Mitigating Salinity Stress in Red Kale (*Brassica napus* L. *var*. *Pabularia* 'KX-1') Through Split-root: Implications for Brackish Water Aquaponics

by

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Abstract

Brackish water aquaponics is a promising approach to expand the scope of aquaculture-hydroponics integration. However, progress on brackish water aquaponics is limited due to low salinity tolerance of most high-value vegetable crops. Salinity can significantly affect plant growth by reducing plant height, decreasing leaf number and leaf size, leading to early leaf senescence, and decreasing biomass accumulation in salt-sensitive plant species. Additionally, salinity can negatively impact plant physiological performance, resulting in reduced stomatal conductance, lower CO_2 assimilation, reduced photosynthetic activity, and ultimately lower plant productivity. To address this limitation in brackish water aquaponics systems, two studies were conducted to investigate the effectiveness of split-root system in deep-water culture (DWC) hydroponics system to mitigate salinity effects on red kale (*Brassica napus* L. *var*. *pabularia*). The focus of the first study was to evaluate the effect of increasing salinity levels (0, 3, 6, and 9 g L^{-1} or ppt) on red kale growth and physiology in whole-root and split-root systems. Results indicated that red kale plants grown in split-root system had a higher growth index than whole-root system when compared to the control treatment (0 ppt) in each condition. Stomatal conductance was similar in split-root system with increasing salinity level but was greatly decreased in the whole-root system as salinity level increased.

The second study evaluated the effect of brackish water aquaculture effluents on the growth and physiology of red kale in two split-root systems: homogeneous and heterogeneous. In homogeneous split-root system, both parts of the plant root system received the same treatment, while in the heterogeneous system, one part was treated and the other exposed to clear water. Treatments included hydroponic solution containing 0 ppt salinity, saltwater-based hydroponics at 11 ppt, and shrimp effluents at 14 ppt. Significant interactions were found between split-root

systems (conditions) and treatments in kale growth traits including height, leaf number, size index, and shoot fresh and dry weights. The heterogeneous split-root system showed positive effects on mitigating salinity stress on red kale growth, compared to the homogeneous split-root system. Plants treated with shrimp effluents showed lower stress levels, evidenced by improved leaf stomatal conductance compared to saltwater-based hydroponic in split-root system.

The split-root system demonstrated in this thesis under the DWC system offers a practical solution to mitigate salinity effects on high-value vegetable crops cultivated with brackish water aquaculture effluents. Further research can explore and refine split-root systems for commercial application in brackish water aquaponics systems.

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List of Abbreviations

DWC	Deep Water Culture
Het	Heterogeneous
Hom	Homogeneous
DW	Dry Weights
FW	Fresh Weights
PPT	Parts Per Thousand
NaCl	Sodium Chloride
DO	Dissolved Oxygen
EC	Electrical conductivity
RAS	Recirculating Aquaculture system
BFT	Biofloc Technology
SS	Suspended Solids
IAA	Integrated Aquaculture and Agriculture

Chapter 1

Literature Review

Limited Freshwater resources

More than 97% of available water resources are in oceans and seas in the form of salt water. Only 2.5% of world's water is freshwater, and, of that, only 0.5% is on the surface (Velmurugan et al., 2020; Gude and Nirmalakhandan, 2009). Amidst the growing concerns posed by climate change and population growth, over 70% of available freshwater resources are consumed in agricultural food production worldwide, significantly contributing to excessive demand for limited water supply (Shemer et al., 2023; Velmurugan et al., 2020; Gude, 2017).

Oceans contain vast saltwater resources that could be used as a sustainable alternative source for agricultural irrigation, thereby helping to reduce pressure on freshwater resources. However, only a limited number of crops can tolerate elevated salinity levels above 45 dS/m or > 35 parts per thousand (ppt) in ocean and sea waters (FAO and AWC, 2023). Brackish water is another alternative water resource that is currently underutilized for agricultural crop irrigation due to its highly saline condition, which only allows successful production of halophytic plant species (FAO and AWC, 2023). Brackish water is considered more salty than freshwater but less saline than ocean or seawater, with salinity levels ranging from 3 to 10 ppt, even up to 35 ppt (NGWA, 2017; FAO and AWC, 2023).

Several management strategies have been developed to make brackish water potable, even for human consumption, such as desalination, and membrane filtration technologies including reverse osmosis (Elimelech and Phillip, 2011). However, these methods remain energy-intensive, which makes them costly and unsustainable (Elimelech and Phillip, 2011;

Gude, 2017). Hence, it's crucial to maximize the use of saltwater resources in ways that are both sustainable and cost-efficient, eliminating the necessity for desalination prior to use.

Brackish water aquaponics implications

Brackish water aquaponics is an emerging system of farming that optimizes the use of available brackish water resources by integrating the soilless (hydroponics) cultivation of salt-tolerant plants (halophytes) alongside saltwater fish culture (Pantanella and Colla, 2013). This technique uses the concept of aquaponics which is the integration of aquaculture (typically freshwater) and soilless production of plants, involving bacterial cycles to convert fish waste to plant nutrients (Goddek et al., 2019; Junge and Antenen, 2020).

Aquaponics is an efficient system for nutrient and water recycling (Junge and Antenen, 2020). Current aquaponics systems are faced with a significant challenge attributed to differing water quality requirements between fish and plants, thus, restricting the viable fish solely to freshwater species (Pinheiro et al., 2020). Most freshwater fish species, especially tilapia, generally have a lower demand in the global market compared to saltwater aquaculture species. In 2020, shrimp, one of the major crustaceans commonly cultivated in brackish water salinity conditions accounted for 16% of the total most traded seafood products globally, while tilapia grouped with other finfish species, represented 7% (FAO, 2022).

This presents a critical concern impacting design, management strategies, and overall economic feasibility of aquaponics systems, particularly in the United States. Therefore, brackish water aquaponics is a promising approach to cultivating economically viable fish to meet market demand for seafood. A key challenge in brackish water aquaponics lies in elevated salinity levels that are managed within systems which limit the number of plant species that can be cultivated within the system (Pinheiro et al., 2020).

While most economically valuable vegetable crops can thrive within salinity thresholds of less than 3 ppt (Agius et al., 2022), most brackish water fish require minimum salinity tolerance thresholds ranging between 3 and 10 ppt for survival (Pinheiro et al., 2020). Hence, integration of halophyte plants in this system is a common practice and emerges as a notable strategy to address this challenge.

Halophytes are defined as a group of plant species that have an exceptional capacity to survive a wide range of salinities (Ventura et al., 2014). These plants are known for their ability to complete their life cycle without economic losses under conditions of soil-water salinity, with a minimum tolerance threshold of at least 200 mM NaCl or an electrical conductivity range of 8–10 dS m^{-1} (Centofanti and Bañuelos, 2019; Custódio et al., 2017). These halophytic plants belong to a wide range of plant species, including trees, saltbushes, and grasses. However, only a small percentage of them are utilized as vegetable crops intended for human consumption (Centofanti and Bañuelos, 2019). Despite their nutrient-rich profiles and potential application as a solution for freshwater scarcity, their current utilization for human consumption remains limited.

Perennial glasswort (*Sarcocornia ambigua* L.) is a halophyte mainly cultivated for its value as a vegetable and for treating brackish water aquaculture effluents (Centofanti and Bañuelos, 2019; Pinheiro et al., 2020). Nonetheless, despite potential benefits it offers, its economic value remains low since it has not yet gained consumer acceptance. Therefore, to address limitations in using halophytic plants for brackish water aquaponics production, increasing salinity tolerance thresholds in vegetables of high economic value is a potential solution.

Salinity effects on plants

Most known economically valuable vegetable crops grown in soil and soilless systems are considered salt-sensitive glycophytes which may not be produced economically with brackish water (Centofanti and Bañuelos, 2019). Halophytic plants can grow with brackish water due to their efficient mechanisms for controlling uptake, transport, and storage of sodium (Na^+) and chloride (Cl^-) ions and root water uptake under root saline conditions (Centofanti and Bañuelos, 2019; Hasanuzzaman et al., 2014). These mechanisms help prevent ion toxicity and other detrimental effects of salinity on plants. However, glycophytes are not able to tolerate soil or media salinity even with an electrical conductivity that exceeds 4 dSm^{-1} (15.7 mM Na^+) is considered detrimental and can severely inhibit growth (Munns and Tester, 2008; Shabala and Munns, 2012).

Research has shown that a reduction in plant growth due to salinity is a result of various physiological responses triggered by water stress. Salinity as a stressor, is known to induce production of reactive oxygen species (ROS), which disrupts nutrient balance in the plant cytosol, causing osmotic damage, and contributing to a notable reduction in water content within plant tissues (Abbasi et al., 2016; Gul et al., 2022; Shahid et al., 2020).

Salinity also causes other physiological responses such as decrease in stomatal conductance, carbon dioxide assimilation rate, and reduced chlorophyll content, which lowers photosynthetic activity of plant. (Jamil et al., 2007; Kwon et al., 2019; Rivelli et al., 2002). These responses result in reduced growth rates, leading to smaller leaves, stunted plants, and a decrease in leaf count (Phogat et al., 2020). Typically, salinity stress occurs in two phases which starts with reduction in stem growth caused by osmotic stress as a result of elevated salt concentrations surrounding roots, affecting water absorption (Läuchli and Grattan, 2007). The second phase involves salt toxicity targeting the plant leaves specifically. The salt toxicity accelerates the

aging (senescence) process in mature leaves, affecting the photosynthetic leaf area, thus, reducing the overall plant growth and productivity (Läuchli and Grattan, 2007; Shannon, M.C. and Grieve, 1998).

Plants have developed different mechanisms to mitigate salinity stress. They use diverse strategies, including regulation of ion homeostasis, activation of osmotic stress pathways, and modulation of plant hormone signaling in response to salt stress (Gul et al., 2022; Shabala and Munns, 2012). Overly sensitive (SOS) pathway and Mitogen-Activated Protein Kinase (MAPK) signal pathway are some of two essential pathways that plants employ in the process of salinity tolerance (Li, 2017).

According to Abbasi et al. (2016), understanding the ability of plants to detoxify radicals (Na^+ and Cl^-) under saline condition may be the most critical requirement for growing salt-sensitive plants, particularly entry of salt to roots. Study by Aroca et al. (2012), also showed that root manipulation to increase water uptake capacity under salinity stress is key to mitigating salinity effects on plants.

Salinity is also known as a chemical eustressor when maintained within tolerable limits for plants (Rouphael et al., 2018). A eustressor is a positive or beneficial stressor that, within certain thresholds, can influence both physical quality and chemical composition of fruits and vegetables (Rouphael et al., 2018). In vegetable production, controlled exposure to salinity can trigger certain physiological responses in plants, leading to high quality produce. The study by Šamec et al. (2021), highlighted the potential of salinity to stimulate the accumulation of phytochemicals in brassicas cultivated in saline soil conditions. These findings demonstrated potential benefits of brackish water in vegetable crop production, particularly for brackish water aquaponic applications.

However, despite potential benefits of moderate salinity highlighted for brassicas, plants are still challenged with maintaining optimal ion homeostasis balance under saline conditions (Šamec et al., 2021; Shabala and Munns, 2012). Therefore, management practices are implemented to mitigate salt toxicity for plants in soilless systems such as increasing leaching fraction, particularly for media-based systems (Katsoulas and Voogt, 2014).

In water-based systems such as deep-water culture (DWC) and nutrient film technique (NFT), salt toxicity is prevented by discarding the whole nutrient solution (Katsoulasand Voogt, 2014). While in brackish water aquaculture, a commonly used approach involves diluting aquaculture effluents to support normal plant growth. This approach is not practical, especially that nutrient concentrations in most aquaculture effluents tend to be low (Yang and Kim, 2019), and supplementing nutrients to maintain an efficient aquaponics system could compromise its fundamental goal.

According to Palm et al. (2018), nutrient supplementation in an aquaponics system should not exceed 50%. This implies that an aquaculture system should ideally provide at least half of the necessary nutrients for plant growth. Therefore, the most effective way to mitigate salt toxicity in plants is by adopting agricultural practices such as split-root system for the DWC systems thereby, avoiding effluent disposal before treatment or dilution under salinity problems.

Split-root system implications on salinity stress mitigation.

Salinity is often non-uniform in natural environments (Celletti et al., 2020). Plants tend to tolerate salinity better in heterogeneous root zone (split-root) conditions than homogeneous (Sun et al., 2016). Split-root system has been extensively studied, particularly in soil and media-based systems, to improve plant water and nutrient uptake under water stress or saline conditions (Kong et al., 2012; Oliveira et al., 2018; Ran, 2014; Valenzuela et al., 2022).

Split-root system allows a single plant to have more than one root zone environment, enabling researchers or producers to manipulate root conditions for a specific investigation (Larrainzar, Gil-Quintana, et al., 2014). Split-root system is widely used for salinity stress management in plants by enabling them to increase root biomass in the non-saline root zone, thereby compensating for water and nutrient uptake for the entire plant (Kong et al., 2012). Sonneveld. C and De Kreij (1999) and Sonneveld and Voogt (1990) are some of the first studies on split-root systems, particularly for vegetable crops like cucumbers and tomato. Their findings indicated that split-root systems effectively minimized yield losses by increasing nutrient uptake in both tomatoes and cucumbers under higher electrical conductivity NaCl (salinity) in the root zone. Higher yields were attained when part of the root section had a lower salinity level than the other. Similar outcomes were observed in recent studies investigating split-root system technique under salinity treatments.

The study by Oliveira et al. (2018) evaluated split-root system as a method to reduce salinity stress in bell peppers when brackish water was used for irrigation. Their findings reported increased plant height by 15 % compared to treatments where both roots were subjected to saline irrigation. The system has also been reported to enhance quality of tomatoes by boosting sugar and acid levels while reducing visual defects in the fruits. This was observed with a split-root electrical conductivity of 2.8/8.0 dS/m (Mulholland et al., 2002).

In tomato seedling production split-root set-up is reported to allow plants to better manage root salt accumulation and regulate nutrients and water for growth and development when part of the root system is under saline conditions (Flores et al., 2002). The study on split-root application in strawberry production with a simulated partial drought condition, resulted in

enhancing fruit size, quality, and reduced fruit yield while improved water use efficiency of the plants was noted (Ran, 2014).

When Zhang et al., (2019) evaluated effects of partial root-zone drying (split-root) and nitrogen availability in strawberry plants, split-root treatment reduced leaf conductance and fruit size. However, fruit quality parameters such as firmness, vitamin C, soluble solids, and sugar content were increased. The study also suggested that split-root and nitrogen availability can jointly improve strawberry fruit quality and water use efficiency under water stress conditions (Zhang et al., 2019).

In sweet basil, split-root nutrition improved plant nutrient uptake and production of essential oils at varying pH in root zone (Abbasov, 2013). Loveys et al. (2000) demonstrated that split-root method can facilitate the understanding of the physiological mechanisms such as stomatal conductance and root capacity for supplying abscisic acids while controlling transpiration and plant vigor without affecting the plant's water status when part of the root system is under drought conditions.

Another often-utilized application of split-root system involves simulating uneven distribution of specific ions, such as iron, which tends to be limited in natural environments or for certain crops like blueberries that lack the ability to acidify their root zone for improved nutrient uptake (Imler et al., 2019). These simulations allow researchers to understand physiological adaptations that plants adopt in response to partial iron availability in the root zones (Celletti et al., 2020; Imler et al., 2019).

Several studies have evaluated various parameters such as physiological performance, growth, and yield to better understand the impact of split-root system on plants. These responses are important in understanding how plants regulate water loss and uptake and nutrient uptake,

which is mainly influenced by factors like soil moisture content, temperature, and light intensity (Sun et al., 2016). Plant responses to stress trigger changes in physiological response of the plant. Researchers have used these responses including root growth, shoot growth, plant water status, nutrient uptake and utilization, hormone concentrations, and signaling, as well as gene expression to evaluate how split-root can mitigate partial salinity stress in the root conditions (Flores et al., 2002; Oliveira et al., 2018).

Split-root application in deep water culture (DWC) system for red kale production.

Research on split-root system has predominantly focused on media-based systems, utilized in production of crops such as trees, small fruits, fruiting vegetables, and herbs (Abbasov, 2013; Celletti et al., 2020; C. Sonneveld and De Kreij, 1999 ; Mulholland et al., 2002 ; Oliveira et al., 2018; Ran, 2014). However, there is still a gap in its application in a water-based system like DWC and for leafy vegetables, such as red kale (*Brassica napus* L. Pabularia).

DWC is the most preferred hydroponic culture system for leafy vegetable productions, notably even within aquaponics research fields and commercial systems (Ayipio et al., 2019). Therefore, a successful implementation of split-root system in a DWC system will provide insights for the cultivation of salt-sensitive leafy vegetables that otherwise cannot be produced economically using brackish water.

