydroponics, glasshouse growing, landscaping, lawn, and stadium turf and in a variety of container applications.

2.7.2.3 Vermiculite

Vermiculite is a hydrous phyllosilicate mineral, which is made when natural mineral rocks are heated at high temperatures and form expandable glass pebbles (Rebel, 2012). It has high water retention and capillary action properties, which make it to be hydrated. It is also very lightweight and sterile in composition. When used in soilless culture, vermiculite is normally mixed with perlite if the media becomes too waterlogged to improve aeration and drainage. According to Rapaka, (2013) vermiculite high water absorption capacity and nutrient holding reserve make this an ideal media beneficial to many growers.

2.7.2.4 Silica sand

Sand more commonly known as quartz sand, industrial or white sand is made up of two elements, silica and oxygen but more specifically silicon dioxide (SiO_2). The SiO_2 in silica sand is quartz rock, just smaller. Because the particle size is smaller than regular rock, moisture doesn't drain out as fast. Silica sand is normally mixed with vermiculite, perlite, and coco coir to retain moisture (Ventura & Sagi, 2013). Fine sand, such as building sand is not recommended for hydroponics as it holds water and prevents aeration in the root zone. One big downside in using sand as a growing media for hydroponics is that it is very heavy (Rebel, 2012). It remains unknown how successful hydroponic cultivation of *T. decumbens* will be in any of the media reported. Therefore, further studies are necessary to determine which media could be more beneficial in cultivating the species.

2.8 Conclusion

Climate change, expanding soil salinization and the developing shortages of freshwater have negatively affected crop production in many countries including South Africa. Current attempts to adapt to these conditions include the use of salt-tolerant plant species with potential economic value. Currently, there is a worldwide interest of edible salt-tolerant plant species due to their adaptive nature to withstand salinity more than conventional vegetable crops. Dune spinach has been reported to withstand biotic and abiotic stresses and thus having the potential to be used as a leafy green vegetable in areas facing the adverse effect of drought and salinity. Reports on the edibility of this species have shown that the leaves and soft stem can be used like green spinach, eaten raw in grain salads, or cooked with other vegetables. However, due to its underutilization by local communities and commercial growers, it is recommended that further studies be conducted on its propagation and cultivation methods to support its commercial viability as a green vegetable.

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CHAPTER THREE

THE EFFECT OF ROOTING MEDIA AND HORMONE ON ROOTING SUCCESS OF SOUTH AFRICAN DUNE SPINACH, *TETRAGONIA DECUMBENS*

PAPER SUBMITTED TO: HORTSCIENCE

The effect of rooting media and hormone on rooting success of South African Dune spinach, *Tetragonia decumbens*

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3.1 Abstract

Tetragonia decumbens is an important species with potential economic output as a novel vegetable, however, its propagation and cultivation methods for commercial purposes are not documented. The close relatives *Tetragonia tetragonoides* and *Tetragonia expansa* are widely utilized in eastern Asia, Australia and Brazil, and sold in fresh vegetable markets as substitute for spinach. This prompted an investigation into the effect of rooting media and commercial rooting hormone on the survival, rooting and growth of stem cuttings of *T. decumbens*. The nodal stem cuttings were obtained from a single stock plant growing along the coast at the Granger Bay campus of Cape Peninsula University of Technology. Only cuttings taken using homogeneous methods, i.e. stem cuttings with about two-thirds of leaves removed, ± 15 cm long with a stem thickness of approx. 8 mm were used for the experiment. The nodal stem cuttings were treated with different concentrations of rooting hormone (Dynaroot 0, 1, 2 and 3) and planted into four different rooting media: Sand (S), Sand: Peat (SP) (1:1), Perlite: Peat (PP) (1:1) and Peat: Perlite: Vermiculite (PPV) (1:1:1) and placed on a specialized propagation bed with heating cables/rod underneath at 26 ºC. A completely randomised design was utilized for the experiment and replicated three times. The results indicated significant growth differences in various rooting media, whereas the rooting hormone and its interaction with rooting media were not significant in most variables assessed. Cuttings planted on Sand: Peat (1:1) mix without hormone treatment had the best results in all variables assessed. This study showed that dune spinach does not require additional rooting hormones which could be a cost-saving option for potential commercial growers of the species.

Keywords: Halophyte**;** Nodal stem cuttings; Vegetative propagation

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3.2 Introduction

The Cape Floristic Region contains many edible species, which were historically foraged rather than cultivated. These species were part of the food cultures of nomadic people, for example, the Bushmen like the San people who were mainly hunter-gatherers. Colonization, apartheid and urbanisation have led to the removal of indigenous people from cultural lands which resulted in societies completely disassociated from the land and knowledge of the useful plants it offers (Cavanagh, 2013; La Croix, 2018). Rediscovering local knowledge and resources such as indigenous foods that are well adapted to their local environments is a vital mitigation strategy against various environmental challenges (Even-Zahav, 2016; Vivus, 2016). These indigenous vegetables have great agronomic potential in regions experiencing the adverse effects of drought, high temperatures, soil salinity and sodicity (Laurie *et al.,* 2017).

The valuable adaptive nature of *T. decumbens* Mill to withstand biotic and abiotic stresses makes it an ideal plant to propagate. This wild vegetable belongs to the family Aizoaceae and is largely distributed along the coast from southern Namibia to the Eastern Cape. It is an endemic sprawling perennial shrub with branches (runners) that can grow up to 1 m long (Forrester, 2004). The leaves and soft stems of dune spinach have a salty taste and can be used like spinach, served raw in grain salads, or cooked with other vegetables. Dune spinach can also be fermented, pickled and used in stews and soups and particularly tasty in a stir fry (Rusch, 2016). It is also served in restaurants such as Wolfgat and Oep ve Koep in Paternoster, West Coast and Table Bay Hotel in Cape Town, respectively (Van der Merwe & De Villiers, 2014; Black, 2015; Mckeown, 2017). However, its propagation and cultivation methods for commercial use are not documented whereas its close relatives*Tetragonia tetragonoides* and *Tetragonia expansa* are widely utilized in eastern Asia, Australia and Brazil and sold in fresh vegetable markets as substitute for spinach. The potential of *T. decumbens* for commercial markets as a novel vegetable in South Africa is promising and strategies to popularize this plant should be put in place, as its cultivation has the potential to be expanded in the coming years.

Recordings on vegetative propagation of *T. decumbens* are limited. As the species is adapted to well-drained coastal sand, this media is popular in the cultivation of other halophytes such as *Salicornia* in North Africa, America and Mexico. In vegetative propagation system, rooting media is considered as a fundamental tool since it directly influences the rooting percentage and the quality of roots. In nursery plant production, the selection and preparation of the media is very important in terms of root development and plant quality. The combination of a good rooting media and hormone would hasten root initiation (Sardoei, 2014; Ibironke & Victor, 2016). This study aimed at assessing the effect of rooting media and commercial rooting hormone on root-initiation and morphology of *T. decumbens* cuttings under greenhouse conditions, in order to formulate a viable propagation protocol.

3.3 Materials and methods

3.3.1 Experimental location

The experiment was conducted in the research greenhouse of the Department of Horticultural Sciences at the Cape Peninsula University of Technology, Bellville campus, Cape Town, South Africa, located at 33°55'56" S, 18°38'25" E. The research greenhouse was equipped with mist, a heating bed and environmental control with temperatures set to range from 21–26 ºC during the day and 12–18 ºC at night with Relative Humidity averages of 60%.

3.3.2 Plant preparation and treatments

Cuttings of *T. decumbens* were harvested from a single stock plant on 1st June 2019 from a wild plant growing along the coast at the Granger Bay campus (CPUT) located at 33°53'58.2"S, 18°24'41.4"E. Only cuttings taken using homogeneous methods, i.e. stem cuttings with about two-thirds of leaves removed, ± 15 cm long with a stem thickness of approx. 8 mm were used for the experiment. The cuttings were then rinsed in 0.1 % Sporekil™ for precaution against fungal infection, thereafter, were dipped in different concentrations of commercial rooting hormone Dynaroot™ (containing 0.1 % IBA for 1 softwood, 0.3 % for 2 semi-hardwood and 0.8 % for 3 hardwood) for two seconds. Stem cuttings with no hormone treatment acted as control. The cuttings were planted into four different rooting media: Sand (S), sand and peat (SP) (1:1), perlite and peat (PP) (1:1) and peat, perlite and vermiculite (PPV) (1:1:1) arranged in a completely randomized design on a specialized propagation bed with heating cables/rod underneath at 26 ºC. The cuttings were then irrigated by an intermittent sprayer at 18 s per 40-minute intervals. The randomize design consisted of 528, a total number of cuttings used for 16 treatments in three replicates (11 cuttings per treatment x 16 treatments x 3 replicates).

3.3.3 Data collection and statistical analysis

Data collection (Figure 3.1) included plant height, leaf number, root length and root number and percentage of rooting after 60 days. All percentage data were subject to angular transformation (arcsin$\sqrt{X}$) and SE number data to square root transformation prior to analysis. The experimental data were analysed using one and two-way analyses of variance (ANOVA), and Fisher's least significant difference was used to compare means at P ≤0.05

level of significance between treatments. All calculations were done on the computer software program, STATISTICA version 13.5.0.17.

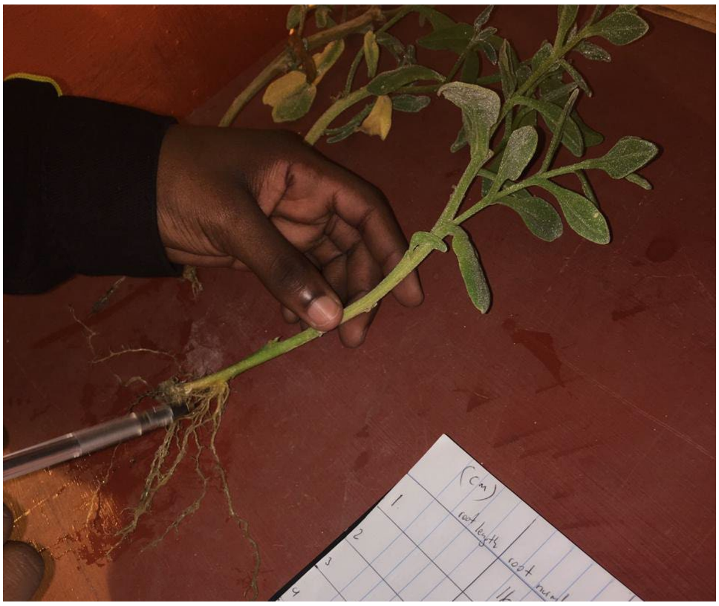

Figure 3.1 *T. decumbens* cutting showing root development after 8 weeks (Sogoni, 2019).

3.4 Results

3.4.1 Rooting percentage (%)

The rooting media in which the nodal stem cuttings were planted significantly (P ≤0.05) affected the rooting percentage (Table 3.1). The highest rooting percentage (84.2 %) was recorded on cuttings planted in SP followed by S at 76.8 %. These percentages were significantly higher than the percentages recorded in PP (66.3 %) and PPV (57.5 %) respectively. Conversely, the rooting hormone had no significant effect on rooting percentage although Dynaroot 2 produced the highest rooting (75.4 %), which did not differ significantly from that obtained with Dynaroot 1(72.6%), 3 (66.8%) and the control (69.8%) (Table 3.2). The interaction of rooting media and hormone treatment also had a significant effect on rooting percentage (Table 3.3). S/D/1 (84.7%), S/D/2 (84.1%), S/D/3 (82.6%), SP/D/0 (87.9), SP/D/1 (82.4%) and SP/D/2 (84.5%) had significantly higher rooting percentages compared to S/D/0 (55.5%), PPV/D/3 (36.3%), PPV/D/2 (69.4%), PPV/D/1 (60.5%) and PP/D/0 (72.3%) (Table 3.3).

3.4.2 Root length (cm)

The root length was significantly (P ≤0.05) reduced by the media PPV to 3.62 cm. Longer root length was recorded in SP at 7.76 cm but this was not significantly different from S (6.21 cm) and PP (5.93 cm) respectively (Table 3.1). The rooting hormone and its interaction with rooting media had no significant effect on root length (Table 3.2 and Table 3.3), although

PP/D/1 produced a root length of 7.59 cm, which did not differ significantly from 3.05 cm obtained in PPV/D/3.

3.4.3 Number of roots (n)

The rooting media significantly increased (P ≤0.05) the mean number of roots per cutting in SP (3.75) and S (2.66) compared to PP (1.52) and PPV (2.41) which had lower numbers of roots (Table 3.1). The rooting hormone and its interaction with rooting media had no significant effect on the number of roots although SP/D/0 recorded the highest number (4.64) of roots in all treatments (Table 3.2 and Table 3.3).

3.4.4 Number of leaves (n)

Although most media had higher numbers of leaves, PPV (8.14) significantly reduced the number of leaves in cuttings. The SP media recorded the highest number of leaves (20.18); this was significantly higher than PP (13.45) and PPV (8.14) except S (Table 3.1). The rooting hormone and its interaction with rooting media had no significant on the number of leaves (Table 3.2 and Table 3.3).

3.4.5 Plant height (cm)

Rooting media had a significant influence on plant height. The SP treatment recorded the highest mean value of height at (19.69 cm) and was significantly higher (P ≤0.05) than PPV (12.43 cm) and PP (14.33 cm) except S (18.45 cm) (Table3.1). Rooting hormone had no significant effect on plant height (Table 3.2) but its interaction with rooting media was significant (Table 3.3). S/D/1 (24.82), SP/D/3 (22.45), SP/D/2 (18.77) and SP/D/0 (18.73) had a significantly higher mean of plant height, which was similar to S/D/3 (18.55) and S/D/2 (17.82) but significantly higher than PP/D/3 (11.59).

Table 3.1 Effect of rooting media on rooting percentage, root length, number of roots, and number of leaves and plant height of *T. decumbens* stem cuttings.

Treatment	Rooting %	Root Length (cm)	Number of roots (n)	Number of leaves (n)	Height (cm)
Sand	76.8±3.8a	6.21±0.92a	2.66±0.31b	18.05±1.70bc	18.45±1.6ab
Sand:Peat(1:1)	84.2±1.6a	7.76±0.86a	3.75±0.45a	20.18±1.88b	19.69±1.38a
Peat:Perlite(1:1)	66.3±2b	5.93±0.94ab	1.52±0.20c	13.45±1.71c	14.33±1.62bc
Peat:Perlite:Vermiculite(1:1:1)	57.5±4.2b	3.62±0.66c	2.41±0.36bc	8.14±1.21a	12.43±1.50c
F- statistic	14.1*	4*	7.2*	10.5*	5*

Values (Mean ±SE) followed by dissimilar letters in each column are significantly different at P ≤0.05 (*)

Table 3.2 Effect of Dynaroot rooting hormone on stem cuttings of *Tetragonia decumbens*

Treatment	Rooting %	Root Length (cm)	Number of roots (n)	Number of leaves (n)	Height (cm)
No rooting hormone (C)	69.8±4a	6.52±0.88a	2.80±0.44a	16.50±2.a	16.64±1.45a
Dynaroot 1	72.6±3.4a	6.99±1.07a	2.89±0.34a	14.86±1.69a	17.68±1.66a
Dynaroot 2	75.4±3.1a	4.72±0.71a	2.39±0.33a	14.16±1.65a	16.26±1.50a
Dynaroot 3	66.8±5.9a	5.30±0.77a	2.27±0.33a	14.30±1.78a	14.33±1.71a
F- value	0.75ns	1.5ns	0.7ns	0.4ns	0.8ns

Values (Mean ±SE) followed by similar letters in each column are not significantly different as calculated by Fisher's least significant difference. D: Dynaroot, C

Table 3.3 Effect of rooting media and hormone on rooting percentage, root length, number of roots, and number of leaves and plant height of *T. decumbens* stem cuttings.

Treatment	Rooting %	Root Length (cm)	Number of roots (n)	Number of leaves (n)	Height (cm)
S/D/0	55.5±0.6e	4.68±1.52bc	2.45±0.74bcde	12.73±3.72bcd	12.64±3.68cd
S/D/1	84.7±3.2a	9.41±2.48ab	3.55±0.71abc	24.45±3.23a	24.82±2.69a
S/D/2	84.1±1.5a	5.22±1.41bc	2.18±0.48bcde	17.82±3.23abc	17.82±2.72abc
S/D/3	82.6±1.7a	5.55±1.64bc	2.45±0.5bcde	17.18±2.89abc	18.55±2.88abc
SP/D/0	87.9±3a	10.32±2.06a	4.64±1.34a	22.91±4.96a	18.73±2.88abc
SP/D/1	82.4±0.5ab	7.23±1.88abc	3.18±0.64abc	15.82±2.96abc	18.82±3abc
SP/D/2	84.5±2.7a	6.00±1.38abc	3.09±0.78abc	18.82±3.73ab	18.77±2.92abc
SP/D/3	81.9±5.1ab	7.50±1.43abc	4.09±0.74ab	23.18±3.17a	22.45±2.47ab
PP/D/0	72.3±5.4bc	6.91±1.63abc	1.36±0.31de	19.18±4ab	18.55±2.98abc
PP/D/1	62.9±0.6cde	7.59±2.74abc	1.73±0.47cde	10.55±2.92bcd	13.55±3.34c
PP/D/2	63.5±5cde	4.14±1.56c	1.36±0.41de	10.36±3.03bcd	13.64±3.34c
PP/D/3	66.4±2.8cd	5.09±1.36bc	1.64±0.45cde	13.73±3.42bcd	11.59±3.361cd
PPV/D/0	63.5±5.5cde	4.18±1.32c	2.73±0.57abc	11.18±2.32bcd	16.64±1.76abc
PPV/D/1	60.5±3de	3.73±1c	3.09±0.79abc	8.64±2.56de	13.55±3.4c
PPV/D/2	69.4±2.9cd	3.52±1.4c	2.91±0.8abc	9.64±2.5cde	14.82±3.12bc
PPV/D/3	36.3±5.2f	3.05±1.62c	0.91±0.51e	3.09±1.77e	4.73±2.45d
F-statistic	10.7*	0.7 ns	1.1 ns	1.9 ns	2.2 *

Values (Mean ±SE) followed by dissimilar letters in each column are significantly different at P ≤0.05 (*), ns = not significant. S: Sand, SP: Sand and peat, PP: Peat and perlite, PPV: Peat, perlite and vermiculite, D: Dynaroot, 0: control

3.5 Discussion

Propagation media is considered as one of the most significant factors affecting root initiation in cuttings (Jaleta and Sulaiman, 2019), and this experiment has provided evidence of its significance on root initiation of the nodal stem cutting of dune spinach. The application of growth regulators for root initiation of dune spinach is optional, as cuttings treated with media only rooted better than those treated with growth regulators. These findings substantiate those of Akinyele (2010) on *Bucholzia coriacea* stem cuttings, Mukhtar (2019) on juvenile stem cuttings of *B. aegyptiaca* and Jiang *et al.* (2016) on nodal stem cuttings of *Silene coronaria,* where cuttings planted in propagation media without hormone treatment showed improved results on tested variables.

The highest cutting survival, rooting rate, number of roots, length of roots and number of leaves were achieved on Sand and Peat mix followed by Sand. Root development was significantly lower in peat, perlite, and vermiculite mix. Several interacting factors within a growth medium such as oxygen, water and nutrient availability are known to affect the rooting success of cuttings (Alikhani *et al.,* 2011, Bhardwaj, 2014). Insufficient of one or more of these beneficial factors leads to lower or reduced rooting percentage (Mehri *et al.,* 2013). The rooting success of *T. decumbens* stem cuttings utilizing sand and peat could be ascribed to the positive interaction of aeration and water-holding capacity, as compared to the combination of peat, vermiculite, and perlite. These results are in agreement with the findings of Ibrahim (2008) on *Voacanga africana* stem cuttings and Dvin *et al.* (2011) on apple hardwood cuttings (MM1111), where the authors reported high rooting success in sand and peat mixture than sand, fibre or peat moss alone. Akakpo *et al.* (2014) also reported that a well-aerated media increases respiration at the base of the cuttings and thus promoting root initiation. Poor rooting performance in peat, vermiculite and perlite mix might be caused by poor aeration since both peat and vermiculite have high water retention capacity. This led to delayed rooting and decaying of cuttings. These results concur with the statement made by Schmitz *et al*. (2013), where the authors indicated that an increase in moisture content within a media reduces the oxygen diffusion efficiency in the root zone, thus resulting to delay rooting.