The red kale 'KX-1' belongs to Brassicaceae family and is a recent invention of a kale variety developed from crossing *Brassica napus* L. *var. pabularia* 'Siberian Kale' with itself (Courtney Michael and Shamrock Seed, 2017). Brassica vegetables are widely known for their high-yielding and excellent agronomic characteristics, such as heat, drought, and moderate salinity tolerance (Soengas et al., 2021; Šamec et al., 2021). They are primarily cultivated for

their leaves which are used for human consumption and sometimes for animal feed (oldest leaves), providing dual benefits to growers (Soengas et al., 2021).

Additionally, most kales are known to contain antioxidants, phenolics, and glucosinolates (GSLs) mainly in their leaves and plant tops which are beneficial to human health in preventing certain diseases such as cancers, cardiovascular diseases, aging, and many other related ailments (Francisco et al., 2017). Red and purple-pigmented vegetables have been reported to have a direct correlation with higher antioxidants, specifically anthocyanin content (Li et al., 2012). Therefore, red kale variety used in this experiment is an important variety that should be cultivated and included in the human diet and its application for brackish water aquaponics is crucial.

Conclusion

The current aquaponics systems are faced with challenges in system design and management especially, maintaining optimal conditions for both fish and plants without affecting one or the other. In brackish water aquaponics systems, salinity further complicates this balance because many economically valuable vegetables are salt-sensitive glycophytes, while halophytes, which have higher salinity tolerance, have not yet gained consumer acceptability. The common approach of diluting brackish water aquaculture effluents to lower salinities may prove ineffective, as it also dilutes nutrient concentrations, leading to a higher need for supplementation, which defeats the goal of aquaponics.

For this reason, most existing aquaponics system are limited to freshwater fish species like tilapia. Shrimp, one of the most consumed seafood in US, holds a higher market value globally than tilapia, and is predominantly farmed in brackish water salinities exceeding 10 ppt, while most vegetables with market have a salinity tolerance below 3 ppt. Split-root system provides a

viable solution to utilize brackish water aquaculture effluents for plant production without need for dilution. Although this technique has primarily been utilized in soil and substrate-based systems, there is a compelling need to explore its potential application in DWC systems. This would significantly enhance adaptability of split-root systems for commercial production in brackish water aquaponics. While gaps still exist in application of split-root systems in water-based systems, studies have demonstrated its effectiveness in mitigating salinity and drought stress in various high-value horticultural crops, including cucumbers, tomatoes, bell peppers, basil, strawberries, and blueberry trees.Therefore, understanding plant responses to different salinity levels in DWC hydroponics split-root system conditions is crucial to effectively address challenges posed by salinity on plant growth and productivity. This could facilitate cultivation of salt-sensitive vegetables like red kale in brackish water aquaponics systems, offering sustainable solutions to optimize limited freshwater use and aquaculture effluent management.

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Chapter 2

Effect of Split-Root on Growth Performance and Physiological Response of Red Kale at Different Salinity Levels in Hydroponic Deep Water Culture

Abstract

The study was conducted to evaluate the effect of increasing salinity levels on red kale (*Brassica napus* L. var Pabularia) growth and physiology in split-root and whole-root growing conditions under hydroponics DWC system. Three salinity levels of 3, 6, and 9 ppt (or g L^{-1}) and a control treatment of 0 ppt were applied for each growing condition. Results showed greater size index, height, and leaf number in red kale plants grown in split-root than whole-root. In the split-root system, increasing salinity resulted in 23% for 3 ppt, 22% for 6 ppt, and 34% for 9 ppt reductions in plant height, while in whole-root system, the reductions were 37%, 52%, and 52% compared to the control (0 ppt). Stomatal conductance remained unaffected by the increased salinity in split-root system. However, it decreased as salinity increased in whole-root system. In whole-root system, reductions in shoot dry weight was 1.15 times greater compared to split-root system. Tissue sodium (Na) concentrations generally increased with increasing nutrient solution salinity and were negatively correlated with tissue concentrations of potassium(K), calcium (Ca), and boron(B). High leaf number, shoot fresh and dry weights, root dry weight, size index, and height in split-root also had low tissue Na content. In summary, split-root treatment showed positive effects on kale growth and nutrient uptake under increased salinity, likely by reducing Na uptake into plant tissue.

Introduction

Salinity is a known abiotic stressor for soil and soilless plant production. When electrical conductivity of a soil solution exceeds 4 dS m^{-1}, equivalent to 15.7 mM Na^{+}, it is considered saline (Munns and Tester, 2008; Shabala and Munns, 2017). In media-based production, salinity is managed by increasing leaching fraction, which means increasing the volume of water draining from the growing medium to remove excess salts. (Katsoulas and Voogt, 2014). In the case of water-based systems such as deep-water culture (DWC) or the nutrient film technique (NFT), the whole nutrient solution is discarded to prevent increased salinity (Katsoulas and Voogt, 2014).

Aquaponics integrates aquaculture production with soilless plant production and presents enormous opportunity for scalability (Goddek et al., 2019). However, current aquaponics systems need to meet different water quality requirements for fish and plants, which poses challenges to system design and management (Pinheiro et al., 2020). Take the brackish water aquaponics for example, the lowest salinity threshold for fish production is 10 ppt, while the highest threshold for most vegetable crops of high economic value is less than 3 ppt (Agius et al., 2022; Roy et al., 2010; Agius et al., 2022). As few crops can tolerate high salinity levels, aquaponics is typically limited to freshwater fish species.

Halophytic plants such as perennial glasswort (*Sarcocornia ambigua* L.), which can tolerate high salinity , are usually of low economic value and have not received consumer acceptability (Pinheiro et al., 2020). If the salinity tolerance of vegetables could be increased to at least match the lowest salinity threshold of brackish water fish species, it would open up new possibilities for aquaponics, enabling the production of commercially appealing fish species.

Salinity stress tends to reduce growth rate, plant size, leaf size, and leaf number (Phogat et al., 2020; Shannon and Grieve, 1998). Increased salinity also causes reduced root length and mass thereby reducing nutrient uptake (Munns and Tester, 2008).

Depending on the composition of a brackish water solution, plant nutrient toxicities or deficiencies can also occur (Munns and Tester, 2008). Although moderate salinity levels can elicit some beneficial responses, such as synthesis of beneficial phytochemicals in brassicas, a challenge still exists for plants to maintain ion homeostasis under such conditions (Šamec et al., 2021).

It is desirable to create growing conditions to facilitate nutrient uptake while reducing harmful effects of high salinity. A typical approach that dilutes brackish water aquaculture effluent is not ideal for aquaponics because it also leads to the dilution of plant nutrients. Because most aquaculture effluents are already low in nutrient concentrations for plant production, dilution would increase the need for nutrient supplementation (T. Yang and Kim, 2019). Although nutrient supplementation of over 50% could be possible and, indeed, necessary in some cases, it is not preferable. Some plant production systems, for example, could use aquaculture effluent that is low in nutrients due to the inherent nature of the system (like a single-pass system). Those systems will need more than 50% nutrient supplementation and would most likely be classified as integrated aquaculture systems and not aquaponics. Aquaponics systems do not and should not require more than 50% nutrient supplementation (Palm et al., 2018).

To address these challenges, split-root system was proposed which allowed for manipulation of root system in such a way that enhances nutrient uptake under brackish water conditions. In split-root system, roots are manipulated to grow into two different compartments,

allowing for different root zone environments for a single plant (Larrainzar, Gil-Quintana, et al., 2014). In the case of salinity, one compartment contains saline solution, while the other contains an ameliorating solution that acts as a buffer (water) for the roots. Split-root systems have been applied to investigate salinity effects in hydroponic tomato production and showed benefit for improving yields and quality under increasing salinity (Koushafar et al., 2011; Tabatabaie et al., 2003). The system has been reported to enable the use of brackish water for irrigating bell peppers without significant fruit yield losses, and it also improved water use efficiency when applied as a partial root drying system in tomato plants (Oliveira et al., 2018; L. Yang et al., 2012).

Split-root system has not been reported in red kale (*Brassica napus* L. *var. pabularia*) for a DWC system. DWC is a preferred production technique for leafy vegetables, including kale, which are a predominant crop in aquaponics (Ayipio et al., 2019). Therefore, the potential success of implementing a split-root system in such hydroponic cultivation could offer wide applicability and prospects for commercial use.

In this investigation, kale was selected as a test crop for its nutritional and health benefits. Red pigmented cultivars are shown to have high antioxidant properties which is beneficial for cancer remediation (Francisco et al., 2017; Li et al., 2012). Kale is also moderately tolerant to salinity stress with potential for adoption in brackish aquaponics (Pavlović et al., 2019) .

The salinity levels used in this study fall within slightly to moderately saline haloponics conditions. The term 'haloponics' is used to describe saline aquaponics systems that utilize salinity levels below those found in seawater (Benz, Coelho, et al., 2019). Haloponics can be classified into 4 groups of salinities namely slightly saline (1-3 g L^{-1}), moderately saline (3-10 g L^{-1}), and high salinity (10-35 g L^{-1}) (Benz, Coelho, et al., 2019). In this study, we use the term

'brackish water' as it is gaining popularity and describes the conditions across a wide range of salinity that is well below seawater levels and the results are therefore applicable to brackish water aquaponics.

Materials and Methods

Location

Three experimental runs were conducted for this study. First and second runs were conducted in summer season from 05/02/2022 to 07/01/2022 and 07/10/2022 through 09/02/2022, respectively, at the Paterson Greenhouse Complex at Auburn University (AU) (lat. 32.59703°N, long. 85.48810°W). The third run was conducted at AU Aquaponics Facility located at E.W. Shell Fisheries Research Center (lat. 32.648935°N, long. 85.486828°W) in the Fall season from 09/19/2022 to 11/16/2022.

Plant cultivation

On May 2nd, 2022, 98 pelleted Red kale (*Brassica napus* L. *var*. *pabularia* 'KX-1') seeds (Johnny's Selected Seeds, Winslow, ME) were sown, one seed per cell, in a 98-cell rockwool plug tray. Two weeks after germination, 49 uniform seedlings were randomly selected and transferred into a 10.16 cm^3-sized rockwool split blocks, where they continued to grow for another two weeks to allow further root development for split-root implementation. Seedlings were sub-irrigated with 0.5x strength nutrient solution containing no additional salinity, from three water-soluble fertilizers [Gramp's Original Hydroponic Lettuce Fertilizer (8N-6.5P-29.9K), calcium nitrate fertilizer (15.5N-0P-0K, 19% Ca), and Epsom salts (0N-0P-0K, 10% Mg)] daily for four weeks before they were transplanted. Concentrations of nutrients in solution are presented (Table 2.1).

Transplants were transferred to a hydroponics DWC system on May 16th, 2022. Twelve 24-L containers were used for split-root system and twelve 7-L capacity containers were used for whole-root system. Three plants were grown in each test container. Aeration was supplied using aquarium air pumps at a rate of 70 L/min (Vivosun, C.L.L Pet Supplies, 702 Watson Ave, Madison, Wisconsin, U.S.A).

Treatments

Four salinity treatment levels in parts per thousand (ppt) were applied to each nutrient solution in test containers. Salinity levels were achieved using red sea salt (RSS) containing Ca^{2+}, Mg^{2+}, and KH formulated at 7 g L^{-1} RSS for 3 ppt, 10 g L^{-1} RSS for 6 ppt and 15 g L^{-1} RSS for 9 ppt salinity levels. The actual sodium levels as well as Ca^{2+}, NO_3-N, K^+, EC, pH, and DO of each salinity level are shown (Table 2.2). There was a 'control' nutrient solution with no salt added represented as 0 ppt.

Water-soluble fertilizer 8N-6.5P-29.9K, (Gramp's Original Hydroponic Lettuce Fertilizer), calcium nitrate (15.5N-0P-0K), and magnesium sulfate (10% Mg) were used as sources for all the nutrient solution (Table 2.1). Nutrient solution composition varied depending on the salinity level required to reach each treatment because the red sea salt contained calcium, magnesium, and potassium (Table 2.2).

Split-root implementation

The split-root system was set up using 24-L capacity rectangular containers, rectangular-shaped Styrofoam boards (55 × 37 cm and 55 × 18 cm), and heavy-duty 151.42-L plastic bags (Fig. 2.1). Two separate, equally sized compartments (side A and side B) were created in each container by placing a 55 × 18 cm polystyrene board in the middle of the container (Fig. 2.1C). Heavy-duty 151.42-L plastic bags were cut in half and the sealed half was used to cover each

side of the container to hold the solution on each side and prevent cross-contamination within the test container (Fig. 2.1D). A 55 × 7 cm, Styrofoam board was laid on top, each one having two openings for each side (Fig 2.1E). Split-root rockwool blocks were used for the split-root development of kale seedlings(Fig 2.1F). This was done by allowing the seedlings to grow enough roots on both sides of rockwool to access the nutrient solution and water before they were transplanted into the system.

Twelve liters of designated treatment solution was placed on side A of the container and 12 liters of fresh water ("clear water") was placed on side B, regardless of the salinity treatment level. Whole-root system was implemented in 7-L containers with three plants in each test container (Fig.2.2). Seven liters of nutrient solution was added to each container and the corresponding treatment was applied.

Measurements

Leaf greenness, measured as SPAD index to estimate leaf chlorophyll was recorded weekly on newly and fully expanded leaves of each plant starting at 0 DAT (Soil Plant Analysis Development meter; Minolta SPAD 502®, Konica Minolta Business Solutions, Europe GmbH). Plant height, widest width, and perpendicular width, which were used to calculate a size index ([height+width1+width2]/3) were measured weekly.

Shoots and roots were separated and weighted to determine shoot fresh weight and root dry weight. Root and shoot samples were dried in a forced air oven (Grieve, The Grieve Corporation, Round Lake, Illinois, USA) for one week at 60 °C. After drying, root and shoot dry weights were recorded and samples of each replicate were analyzed for macro and micronutrient concentrations using ICP-MS (Waters Agricultural Laboratory, Camilla, Georgia, USA).

Leaf physiology

Leaf physiology of newly, fully expanded leaves were measured for leaf stomatal conductance to water vapor ($g_{w,}$ mol [H_2O] m^{-2} s^{-1}), leaf apparent transpiration ($E_{apparent}$, mmol m^{-2} s^{-1}), leaf temperature (°C), leaf vapor pressure deficit (VPD, kPa), photosystem II (PSII) quantum efficiency (F_{PSII}), and leaf electron transport rate (ETR, mmol m^{-2} s^{-1}) (Portable leaf fluorometer; LI-600, LI-COR, biosciences, Lincoln, Nebraska, USA). Maximum (Fm¢) and steady state (Fs) fluorescence from the device to help understand the leaf physiological responses.

Water quality

Water quality was monitored and recorded three times per week for the entire trial duration. Nutrient solution pH, electrical conductivity (EC), nitrates (NO_3), potassium ($[K^+]$), calcium ($[Ca^{2+}]$), sodium ($[Na^+]$), salinity, and dissolved oxygen (DO) were monitored to ensure a proper range to maintain quality. Water and nutrient solutions were restored in each test container when there was a decrease below half the level. A portable pH/EC/TDS/temperature meter (HI9813-6; Hanna Instruments, Smithfield, Rhode Island) was used for pH and EC measurements. Ion concentration measurements for $[NO_3^-]$, $[K^+]$, $[Ca^{2+}]$, and $[Na^+]$ were recorded routinely with handheld meters (L- AQUA Twin; Horiba, Kyoto, Japan).

Data analysis

The trial was organized as a completely randomized design with four treatments (0 ppt, 3 ppt, 6 ppt, and 9 ppt) and three replications. Experimental units were represented by each test container containing three plants and a total of twelve experimental units each for split and whole-root systems, respectively.

Data were analyzed with analysis of variance (ANOVA) using SAS 9.4 software (SAS Institute, Cary, North Carolina, USA). One-way ANOVA was conducted after testing for

normality and homoscedasticity of the data for each condition. The approach of relative effect was adopted when comparing the salinity levels of each condition to account for the difference in salinity stress exposure between the whole-root and split-root systems.

A full factorial analysis for salinity levels and conditions was not considered due to differences in water quality measurements and container types between whole-root and split-root systems, which could have affected the treatments. Instead, Dunnett mean comparison was performed to compare conditions when there was a significant treatment effect ($P = 0.05$) in each condition type, allowing for relative effect comparison.

The generalized mixed effects procedure (PROC GLIMMIX) in SAS was used for all ANOVA. The random statement was used to account for error and nest time variables. In the case of variables such as growth, a repeated measures design approach was adopted to account for autocorrelation using the ante dependent 1 (ante (1)) covariance correction. A contrast argument was added to the tissue analysis code to compare the average salinity effect against the control in each condition. A factor and principal component analysis were conducted using PROC FACTOR and PROC PRINCOMP functions, respectively, to identify groupings in measurements and assess if they aligned with the salinity treatments. This analysis's purpose is to describe the samples using traits measured.

A pair-wise Pearson correlation was conducted using R software (version 4.3.2), RStudio (RStudio Team 2018, Boston, MA, USA) with packages GGally and ggploS-H for analysis and visualization. Correlation was conducted separately for growth traits and biomass and for tissue nutrient content analysis. Tissue sodium content was featured in both sets of correlation analyses.