Time at which the cuttings were collected might have also played an impact on the rooting success of nodal cuttings. Soundy *et al.* (2008) reported that *Lippia javanica* stem cuttings collected in summer and spring had a major effect on rooting success. Same results were reported by Haile *et al.* (2011) on *Boswellia papyrifera* leafless cuttings and Mabizela *et al.* (2017) on *Cyclopia subternata* stem cuttings, proving that indeed seasonal effect does play a significant role in root initiation of plant species. The rooting success of dune spinach cuttings collected during the rainy season (June) may be attributed to carbohydrate reserves in mother plants, a common phenomenon in many fynbos species during the dry, hot summers

(Snijman, 2013). This is caused by low physiological activities within the plant which act as an adaptation strategy to withstand dry hot summers (Manning, 2013).

5. Conclusion and recommendations

T. decumbens is an important species with potential commercial value as a novel vegetable and its cultivation has the potential to be expanded in the coming years. The present study demonstrated that nodal stem cuttings of dune spinach may be propagated vegetatively with or without the use of rooting hormones during the winter period. Improved rooting was observed from nodal cuttings planted in sand: peat mixture with or without the use of rooting hormone. This study showed that dune spinach does not require additional rooting hormones which could be a cost-saving option for potential commercial growers of the species. The fact that plant growth parameters were significant in the growth media showed that species is relative fast-growing which could further benefit commercial cultivation, especially where both leaves and stems are eaten. Observations in its natural habitat have shown that this species is adaptive to withstand biotic and abiotic stresses, which could add to a robustness to withstand a range of environmental conditions during cultivation. Therefore, further studies on fertigation and salt stress responses of this species during cultivation are recommended to improve larger-scale production of the species.

3.7 Acknowledgements

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CHAPTER FOUR

EFFECT OF SALT STRESS ON PLANT GROWTH, NUTRIENT UPTAKE AND CHLOROPHYLL CONTENT IN *TETRAGONIA DECUMBENS* (DUNE SPINACH*)*

Effects of salt stress on plant growth, nutrient uptake and chlorophyll content in *Tetragonia decumbens* Mill (Dune spinach)

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4.1 Abstract

Climate change, expanding soil salinization and the developing shortages of freshwater have negatively affected crop production around the world. Seawater and salinized lands represent potentially cultivable areas for edible salt tolerate plants. In the present study, the effect of salinity stress on plant growth, nutrient uptake and chlorophyll content of *T. decumbens* was evaluated. Salt concentrations were set up on three treatments by adding increasing concentrations of NaCl in the nutritive solution (50, 100 and 200 mM). A total of 300 mℓ nutrient solution was prepared for each plant with and/or without NaCl addition, the plants were then watered every three days using a plastic beaker. The control treatment was sustained and irrigated only by the nutritive solution. Results revealed that the use of nutritive solution incorporated with 50 and 100 mM NaCl had a positive effect on plant growth, nutrient uptake and chlorophyll content in the leaves. Nitrogen and phosphorus uptake were more prominent in plants irrigated with 200 mM NaCl, even though this treatment negatively affected plant growth. The results of this study indicate that there is potential for seawater cultivation of *T. decumbens* for consumption.

Keywords: Dune spinach; halophytes; NaCl; nutrient uptake; plant growth; salt stress; *Tetragonia decumbens*

4.2 Introduction

Climate change, expanding soil salinization and the developing shortages of freshwater have negatively affected agricultural production on arable lands throughout the world (Loconsole *et al*., 2019).The world bank development report has estimated that crop production should increase by 70% to 100% by 2050 to meet global food demand (World Bank, 2007) However, increasing crop production has led to a loss of soil fertility and the phenomena of salinization and desertification, which make soils unsuitable for cultivation. This is caused by the accumulation of soluble salts in the soil moisture of the root zone. These salts restrict the absorption of water by the plant roots, which leads to osmotic stress and thus nutritional imbalance due to the high concentrations of toxic salts in soil and plant cells (Hussain *et al*., 2013; Shrivastava & Kumar, 2015). In addition, the accumulation of toxic ions inhibits the physiological processes such as photosynthesis, respiration, and nitrogen fixation (Farooq *et al*., 2015).These effects can result in reduced leaf area, plant biomass production and yield (Atieno *et al*., 2017; Huang *et al*., 2017).

Thus, the need for a creative, sustainable, and sufficient crop production method is inevitable given the rising population and increasing demand for plant-based food (Jimoh *et al.*, 2020b; Salami & Afolayan, 2020). With this in mind, numerous researchers have pointed out the use of salt tolerant plant species with possible commercial value as a proposed upfront strategy for saline lands without compromising the yield and quality of the final product (Ventura & Sagi, 2013; Debez *et al.*, 2010).Currently, underutilised edible halophytes are fast becoming a viable alternative to popular crops for addressing food and nutritional challenges. The use of native halophytes for food production in South Africa could be a climate change adaptation strategy as fresh water continues to become scarce and the rain, particularly in Sub-Saharan Africa becomes more sporadic (Bartels & Sunkar, 2005; Glenn *et al.*, 1999). Seawater and salinized lands represent potentially cultivable areas for edible salt tolerant plants.

*Tetragonia decumbens (*Aizoaceae) can be found on coastal sand dunes from southern Namibia to the Eastern Cape (Bittrich, 1990; Klak *et al*., 2017). It is a spreading shrub with dark green, sessile, glistening leaves (Forrester, 2004). It usually starts flowering from August to November. This widespread dune performs an important role in stabilizing the sand, enabling the dune to develop into a friendly environment for other plants (Mucina *et al*., 2006; Lubke & Strong, 1988). The leaves and soft stems of dune spinach have a salty taste and can be used like spinach, eaten raw in grain salads, or cooked with other vegetables (Mncwango *et al*., 2020; Forrester, 2004). Dune spinach can also be fermented, pickled and used in stews and soups and particularly tasty in a stir fry. It is also served in restaurants such as Wolfgat and Oep ve Koep in Paternoster, West Coast and Table Bay Hotel in Cape Town, respectively (van Der Merwe & de Villiers, 2014; Forrester, 2004). However, it is still unpopular among South Africans whereas its close relative *Tetragonia tetragonioides* is widely utilized in New Zealand, Australia and Brazil and sold in fresh vegetable markets as a substitute for spinach.

The potential of *T. decumbens* for commercial markets as a novel vegetable in South Africa is promising and strategies to popularize this plant should be put in place, as its cultivation has potential to be expanded in the coming years. It is well adapted in sand dunes, which are characterized by low soil fertility and require minimal cultivation needs. Majority of the accessible fields with these qualities in South Africa are located close to coastal areas and suffer from salinity. Therefore, understanding the responses of this species to salinity may be very vital in enhancing its overall performance and planning new regions for its expansion. Furthermore, it is to our knowledge that this is the first study examining the salt tolerance of *T. decumbens*.

4.3 Materials and methods

4.3.1 Experimental location

The experiment was conducted in the greenhouse of the Department of Horticultural Sciences at the Cape Peninsula University of Technology (CPUT), Bellville campus, Cape Town, South Africa, located at 33°55’56” S, 18°38’25” E. The greenhouse was equipped with environmental control with temperatures set to range from 21–26 ºC during the day and 12–18 ºC at night with relative humidity averages of 60%.

4.3.2 Plant preparation, irrigation, and treatments

Cuttings of *T. decumbens* were harvested on the 1st of August 2019 from a selected plant population growing along the coast at the Granger Bay campus (CPUT) located at 33°53'58.2"S, 18°24'41.4"E. Only cuttings taken using homogeneous methods, i.e. stem cuttings with about two thirds of leaves removed, ± 15 cm long with a stem thickness of approximately 8 mm were used for the experiment. One hundred cuttings were made to ensure the minimum number of 60 rooted plants required for the experiment was available. The cuttings were then soaked in 0.1 % Sporekil^{TM} for precaution against fungal infection, thereafter, were dipped on a rooting hormone (Dynaroot^{TM} No. 1 with active ingredient 0.1 % I.B.A) for two seconds. The cuttings were then placed in cutting trays containing washed and sterilized coarse river sand and peat (V: V). The cutting trays were then placed in the main greenhouse heated propagation beds. Once rooted, eighty *T. decumbens* plants of uniform size were individually transplanted in 12.5 cm plastic pots containing a mixture of commercial peat and sand (V: V) and were placed in a greenhouse to acclimatize. Only cuttings showing the strongest growth were selected and left to grow for two weeks. During this period, rooted cuttings were irrigated by a nutrient solution three times a week. The nutrient solution was formed by adding NUTRIFEED™ (Manufactured by STARKE AYRES Pty.Ltd. Hartebeesfontein Farm, Bredell Rd, Kaalfontein, Kempton Park, Gauteng, 1619) to municipal water at 10 g per 5 ℓ. The nutrient solution contained the following ingredients: N (65 mg/kg), P (27 mg/kg), K (130 mg/kg), Ca (70 mg/kg), Cu (20 mg/kg), Fe (1500 mg/kg), Mo (10 mg/kg), Mg (22 mg/kg), Mn (240 mg/kg), S (75 mg/kg), B (240 mg/kg) and Zn (240 mg/kg). After fourteen days of plant growth, plants were organized into four treatments each containing fifteen replicates (Figure 4.1). Salt concentrations were set up on three treatments by adding increasing concentrations of NaCl in the nutritive solution (50, 100 and 200 mM). About 300 mℓ nutrient solution each was prepared for each plant with and/or without NaCl. The plants were then watered every three days using a plastic beaker. The control treatment was sustained and irrigated only by the nutritive solutions. In all treatments the pH was maintained at 6.0. Ten weeks after salt treatments (Figure 4.2), all plants were harvested, and various postharvest measurements were made. Parameters measured included shoot length, shoot wet and dry weight, stem and root wet and dry weight.

Figure 4.1 *Tetragonia decumbens* plants at week 1 in the greenhouse. Plants labelled in a randomised block design. (Sogoni, 209).

4.3.3 Determination of plant growth

4.3.3.1 Plant weight

The weight of plants was measured using a standard laboratory scale (RADWAG® Model PS 750.R2) before planting out to ensure homogeneity within the samples. Post-harvest, shoots, stems and roots were separated and individual samples fresh/ wet weights were recorded. The plant material was then oven dried at 55 ˚C in a LABTECH™ model LDO 150F (Daihan Labtech India. Pty. Ltd. 3269 Ranjit Nagar, New Dehli, 110008) to a constant weight and were measured and recorded. The difference between the fresh and dry weights compares with the amount of water held within the plants' tissues (Olatunji & Afolayan, 2019).

4.3.3.2 Shoot length and branch number

The shoot length and branch number was used as a variable to determine new growth. Shoot length was measured every two weeks with a metal tape measure from the substrate level to the tip of the tallest shoot, while branch number was counted manually.

4.3.3.3 Nutrient analysis

In order to determine the nutrient uptake of each set of replicates in the experiment, three plants (shoots/leaves) were randomly selected from each treatment at the end of week 10 as seen in (Figure 4.2). The vegetative material was then removed, labelled, and sent to Bemlab Laboratory, 16 van der Berg Crescent, Gant's Centre, Strand, Cape Town for proximate analysis. The methodology to determine macronutrients (N, K, P, Ca, Mg and Na) and micronutrients (Cu, Zn, Mn, Fe, Al and B) was conducted by ashing 1g ground sample of plant material in a porcelain crucible at 500°C overnight. This was followed by dissolving the ash in 5mL of HCl and placing it in an oven at 50°C for 30 min. Thirty-five millilitres of deionised water was then added and the extract filtered through Whatman No. 1 filter paper. Nutrient concentrations in plant extracts were determined using an inductively coupled plasma (ICP) emission spectrophotometer (IRIS/AP HR DUO Thermo Electron Corporation, Franklin, Massachusettes, USA), (Jimoh *et al.,* 2020a; Hoenig *et al.*, 1998; Idris *et al.*, 2019).The concentrations of N, P, K, Ca, and Mg were all converted from percentages to mg/ kg using 10 000 as a conversion factor.

4.3.3.4 Chlorophyll content

The chlorophyll content was measured every two weeks using a Soil Plant Analysis Development (SPAD-502) meter supplied by Konica-Minolta. This device measures transmission of red light at 650 nm, the frequency at which chlorophyll absorbs light and transmission of infrared light at 940 nm, a wavelength at which no absorption occurs (Ingarfield, 2018). Using these two transmission values, the instrument calculates a SPAD level, which is indicative of chlorophyll content. The readings of two fully formed leaves were taken from each plant and the figures were averaged out by the SPAD-502 meter to produce a final number. The readings were taken between 11 am and midday from week 4 to 10 of the experiment.

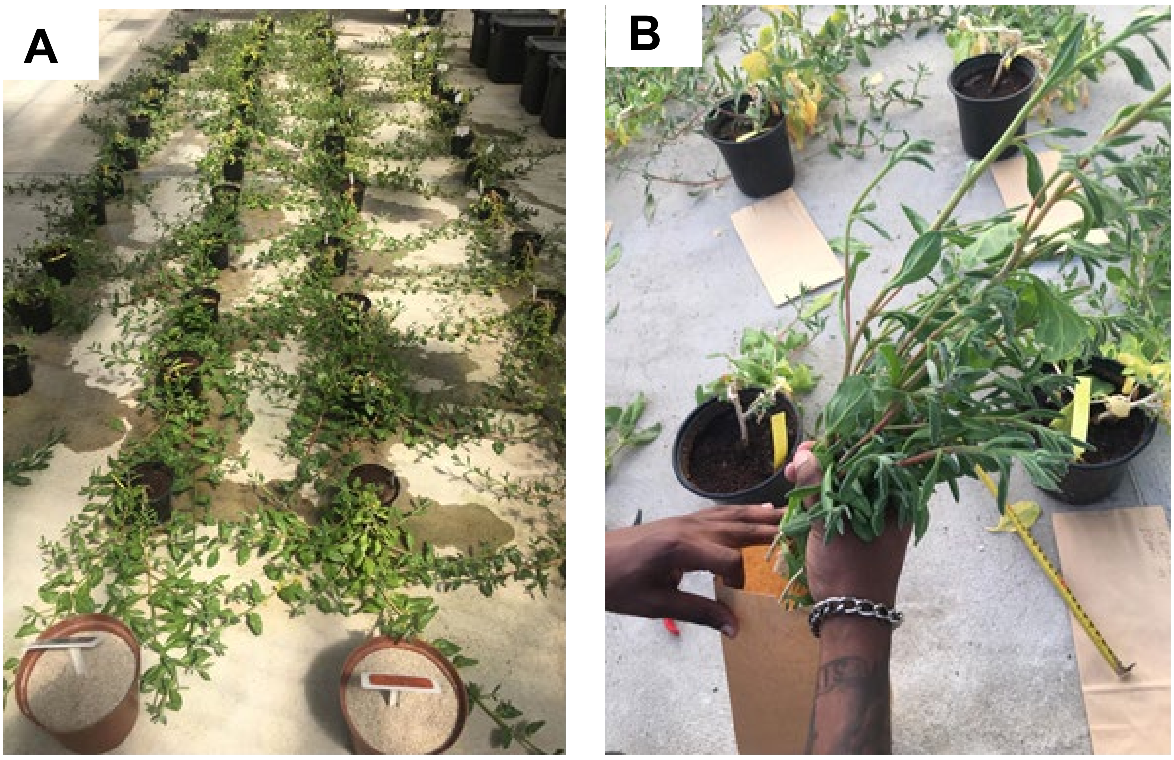

Figure 4.2 (A) Plant growth respose to salt stress at the end of week 10. (B) Harvested shoots collected and then dried for mineral analysis. (Sogoni, 2019).

4.3.4 Statistical analysis

Where applicable, the data were subjected to statistical analysis using STATISTICA version 13.5.0.17. A one-way Analysis of Variance (ANOVA) was used to compare the means among the growth parameters. Means were compared using Fisher's least significant difference at $P \leq 0.05$ significance level.

4.4 Results

4.4.1 Effects of salinity stress on plant growth

4.4.1.1 Shoot length and lateral branch number

The results showed that *T. decumbens* growth parameter's response to NaCl were variable (Table 4.1). Shoot length and lateral branch number were significantly affected by salinity concentrations at $P \leq 0.05$. The control had the highest shoot length and this was significantly higher than 100 mM NaCl and 200 NaCl mM concentrations respectively, but did not differ significantly from 50 mM NaCl concentration. However, this was not the case with the lateral branch number where 50 mM NaCl concentration had the highest number of branches compared to the control.

4.4.1.2. Fresh and dry weight of shoots

Both fresh and dry weights of shoots significantly differed between treatments (Table 4.1). The highest fresh weight was obtained at 50 mM NaCl concentration. This was significantly higher than all NaCl concentrations including the control. The lowest fresh weight was obtained at 200 mM NaCl concentration; however, this was not significantly different from the control. As for the dry weight, the highest mean value was again obtained at 50 mM NaCl concentration; this was significantly higher than all other treatments including the control. The lowest dry weight was obtained at 200 mM NaCl concentration and this was significantly lower compared to other treatments including the control.

4.4.1.3 Fresh and dry weight of stem and roots

NaCl concentrations positively influenced the fresh and dry weights of stem and roots. The highest fresh weight of stem and roots was obtained at 50 mM NaCl concentration. This was significantly higher than the control but did not differ significantly to 100 mM and 200 mM NaCl concentrations respectively. The highest dry weight of stem and roots was again recorded at 50 mM concentration. This was significantly higher than the control and 200 mM NaCl, but did not differ significantly from the 100 mM NaCl concentration.

4.4.1.4 Total fresh and dry weight

Experimental results also showed that NaCl concentrations significantly ($P \leq 0.05$) affected the total fresh and dry weight of dune spinach (Table 4.1). Plants exposed to different salt concentrations had fresh weights that were higher than the control. The highest measurement of fresh weight was obtained at 50 mM NaCl concentration and this was significantly higher than all treatments including the control. Although, plants exposed to 200 mM NaCl concentration had higher fresh weight than the control, they did not differ significantly from each other. This was not the case in total dry weight, where 200 mM NaCl concentration recorded the lowest dry weight compared to all treatments including the control. The highest total dry weight was again obtained at 50 mM NaCl concentration and this was significantly higher than all other treatments including the control.

Table 4.1: Effect of salt stress on growth parameters of *T. decumbens*

Treatment **NaCl conc.**	**SL (cm)**	**BN (n)**	**FWS (g)**	**DWS (g)**	**FWSR (g)**	**DWSR (g)**	**TFW (g)**	**TDW (g)**
Control	101.07±3.8^{a}	3.40±0.2^{c}	136.86±6.7^{c}	23.09±1.2^{c}	61.40±5.4^{b}	14.54±1^{b}	198.26±8.3^{c}	37.62±1.1^{c}
50 mM	96.53±3.1^{ab}	5.40±0.4^{a}	210.84±10.4^{a}	32.13±1.3^{a}	89.33±6.7^{a}	18.46±1^{a}	300.18±12.8^{a}	50.58±1.6^{a}
100 Mm	90.73±2.2^{b}	4.67±0.2^{ab}	186.38±6.8^{b}	26.86±0.7^{b}	78.01±3.8^{a}	17.27±0.8^{ab}	264.39±6.6^{b}	44.13±0.9^{b}
200 Mm	73.73±3.1^{c}	3.87±0.3^{bc}	119.67±6.3^{c}	15.62±0.8^{d}	79.48±6.2^{a}	15.26±1.1^{b}	199.15±7.8^{c}	30.87±1.2^{d}
F-statistic	14.6*	7.6*	29.8*	45*	4.2*	3.2*	30*	45*

SL: Shoot Length; BN: Branch Number; FWS: Fresh Weight of Shoots; DWS: Dry Weight of Shoots; FWSR: Fresh Weight of Stem and Roots; DWSR: Dry Weight of Stem and Roots; TFW: Total Fresh Weight; TDW: Total Dry Weight. The values (Mean ±SE) followed by dissimilar letters in each column are significantly different at P ≤0.05 (*).

4.4.2 Effect of salinity stress on nutrient uptake

4.4.2.1 Macronutrients

Salinity stress significantly increased macronutrients (N, P and Na) uptake in the leaves of dune spinach. The highest mean values of N, P and Na were all obtained from the highest salt concentration treatment (200 mM). Conversely, the accumulation of NaCl in the leaves significantly ($P \leq 0.05$) lowered other macronutrients such as K, Ca, and Mg, in comparison to the control (Table 4.2).

4.4.2.2 Micronutrients

Salt stress positively influenced the micronutrients (Mn, Fe, and Cu) in the leaves of dune spinach. The highest mean values of Mn, Fe and Cu were all obtained from the moderate salt concentration (100 mM). However, the highest mean values in Mn and Cu were not significantly ($P \leq 0.05$) different from all other treatments including the control. But, the opposite was true for Fe. Conversely, salt stress negatively influenced Zn and B accumulation in the leaves. The control had the highest mean values in both Zn and B, and these were significantly different from all salt treatments (Table 4.3).