Results

Water Quality

Salinity levels used in this study resulted in sodium concentrations of 93.52-, 138.32-, and 210-mM Na for 3, 6, and 9 ppt, respectively, for the split-root system (Table 2.3). Also, for the whole-root system, the nutrient solution contained 126.14, 169.64, and 252.28 mM of Na for 3, 6, and 9 ppt of salinity, respectively. Concentrations of cations such as sodium ($[Na^+]$), potassium ($[K^+]$), and calcium ($[Ca^{2+}]$), including electrical conductivity (EC), which is an indication of nutrient solution's total ionic strength, were all found to be within similar ranges in each salinity level under both split-root and whole-root systems. Dissolved oxygen maintained in similar ranges in both conditions across all treatments (Table 2.3).

Plant growth

As salinity increased, the size index tended to decrease over time progression (Table 2.3). Higher salinity levels led to smaller plants which resulted in earlier growth reduction over sampling dates than the control (Fig 2.2). Size index decreased by 33% for 3 ppt, 46% for 6 ppt, and 56% for 9 ppt in whole-root, while in split-root only reduced by 13%, 22% and 33% for 3 ppt, 6 ppt and 9 ppt in relative to control, recording lower reductions than whole-root system (Table 2.3). Similarly, decrease in plant height under whole-root system was 37%, 52%, and 52% for 3 ppt, 6 ppt, and 9 ppt, compared to control (0 ppt), while in split-root were 23%, 22%, and 34% for the same salinity levels (Table 2.3).

Increasing salinity decreased leaf count by 25% for 3 ppt, 38% for 6 ppt, and 50% for 9 ppt in whole-root compared to control, but leaf count did not decrease by increasing salinity under split-root system respectively. Salinity level did not affect chlorophyll content of plants grown in split-root system. Chlorophyll content in the whole-root system increased by 15%, 10 % and 8% for 3 ppt, 6 ppt, and 9 ppt when compared to control (Table 2.3).

Leaf physiology

Increasing salinity levels in whole-root system resulted in significant reductions in leaf stomatal conductance, leaf transpiration rate, and increased leaf vapor pressure deficit (VPD_{Leaf}) and leaf temperature (T_{Leaf}). Salinity levels of 3 ppt, 6 ppt, and 9 ppt reduced stomatal conductance by 13%, 39%, and 69% when compared to the control. Similarly, leaf transpiration was reduced by 2.5% for 3 ppt, 21% for 6 ppt, and 51% for 9 ppt. Leaf VPD increased by 6.8%, 10%, and 31% for 3 ppt, 6 ppt, and 9 ppt salinity levels compared to control, while temperature increased by 1.2% for 3 ppt, 1.1% for 6 ppt and 3.9% for 9 ppt compared to control respectively (Table 2.4). However, increasing salinity did not affect stomatal conductance (P=0.30), leaf VPD (P = 0.87), and leaf transpiration rate (P = 0.15) in the split-root system.

Biomass

Increasing levels of salinity led to a significant reduction in shoot fresh and dry weights, root dry weight, and shoot-to-root ratio in both split and whole-root systems (Table 2.5). However, shoot dry matter content as a percentage of fresh weight was only significantly lower in whole-root by 65 % for 3 ppt, 82 % for 6 ppt and 89 % for 9 ppt but not in split-root system, respectively (Table 2.5). The effect of salinity on plant biomass was higher in the whole-root than in the split-root system (Fig 2.4). Increasing salinity reduced shoot fresh weight in the whole-root system by 1.13 times lower than in the split-root system. Also, the decrease in shoot dry weight due to salinity was 1.15 times greater in the whole-root system than in the split-root system respectively. However, the effect of salinity levels on shoot dry matter content relative to their controls was similar for both split and whole-root systems (Table 2.5).

Root dry weight was much less impacted by salinity levels in the whole-root than was in the split-root system. Also, there was more biomass distributed to the root than to shoots due to salinity levels in both split-root and whole-root systems. The increase in root-to-shoot ratio due

to increased salinity level in the whole-root system was 3 times as high as it was in the split-root system. Root partitioning in split-root system showed root dry weight reduced in the nutrient solution side as salinity increased in the alternative side. In the split-root system, allocation of roots to the nutrient solution side was 81%, 49%, 45%, and 42% of the total root dry weight for the control 0 ppt, 3 ppt, 6 ppt, and 9 ppt, respectively.

Nutrient uptake

Plant uptake of potassium (K) was improved by the split-root system (Table 2.6). Calcium (Ca) uptake was similar in both split-root and whole-root systems. However, split-root salinity treatments reduced leaf tissue Ca relative to the control to a greater extent than salinity treatments in the whole-root system. There was higher content of sodium (Na) in the whole-root system than in split-root. The Na content of the salinity treatments compared to their controls in the whole-root system was twice that of the split-root system.

Phosphorus (P) uptake was not affected in split-root system with increasing salinity level but was slightly increased in the whole-root system. This might be due to the need for ionic balance between cations and anions. Nitrogen (N) uptake was reduced in both whole-root and split-root systems. A similar reduction of tissue N was observed between salinity levels and their controls in both conditions. Increasing salinity level increased tissue magnesium (Mg) content in both conditions. Increase in tissue Mg between salinity treatment and their controls was higher in whole-root than in split-root. There was a significant reduction in tissue sulfur (S) content between the control and salinity treatments in both split-root and whole-root systems. A higher decrease of tissue S was observed between the control and salinity treatments in split-root than in whole-root system.

Tissue boron (B) was reduced to due to increased salinity levels in both split-root and whole-root systems. However, the reduction in tissue B was higher in whole-root than in split-root system (Table 2.7). Zinc (Zn) content was increased in both split-root and whole-root systems due to increased salinity. The increase in tissue Zn was highest in the split-root than in the whole-root system due to a significantly higher tissue Zn recorded at 9 ppt for the split-root system. Tissue manganese (Mn) was significantly reduced due to salinity in split-root system but was increased in the whole-root system albeit not significant ($P > 0.05$).

In whole-root system, tissue Mn was highest at 3 ppt while in split-root system, tissue Mn decreased linearly with increasing salinity. Tissue iron (Fe) content was increased in the split-root but reduced in the whole-root due to increased salinity. Also, tissue copper (Cu) was increased in both split-root and whole-root systems due to the salinity. The increase in tissue Cu was slightly higher in the split-root than in the whole-root between the salinity treatments and their control.

Multivariate analysis

Factor and principal component Analysis

Factor analysis was used to identify groupings that could describe variables measured. Two latent factors explained a total of 72.18% of variation in samples irrespective of condition (Fig. 2.7). Factor 1 represented samples with more tissue K, Ca, and moderate tissue B, Fe, and S (Fig. 2.7A). Factor 1 also had higher SPAD index, shoot dry weight, leaf count, and moderate size index(Fig. 2.7A). Factor 1 was a sample with reduced tissue N, Mn, Cu, P, Mg, Na. Moreso, Factor 1 samples had less percent dry matter content and lower root: shoot (Fig. 2.7A).

On the other hand, factor 2 could be described as a sample with more tissue Mn, Cu, N, S, B, Ca, K and moderate amounts of tissue Mg, P, Zn, and Fe (Fig. 2.7B). Factor 2 also

represented samples with moderate amounts of plant height, root dry weight, shoot fresh weight, and almost no SPAD (Fig. 2.7B). Factor 2 samples had no tissue Na, percent dry matter content, size index, leaf count, and shoot dry weight (Fig. 2.7B).

Principal component analysis was conducted for each condition (Fig. 2.8). In the whole-root system, the first principal component explained 60.09% and the second principal component explained 25.15% of the variation in measured variables (Fig. 2.8A). In the split-root system, the first principal component explained 50.23%, and the second principal component explained 34.06% of the variation (Fig 2.8B). In the whole-root system, the control treatment (0 ppt) correlated with shoot dry and fresh weight, size index (SI), K content, Ca, and leaf count (Fig. 2.8A). However, the highest salinity level (9 ppt) correlated with shoot Na content, percent dry matter content and root-to-shoot ratio (Fig. 2.8A).

Correlation analysis

Pearson correlation analysis was used to explore relationships between measured variables. The focus on the relationship between tissue Na and other plant traits in this discussion. Increased levels of salinity resulted in increased tissue Na content, especially in the whole-root system (Table 2.6). Here, the Pearson correlation results showed that increased tissue Na is negatively correlated with most growth traits measured. However, the relationship between tissue Na and growth traits was dependent on salinity level (Fig 2.7).

Results showed that an increase in tissue Na content is associated with decreased plant height (r = -0.77), size index (r = -0.77), leaf count (r = -0.70), shoot fresh weight (r = -0.73), shoot dry weight (r = -0.70), and root dry weight (r = -0.72). The relationship between tissue Na and size index was strongest for 3 ppt (r = -0.95) and 6 ppt (r = -0.95). Similarly, the relationship

was strongest in 3 ppt (r = -0.83) and 6 ppt (r = -0.86) for shoot fresh and dry weights, respectively.

Although there was an overall weak correlation between tissue Na concentration and chlorophyll content (SPAD index) (r = 0.032), the relationship was strongly negative at 6 ppt (r = -0.54). Also, the relationship between tissue Na and root: shoot ratio was positive and weak (r = 0.29) at 9 ppt, but strong at 3 ppt (r = 0.82) and 6 ppt (r = 0.84). When partial correlations were considered by controlling for confounding variables, tissue Na concentration had a moderately strong positive correlation with only shoot dry matter content (r = 0.51) and a negative correlation with root dry matter (r = -0.52).

Tissue Na content had a stronger relationship with K than with Ca which shows expected substitution of Na for K (both monovalent cations) when salinity levels increase. However, it was observed that the relationship between Na and Ca was strongest only in the control treatment (0 ppt, r = -0.706, $P < 0.001$). As salinity level increased, Na/Ca relationship became poor (Fig 2.8). Other macronutrients that were expected to have a negative relationship with tissue Na uptake were other cations such as Mg. However, rather a significant positive relationship (r = 0.58) appeared between Na and Mg. This means that uptake was not a simple ionic balance function where plant tries to balance its cations and anions.

Increased uptake of Mg and Na might be due to a need to create more chlorophyll molecules to keep up with photosynthesis and mitigate the harmful effects of Na. The relationship between tissue Na content and N was poor globally but was strong and significant at 3 ppt (r = 0.776, $P < 0.01$), 6 ppt (r = 0.626, $P < 0.05$) and 9 ppt (r = 0.652, $P < 0.05$) showing effect of salinity level on this relationship. The relationship between Na and P was positive and

highly significant ($r = 0.601$, $P < 0.001$) with the strength of the relationship decreasing as the salinity level increased.

Tissue Na content had a weak relationship with most micronutrients. Tissue Na had a significant positive relationship with tissue copper ($r = 0.352$, $P < 0.05$). Although the relationship between tissue Na and boron was negative globally, there were strong significant positive relationships at 3 ppt ($r = 0.931$, $P < 0.001$), and 6 ppt ($r = 0.749$, $P < 0.001$). Also, Na/zinc relationship was generally weak but strong at 0 ppt ($r = 0.636$, $P < 0.05$) and 3 ppt ($r = 0.602$, $P < 0.05$) implying a diminishing relationship as salinity levels increase.

Discussion

The slightly higher Na in the whole-root than in the split-root might be due to water moving from clear the water side towards the nutrient solution side under salinity treatment of split-root system through transfer by the rockwool block. However, the salinity dilution was not large enough to affect the results of this study.

Salinity condition was formulated based on brackish water aquaculture requirements; therefore, the focus was on Na and overall salinity level rather than NaCl. Red sea salt used for this study contained other cations such as K, Mg, and Ca which contributed to the overall salinity/electrical conductivity level, but Na was the predominant ion (Table 2.2). Indeed,Na is the main driver of osmotic stress resulting from reduced water uptake. Increased levels of Na would also lead to oxidative stress due to increased reactive oxygen species (ROS) (C. Sonneveld and Voogt, 1990).

Species that are tolerant to salinity stress can adjust their cell turgor by accumulating Na and Cl ions in their roots and leaves. Na concentration of leaves increased in kale plants as salinity level increased except in whole-root system where increasing salinity level to 9 ppt

decreased tissue Na content to levels similar to 3 ppt. This pattern in tissue Na concentration suggests that kale has some tolerance to salinity stress. In a study comparing kale, Chinese cabbage, and white cabbage, Pavlovic et al. (Pavlović et al., 2019) concluded that kale has higher tolerance to salinity than other two *Brassicaceae* species. Authors found that kale maintained high Na/K ratios without increasing stress compound malondialdehyde. In this study, kale plants were exposed for an extended period (30-31 d), and therefore better response patterns were elicited than if plants were exposed for a brief period. Split-root system further enhanced salinity tolerance capability of kale. Plants increased root biomass allocation to clear water side as salinity level in the nutrient solution side increased (Fig 2.5), thereby reducing harmful effects of salinity.

Further, from increased SPAD index with increasing salinity (Table 2.3), it is clear kale cultivar is tolerant of moderate salinity conditions. Salt-tolerant species usually show increased or unchanged chlorophyll content under higher-than-normal salinities. which suggests leaf chlorophyll could be a biochemical marker of salt tolerance (Stepien and Johnson, 2009). The trend in SPAD index value is also supported by the correlation between Na and Mg (Fig 2.8). The strength of the relationship between Na and Mg decreased as salinity level increased. Since Mg is a key component of chlorophyll, it can be concluded that the kale plants were able to adapt to salinity conditions by upregulating their Mg uptake and thus their chlorophyll (Fig 2.8). This is why the increase in SPAD value between the control and salinity levels indicated by the contrast was higher in whole-root than in split-root system (Table 2.3).

Root inhibition is an indication of osmotic stress and is defined as the ratio of biomass under salinity conditions to biomass under control conditions (Soda et al., 2017). Root inhibition increased in whole-root system as salinity level increased (Table 2.5). However, in split-root

system, root inhibition leveled out only after 3 ppt of salinity and remained similar even up to 9 ppt. Plants in the split-root system were able to decrease effect of increasing salinity level on their roots because they were able to draw water from non-saline side and nutrients from saline side (Parihar et al., 2015; Soda et al., 2017). Osmotic stress reduces growth by reducing root hydraulic conductivity (Kaneko et al., 2015). In this study root dry weight decreased with increasing salinity in nutrient solution, whole-root system. Root hydraulic conductivity is normally based on root dry weight. Therefore, shoot dry weight results could be used as a proxy indication that there was a reduction in root hydraulic conductivity. Indeed, reduction in root growth leads to reduced water uptake. Plants can uptake water and nutrients differently because there are different pathways for those two activities. Indeed, Sonneveld and Voogt, (1990), showed that tomato plants growing in a split-root system with one side having high EC and the other side having low EC were able to do the same. That is, by splitting the root system, plants drew water from low EC side and nutrients from the high EC side (C. Sonneveld and Voogt, 1990). Thus, in line with the earlier study, split-root mitigates the effect of salinity stress on root growth which affects nutrient uptake. Nutrient and water uptake are important for the growth of the plants. Therefore, the ability to balance them gives an advantage to plant performance. As observed (Fig 2.3), growth metrics of split-root system were better than those in whole-root system. In both trials 2 and 3, growth was reduced with increasing salinity level especially compared in whole-root system than in the split root compared to control. The reduction in difference in growth between control and salinity levels in split-root is an indication that split-root improved the overall performance of the kale plants and reduced the growth limiting effect of the salinity treatments.

One of most important plant growth limitations imposed by increased salinity is nutrient solution Na/K ratio. Generally, Na competes with K for binding sites because both are monovalent cations. Although K uptake is given preference over Na, when Na/K ratio is too high in nutrient solution, the balance shifts towards Na uptake. Our results showed that as salinity increased from 3 to 9 ppt, Na/K ratio of nutrient solution increased from 11.5 to 18.3 for split root and 11.5 to 19.7 for whole-root (Table 2.3). Increased uptake of Na instead of K would lead to increased leaf and root Na/K ratios. In kale plants, leaf Na/K ratio was reported to reach 10x for the highest salinity level of 200 m*M* NaCl while root contained 600 times compared to control (Pavlović et al., 2019). Results showed that tissue Na/K values were 8 times that of control for both 3 and 6 ppt and 9 times of the control in split-root system. In whole-root system, Na/K ratio compared to the control were 15, 23, and 16 for 3, 6, and 9 ppt, respectively. This shows the ability of the split-root system to reduce the effects of Na on K uptake by ameliorating increased relative K uptake.