Table 4.2: Effect of salt stress on macronutrient uptake in the leaves of *T. decumbens*

Treatment	**N**	**P**	**K**	**Ca**	**Mg**	**Na**
NaCl conc.	mg/ kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Control	23466.6±536.4^{a}	4733.3±676.6^{a}	44133.3±1581.5^{a}	6966.3±696.3^{a}	6966.7±466.6^{a}	6930.0±1977.5^{b}
50 Mm	20833.3±120.2^{b}	3300.0±493.3^{a}	26800.0±1137.3^{b}	3666.7±176.4^{b}	3333.3±88.2^{b}	35896.7±2315.3^{a}
100 Mm	20900.0±608.3^{b}	3433.3±584.0^{a}	22566.7±1026.9^{c}	3733.3±523.9^{b}	3166.7±240.3^{b}	54683.3±5299.9^{c}
200 Mm	24433.0±466.4^{a}	5033.3±800.7^{a}	21933.3±688.8^{c}	2466.7±88.2^{b}	2800.0±100^{b}	59043.3±927.6^{c}
F-statistic	14.9*	1.9ns	81.3*	18.6*	18.6*	58.8*

Values (Mean ±SE) followed by dissimilar letters in each column are significantly different at P ≤0.05 (*); ns = not significant

Table 4.3: Effect of salt stress on micronutrient uptake in the leaves of *T. decumbens*

Treatment	**Mn**	**Fe**	**Cu**	**Zn**	**B**
NaCl conc.	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Control	74.30±14.3[a]	66.40±2.2[b]	1.7 ± 0.3[a]	56.7 ±4.8[a]	35.1±5[a]
50 Mm	67.50±14.9[a]	61.30± 0.83[bc]	1.7 ±0.2[a]	35.4 ±0.5[b]	20.7±0.7[b]
100 Mm	81.30±8.9[a]	89.20± 2.7[a]	4.2 ±1.9[a]	51.6 ±1.4[a]	24.7±2.4[b]
200 Mm	64.40±4.2[a]	57.90± 2.2[c]	2.3± 0.5[a]	36 ±0.3[b]	22.1±0.9[b]
F-statistic	0.4ns	44.9*	1.2ns	17.9*	5.2*

Values (Mean ±SE) followed by dissimilar letters in a column are significantly different at P ≤0.05 (*); ns = not significant

4.4.3 Effect of salinity stress on Chlorophyll content

As shown in Figure 4.3, the total chlorophyll contents were negatively affected by 50 mM, 100 mM, and 200 mM salinity concentrations during the 4th week of growth. However, plants exposed to lower salinity (50 mM NaCl) had the highest SPAD-502 values, which was significantly higher at P ≤0.05 from all other treatments including the control. During the 6th week, salinity concentrations positively affected the chlorophyll values and were significantly different from one another at P ≤0.05. The highest SPAD-502 values were obtained at 200 mM NaCl concentration followed by 100 mM, 50 mM and the control. During the 8th week, chlorophyll values were negatively affected by salinity as all treatments, including the control had lower chlorophyll values when compared to week 6. However, higher concentration (200 mM NaCl) had the highest SPAD-502 value but it was not significantly different from other treatments except the control. During the 10th week, salinity stress further reduced the chlorophyll values of all treatments except the control. The control had the highest mean value, which was significantly higher from all salt treatments.

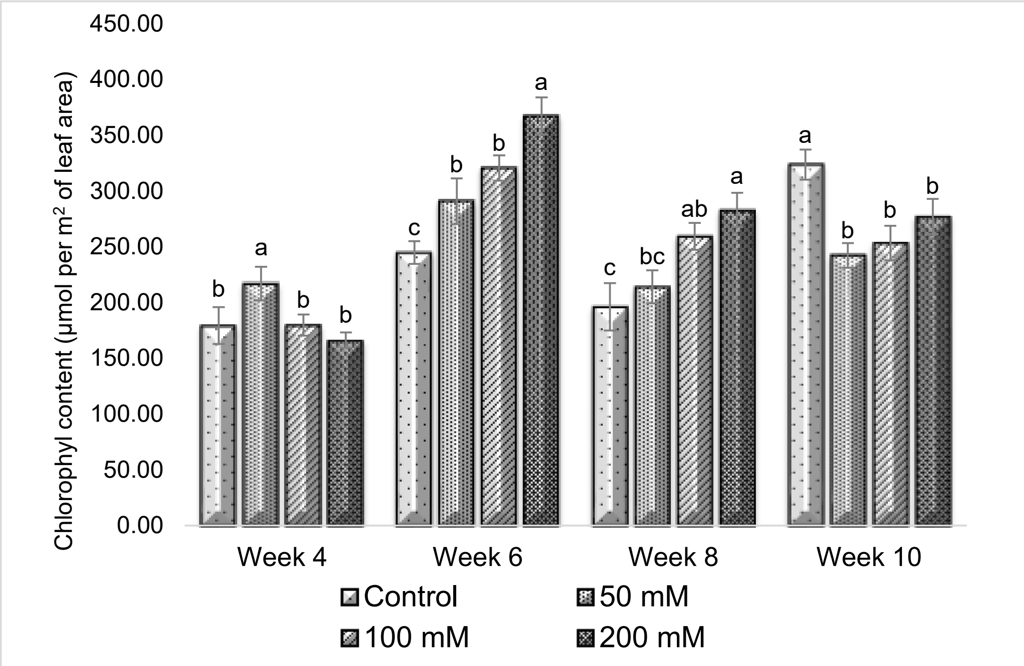

Figure 4.3 The effect of NaCl concentrations on the chlorophyll readings of *T. decumbens* leaves measured from weeks 4 to 6.

4.5 Discussion

4.5.1 Effect of salinity stress on plant growth

There is extensive literature on the reduction of growth caused by salt stress in large number of plants. Nevertheless, the effect of salt stress varies among plants, thus requiring crop specific information for modelling and predictions. In the present study, increasing NaCl concentrations led to a significant decrease in plant height. Height reduction as a result of salinity stress has been reported in several plant species and has been mainly associated to the osmotic stress and ion toxicity that causes a reduction in plant growth (Fageria *et al.,* 2012; Munns &Tester, 2008; Petretto *et al*., 2019; Rodrigues *et al*., 2019). Nevertheless, when comparing the number of branches (compactness) among the treatments, all plants irrigated with NaCl had more branches compared to the control. This might be caused by the natural adaptation of the species to stabilise the coastal sand dunes. The increase in branch numbers resulted in higher total fresh weight. These findings contradict those reported by Grigore *et al.* (2012), where the authors observed that after 12 weeks of cultivating some halophytes such as *Inula crithmoides* L., *Plantago crassifolia* Forssk. and *Medicago marina* L., the plants whose irrigation water had not been spiked with salt showed better productivity and growth rates. However, the results of the current work agree with the findings of Van Puijenbroek *et al.* (2017), where the halophyte *Ammophila arenaria* showed increased plant biomass in lower to moderate soil salinity. The ability of dune spinach to withstand these varying salt concentrations could be attributed to osmotic, ion, and tissue tolerance. Furthermore, high concentrations of salt reduced growth of dune spinach. This has been reported on numerous studies (Guo *et al.,* 2018; Orlovsky *et al.,* 2016; Rahneshan *et al.,* 2018) conducted on halophytes, where increasing salinity negatively affected plant growth performance, causing a reduction in biomass, leaf number and plant height. Tian *et al.* (2020), also reported that longer salt exposure in the root zone restricts the flow of water and nutrients into the plant by direct injury to plant cells through the accumulation of toxic ions causing a decline in plant growth.

4.5.2 Effect of salinity stress on nutrient uptake

Salt stress is one of the major environmental factors affecting nutritional value of many edible plants. In the present study, salt stress increased the uptake of N, P and Na in the leaves, while K, Ca and Mg were reduced drastically. The reduction of these elements may be directly linked to excessive Na^+ absorption by the roots as reported by Benito *et al.* (2014). However, sufficient K, Ca and Mg are required to meet basic metabolic processes such as intracellular K homeostasis, which is essential for optimal functioning of the photosynthetic machinery and maintenance of stomatal opening (Shabala & Pottosin, 2014). These results suggest that dune spinach has the ability to transport K, Ca and Mg to new shoots and leaves under salt stress, and maintain a suitable ratio needed for normal metabolism; hence, the chlorophyll content was not affected for 8 weeks. This could be attributed to the water use efficiency and carbon fixing capacity of this species, which uses Crassulacean Acid Metabolism (CAM) to adapt to harsh conditions. Our results agree with those conducted on quinoa genotype A7 (*Chenopodium quinoa* Willd.) (Parvez *et al*., 2020) and Spiny chicory (*Cichorium spinosum* L.) (Petropoulos *et al*., 2017) in saline conditions, where it was reported that higher capacity of K and Ca transport into new shoots and leaves contributed in mitigating ion toxicity in leaf cells.

Moreover, salinity stress also increased the Mn, Fe and Cu contents in the leaves, while Zn and B were negatively affected. Similar findings were reported by Lima *et al.,* (2020) on slender glasswort (*Salicornia ramosissima* J. Woods), where salinity stress increased Mn, Fe and Cu. These results indicate that salt stress caused Zn and B deficiency in the leaves of dune spinach, but since they are required in small quantities, visual symptoms of nutrient deficiency did not occur.

4.5.3 Effect of salinity stress on chlorophyll content

It has been reported in previous literature that salinity stress damages nutrition and promotes senescence mechanisms in plants, causing a reduction of chlorophyll content in the leaves (Munns & Tester, 2008; Petretto *et al.,* 2019). However, the extent of reduction depends on the salt tolerance of the plant species (Gong *et al.,* 2018). Stepien *et al.* (2009) reported that salt-tolerant species (halophyte) such as *Thellungiella halophila* indicated more or unchanged chlorophyll content when exposed to 0-500 mM NaCl, while salt-sensitive species (glycophyte) such as *Arabidopsis thaliana* had lower chlorophyll content. In the present study, the chlorophyll content was used as a biochemical marker to screen the salt tolerance of dune spinach. SPAD values (chlorophyll content) varied among treatments during the growing weeks, with salt treatments having higher SPAD values on week 6 and 8 when compared to the control. The findings of this study agree with results obtained by Sui *et al*. (2015) in M-81E sweet sorghum (salt tolerant genotype), where the chlorophyll content was not affected by 50 mM NaCl. In another study conducted by Ferreira *et al*. (2015), spinach cultivar raccoon treated with saline irrigation water maintained SPAD chlorophyll levels, but had reduced photosynthetic rate, stomatal conductance and transpiration rate. This interaction in dune spinach remains unknown and further studies are necessary to better understand the salt tolerance of this species. However, outcomes of this study have demonstrated that *T. decumbens* can withstand varying amounts of salinity (50, 100, 200 mM) for up to 8 weeks, even though plant growth reduces on higher salt concentration.

4.6 Conclusion

Although poor performance in most assessed variables were observed under increased salinity stress (200 mM), nutrient uptake and total fresh weight of leaves were very high under low (50 mM) and moderate (100 mM) salinity concentrations. Base on this study it is evident that there is potential for seawater cultivation of the leafy vegetable *T. decumbens*. This could be a water-saving option in provinces experiencing the adverse effect of drought and salinity, where seawater or underground saline water could be diluted with freshwater and used as irrigation water in the production of this vegetable. These results would be encouraging to future growers of novel fresh leafy green vegetables which in time could become sustainable commercial crops. Further studies are necessary in evaluating the effect of salt stress on the re-sprouting ability and nutritional properties of this plant when continuously harvested from mature plants. This could further support the species commercial viability as a suitable vegetable for consumption.

4.7 Acknowledgments

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CHAPTER FIVE

EFFECT OF SALT STRESS ON THE POLYPHENOLIC CONTENT AND ANTIOXIDANT-CAPACITY OF *TETRAGONIA DECUMBENS* (DUNE SPINACH)

Effects of salt stress on the polyphenolic content and antioxidant-capacity of *Tetragonia decumbens* Mill (Dune spinach)

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5.1 Abstract

Vegetables including spinach are known to be rich in important phytochemicals, such as phenolic compounds, vitamins, antioxidant compounds, carotenoids and nutritive elements. When exposed to salinity stress secondary metabolites of some vegetable species increases. In this study, the antioxidant activities and accumulation of metabolites within the leaves exposed to salt stress were assessed using assays for total polyphenols, ferric reducing antioxidant power (FRAP) and Trolox equivalent antioxidant capacity (TEAC/ABTS). The result showed that salinity positively affected polyphenolic compounds and antioxidant capacity (FRAP excluding ABTS) in the leaves. Polyphenolic compounds increased with an increase in concentration of NaCl with the highest content found in 200 mM. This was not the case when FRAP capacity of the leaves were assessed, where the lower and moderate NaCl concentrations proved to strengthen the FRAP capacity more than the highest concentration. As for the ABTS capacity, salt stress did not have any significant effect since the control had a slightly more capacity than all NaCl concentrations. Furthermore, a strong correlation was evident in the total polyphenols and FRAP capacity of the leaves exposed to moderate NaCl concentrations. These results have shown that the leaves of dune spinach may be used as a natural source of nutritional antioxidants.

Keywords: Dune spinach; Ferric reducing antioxidant power (FRAP); Trolox equivalent antioxidant capacity (TEAC/ABTS); Reactive oxygen species (ROS).

5.2 Introduction

Salinity and drought stress are well-known factors to induce oxidative stress in plants through the production of superoxide radicals by the process of Mehler reaction (García-Caparrós *et al.*, 2020). These free radicals initiate the chain of reactions that produce more harmful oxygen radicals (Chao *et al.,* 2009). These reactive oxygen species (ROS) are continuously generated during normal metabolic processes in mitochondria, peroxisomes and cytoplasm which disturb normal metabolism through oxidative damage of lipids, proteins, and nucleic acids when produced in excess (Hernandez *et al.,* 2001; Ahmad *et al.,* 2010). Plants throughout their lives are prone to oxidative damage caused by environmental factors due to their sessile nature (Hippeli & Elstner, 1996). There is a constant need for efficient mechanisms to compensate the possible oxidative damage to cellular components. Plants have evolved efficient systems for ROS removal, which include specific ROS-scavenging anti-oxidative enzymes and small non-enzymatic molecules that act as ROS scavengers such as ascorbate, glutathione, α tocopherol, flavonoids, anthocyanines, polyphenolic compounds and carotenoids.

To overcome salt-mediated oxidative stress, plants detoxify ROS by up-regulating anti-oxidative enzymes which includes the superoxide dismutase (SOD) found in various cell compartments. This enzyme catalyses a conversion from two O_2 radicals to H_2O_2 and O_2 (Scandalios, 1993). In alternative ways, several antioxidant enzymes can also eliminate the H_2O_2 such as catalases (CAT) (Scandalios, 1993) and peroxidases (POX) by converting it to water (De Gara *et al.,* 2003). During this process antioxidant capacity of some species increases when exposed to salinity stress to eliminate or reduce the ROS. However, this process varies among species. For example, *Beta vulgaris* (sugar beet) is known to be salt tolerant at later stages of growth whilst it is sensitive at germination phases. Some wild greens such as *Salicornia*, *Sarcocornia* and *Cichorium* tolerate salt stress throughout their growing process without significant effects on plant growth and yield (Petropoulos *et al.,* 2017). These wild greens are used in human diets in other countries with several studies confirm their nutritional value and antioxidant activity (Morales *et al.,* 2012). These antioxidants are very useful against free radicals that predispose humans to sickness and diseases. Thus, research concerning the impact caused by salinity on secondary metabolism of underutilised edible halophytes is needed.

Tetragonia decumbens Mill. which belongs to the family Aizoaceae is an edible halophyte that is distributed along the coast from southern Namibia to the Eastern Cape. It is a spreading shrub with dark green, sessile, glistening leaves. The leaves and soft stems have a salty taste and can be used like spinach, served raw in grain salads, or cooked with other vegetables. The leaves can also be fermented, pickled and used in stews and soups and particularly tasty in a stir fry (Rusch, 2016). It is also served in restaurants such as Wolfgat and Oep ve Koep in Paternoster, West Coast and Table Bay Hotel in Cape Town, respectively (Van der Merwe & De Villiers, 2014; Black, 2015; Mckeown, 2017). Even though the species is still unknown amongst most South Africans it has the potential to be used as an alternative source of nutrition. However, understanding the biochemical responses of this species to salinity could potentially influence its commercial cultivation. Hence, in this study, we aimed to investigate the antioxidant capacity of *T. decumbens* in response to salt stress. Furthermore, it is to our knowledge that this is the first study examining the influence of salt stress on antioxidant status and oxidative damage in *T. decumbens* 'Dune spinach'.

5.3 Methods and material

5.3.1 Experimental location

The experiment was conducted in the research greenhouse of the Department of Horticultural Sciences at the Cape Peninsula University of Technology, Bellville campus, Cape Town, South Africa, located at 33°55'56" S, 18°38'25" E. The research greenhouse was equipped with mist, a heating bed and environmental control with temperatures set to range from 21–26 ºC during the day and 12–18 ºC at night with Relative Humidity average of 60%.

5.3.2 Plant preparation, irrigation and treatments

Cuttings of *T. decumbens* were harvested on the 1st of August 2019 from a single stock plant growing along the coast at the Granger Bay campus (CPUT) located at 33°53'58.2"S, 18°24'41.4"E. Only cuttings taken using homogeneous methods, i.e. stem cuttings with about two thirds of leaves removed, ± 15 cm long with a stem thickness of approximately 8 mm were used for the experiment. One hundred cuttings were made to ensure the minimum number of 60 rooted plants required for the experiment was available. The cuttings were then soaked in 0.1 % Sporekil™ for precaution against fungal infection, thereafter, were dipped on a rooting hormone (Dynaroot™ No. 1 with active ingredient 0.1 % I.B.A) for 2 seconds. The cuttings were then placed in cutting trays containing washed and sterilized coarse river sand and peat (v:v). The cutting trays were then placed in the main greenhouse heated propagation beds. Once rooted, eighty *T. decumbens* plants of uniform size were individually transplanted in 12.5 cm plastic pots containing a mixture of commercial peat and sand (v:v) and were placed in a greenhouse to acclimatize. Only cuttings showing the strongest growth were selected and left to grow for 2 weeks. During this period, rooted cuttings were irrigated by a nutrient solution three times a week. The nutrient solution was formed by adding

NUTRIFEED™ (Manufactured by STARKE AYRES Pty.Ltd. Hartebeesfontein Farm, Bredell Rd, Kaalfontein, Kempton Park, Gauteng, 1619) to municipal water at 10 g per 5ℓ. The nutrient solution contained the following ingredients: N (65 mg/kg), P (27 mg/kg), K (130 mg/kg), Ca (70 mg/kg), Cu (20 mg/kg), Fe (1500 mg/kg), Mo (10 mg/kg), Mg (22 mg/kg), Mn (240 mg/kg), S (75 mg/kg), B (240 mg/kg) and Zn (240 mg/kg). After fourteen days of plant growth, plants were organized into four treatments each containing fifteen replicates (Figure 4.1). Salt concentrations were set up on three treatments by adding increasing concentrations of NaCl in the nutritive solution (50, 100 and 200 mM). About 300 mℓ nutrient solution each was prepared for each plant with and/or without NaCl. The plants were then watered every three days using a plastic beaker. The control treatment was sustained and irrigated only by the nutritive solution. In all treatments the pH was maintained at 6.0. Ten weeks after salt treatments were completed, five plants per treatment were randomly selected, measured, and analysed.

5.3.3 The antioxidant analysis

5.3.3.1 Sample preparation

Harvested shoot materials were immediately dried in a fan-drying laboratory oven (Oxidative Stress Research Centre, Faculty of Health and Wellness Sciences at CPUT, Bellville, South Africa) at 40 °C for 7-14 days. The dried plants were grounded into fine powder using a Junkel and Kunkel model A 10 mill. Shoot, material was extracted by mixing 100mg of the dried powdered material with 25 ml of 70% (v/v) ethanol (EtOH) (Merck, South Africa) for 1 hour. It was centrifuged at 4000 rpm for 5 min and the supernatants were used for all analyses.

5.3.3.2 Determination of antioxidant capacity and content

Antioxidant activity and accumulation of metabolites within the leaves were assessed using assays for total polyphenols, ABTS and ferric reducing antioxidant power (FRAP).