Effects on leaf fluorescence and porometer traits

Most field measurements focus on F_{PSII} as a key fluorescence parameter. Our results suggest that increasing level of salinity had no effect on F_{PSII} or electron transport rate. The following could be explained. First, it was concluded that steady state fluorescence parameter (Fs) was negatively correlated with F_{PSII} while maximum fluorescence parameter (Fm¢) had a poor relation. Second, it was observed that Fm¢ decreased significantly as salinity level increased while Fs was unaffected by increasing salinity level. Therefore, since parameter in which salinity had greater effect had no relationship with F_{PSII}, the kale plants were not bound to decrease their F_{PSII} with increasing salinity. Although this presents no biochemical explanation based on theory, these results are not isolated occurrences. Indeed, Lu et al (2003) found that

salinity stress had no effect on photosystem II photochemistry. The authors found specifically F_{PSII} was not affected by increased salinity with temperatures below 35 °C. Results in the current study show that leaf temperature was not affected by increasing salinity levels in split-root system and only increased by 1 °C at 9 ppt in the whole-root system. Therefore, it can be argued that the kale plants acclimated their photochemical apparatus to the increased salinity levels thereby maintaining similar performance with the control plants. This explains why the plants survived the trial, although they had reduced growth rates and other morphological reductions. Hence, it is recommended to elicit the potential response in future studies, measurements should be taken a few days after exposure to salinity.

Conclusion

The split-root system is a practical solution to mitigating salinity stress in kale and could be applied to various salt-sensitive high-value vegetables in brackish water aquaponics. Split-root system reduced the effect of accumulated sodium in kale tissue by reducing the Na/K ratio, increasing plant growth and biomass. In split-root system, plants were able to draw nutrients from the high-saline side and water from non-saline (clear water) side. Plants in split-root system grew more roots in non-saline side as salinity increased enabling them to balance nutrients and water uptake. It can be concluded that with this technique, it is possible to combine fresh and brackish water aquaculture effluent side by side to obtain synergistic production. Thus, future research can examine the possibility of varying salinity levels of brackish water aquaculture effluents on one side and nutrient-rich freshwater on the other side

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Tables

Fertilizer Formulations

Table 2.1. Fertilizer and sea salt parts are used for nutrient solution and salinity formulation

Expected salinity level	Sea Salt Weight	Gramps® (8-15-36) Fertilizer	Calcium nitrate Fertilizer	Magnesium Sulfate fertilizer
Parts Per Thousand		gL^{-1}		
0 (Control)	0	0.63[z]	0.68	0.4
3	7	0.42	0.45	0.26
6	10	0.37	0.37	0.21
9	15	0.34	0.22	0.18

[z] Actual concentrations of NO3-N, K, and Ca for 0 ppt was 500 mgL^{-1}, 180 mgL^{-1}, and 230 mgL^{-1}, for 3 ppt was 380 mgL^{-1}, 120 mgL^{-1}, and 190 mgL^{-1}, for 6 ppt was 310 mgL^{-1}, 100 mgL^{-1} and 170 mgL^{-1}, for 9 ppt was 210 mgL^{-1}, 94 mgL^{-1} and 110 mgL^{-1}

Water Quality

Table 2.2. Nutrient solution water quality measured one week after transplant

Water quality parameter	Unit	Root condition	Salinity Level				
			0 ppt	3 ppt	6 ppt	9 ppt	P-value[z]
Ca	mgL^{-1}	Split	125	186.67	216.67	241.67	<.0001
		Whole	161.7	230	225	255	0.0008
K	mgL^{-1}	Split	135	186.67	218.33	268.33	<.0001
		Whole	185	248.3	256.7	291.7	<.0001
Na	mgL^{-1}	Split	92	2150	3183	4833	<.0001
		Whole	55.2	2866.7	3883.3	5750	<.0001
Electrical Conductivity	$mS\ cm^{-1}$	Split	1.6	7.4	8	8.6	<.0001
		Whole	1.9	7.7	8.4	8.8	<.0001
Na to Ca ratio		Split	0.7	11.5	14.7	20	<.0001
		Whole	0.5	12.6	17.5	23.1	<.0001
Na to K ratio		Split	0.7	11.5	14.6	18.3	<.0001
		Whole	0.3	11.5	15.1	19.7	<.0001
Dissolved Oxygen	mgL^{-1}	Split	7.7	7.8	7.8	7.8	0.8735
		Whole	7.6	7.6	7.6	7.6	0.9997

[z]Test of significance was done using the GLIMMIX procedure in SAS. Each condition was analyzed separately. Mean comparison was not proceeded afterwards

Growth Traits at Harvest

Table 2.3. Growth traits of Kale 'KX-1' at different salinity levels in Split and whole-root systems. Main effects of the salinity treatments are compared to control using the Dunnett mean separation at alpha = 0.05. Contrast value is the control minus mean across the three salinity levels

Measurement	Unit	Root Condition	Salinity Level (g /L, ppt)[z]				ANOVA	
			0	3	6	9	P-Value	Contrast
Plant Height	cm	Split	12.2	9.4*	9.5*	8.1***	0.0031	3.2***
		Whole	12.6	8***	6.6***	6.1***	<.0001	5.7***
Leaf number	count	Split	9	8	8	7	0.1489ns	1.1ns
		Whole	8	6**	5***	4***	<.0001	2.8***
Leaf Greenness	SPAD index	Split	38	40	38.5	40.1	0.6152ns	-1.5ns
		Whole	37.8	44.6**	41.8	41	0.0284	-4.7*
Size Index		Split	33	28.7	25.8*	22.2***	0.0008	7.6***
		Whole	27.6	18.6***	15***	12***	<.0001	12.3***

[z] Means (replications = 6) in salinity levels are compared to their respective controls (0 ppt) using the Dunnett test where "ns" = not significant ($P > 0.05$); "*" = significant at 5% ($0.01 < P < 0.05$)," **" = significant at 1% ($0.001 < P < 0.01$), and "***" = significant at 0.1% ($P < 0.001$).

Leaf Physiology

Table 2.4. Midday leaf physiological responses of Kale 'KX1' at different salinity levels in Split and Whole-root systems. Measurements were taken in newly fully expanded leaves at three weeks after transplanting.

Measurement	Unit	Root condition	Salinity Level (g L^{-1}, ppt)[z]				ANOVA	
			0	3	6	9	Salinity	Salinity*Trial
Stomatal conductance	mol [H_2O] m^{-2} s^{-1}	Split	0.82	0.86	0.74	0.79	0.3036ns	0.0133
		Whole	0.77	0.67	0.47***	0.24***	<.0001	0.0662ns
Transpiration	mmol [H_2O] m^{-2} s^{-1}	Split	5.4	6	5.1	5.4	0.1485ns	0.0464
		Whole	5.23	5.1	4.13	2.56***	<.0001	0.1497ns
Leaf vapor pressure deficit	kPa	Split	0.79	0.82	0.83	0.82	0.8676ns	0.9797ns
		Whole	0.82	0.88	0.91	1.19***	<.0001	0.0015
PSII efficiency (FPSII)	-	Split	0.69	0.68	0.74	0.74	0.0785ns	0.2417ns
		Whole	0.71	0.76	0.72	0.72	0.4767ns	0.9983ns
Electron transport rate	mmol m^{-2} s^{-1}	Split	114.54	139.48	130.13	114.91	0.8443ns	0.8415ns
		Whole	90.52	159.87	135.29	79.29	0.0632ns	0.1049ns
Leaf temperature	°C	Split	28.16	28.36	28.29	28.32	0.9014ns	0.8353ns
		Whole	28.34	28.67	28.66	29.49***	0.0013	0.0135

[z] Means (replications = 9) in salinity levels are compared to their respective controls (0 ppt) using the Dunnett test where "ns" = not significant ($P > 0.05$); "*" = significant at 5% ($0.01 < P < 0.05$)," **" = significant at 1% ($0.001 < P < 0.01$), and "***" = significant at 0.1% ($P < 0.001$).

Biomass at Harvest

Table 2.5. Shoot and root Biomass of Kale 'KX-1' at different salinity levels in Split and Whole-root systems

Measurement	Unit	Root Condition	Salinity Level (g L^{-1}, ppt)[z]				ANOVA	
			0	3	6	9	Salinity	Salinity*Trial
Shoot fresh weight	g $plant^{-1}$	Split	256.67	145.22*	110**	122.72**	0.0033	0.1244
		Whole	173.33	41***	22.67***	11.67***	<.0001	0.3585
Shoot dry weight	g $plant^{-1}$	Split	19.57	12.72*	10.02**	10.24**	0.0039	0.0412
		Whole	12.6	4.43***	2.31***	1.43***	<.0001	0.3827
Shoot Dry matter Content	%	Split	7.53	8.47	8.86	8.73	0.0716	0.256ns
		Whole	7.31	10.50**	9.82**	12.25***	<.0001	0.5142
Root dry weight	g $plant^{-1}$	Split	1.47	0.66**	0.53**	0.55**	0.0018	0.1836
		Whole	1.19	0.51***	0.3***	0.04***	<.0001	0.001
Root to shoot ratio	%	Split	7.76	5.19*	5.12*	4.04**	0.0091	0.314
		Whole	9.93	11.77	11.98	2.54*	0.0056	0.5464

[z]Means (replications = 9) in salinity levels are compared to their respective controls (0 ppt) using the Dunnett test where "ns" = not significant ($P > 0.05$); "*" = significant at 5% ($0.01 < P < 0.05$)," **" = significant at 1% ($0.001 < P < 0.01$), and "***" = significant at 0.1% ($P < 0.001$).

Tissue Macronutrient Concentration

Table 2.6. Tissue macronutrient content of Kale 'KX-1' tissue at different salinity levels in split and whole-root systems. Units are % of total dry matter of final harvest. Whole above ground tissue was analyzed

Nutrients	Units	Root Condition	Salinity Levels (g L^{-1}, ppt)				Statistic	
			0	3	6	9	S.E.D[x]	Contrast Estimate[y]
Ca	%	Split	2.5	1.4***[z]	1.3***	1.1***	0.08	1.28***
		Whole	2.3	1.5*	1.3**	1.5*	0.14	0.92***
Na	%	Split	0.3	1.7***	1.8***	2.0***	0.09	-1.55***
		Whole	0.4	3.3***	4.3***	3.3***	0.4	-3.27***
K	%	Split	6.1	4.3***	4.7***	4.4***	0.18	1.64**
		Whole	6.6	3.9***	3.3***	4.2***	0.3	2.73***
P	%	Split	0.7	0.6ns	0.6ns	0.6ns	0.03	0.08 ns
		Whole	0.7	0.8*	0.9***	0.9**	0.03	-0.18***
N	%	Split	4.5	3.7***	3.9**	3.9*	0.12	0.64***
		Whole	5.4	5.0ns	4.8*	4.6**	0.15	0.63**
Mg	%	Split	0.7	0.9***	0.9***	0.9***	0.03	-0.20***
		Whole	0.7	1.1***	1.1***	0.9***	0.04	-0.33***
S	%	Split	1.7	1.4**	1.4*	1.3***	0.06	0.33***
		Whole	1.6	1.6ns	1.4*	1.3*	0.06	0.20*

[x]S.E.D is the standard error of mean difference. This value shows the variability around the mean of the effect. It can be used to compare two means by subtracting them from each other and comparing the value to the S.E.D. if the difference between the two means is greater than the S.E.D, it means the two means are different.

[y]Contrast effect is the difference between the control and average salinity treatments. If the value is positive, control was higher than the average of the salinity treatments whilst a negative value implies the control was lower.

[z]Means (replications = 6) in salinity levels are compared to their respective controls (0 ppt) using the Dunnett test where "ns" = not significant ($P > 0.05$); "*" = significant at 5% ($0.01 < P < 0.05$)," **" = significant at 1% ($0.001 < P < 0.01$), and "***" = significant at 0.1% ($P < 0.001$).

Tissue Micronutrient Concentration

Table 2.7. The tissue micronutrient content of Kale 'KX-1' at different salinity levels in split and whole-root systems.

Nutrient	Units	Root Condition	Salinity Levels (g L^{-1}, ppt)				Statistic	
			0	3	6	9	S.E.D[x]	Contrast Estimate[y]
B	mg kg^{-1}	Split	84.5	51.2***	50.3***	49.0***	3	34.33***
		Whole	134.8	82.3***	77.5***	83.0***	6	53.89***
Zn	mg kg^{-1}	Split	46.5	59.8ns	56.8ns	89.2*	8.97	-22.11*
		Whole	39.2	50.3**	45.2ns	43.5ns	2.24	-7.17*
Mn	mg kg^{-1}	Split	208.8	197.5ns	152.5**	104.2***	10.28	57.44***
		Whole	186.8	290.7**	219.2ns	185.5ns	19.17	-44.94ns
Fe	mg kg^{-1}	Split	51.2	53.8ns	51.5ns	56.8ns	2.09	-2.89ns
		Whole	81	59.7ns	63.3ns	60.0ns	11.2	20.0ns
Cu	mg kg^{-1}	Split	6.3	10.2***	8.5*	9.5***	0.49	-3.06***
		Whole	6.8	10.7*	10.5*	7.7ns	0.8	-2.78**

[x]S.E.D is the standard error of mean difference. This value shows the variability around the mean of the effect. It can be used to compare two means by subtracting them from each other and comparing the value to the S.E.D. if the difference between the two means is greater than the S.E.D, it means the two means are different.

[y]Contrast effect is the difference between the control and average salinity treatments. If the value is positive, it means the control is higher than the average of the salinity treatments. A negative value means the control is lower.

[z]Means in salinity levels are compared to their respective controls (0 ppt) using the Dunnett test where "ns" = not significant ($P > 0.05$); "*" = significant at 5% ($0.01 < P < 0.05$)," **" = significant at 1% ($0.001 < P < 0.01$), and "***" = significant at 0.1% ($P < 0.001$).

Figures

Split-root System Setup

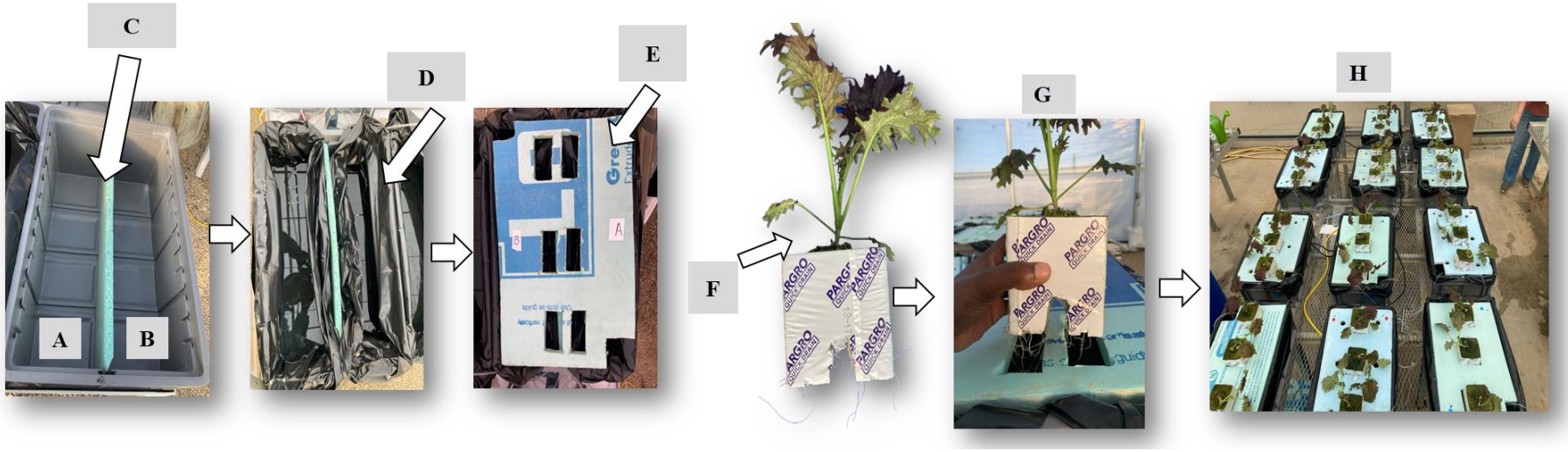

Fig. 2.1. Schematic of the split-root system implementation. Styrofoam divider (C) was used to create two sides (A and B), black plastic (D) covered each side of the container, while 46tyrofoam board € was used to suspend kale seedling in a split-root rock wool(F) when transplanted into the system (G) and the final split-root system (H) with each container holding three plants per container.

Whole-root System Setup

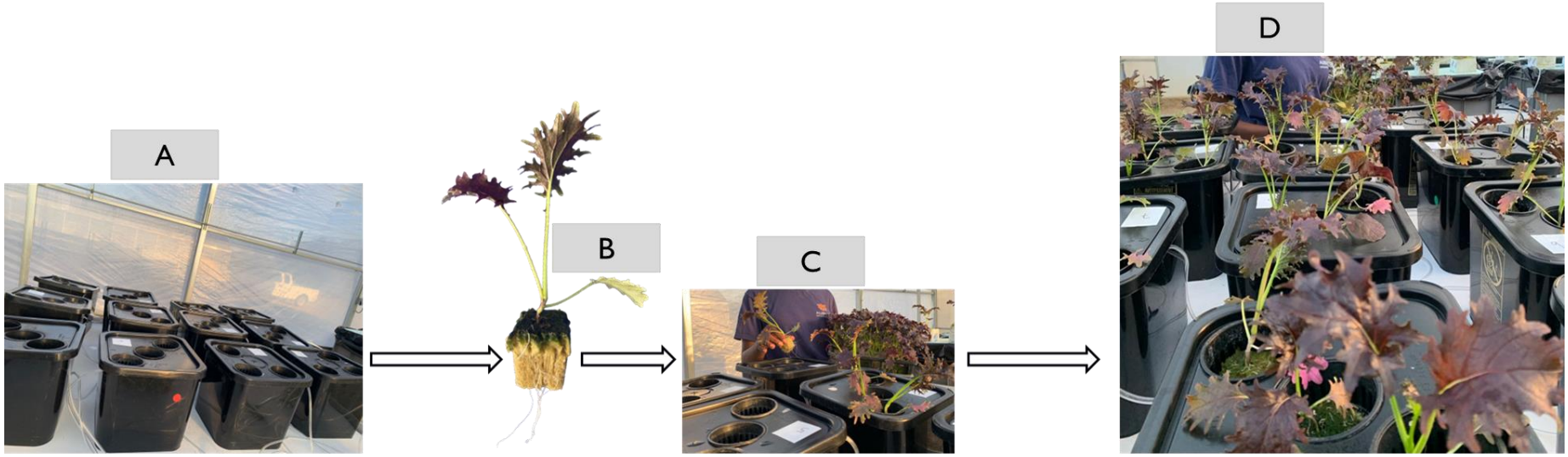

Fig. 2.2. Schematic of the whole-root system: 7-liter black containers (A) kale seedlings (B) were transplanted in each container (C). The complete system features each container supporting three plants (D).

Size Index

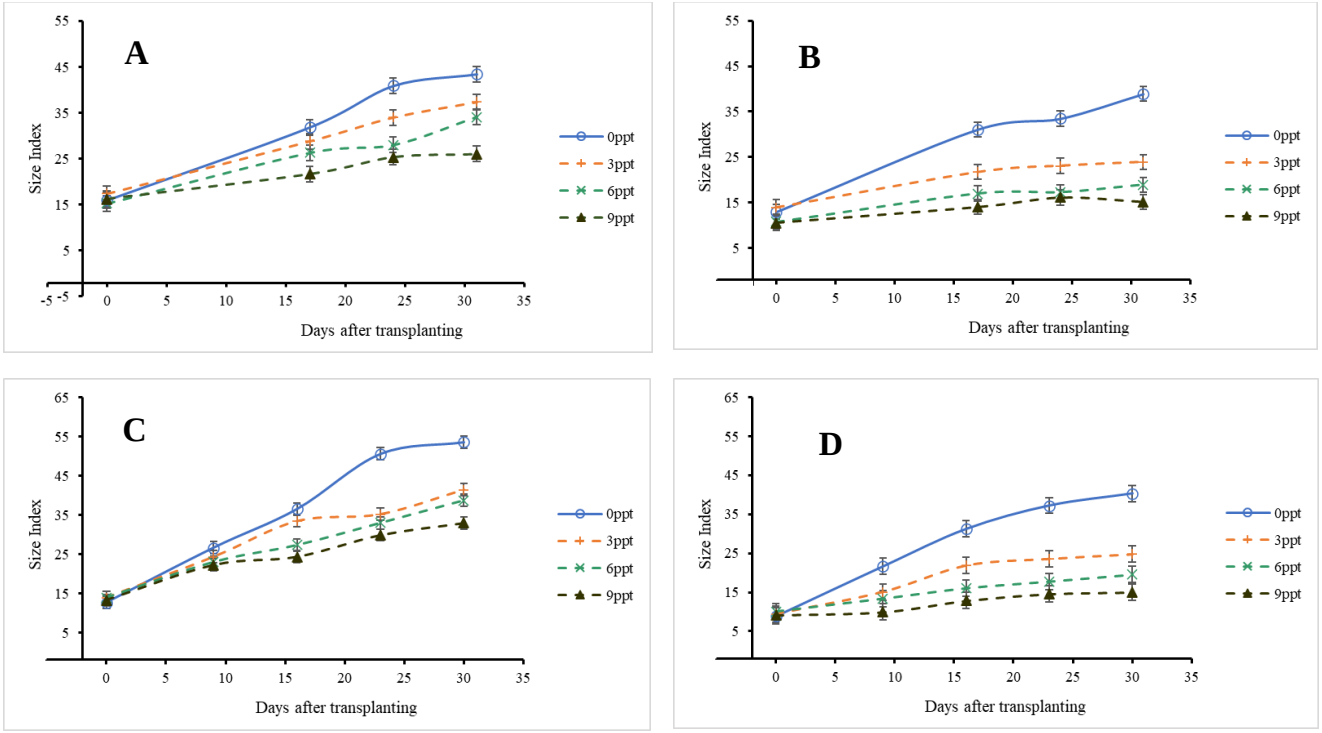

Fig. 2.3. Growth index of Kale 'KX1' over time in split-root system trials 1 (A) and 2 (C) and in whole-root system trials 1 (B) and 2 (D) with increasing salinity levels. Size index was estimated as the average plant height, and two broad lengths. The values are on a per plant basis.

Red Kale Shoot Biomass at Harvest

Fig 2.4. Size comparison of red kale in split-root and whole-root systems at 0 ppt (A), 3 ppt (B), 6 ppt (C), and 9 ppt (D) salinity levels.

Root Partitioning Ratios

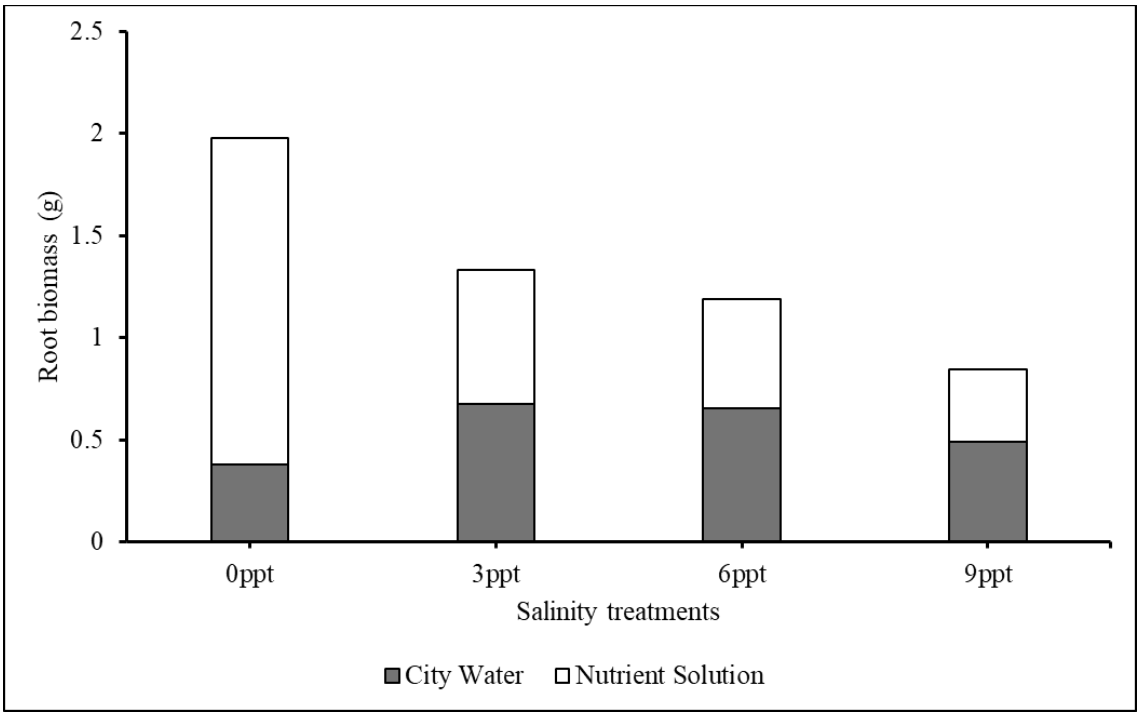

Fig. 2.5. Root partitioning ratios between nutrient solution and clear water sides in a split-root system at increasing salinity is illustrated in a stacked graph.

Red Kale Root Biomass at Harvest

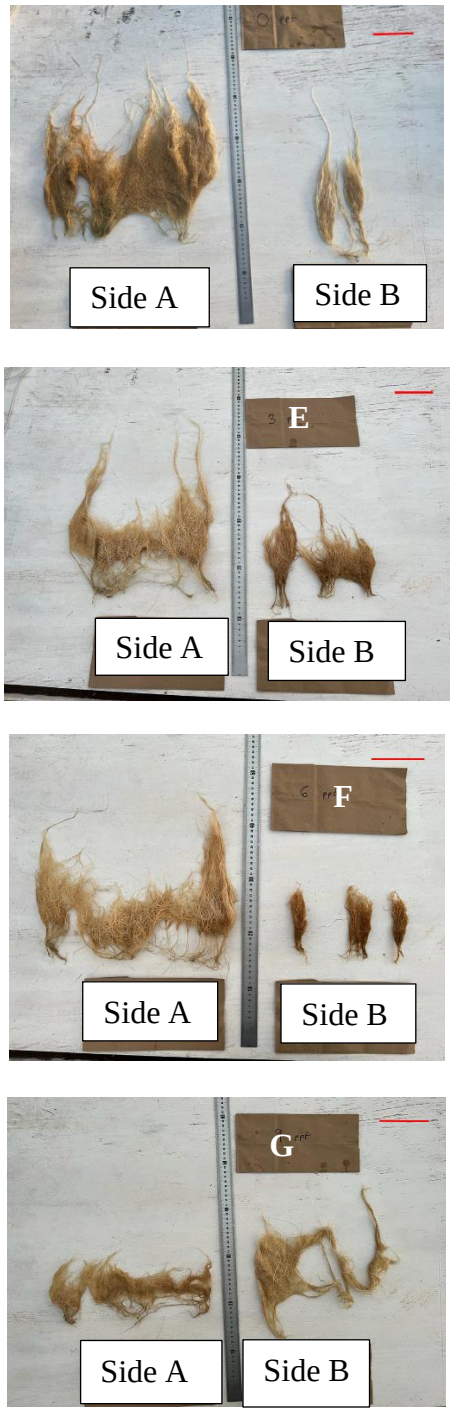

Fig. 2.6. The left panel on the image shows side A with kale root biomass allocated to nutrient solution and side B with root biomass allocated to clear water for 0 ppt (D), 3 ppt (E), 6 ppt (F), and 9 ppt (G) under split-root system.

Tissue nutrient analysis

Multivariate Analysis

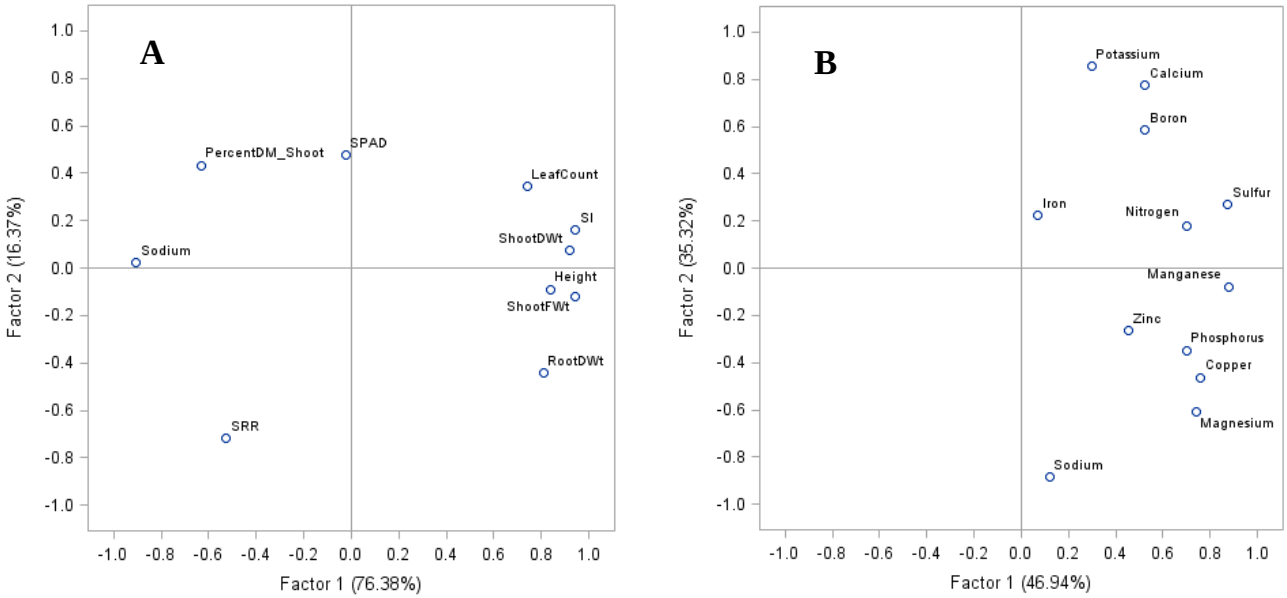

Fig. 2.1. Factor analysis of plant biomass traits (A) and tissue nutrients (B) to assess groupings in the measured variables. The percentage DM_shoot, shoot DWt, Root DWt, and SI in the figure refer to percentage dry weights of the shoot, shoot dry weights, root dry weights, and size index.

Principal Component Analysis

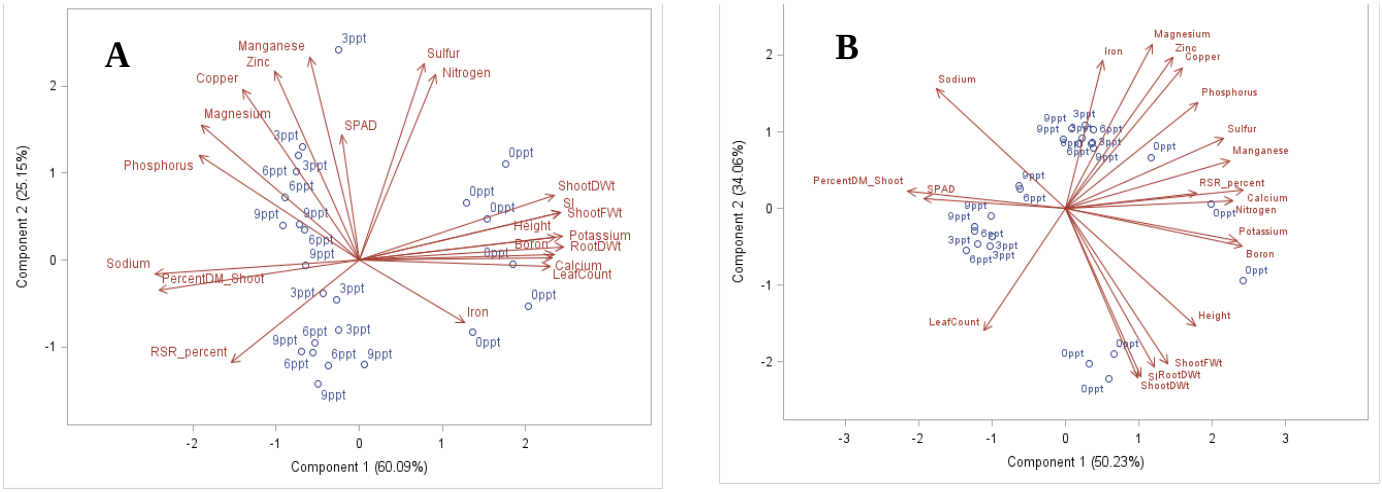

Fig. 2.8. Principal component analysis using the multidimensional preference analysis approach. Whole-root (A) and split-root (B) refer to plants growing in whole and split-root systems, respectively**.** The RSR_percentage in the figure refers to root shoot ratios, while the percentage DM_shoot, shoot DWt, Root DWt, and SI refer to the percentage dry weights of the shoot, shoot dry weights, root dry weights, and size index.

Chapter 3

Effect of Brackish Water Aquaculture Effluents on Red Kale (*Brassica napus* L. *var. pabularia* 'KX-1') on Growth and Physiology Performance in Two Split-root Growing Conditions.

Abstract

The study evaluated growth performance and physiological response of red kale cultivated with different nutrient solution treatments: hydroponics (H; control_0 ppt), saltwater-based hydroponics (S-H_11 ppt), and brackish water biofloc shrimp aquaculture effluents (S-Eff_14 ppt), under homogeneous and heterogeneous deep water culture (DWC) split-root systems. Heterogeneous split-root system with S-H and S-Eff treatments resulted in lower reductions in plant height (22% and 6% in trial 1, and 12% and 18% in trial 2, respectively) compared to homogeneous systems (32% and 29% in trial 1, and 38% and 42% in trial 2). Additionally, homogeneous systems significantly decreased shoot fresh weights by up to 95% for S-H and 92% for S-Eff across both trials, relative to control. In contrast, heterogeneous systems showed smaller declines, with reductions ranging from 65% to 81% for S-H and 72% to 76% for S-Eff. S-Eff treated plants exhibited less stress compared to S-H in both systems, achieving similar leaf greenness and Mg uptake as the control. CO2 assimilation rates were twice as high in shrimp effluent-treated plants as in those treated with saltwater-based hydroponics during trial 1.