5.3.3.2.1 Polyphenol assay

The total polyphenols assay (Folin assay) was performed as described by Ainsworth and Gillespie (2007). Folin and Ciocalteu‟s phenol reagent (2N, Sigma South Africa) was diluted 10 times with distilled water and a 7.5% sodium carbonate (Sigma, South Africa) solution was prepared. In a 96-well plate, 25µl of the crude extract was mixed with 125µl Folin and Ciocalteu‟s phenol reagent and 100µl sodium carbonate. The plate was incubated for 2 hours at room temperature. The absorbance was then measured at 765 nm in a Multiskan spectrum plate reader (Thermo Electron Corporation, USA). The samples polyphenol values were calculated using a gallic acid (Sigma, South Africa) standard curve with concentration varying between 0 and 500 mg/L. The results were expressed as mg gallic acid equivalents (GAE) per g dry weight (mg GAE/g DW).

5.3.3.2.2 ABTS assay

The ABTS assay was performed following the method of (Re et al., 1999). The stock solutions included a 7 mM ABTS and 140 mM Potassium–peroxodisulphate ($K_2S_2O_8$) (Merck, South Africa) solution. The working solution was then prepared by adding 88 µL $K_2S_2O_8$ to 5 mL ABTS solution. The two solutions were mixed well and allowed to react for 24 h at room temperature in the dark. Trolox (6-Hydrox-2,5,7,8-tetramethylchroman-2- 20 carboxylic acid) was used as the standard with concentrations ranging between 0 and 500 µM. Crude sample extracts (25 µL) were allowed to react with 300 µL ABTS in the dark at room temperature for 30 min before the absorbance was read at 734 nm at 25 °C in a plate reader. The results were expressed as µM/Trolox equivalent per g dry weight (µM TE/g DW).

5.3.3.2.3 Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed using the method of Benzie and Strain (1999). FRAP reagent was prepared by mixing 30 mL Acetate buffer (0.3M, pH 3.6) (Merck, South Africa) with 3 mL 2,4,6- tripyridyl-s-triazine (10mM in 0.1M Hydrochloric acid) (Sigma, South Africa), 3 mL Iron (III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$) (Sigma, South Africa) and 6 mL of distilled water. In a 96-well plate, 10 µL of the crude sample extract was mixed with 300 µL of the FRAP reagent and incubated for 30 min at room temperature. The absorbance was then measured at 593 nm in a Multiskan spectrum plate reader (Thermo Electron Corporation, USA). The samples FRAP values were calculated using an L-Ascorbic acid (Sigma-Aldrich, South Africa) standard curve with concentrations varying between 0 and 1000 µM. The results were expressed as µM ascorbic acid equivalents (AAE) per g dry weight (µM AAE/g DW) (Ainsworth & Gillespie, 2007).

5.3.4 Statistical analysis

The data was statistically analysed using one-way Analysis of Variance (ANOVA). The statistical significance between antioxidant activities values of the various crude plant extracts at $P \leq 0.05$ were compared using Fisher's least significant difference. All the calculations were done on a computer software program called STATISTICA version 13.5.0.17.

5.4 Results

5.4.1 Polyphenol content

The polyphenol content in the leaves vary significantly at P ≤0.05, when different NaCl concentrations were compared with each other and with the control (Figure 5.1 and Table 5.1). Plants exposed to higher NaCl concentration (200 mM) had the highest mean value of polyphenol content (2.6 mg GAE/g dry weight**)** in their leaves as compared to the control and other treatments. This was significantly different from the control and the lower NaCl (50 mM) concentration but did not differ significantly to the moderate NaCl (100 mM) concentration.

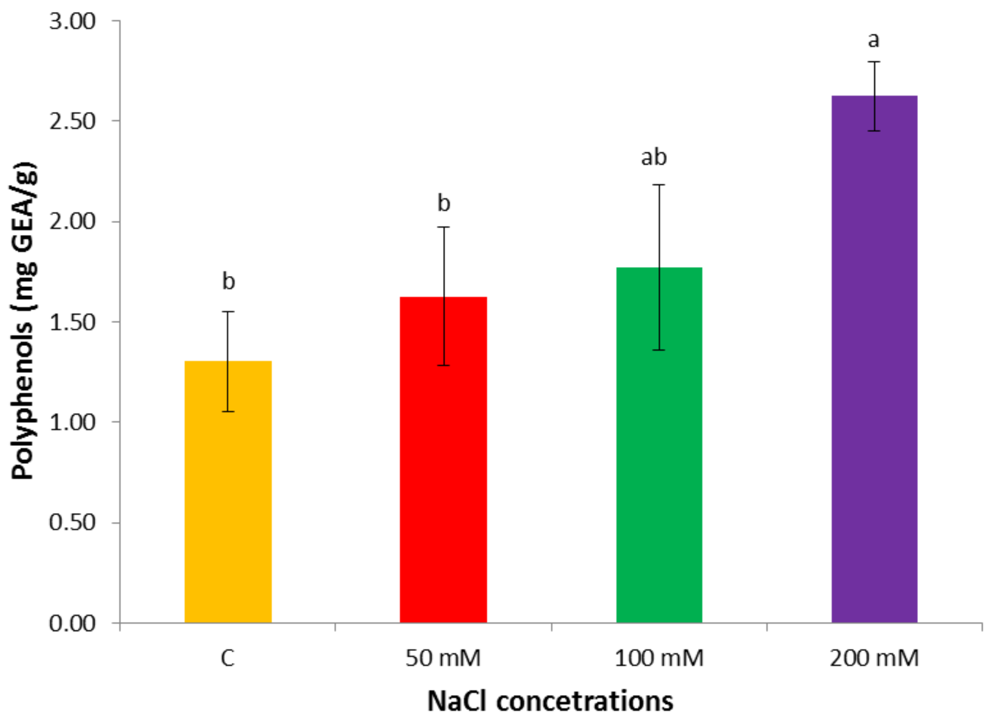

Figure 5.1 The effect of NaCl concentrations on total polyphenol (mg GAE/g dry weight) content in the leaves of *T. decumbens*

5.4.2 ABTS capacity

Salt stress negatively influenced the ABTS capacity in the leaves of *T. decumbens*. The control had the highest mean value when compared to all treatments; however, this was not significantly different from all other treatments (Figure 5.2).

5.4.3 FRAP capacity

The total FRAP capacity in the leaves of *T. decumbens* was significantly influenced by the NaCl concentrations at P ≤0.05. The lower NaCl (50 mM) concentration had the highest mean value (13.3 μM AAE/g dry weight) compared to other treatments including the control. This was significantly higher than other treatments including the control.

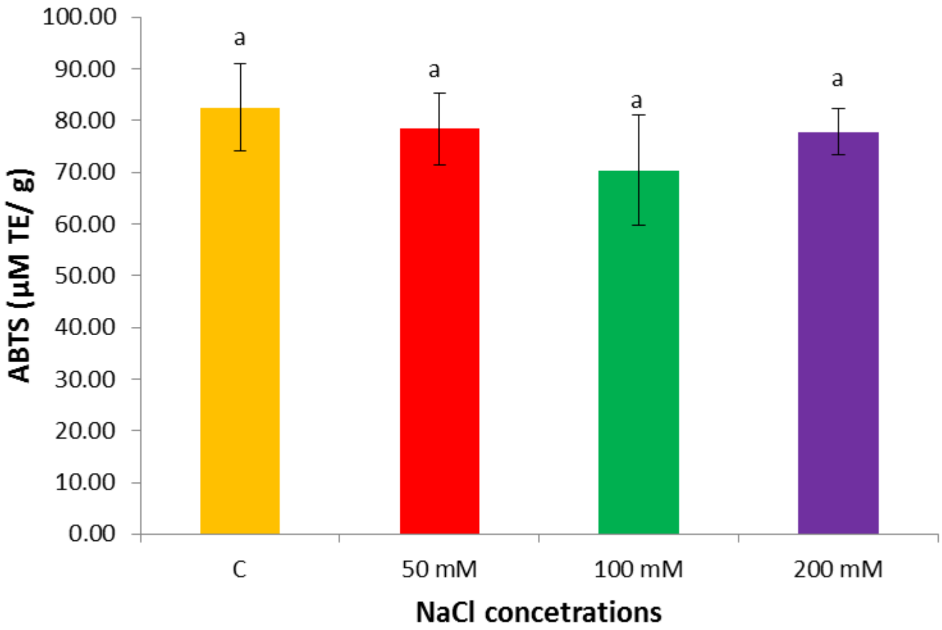

Figure 5.2 The effect of NaCl concentrations on total ABTS (µM TE/g dry weight) content in the leaves of *T. decumbens*

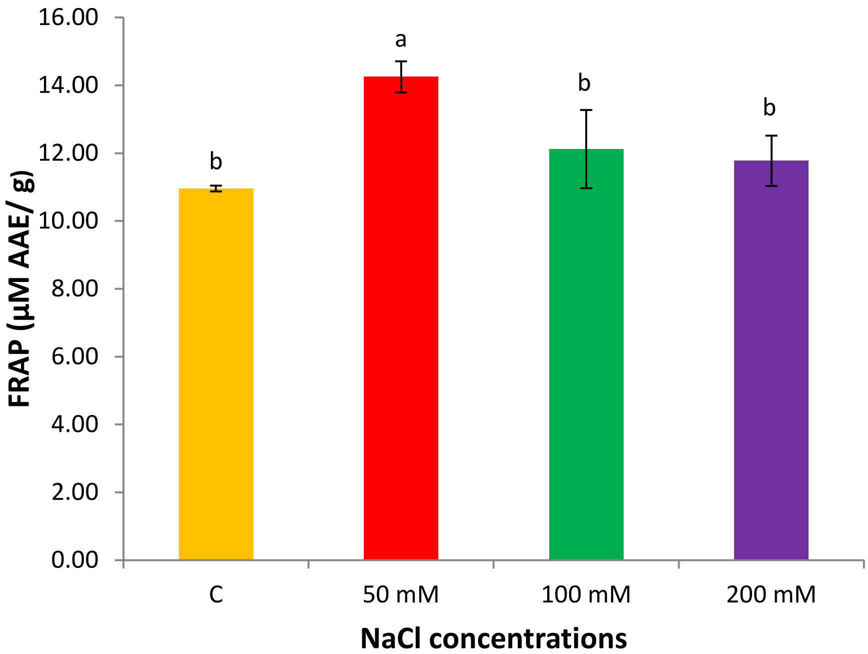

Figure 5.3 The effect of NaCl concentrations on total FRAP (µM AAE/g dry weight) content in the leaves of *T. decumbens*

Table 5.1 Effect of salt stress on total polyphenolic content and antioxidant properties of *T. decumbens* leaves.