Introduction

Facing an increasing global demand for seafood, integrated aquaculture and agriculture (IAA) offers a sustainable alternative to reduce overfishing while fostering sustainable water use in food production systems. Aquaculture contributes to over 52% of aquatic animal food for

human consumption, with shrimp as one of the most farmed and consumed seafood globally (Boyd et al., 2022; FAO, 2022; Iber and Kasan, 2021). Amidst a rapidly growing aquaculture industry, there is increasing concern over the environmental impact of the industry, particularly regarding effluent treatment before disposal (Iber et al., 2021).

Aquaculture effluents contain dissolved and suspended solid (SS) waste containing biochemical oxygen demand and nutrients such as phosphorus and nitrogen generated from fish waste, feces, and uneaten fish feeds (Song et al., 2016; Summerfelt, 2003). When effluents are disposed of without proper management, it significantly contributes to eutrophication which affects the quality of freshwater resources. Biofloc technology (BFT) and clear water recirculating aquaculture systems (RAS) have emerged as vital approaches for managing effluents and ensuring sustainable water use (de Sousa Leite et al., 2017; Khanjani et al., 2024). BFT allows accumulation of suspended aquaculture solid waste and their associated microbial communities serving as food for cultured aquatic animals in the system (Khanjani et al., 2024). BFT offers a wide range of benefits including improved water quality of effluents, reduced environmental impact, and enhanced disease control in aquatic animals (Nisar et al., 2022; Bossier and Ekasari, 2017). According to Ray et al. (2017), BFT systems may not be as efficient as clear water RAS systems in treating solid waste from effluents and maintaining optimal water quality. However, BFT systems are reported to promote high yields and quality in both fish and plants when integrated in an aquaculture-agriculture system (Pinho et al., 2017).

Aquaponics, a form of integrated recirculating aquaculture combined with hydroponics (soilless culture), offers a solution by efficiently utilizing biofloc aquaculture effluents for effective crop production (Benz, Maurício, et al., 2019; Pinho et al., 2017). In aquaponics, combination of aquatic animals and plant production enables efficient reuse of water and

nutrients, thereby reducing production costs while fostering environmental sustainability (Stevenson et al., 2010). Aquaponics is recognized as an efficient system for nutrient and water recycling. However, research gaps still exist, particularly in its application for brackish water aquaculture systems (Junge and Antenen, 2020; Pinheiro et al., 2020; Joesting et al., 2016).

The main challenge in adopting aquaponics for brackish water aquaculture effluents management is elevated salinity levels, which limit the number of vegetable species with established markets that can be cultivated within the system (Pinheiro et al., 2020). For instance, shrimp, a valuable crustacean, commonly farmed in the United States, is managed within salinity levels ranging between 5 and 10 parts per thousand (ppt) as minimum tolerance salinity thresholds (Ray and Lotz, 2017; Pinheiro et al., 2020). However, vegetables considered salt-sensitive glycophytes are affected even by salinities exceeding 3 ppt (Agius et al., 2022; Horie et al., 2012). Common practice often utilized to maximize the use of nutrient-rich brackish water aquaculture effluents for salt-sensitive plant production, is effluent dilution to cope with salinity (de Sousa Leite et al., 2017). This practice results in reduced nutrient concentrations in effluents which becomes a limiting factor in plant productivity.

Split-root system involves a single plant having more than one root zone environment, allowing for manipulation of plant water and nutrient uptake. This is achieved by partially submerging root system in saline nutrient solution or drought conditions (Larrainzar et al., 2014; Oliveira et al., 2018). The system has shown efficiency in managing salinity stress on red kale in a hydroponic DWC system. Therefore, the current study focuses on applying split-root using biofloc brackish water from shrimp aquaculture system to cultivate red kale Kale (*Brassica napus* L. *Pabularia*), in a DWC system.

Materials and Methods

Location and Seedling Production

The first trial began in May to July 2023, and the second trial was carried out from August to November 2023. The trials were performed on the west side of a double-covered inflated polyethylene greenhouse located at Auburn University aquaponics Facility at E.W.Shell Fisheries Research Center (lat.32.648935°N, long. 85.486828°W). Greenhouse environment was controlled by an environmental computer (Bartlett GHK12X2GH; Bartlett Controllers, Fort Madison, Iowa) with the average temperature ranging between 20 and 25 ^{o}c.

Pelleted seeds of red kale (Johnny's Selected Seeds, Winslow, ME) were sown (May 13th, 2023), in a 98-cell rockwool plug tray four weeks before the start of the experiment. On June 1st, 2023, seedlings were transferred into a 10.16 cm^{3}-sized rockwool split blocks for root development. Hydroponic (control) nutrient solution at ½ strength with salinity of 0 ppt, was exclusively utilized for daily irrigation until seedlings were ready for transplanting into split-root system (Table 2.1).

Split-root System Setup and Plant Cultivation.

Split-root system was set up using 70-L gray containers (Straight Wall Container, BUCKHORN, Tipton, PA), thick black plastic liners, plywood sheets (121.92 × 38.1 cm), and Styrofoam boards (122× 38 cm). Plywood was used to divide test containers to make two separate sides (A and B), by positioning it horizontally in the middle of the container, (Fig 2.1). The purpose of thick black plastic liners was to completely cover each container, preventing any leakage or mixing of solutions between divided sides. Styrofoam boards were used to support and suspend plants within each container. Four openings were made on each side of the board to allow plant access to water or nutrient solution (Fig 1). This setup aimed to mimic DWC system while integrating the concept of a split-root system. On June 5th, 2023, seedlings were

transplanted into split-root DWC system. Aeration was maintained on each container side (two air stones per side; aquarium air pump, 70 L/min; Vivosun, C.L.L Pet Supplies, 702 Watson Ave, Madison, Wisconsin, U.S.A).

Treatments

Treatments included a hydroponic solution (H or control) at 0 ppt salinity with 0 g L^{-1} of NaCl, saltwater-based hydroponic solution (S-H) with 15.05 g L^{-1} RSS for 10 ppt salinity (Table 2.1), and brackish water shrimp aquaculture effluent (S-Eff), which ranged between 13-14 g L^{-1} of NaCl between trial 1 and 2 (Table 3.1). The saltwater-based hydroponic solution was formulated to mimic minimum salinity levels used for higher productivity in shrimp aquaculture biofloc systems in the southeast of United States (Roy et al., 2010).

During trial 1 shrimp aquaculture effluent treatment was sourced from a six-week-old and in trial 2 from an eight-week-old recirculating biofloc system located in the wet laboratory of the E.W. Shell Fisheries Center at Auburn University. The initial nutrient concentrations of all treatments were analyzed using ICP-MS (Waters Agricultural Laboratory; Camilla, GA) and are detailed in Table 3.1. In homogeneous split-root systems, both parts of the plant root system received the same treatment, while in heterogeneous split-root systems, one side (A) of same plant root system was under treatment, and side B was maintained with clear water.

Growth and biomass accumulation measurements

Plant height, widths, leaf count, and size index (SI) (calculated; [height+width1+width2]/3) were measured weekly starting at 0 days after transplant (DAT). Leaf greenness was recorded weekly (Soil Plant Analysis Development meter; Minolta SPAD 502®, Konica Minolta business solutions, Europe GmbH) on newly fully expanded leaf of each plant. On July 6th, red kale was harvested, and destructive measurements such as shoot and root

fresh and dry weights were recorded (Sensitive scale, Ohaus ranger 3000, Parsippany, NJ, USA). Plant samples were dried for a week at 60 ^{o}C (forced air oven, Grieve, The Grieve Corporation, Round Lake, Illinois, USA). Once completely dried, samples were weighed and sent for tissue analysis (Waters Agricultural Laboratory, Camilla, Georgia, USA).

Diurnal measurements: Leaf physiology

Leaf physiology measurements were performed on newly fully expanded leaves. Diurnal measurements were conducted every 2 hours over a 12-hour period to capture variations in physiological parameters during the day (Portable photosynthesis system; LI-6800, LI-COR Biosciences, Lincoln, Nebraska, USA). Physiological parameters observed were leaf CO_2 Assimilation rate ($\mu mol \cdot m^{-2}\ s^{-1}$) and leaf stomatal conductance to water vapor ($g_{sw,}$ mol [H_2O] $m^{-2}\ s^{-1}$).

Water quality

Water quality was monitored and recorded thrice per week for the entire trial duration. Nutrient solution pH, electrical conductivity (EC), salinity, and dissolved oxygen (DO) were monitored to ensure a proper range to maintain quality. Water and nutrient solutions were restored on each side of container when there was a decrease below half. pH and EC were monitored weekly (pHI9813-6; Hanna Instruments, Smithfield, Rhode Island). A handheld salinity meter (Cole-Parmer, Vernon Hills, Illinois, USA) was used to monitor salinity levels in each treatment during both trials.

Data analysis

The study was organized in completely randomized design consisting of three nutrient solution treatments applied under two split-root systems: homogeneous (Hom) and heterogeneous (Het) at three replications. Eighteen test containers were used as experimental

units. Four red kale seedlings were cultivated in each replicate and data was collected on two middle plants, in consideration of border effect. Data was organized in Microsoft Excel and subjected to test for homogeneity of variance and normality before analysis of variance (ANOVA) was performed using PROC GLIMMIX in SAS (SAS 9.4, Cary, North Carolina, USA). Initial water quality data, tissue analysis, and shoot fresh and dry weight data were analyzed using a two-way analysis of variance (ANOVA) with treatment and condition. A three-way ANOVA was considered for root partitioning ratio data with treatment, condition, and side. Least-square means for treatments within each condition were compared using multiple comparisons by Tukey HSD test whenever there was a significant effect ($P \leq 0.05$) in the ANOVA. Due to variations in nutrient solution condition of shrimp effluent treatment, trial 1 and 2 were analyzed separately. Experimental unit (EU) within treatments and conditions was used as a random effect. In variables such as growth and diurnal measurements for leaf physiology, a repeated measures design approach was adopted.

Results and Discussion

Initial nutrient composition

Nutrient concentrations in H and S-H solution treatments were similar during both trials since they were formulated the same way using fertilizer recommendations for hydroponic lettuce (Table 3.1). In contrast, S-Eff showed variability in nutrient concentration between the two trials. For instance, Ammonia-Nitrogen levels were considerably higher in trial 2 (91.2 mg L^{-1}) compared to trial 1 (0.08 mg L^{-1}), and Phosphorus also showed a notable increase from 8.46 mg L^{-1} in trial 1 to 18.9 mg L^{-1} in Trial 2, (Table 3.1). The disparities are likely due to the age difference in biofloc systems when effluents were utilized (Trial 1 utilized at six weeks old; Trial 2, at eight weeks old). Older biofloc systems typically show enhanced efficiency in carbon and

nitrogen balance, leading to better nutrient retention than newer systems (Crab et al., 2012). Sodium (Na) and chloride (Cl) concentrations, however, remained consistent across the trials (Table 3.1). Average Na and Cl concentrations between trials were 0.0257 g L^{-1} of Na and 0.034 g L^{-1} of Cl for H, 4.095 g L^{-1} Na and 6.620 g L^{-1} of Cl for S-H, and 4.823 g L^{-1} of Na and 8.750 g L^{-1} of Cl for shrimp effluents respectively. Since salinity is a measure of total Na and Cl ions present in solutions, shrimp effluent treatment had a higher salinity level of 13.57 ppt than saltwater-based hydroponic (10.71 ppt). Both S-Eff and S-H salinity levels fall within salinity ranges commonly utilized in brackish water aquaculture systems in the southeast (Roy et al., 2010), while hydroponics (control) solution could be classified as freshwater solution (Benz et al., 2019).

Water quality

There was no significant interactions between treatments and conditions for water quality parameters, including electrical conductivity (EC) (Table 3.2). However, significant differences were observed between the homogeneous and heterogeneous split-root systems (Table 3.2). Homogeneous systems resulted in salinity levels approximately twice as high as those in heterogeneous systems during both trials.

Similarly, EC was higher in homogeneous systems compared to heterogeneous split-root system. Specifically, for S-H and S-Eff treatments, EC was 1.76 mS cm^{-1} and 1.85 mS cm^{-1} higher in homogeneous systems than in heterogeneous systems during both trials respectively. The mean EC values for S-Eff treatment were 7.35 mS cm^{-1} (homogeneous) 3.30 mS cm^{-1} (heterogeneous) in trial 1, and 8.69 mS cm^{-1} (homogeneous), and 4.75 mS cm^{-1} (heterogeneous) in trial 2, based on averages between each container side. For the S-H treatment, EC values were 7.27 mS cm^{-1} (homogeneous) and 3.28 mS cm^{-1} (heterogeneous) in trial 1, with 8.33 mS cm^{-1}

(homogeneous) and 4.46 mS cm^{-1} (heterogeneous) in trial 2. Given that EC measures ionic strength and salinity levels in solutions (Schmidt et al., 2018), the higher salinity and EC values observed in homogeneous systems suggest greater plant stress under S-H and S-Eff treatments compared to the heterogeneous split-root system.

Plant growth performance

Nutrient solution conditions with an EC exceeding 5.5 mS cm^{-1} are known to decrease plant yield and productivity in brassica species, leading to significant economic losses (Shannon, M.C.; Grieve, 1998). This study observed a similar effect on red kale, with significant interactions between treatments and split-root systems affecting plant height, leaf number, and size index during both trials (Table 3.3). Leaf greenness also showed significant interactions between these factors in trial 1 (Table 3.3). Homogeneous split-root system resulted in shorter plants under S-H and S-Eff (33% and 34% in Trial 1; 27% and 29% in Trial 2) while, heterogeneous system (21% and 16% in Trial 1; 0.67% and 10% in Trial 2), relative to the hydroponic treatment in each condition (Table 3.3).

The reductions in plant height were likely due to high salinity stress under S-H and S-Eff treatments, especially when both roots (homogeneous system) were exposed the treatment, as compared to when only one part of the root system was grew in (heterogeneous system). This implies that a heterogeneous split-root system was able to reduce this effect on plant height. Oliveira et al. (2018) reported similar findings in bell peppers where lower reductions in plant height were achieved with heterogeneous split-root saline nutrient solution application. Leaf number was reduced by 67% and 63% during Trial 1, and followed a similar trend in Trial 2 (37% and 42%) for S-H and S-Eff treatments under homogeneous systems, while leaf numbers in heterogeneous split-root systems were reduced by 41% and 36% in Trial 1, and 12% and 18%

in Trial 2 (Table 3.3). Salinity has been reported to reduce leaf number and biomass accumulation in basil plants when the nutrient solution was supplemented with 2.9 g L^{-1} of NaCl (Attia et al., 2011). However, the heterogeneous split-root system slightly reduced this effect on leaf number compared to the homogeneous system.

The size index under heterogeneous split-root system decreased by 32% and 25% for S-H and S-Eff during trial 1, and 14% for S-H and 22% S-Eff in trial 2 for S-H and S-Eff, respectively (Table 3.3). In contrast, homogeneous system showed more substantial decreases of 48% and 43% for S-H and S-Eff in trial 1, and 38% and 43% in trial 2 relative to H (Table 3.3). Kale is a moderate salt-sensitive glycophytic plant (Shannon, M.C.; Grieve, 1998). Higher concentrations of Na and Cl ions in the root environment are reported to increase osmotic stress in glycophytic plants since they are less effective at controlling uptake and storage of these ions compared to halophytes (Centofanti and Bañuelos, 2019). In lettuce, NaCl stress caused chemical composition changes by reducing nitrogen uptake resulting in small plants with fewer leaves (Kleiber et al., 2022). Although S-Eff had relatively higher Na and Cl concentrations than S-H (Table 3.1), it resulted in slightly higher plant height, and leaf count values, than S-H in both conditions in trial 1. Additionally, size index means at harvest were also greater in S-Eff treated plants in both trials under each condition (Table 3.3).

S-H and S-Eff significantly increased leaf greenness (15% and 19%, respectively), compared to the control in homogeneous split-root system during trial 2 (Table 3.3). In the heterogeneous split-root system leaf greenness increased by 14% and 12% (of what?) during the same trial (Table 3.3). There was no significant difference in chlorophyll content between shrimp effluents plants and control plants during trial 1 under a homogeneous split-root system, while saltwater-based hydroponics increased by 21% compared to the control (Table 3.3). Salinity

stress is reported to increase chlorophyll content in brassica species specifically in cabbage (Jamil et al., 2007). Increasing chlorophyll is a stress response mechanism that brassicas vegetable use to adapt and tolerate highly saline conditions (Šamec et al., 2021; Stepien and Johnson, 2009).

Therefore, kale plants treated with shrimp effluents were likely to experience lower stress levels compared to saltwater-based hydroponic treated plants. The exact reason for this difference in performance is not fully understood; however, it could be attributed to presence of organic material in shrimp effluents, which may have helped alleviate salinity stress on plants. Jabeen, (2018) reported improved plant growth and biomass accumulation in tomato plants when organic fertilizers were used instead of inorganic fertilizers to reduce salinity stress under high soil salinity conditions. Similarly, Hoque et al. (2022) also noted that organic soil amendments can reduce salt stress and enhance plant productivity by maintaining better ionic balance, boosting antioxidant enzyme activities, reducing osmotic and oxidative stress, and regulating gene expression in plants.

Biomass accumulation

Shoot fresh and dry weights were impacted by the interaction among treatments within root conditions (Table 3.4). S-H and S-Eff homogeneous split-root system reduced shoot fresh weights by 2.2 and 1.9 times (trial 1) and 2.4 and 2.7 times (trial 2) lower than heterogeneous split-root system (Table 3.4). Shoot dry weights were reduced by 93% and 92% during trial 1, and 83% and 87% in trial 2 in homogeneous S-H and S-Eff compared to control (Fig.3.2). In cabbage and Chinese mustard, salinity is reported to reduce shoot dry weight by 40% and 30% when the whole root system was irrigated with 150 m*M* NaCl salinity (Chakraborty et al., 2016). However, in the current study these reductions were lowered under heterogenous condition (77%

and 78%; trial 1, 48% and 60%) when compared to control (Fig.3.2). The shoot dry weight of plants treated with S-H and S-Eff under heterogeneous split-root system were 1.9 and 2.2 times higher than the homogeneous split-root system in trial 1, and 2.2 and 2.5 times higher than the homogeneous split-root system in trial 2 (Table 3.4). In trial 1, shrimp effluent treatment recorded higher average shoot DW than saltwater-based hydroponic treatment, although the effect was not statistically significant (Table 3.4). Observed increases in shoot fresh and dry weights under heterogenous shows that heterogenous split-root system can alleviate negative effects of salinity on plant biomass accumulation. These findings align with a previous study on bell peppers, which reported the positive effect of split-root setupon shoot fresh and dry weight and percentage dry matter content on bell pepper plants under induced salinity stress (Oliveira et al., 2018). Shoot dry matter percentage (%) increased in plants treated with S-H and S-Eff under homogeneous split-root system (Table 3.4). Although no significant interactions were observed between split-root systems (conditions), the mean percentage of dry matter content was higher in homogeneous system compared to heterogeneous system (Table 3.4). This increase in % shoot dry matter can be attributed to significantly higher salinity levels that plants under homogeneous split-root systems were exposed to compared to heterogeneous split-root systems (Table 3.2). However, plants treated with shrimp effluent (S-Eff) showed lower % dry matter content compared saltwater-based hydroponic treatment (1.05 times higher) under both conditions (Table 3.4). This difference suggests that the composition of shrimp effluent may have influenced plant dry matter % differently than saltwater-based hydroponic treatment despite having a higher salinity level.

Tissue nutrient uptake

In trial 1, interactions between treatments and root conditions did not significantly affect tissue uptake of potassium (K), magnesium (Mg), calcium (Ca), sodium (Na), and chloride (Cl) (Table 3.5). However, higher Na and Cl concentrations were observed under homogeneous in S-H and S-Eff treatments than heterogenous split-root system. Trial 2 showed significant interactions between these two factors in tissue Na and Cl concentrations (Table 3.5). Tissue Na concentration was 1.92 times higher in homogeneous S-H compared to heterogeneous S-H, while 1.44 times higher in homogeneous S-EFF compared to heterogeneous S-EFF (Table 3.5). Tissue Cl concentration decreased by 51% and 46% in heterogeneous S-H and S-Eff (trial 1), and by 2.7% and 73% (trial 2) compared to homogeneous split-root systems. Despite higher concentrations of Na and Cl in S-H and S-Eff solutions, no Cl ion toxicity symptoms were observed, likely due to moderate tolerance of *Brassica napus* species to Cl ions toxicity (Chakraborty et al., 2016).

Tissue Ca concentration decreased by 53% to 68% in S-H and S-Eff, while K decreased by 64% in S-H and 34% in S-EFF relative to control (Table 3.5). Similar findings were reported in Marte plants where homogeneous salinity condition resulted in lowering K and Ca uptake (Della et al., 2023). Na is known to compete with K for binding sites because they are both monovalent cations. Since both S-H and S-Eff nutrient solutions had relatively higher Na concentrations than K (Table 3.1), decrease in K observed in tissue K uptake may be attributed to nutrient imbalance caused by this competition.

Tissue Mg uptake increased in S-H and S-Eff compared to control within each condition. Homogeneous shrimp effluents recorded higher tissue Mg uptake of 1.4 times higher than heterogeneous shrimp effluent in trial 1. This was also reported in Martes plants and other

brassicas leafy vegetables, where salinity increased tissue Mg content as an adaptation response (Della et al., 2023; Šamec et al., 2021). Additionally, *Brassica napus* species are known to have moderate tissue tolerance because of their ability to retain higher K and control over the accumulation of Na and Cl ions in leaves (Chakraborty et al., 2016).

Leaf physiology

There was no significant interaction among treatments within root conditions for stomatal conductance and CO2 assimilation rate (Table 3.6). However, salinity impacted both stomatal conductance and assimilation rate (Table 3.6). Increased salinity in treatments reduced stomatal conductance, evident in both S-H and S-EFF treated plants with reductions of 61% and 23% during trial 1, and 91% and 83% in trial 2 when compared to control (Table 3.6). Chakraborty et al. (2016) reported similar results in brassicas species where higher salinity treatments reduced stomatal conductance. Reductions in stomatal conduction is associated with stomatal closure (Chaves et al., 2009). Stomatal closure is a plant response to leaf water deficit activated to mitigate salinity-induced osmotic stress, one of the major physiological responses which limits plant photosynthetic ability under salt stress conditions (Hniličková et al., 2017; Chakraborty et al., 2016).

These responses affect various aspects of leaf photosynthetic activity, particularly CO_2 assimilation rate of plants as observed in this study. Reductions in CO_2 assimilation rate observed in S-H and S-Eff during trial 2, were 52% and 42% compared to control likely due to higher osmotic stress caused by elevated salinity levels in these treatments (Table 3.6). In trial 1, shrimp effluent treated plants were statistically different from S-H, with mean values that were 2 times higher than saltwater-based hydroponic treatment. Additionally, shrimp effluent treated plants were not significant from control in CO_2 assimilation rate of trial 1. On the other hand, S-

H decreased by 29% compared to control during the same trial. In trial 2, CO_2 assimilation rate was significantly affected by S-EFF under both root conditions. However, homogenous S-Eff CO_2 assimilation rate mean values were 2.8 times lower than heterogeneous S-EFF treated plants (Table 3.6).

The beneficial effect of heterogeneous split-root systems on plant growth, biomass accumulation, nutrient uptake, and physiological performance under elevated salinity levels in root zones observed in this study could be attributed to the plant's ability to redirect root development toward less saline or non-saline side (Galvan-Ampudia and Testerink, 2011). This enables plants to sustain productivity in partially saline root conditions. This adaptation was evident in root partitioning ratios observed in this study. A substantial proportion of roots were allocated to clear water (side B) under split-root heterogeneous S-H and S-Eff treatments, with 86% and 59% in trial 1 (Fig. 3.3A), and 67% and 84% in trial 2 (Fig. 3.3B). On the other hand, the control only allocated 17% and 23% of its total roots per plant in trials 1 and 2, respectively (Fig. 3.2 A; Fig. 3.2 B). This root partitioning strategy resulted in increased total root biomass per plant in the heterogeneous split-root system. In trial 1, the root biomass was 2 g $plant^{-1}$ and 1.7 g $plant^{-1}$ higher for S-H and S-Eff treatments when compared homogeneous system under the same treatments (Fig. 3.2A). Similarly, in Trial 2, the increases were 1.8 g $plant^{-1}$ for S-H and 2.8 g $plant^{-1}$ for S-Eff (Fig. 3.2B). A study by Galvan-Ampudia and Testerink, (2011) supports these findings, indicating that glycophytic plants can dynamically redirect their root growth to mitigate detrimental effects of salt stress because they lack permanent morphological adaptations for high salinity. Instead, plants adapt by adjusting root growth and architecture in response to salinity stress.

Conclusions

Heterogeneous split-root growing condition resulted in increasing plant height, leaf number, and size index than homogeneous split-root system under S-H and S-Eff treatments during both trials. Tissue Na and Cl concentrations were also reduced with heterogeneous compared to homogeneous. Although shrimp aquaculture effluents had a higher salinity level than saltwater-based hydroponics solution, stomatal conductance and CO_2 assimilation were higher in plants treated with shrimp effluents in both conditions during both trials. This was also observed in leaf greenness and tissue Mg were similar to control and relatively higher in saltwater-based hydroponics solution. Outcomes of the study support the hypothesis that brackish water aquaculture effluents can be utilized in producing salt-sensitive plants like red kale under heterogeneous salinity conditions without the need for dilution.

The study further confirms that heterogeneous root salinity condition facilitates higher total root weight per plant which results in improved growth traits and physiological performance of plants leading to higher yields and productivity than homogeneous split-root systems. To further comprehend split-root approach for brackish water aquaponics application, future research could explore effect of biofloc aquaculture effluents on plant responses to salinity to understand if the presence of organic material in nutrient solution could lead to reduced salinity stress on plants under DWC system. Additionally, split-root investigation in a decoupled aquaponics system would be ideal.

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Tables

Initial Nutrient Compositions

Table 3.1. Initial nutrient composition and salinity levels in the nutrient solution type for trials 1 and 2

		Nutrient Solution Type[z]					
		Hydroponic		Saltwater-based Hydroponic[y]		Shrimp effluents[x]	
Nutrient	Unit	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Ammonia-Nitrogen	mg L^{-1}	24.1	150	4.16	54.7	0.08	91.2
Nitrate-Nitrogen		198	20.3	74.1	9.22	23.2	0.466
Phosphorus		50.2	41.2	24.3	19.1	8.46	18.9
Boron		1.64	1.31	2.17	1.8	1.42	2.15
Zinc		0.643	0.33	0.356	0.165	0.041	0.261
Manganese		1.75	1.2	0.982	0.67	0.039	0.514
Iron		1.71	2.18	0.388	0.404	0.012	0.146
Copper		0.191	0.14	0.1	0.069	0.06	0.183
Potassium	g L^{-1}	0.221	0.205	0.267	0.256	0.151	0.319
Calcium		0.155	0.131	0.184	0.156	0.137	0.179
Magnesium		0.0535	0.0453	0.496	0.406	0.374	0.478
Sulfate		0.275	0.278	1.1	0.995	1.034	1.333
Chloride		0.045	0.023	6.45	6.79	8.75	8.75
Sodium		0.0182	33.1	4.434	3.755	4.62	5.026

[z]The hydroponic and saline hydroponic solution treatments were formulated using water-soluble fertilizers 8N-6.5P-29.9K, (Gramp's Original Hydroponic Lettuce Fertilizer), calcium nitrate (15.5N-0P-0K) and magnesium sulfate (10% Mg) as sources.

[y]Saltwater-based hydroponic nutrient solution treatment salinity was achieved with red sea salt (RSS) at 15 g L-1

[x]Shrimp aquaculture effluent was sourced from a biofloc system for six-week-old (trial 1) and eight-week-old (trial 2) at E.W. Shell Fisheries center.

Nutrient Solution Water Quality

Table 3.2. Water quality parameters for each nutrient solution treatment in DWC split-root heterogeneous and homogeneous split-root systems recorded at one week after transplant in two trials.

Treatment	Electrical Conductivity (mS cm^{-1})		Salinity (ppt)	
	Het[z]	Hom	Het	Hom
	Trial 1			
H[y]	1.01[x]b	1.71b	0.00c	0.00c
H-S	4.46ab	8.34a	3.99bc	7.91ab
S-Eff	4.75ab	8.70a	4.42bc	9.59a
ANOVA				
Treatment	0.2154		<.0001	
Condition	0.0432		0.0033	
Treatment× Condition	0.2114		0.0902	
	Trial 2			
H	3.30a	2.77a	0.00b	0.00b
H-S	3.28a	7.27a	5.72ab	10.73a
S-Eff	3.30a	7.35a	5.04ab	11.00a
ANOVA				
Treatment	<.0001		<.0001	
Condition	0.0016		0.0025	
Treatment× Condition	0.1998		0.0715	

[z]Het = heterogeneous root condition, Hom = homogeneous root condition H[y]=Hydroponic solution, S-H = Saltwater hydroponic solution, S-Eff = Shrimp effluents

[x]Means followed by same uppercase letters among conditions (column) within treatments (rows) indicate no significant differences based on Tukey test. Means followed same lowercase letters among treatments (rows) within conditions (column) are not significant according to Tukey test.

Growth Performance

Table 3.3. Effect of nutrient solution treatments on red kale growth traits at harvest in DWC split-root heterogeneous and homogeneous split-root systems during trials 1 and 2.

Treatment	Nutrient							
	Height (cm)		Leaf number (count)		Leaf greenness (SPAD index)		Size index	
	Het[z]	Hom	Het	Hom	Het	Hom	Het	Hom
	Trial 1							
H[y]	12.9[x]ab	13.4a	18.7b	29.7a	41.5cd	41.7bc	47.0b	55.2a
S-H	10.0cd	9.1e	7.7cd	5.7e	40.3de	63.4a	27.7cd	21.1e
S-Eff	12.1bc	9.6de	9.2c	6.5de	33.1e	40.4d	33.1c	24.3de
ANOVA								
Treatment	<.0001		<.0001		<.0001		<.0001	
Condition	0.0182		0.6938		<.0001		0.0234	
Treatment× Condition	0.0491		<.0001		<.0001		<.0001	
	Trial 2							
H	14.0ab	15.1a	12.5a	13.3a	37.9d	43cd	51.0a	51.9a
S-H	12.3bc	9.3de	9.7b	6.1c	49.2b	56.1a	34.0b	24.7d
S-Eff	11.4c	8.8e	9.4b	4.6cd	41.2c	56.3a	31.6bc	19.7c
ANOVA								
Treatment	<.0001		<.0001		<.0001		<.0001	
Condition	0.2775		<.0001		<.0001		<.0001	
Treatment× Condition	0.0003		<.0001		0.2099		<.0001	

[z]Het = heterogeneous root condition, Hom = homogeneous root condition, H[y] =Hydroponic solution, S-H = Saltwater hydroponic solution, S-Eff = Shrimp effluents

[x]Means followed by same uppercase letters among conditions (column) within treatments (rows) indicate no significant differences based on Tukey test. Means followed same lowercase letters among treatments (rows) within conditions (column) are not sugnificant according to Tukey test.

Biomass

Table 3.4. Effect of nutrient solution treatments on biomass accumulation of red kale 'KX-1' cultivated in heterogeneous and homogeneous split-root systems in two trials.

Treatment	Nutrient					
	Shoot FW (g plant^{-1})		Shoot DW (g plant^{-1})		Shoot DM (%)	
	Het[z]	Hom	Het	Hom	Het	Hom
	Trial 1					
H[y]	550.5[x] a	842.2a	59.0b	90.2a	9.97b	10.8ab
H-S	102.6c	46.1c	13.2c	6.0c	12.9ab	13.7a
S-Eff	130.6c	60.0c	13.3c	7.2c	9.8b	12.1ab
ANOVA[y]						
Treatment	<.0001		<.0001		0.0038	
Condition	0.1799		0.0619		0.0549	
Treatment× Condition	0.0012		<.0001		0.593	
	Trial 2					
H	623.8b	899.3a	54.5b	71.8a	8.4bc	7.7c
H-S	219.4c	91.3c	28.5c	12.5de	12.53abc	14.28a
S-Eff	176.0c	64.1c	21.6cd	8.7e	11.8abc	13.5ab
ANOVA[y]						
Treatment	<.0001		<.0001		0.0001	
Condition	0.6661		0.1193		0.3634	
Treatment× Condition	<.0001		<.0001		0.5037	

[z]Het = heterogeneous root condition, Hom = homogeneous root condition, H[y] =Hydroponic solution, S-H = Saltwater hydroponic solution, S-Eff = Shrimp effluents

[x]Means followed by same uppercase letters among conditions (column) within treatments (rows) indicate no significant differences based on Tukey test. Means followed same lowercase letters among treatments (rows)within conditions(column) are not significant according to Tukey test.

Tissue Nutrient Content

Table 3.5. Effect of nutrient solution treatments on tissue nutrient uptake of red kale 'KX-1' cultivated under heterogeneous and homogeneous DWC split-root systems of two trials.