Treatments	Total polyphenols (mg GAE/g dry weight)	ABTS (µM TE/g dry weight)	FRAP (µM AAE/g dry weight)
Control	1.3±0.2^{b}	82.5±8^{a}	10.9±0.08^{b}
50 mM	1.6±0.3^{b}	78.4±6^{a}	14.3±0.4^{a}
100 mM	1.7±0.4^{ab}	70.4±4^{a}	12.1±1.1^{b}
200 mM	2.6±0.2^{a}	77.8±4.4^{a}	11.8±0.7^{b}
F- statistic	3.3*	0.41ns	3.6*

Mean values ±SE are shown in columns. The mean values followed by different letters are significantly different at P ≤0.05 (*); ns = not significant as calculated by Fisher's least significant difference.

5.5 Discussions

5.5.1 Polyphenols content

Vegetables including spinach are known to be rich in interesting phytochemicals, such as phenolic compounds (Ismail *et al.,* 2004), vitamins, antioxidant compounds, carotenoids and nutritive elements (Bhattacharjee *et al.,* 1998; Ismail *et al.,* 2004). Among these phytochemicals, phenolic compounds are of great interest due to the relevant role they play in the taste and flavour of food products as well as their health-promoting properties (Tomás-Barberán & Espin, 2001). The total phenolic content in the leaves of plants that were irrigated with high NaCl concentration (200mM) were significantly higher (P≤0.05) than other treatments including the control. These findings validate that of Daiponmak *et al.* (2010), where an increase in the total phenolic content, antioxidant activity, and cyanidin-3-glucoside content was found in Khamdoisaket and KDML 105 Thai rice cultivars subjected to salinity stress. A similar trend was also shown by Jafari and Garmdareh, (2019) on effects of salinity on biochemical characteristics of stock plant (*Matthiola incana* L.), where the phenol contents in severe salt-stressed plants of both cultivars (PanAmerican and Cinderella) were higher than the control. However, in commercial spinach salt stress negatively affect the phenolic compounds as reported by Bunea *et al*. (2008). Kim *et al*. (2008) stated that phenolic compounds may be affected by salinity, but this critically depends on salt sensitivity of a considered species. The results of the study prove that dune spinach is capable of growing under severe salinity concentrations and could be considered as an alternative source of nutrition in areas with higher and problematic saline soils. Further studies on anti-oxidative enzymes/ encoding genes responsible for salt tolerance in dune spinach are required to better understand the salt responses of this species.

5.5.2 ABTS capacity and FRAP capacity

The antioxidant activity (ABTS capacity) in the leaves of plants exposed to various salinity concentrations showed a much weaker antioxidant capacity compared to the control, but the TE values/ g dry weight were not significantly different in all treatments including the control. This contradicts the finding of Chrysargyris *et al.* (2018) on edible flowers, where three antioxidant assays (FRAP, DPPH and ABTS) increased with the application of salinity, with more pronounced impacts at salinity of 100 mM NaCl. Furthermore, these results also substantiate that of Zhou *et al.* (2018) on traditional Chinese herb, where a much weaker antioxidant capacity was found with increasing NaCl concentrations at 50 mM and 100 mM respectively as compared to the control. This was not the case in FRAP capacity as it was positively influenced by salt stress. The leaves of plants exposed to lower and moderate NaCl concentrations had the strongest antioxidant capacity as compared to other treatments including the control. All salinity treatments had a much better capacity of antioxidants than the control. This is supported by the findings of Chrysargyris *et al.* (2018) on edible flower, where three antioxidant assays (FRAP, DPPH and ABTS) increased with the application of salinity.

Changes in the overall results of ABTS and FRAP may be directly linked with phenolic amounts because of their free radical scavenging capacities (Huang *et al*., 2005; Hichem *et al.,* 2009; Taarit *et al*., 2012). On various studies conducted on maize, Chinese medicinal plants and in buckwheat sprout, the total phenolic amounts were significantly correlated with antioxidant capacity (Zhou *et al.,* 2018).

5.6 Conclusion

The results of this study showed that the polyphenol compounds and antioxidant activity (FRAP excluding ABTS) in the leaves of dune spinach were positively affected by salinity stress levels. Based on these experimental results, the leaves of dune spinach may be used as a natural source of nutritional antioxidants. However, increasing salt concentrations in the leaves of some halophytes have been reported to cause nutritional barrier due to high levels of anti-nutrients. Hence further studies are recommended on the nutritional profile of this species to further support its consumption as a leafy vegetable.

5.7 Acknowledgments

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CHAPTER SIX

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

6.1 General discussion

Introduction

Climate change, expanding soil salinization and the developing shortage of freshwater have negatively affected arable lands around the world including South Africa. This indicates the need for a creative, sustainable and sufficient crop production method. With this in mind, numerous researchers have pointed out the use of salt-tolerant plant species with possible commercial value as a proposed upfront strategy for saline lands. The use of halophytic plants for food production could be an adaptation strategy for climate change as freshwater continues to become scarce and the rainfall, particularly in Sub-Saharan African become more sporadic (Glenn *et al*., 1999; Bartels *et al.,* 2005). Seawater and salinized lands represent potentially cultivable areas for edible salt tolerate plants. In South Africa majority of the fields with these qualities are located close to coastal areas and suffer from high salinity conditions. Therefore, saline agriculture with halophytes could enable coastal and salinized lands to be productive. Hence, knowledge on the cultivation of native edible halophytes is needed.

In Chapter 2 it was concluded that there is a worldwide interest of edible salt-tolerant plant species due to increasing soil salinization and water shortages that affects production of conversional crops. Dune spinach (*T. decumbens)* has been reported to withstand biotic and abiotic stresses and thus having the potential to be used as a leafy vegetable in provinces facing the adverse effect of drought and salinity. Reports on the edibility of this species have shown that the leaves and soft stem can be used like green spinach, eaten raw in grain salads, or cooked with other vegetables. However, due to its underutilization by local communities and commercial growers, it was recommended that further studies should be conducted on its propagation and cultivation methods to support the commercial viability of *T. decumbens* as a leafy green vegetable.

In Chapter 3 the investigation into the ideal propagation media and hormone in rooting of nodal stem cuttings of Dune spinach (*T. decumbens)* was conducted. The results indicated that the application of growth regulators for root initiation of dune spinach is optional, as cuttings treated with media only rooted better than those treated with a rooting hormone. The highest cutting survival, rooting rate, number of roots, length of roots and number of leaves were achieved on sand and peat mix followed by sand. Root development was significantly lower in peat, perlite, and vermiculite mix. The rooting success of *T. decumbens* stem cuttings utilizing sand and peat could be ascribed to the positive interaction of aeration and water-holding capacity, as compared to the combination of peat, vermiculite, and perlite. This concurs with the natural growth media of the species on coastal areas*,* which could be an indication that the species prefer a well-drained media. Poor rooting performance in peat, vermiculite and perlite mix might be caused by poor aeration since both peat and vermiculite have high water retention capacity, which led to delayed rooting, and decaying of cuttings. It was thus concluded that nodal stem cuttings could be propagated using a soil mixture of sand: peat (1:1) without hormone treatment for optimal rooting.

In Chapter 4 the investigation into the effect of salt stress on vegetative growth, nutrient uptake and chlorophyll content in Dune spinach (*T. decumbens)* was conducted. The results indicated that the use of nutrient solution incorporated with 50 mM and 100 mM NaCl resulted in plants that have higher yields of material with regards to both wet and dry weights of shoots, stem and roots, total weights and number of branches compared to the plants irrigated with nutrient solution without NaCl. The nutrient uptake of N, P and Na in the leaves was positively affected by salt stress, while K, Ca and Mg were reduced drastically. The reduction of these elements maybe directly linked to excessive Na^+ absorption by the roots as reported by Benito *et al.* (2014). However, sufficient K, Ca and Mg are required to meet basic metabolic processes such as intracellular K homeostasis, which is essential for optimal functioning of the photosynthetic machinery and maintenance of stomatal opening (Shabala & Pottosin, 2014). These results suggested that dune spinach has the ability to transport K, Ca and Mg to new shoots and leaves under salt stress, and maintain a suitable ratio needed for normal metabolism; hence, the chlorophyll content was not affected for 8 weeks. This could be attributed to the water use efficiency and carbon fixing capacity of this species, which uses Crassulacean Acid Metabolism (CAM) to adapt to harsh conditions. Salinity stress also increased Mn, Fe and Cu contents in the leaves, while Zn and B were negatively affected. These results indicate that salt stress caused Zn and B deficiency in the leaves of dune spinach, but since they are required in small quantities, visual symptoms of nutrient deficiency did not occur. It was then concluded that dune spinach could be cultivated under salt stress (50 and 100 mM) for optimal growth.

In Chapter 5 the investigation into the effect of salt stress on the polyphenolic content and antioxidant-capacity of Dune spinach (*T. decumbens*) was evaluated. The results indicated that the use of nutrient solution incorporated with NaCl positively affected polyphenolic compounds and antioxidant capacity (FRAP excluding ABTS) in the leaves. Polyphenolic compounds increased with increasing concentration of NaCl with the highest content found in 200 mM NaCl concentration. This was not the case when FRAP capacity of the leaves were tested where the lower (50 mM) and moderate (100 mM) NaCl concentration proved to strengthen the FRAP capacity more than the highest concentration (200 mM). As for the ABTS capacity, salt stress did not have any significant effect, even though the control had a slightly more capacity than all NaCl concentrations. Changes in the overall results of ABTS and FRAP may be directly linked with phenolic amounts because of their free radical scavenging capacities (Taarit *et al.,*2012; Huang *et al.,* 2005; Hichem *et al.,* 2009). Based on the experimental results, it was concluded that the leaves of dune spinach may be used as a good natural source of dietary antioxidants.

6.2 Conclusion and recommendations

This study found that the use of peat and sand mixture (1:1) as a rooting media had a positive significant effect on the root initiation and growth of nodal stem cuttings of dune spinach. This study concludes that *T. decumbens* can easily be propagated in large quantities for commercial purposes and that no costly seed and or expensive equipment are required to increase crop plants. Conversely, the effects of salt stress on plant growth, chlorophyll content, nutrient uptake and antioxidant potential also showed a significant effect to support healthy plant growth. These results have indicated that there is potential for *T. decumbens* to be cultivated using diluted seawater. However, more research is needed on the effect of salinity on re-sprouting ability of *T. decumbens* when continuously harvested, as well as its effect on antibacterial activity, antifungal activity and nutritional content to support its commercial viability as a leafy green vegetable.

CHAPTER SEVEN

REFERENCES AND APPENDICES

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