Treatment	Nutrient									
	K		Mg		Ca		Na		Cl	
	Het	Hom	Het	Hom	Het	Hom	Het	Hom	Het	Hom
	Trial 1									
H	4.70[x]a	4.99a	0.79b	0.80b	2.45a	2.60a	0.65b	0.51b	0.88bc	0.55c
H-S	1.71c	1.83c	0.92b	0.82b	0.93bc	0.71c	3.58a	3.03a	1.59ab	3.26a
S-Eff	3.34b	2.96c	0.99b	1.38a	1.155b	1.23b	2.93a	3.54a	1.34abc	2.49a
ANOVA										
Treatment	<.0001		0.0002		<.0001		<.0001		0.0011	
Condition	0.9445		0.1516		0.9728		0.9437		0.0215	
Treatment× Condition	0.2391		0.0157		0.0923		0.1611		0.0563	
	Trial 2									
H	5.57a	5.87a	0.61bc	0.57c	2.15a	2.24a	0.17c	0.32c	0.86b	0.81b
H-S	2.62c	3.31bc	0.73abc	0.85ab	0.90b	0.87b	1.24b	2.40a	2.85ab	2.77ab
S-Eff	2.68c	4.04b	0.88a	0.71abc	0.93b	0.87b	1.79ab	2.59a	1.70b	6.29a
ANOVA										
Treatment	<.0001		0.003		<.0001		<.0001		0.0004	
Condition	0.0001		0.5451		0.9599		0.0019		0.0175	
Treatment× Condition	0.0633		0.0838		0.7581		0.0193		0.0137	

[z]Het = heterogeneous root condition, Hom = homogeneous root condition, H[y] =Hydroponic solution, S-H = Saltwater hydroponic solution, S-Eff = Shrimp effluents

[x]Means followed by the same uppercase letters among conditions (column) within treatments (rows) indicate no significant differences based on Tukey test. Means followed same lowercase letters among treatments (rows)within conditions(column) are not sugnificant according to Tukey test.

Leaf Physiology

Table 3.6. Effect of nutrient solution treatments on midday stomatal conductance and CO_2 Assimilation rate of red kale cultivated in DWC split-root heterogeneous and homogeneous split-root systems in two trials.

Treatment	Trial 1			
	GSW[w] ([H2O] m^{-2} s^{-1})		A[v] (CO_2 m^{-2} s^{-1})	
	Het[z]	Hom	Het	Hom
	Trial 1			
H[y]	1.34[x]a	1.30a	22.50ab	19.64ab
S-H	0.72ab	0.29ab	17.98ab	12.04b
S-Eff	1.02ab	1.01ab	20.04ab	24.56a
ANOVA				
Treatment	0.0012		0.0153	
Condition	0.3319		0.4903	
Treatment× Condition	0.5216		0.1272	
	Trial 2			
H	1.78a	2.02a	25.10a	21.13a
S-H	0.22b	0.10b	13.19abc	8.86bc
S-Eff	0.55ab	0.06b	19.56ab	6.85c
ANOVA				
Treatment	<.0001		0.0002	
Condition	0.6705		0.0031	
Treatment× Condition	0.592		0.1977	

[z]Het = heterogeneous root condition, Hom = homogeneous root condition, H[y] =Hydroponic solution, S-H = Saltwater hydroponic solution, S-Eff = Shrimp effluents

[x]Means followed by same uppercase letters among conditions (column) within treatments (rows) indicate no significant differences based on Tukey test. Means followed same lowercase letters among treatments (rows)within conditions(column) are not significant according to the Tukey test.

[w]Stomatal Conductance to water vapor

[v]Leaf CO_2 Assimilation rate

Figures

Size Index

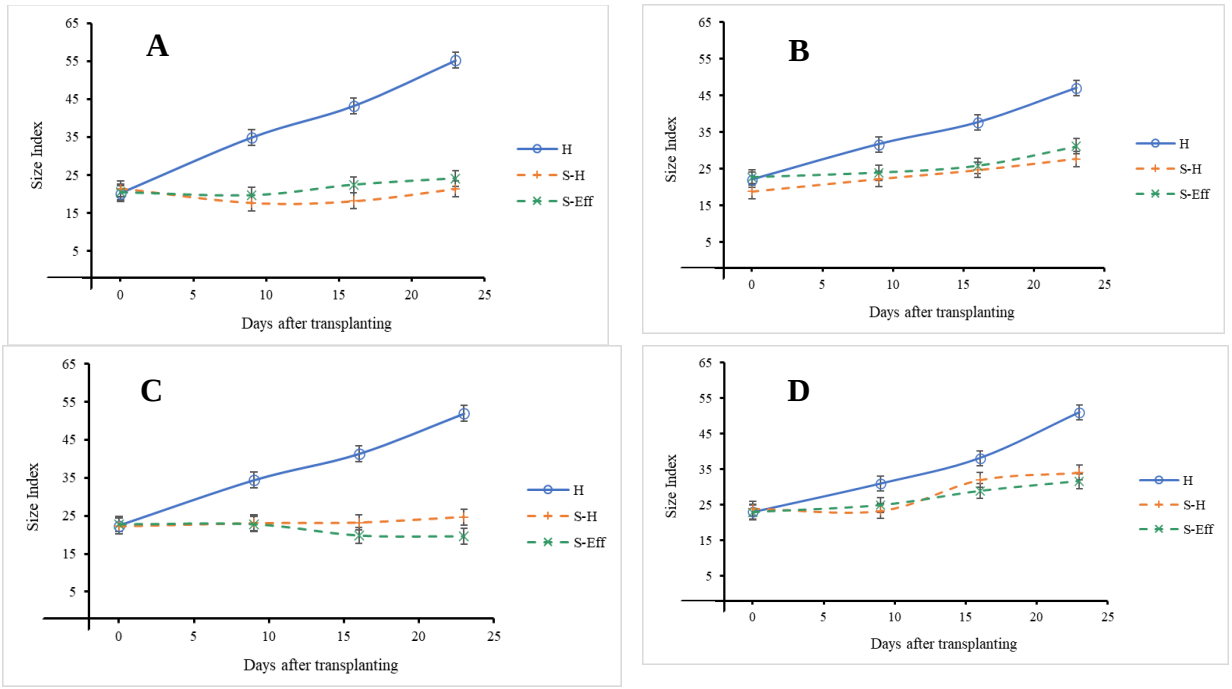

Fig. 3.1. Red kale 'KX1' size index over time in homogeneous split-root systems in trials 1 (A) and 2 (C) and heterogeneous split-root systems for trials 1 (B) and 2 (D) under hydroponic (H), salt-water hydroponic (S-H), and shrimp effluents (S_Eff) treatments. Size index was estimated as the average plant height, widest width, and perpendicular width for each plant.

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Red Kale Shoot Biomass at Harvest

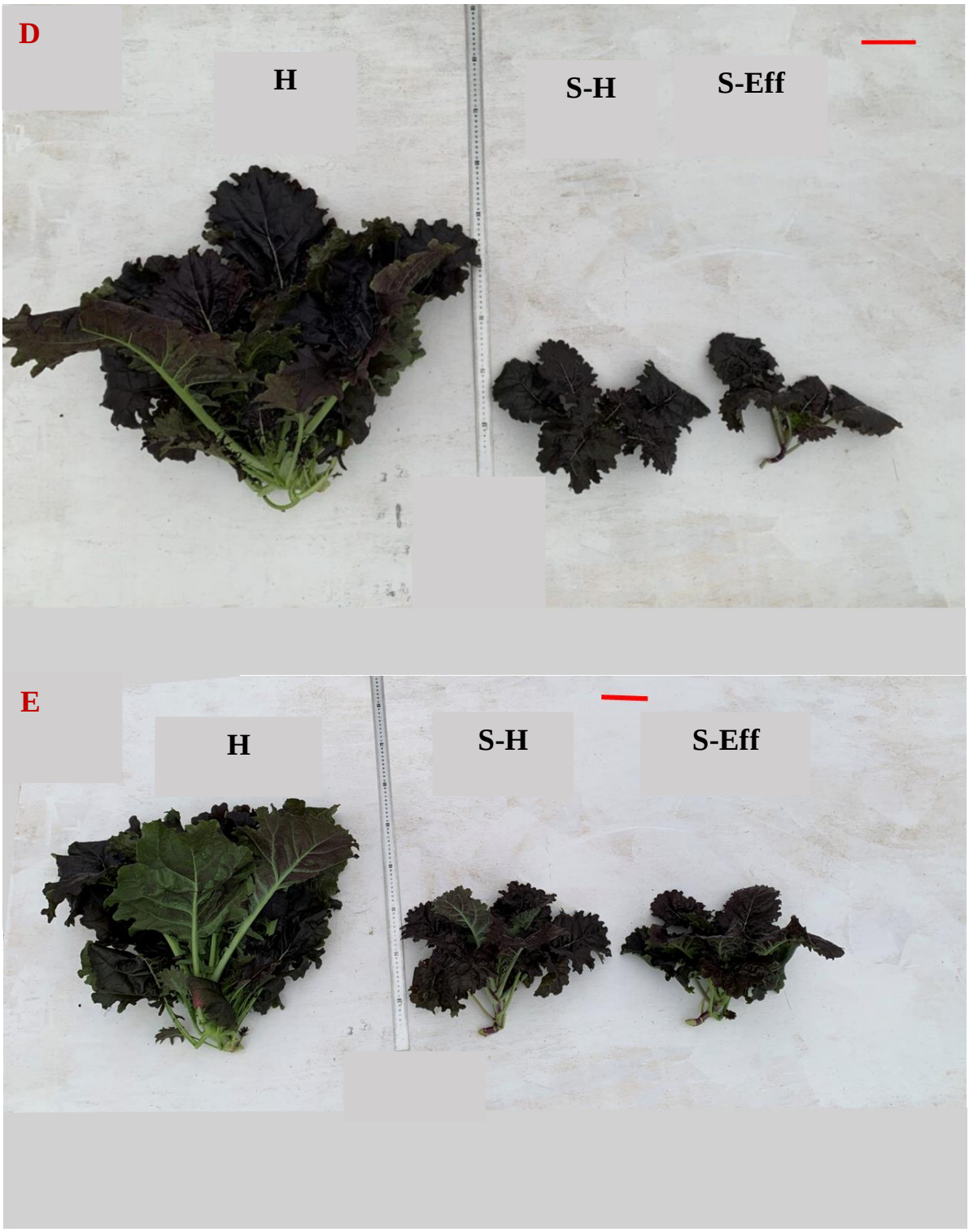

Fig 3.2. Photos of red kale shoot biomass at harvest in homogeneous (D) and heterogeneous (E) split-root systems under hydroponic (H), saltwater hydroponic (S-H), and shrimp effluents (S-Eff) treatments.

Root Partitioning Ratios

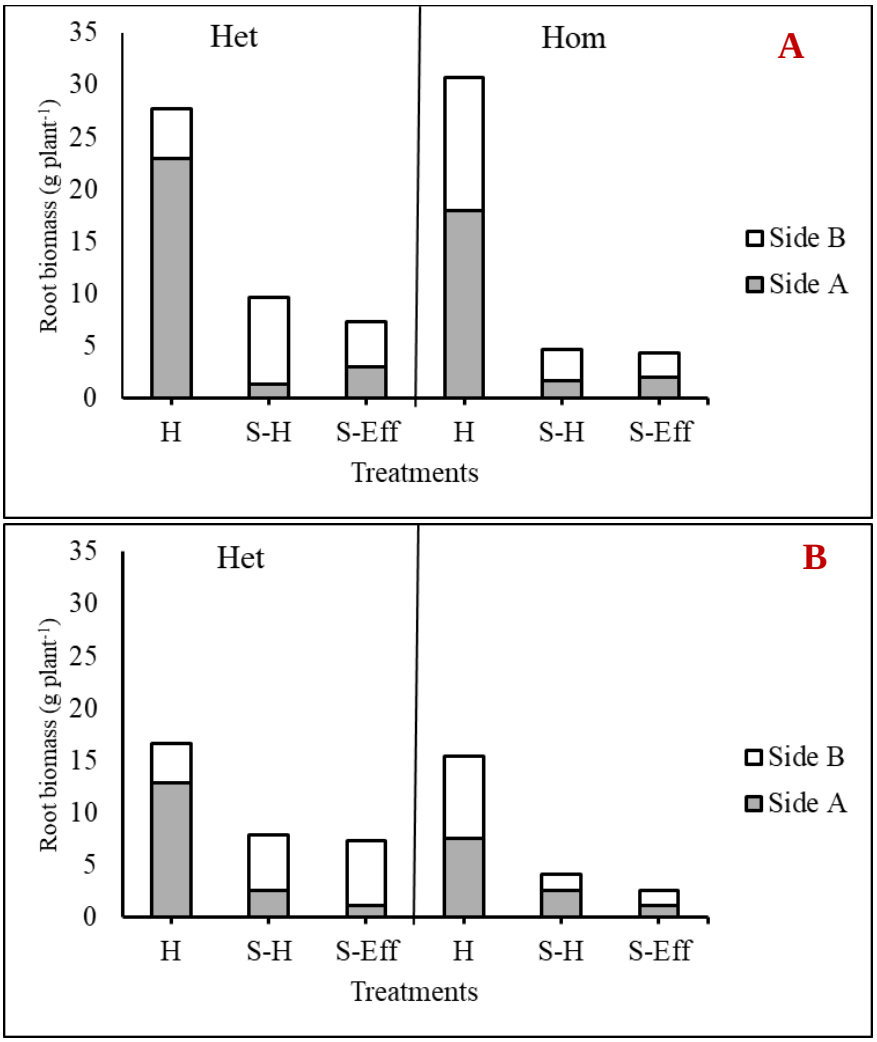

Fig. 3.3. Root partitioning ratios of red kale in trials 1 (A) and 2 (B) under different treatments: Hydroponic (H), saltwater hydroponic (S-H), shrimp effluents (S-Eff) in heterogeneous (Het) and homogeneous (Hom) split-root systems. In heterogeneous system, side A had nutrient solution, and side B had clear water, while in homogeneous system, both sides A and B had the same treatment.

Root Biomass

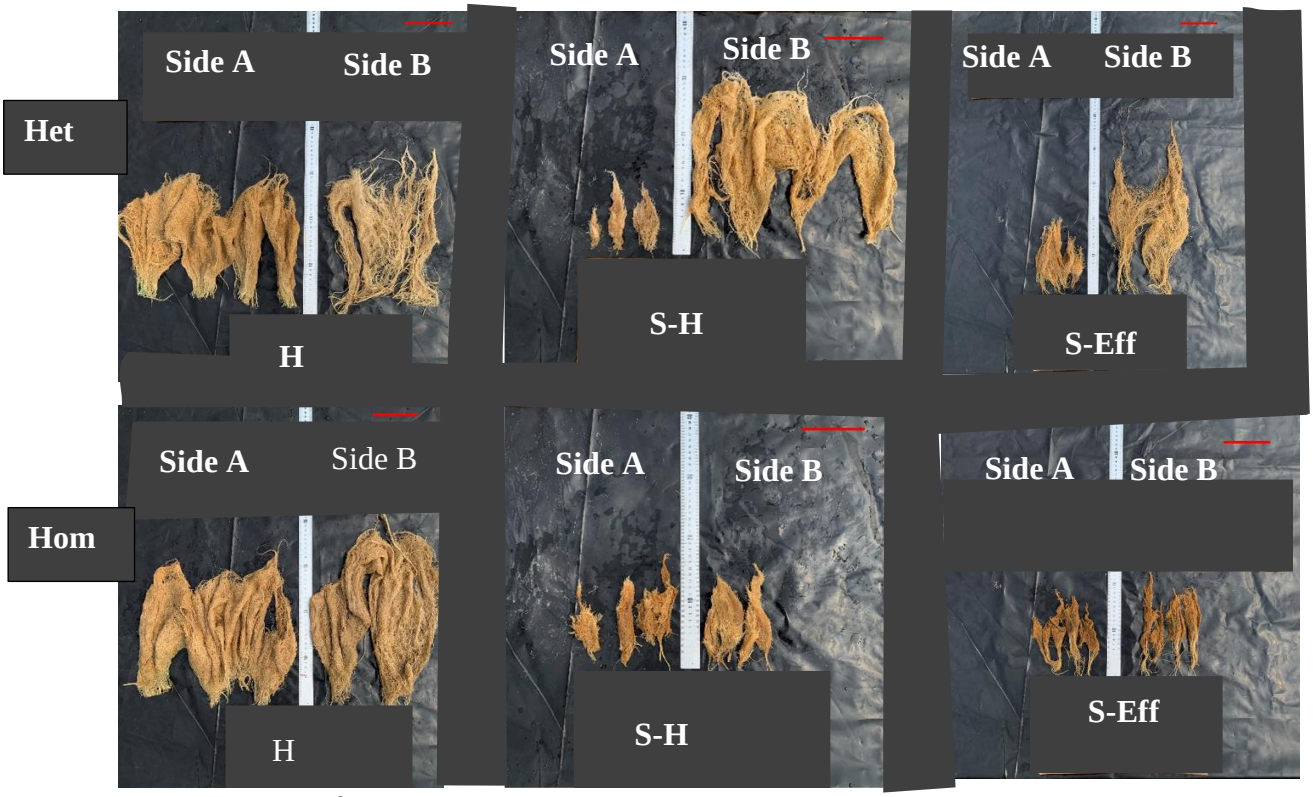

Fig 3.4. Photos of root biomass partitioning ratios to sides A and B in heterogeneous (Het) and homogeneous (Hom) split-root systems under hydroponic (H), saltwater-based hydroponic (S-H), and shrimp effluents (S-Eff) treatments.