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SUSTAINABLE PRODUCTION STRATEGIES FOR UNDERUTILIZED LEAFY VEGETABLES UNDER SALT STRESS FOR THE FRESH-CUT INDUSTRY

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Summary

1. Introduction

Water is a critical resource for agriculture, representing roughly 70% of global freshwater withdrawals (Chartzoulakis and Bertaki, 2015). In recent decades, escalating climate change, prolonged droughts, and progressive soil degradation have sharply intensified water scarcity, simultaneously reducing freshwater availability and accelerating soil salinization, particularly in arid and semi-arid regions (Ondrasek and Rengel, 2021). These trends pose a severe risk to agricultural sustainability, threatening water use efficiency, crop productivity, and global food security (Munns and Tester, 2008). Researchers and farmers are increasingly exploring alternative water management strategies, including the use of suboptimal water sources such as brackish and saline waters (Qadir et al., 2007). These water sources are classified by their dissolved salt content: freshwater typically used in agriculture contains <1 g L^{-1} of salts (electrical conductivity, EC <0.7 dS m^{-1}), brackish water ranges from 1–30 g L^{-1} (EC from 0.7 to 25 dS m^{-1}), and saline water exceeds 35 g L^{-1} (EC >25 dS m^{-1}) (Horita, 2005; Musie and Gonfa, 2023). High salinity induces salt stress, triggering morpho-physiological and metabolic responses that may impair plant growth, productivity, and survival (Zhu, 2007). Tolerance to salinity varies widely among species: most cultivated crops are classified as salt-sensitive glycophytes, while halophytes possess specialized adaptive mechanisms enabling survival and growth under saline conditions (Geissler et al., 2009; Van Zelm et al., 2020). Salt tolerance can be quantified using several criteria, including: the ratio of biomass accumulation and yield under saline conditions versus optimal conditions; the salt concentration causing a 50% reduction in biomass or yield compared to the control; the threshold salinity and associated yield-decline slope; and the percentage loss of yield or biomass (El-Hendawy et al., 2017; Hu and Schmidhalter, 2004; Hussin et al., 2023; Negrão et al., 2017). Several studies in plant physiology and agronomy have examined the ability of different plant species to tolerate salinity stress and its effects on plant performance, leading to widely adopted classifications such as salt-sensitive, moderately salt-sensitive, moderately salt-tolerant, and salt-tolerant (Grieve et al., 2012; Katerji et al., 2003). In particular, Shannon and Grieve (Shannon and Grieve, 1998) compiled an extensive list of vegetable crops, assigning each to a salinity tolerance class and reporting the corresponding EC thresholds. However, it is important to note that salt tolerance varies not only among species but also among cultivars and developmental stages (Cai and Gao, 2020; Yadav et al., 2019). The negative effects of salinity on plants arise from an interplay of primary stress (osmotic and ionic) and secondary stress (oxidative) (Hao et al., 2021; Munns and Tester, 2008). Osmotic stress occurs first, due to reduced soil water potential and limiting water and nutrient absorption (A. Kumar et al., 2020). Plants respond through stomatal closure and the accumulation of osmolytes (such as proline and soluble sugars) to maintain osmotic balance (Rasool et al., 2013; Yang and Guo, 2018). Subsequently, ionic stress develops as excessive Na^+ and Cl^- accumulate, disrupting metabolic processes and impairing

photosynthesis (Ashraf and Harris, 2013; Tavakkoli et al., 2011). These primary stresses ultimately lead to secondary oxidative stress, characterized by the overproduction of reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2), superoxide ($O_2^{\bullet -}$), and hydroxyl radicals ($OH^{\bullet}$), which can cause severe structural and functional damage if not neutralized (Bhattacharjee, 2019; Mushtaq et al., 2020). Plants counter these effects through antioxidant defense systems involving enzymatic components such as superoxide dismutase (SOD), catalase (CAT), and various peroxidases (e.g., APX, GPX) (Abeer et al., 2014; Qi et al., 2023), as well as non-enzymatic antioxidants such as ascorbate, glutathione, proline, carotenoids, and flavonoids (Das and Roychoudhury, 2014; He et al., 2017).

Given the increasing pressures from freshwater scarcity and salinity, the development of effective strategies to mitigate the effects of suboptimal water and sustain crop productivity is essential. Among these strategies, the agricultural application of biostimulants and elicitors has gained significant attention. According to Regulation (EU) 2019/1009 a plant biostimulant is defined as products that stimulate plant nutrition processes independently of their nutrient content, with the primary aim of improving nutrient use efficiency, abiotic stress tolerance, quality traits, or the availability of nutrients in the soil or rhizosphere.

In contrast, elicitors are substances that trigger natural defenses by simulating biotic stress (Abdul Malik et al., 2020). Numerous studies have demonstrated that exogenous protective compounds, such as antioxidants, nitric oxide (NO) donors, metabolic precursors, phytohormones and their derivatives, can enhance plant growth by improving nutrient and water uptake (Sun and Shahrajabian, 2023; Wise et al., 2024), increasing yield and quality traits (Paradiković et al., 2011; Popko et al., 2018), enhancing photosynthetic performance and gas exchange (Baltazar et al., 2021; Raza et al., 2022), modulating hormonal signaling pathways (Moncada et al., 2022), and regulating metabolic and enzymatic activities (Calvo et al., 2014; Mattarozzi et al., 2020). Under stress conditions, they can alleviate oxidative damage through the accumulation of osmo-protectants and antioxidant compounds (Hasanuzzaman et al., 2021; Miceli et al., 2021).

Parallel to agronomic inputs, crop diversification represents another promising strategy to enhance the resilience and sustainability of agro-food systems. In recent decades, consumer dietary behavior has evolved toward healthier eating patterns, with increasing demand for fresh, nutraceutical-rich leafy vegetables and ready-to-eat (RTE) products (Aschemann-Witzel et al., 2013; López-González et al., 2021). These products provide significant amounts of vitamins, minerals, antioxidants, fibers, carotenoids, and flavonoids, and are widely recognized for their contribution to health promotion and disease prevention (D. Kumar et al., 2020; Saini et al., 2017; Zeng et al., 2025). To meet this demand and expand the fresh-cut sector, there is increasing scientific and commercial interest in underutilized vegetable species, including edible wild plants, halophytes, and local landraces. These species often

exhibit enhanced tolerance to abiotic stress due to their broad genetic variability and specialized defense mechanisms (Ali and Yun, 2017; Kapazoglou et al., 2023; Mishra and Tanna, 2017), as well as a higher content of bioactive compounds compared to conventional cultivars (Esposito et al., 2025). However, their diffusion remains limited by scarce availability of propagation materials and the lack of agronomic knowledge regarding cultivation practices (Jaenicke and Höschle-Zeledon, 2006; Jena et al., 2018), particularly for controlled and soilless environments.

Hydroponic cultivation systems provide a key technological platform to support the development of both conventional and novel leafy vegetables for RTE production. Hydroponic systems are widely used for leafy vegetables such as lettuce, spinach, rocket, and valerian (Acharya et al., 2021; Majid et al., 2021; Petropoulos et al., 2016; Tabatabaei, 2008), ensuring rapid growth, multiple production cycles, high yield, superior quality, efficient resource use, and enhanced food safety (Kumar, Vikanksha and Singh, 2023; Tüzel et al., 2019). Hydroponics is a soilless cultivation system in which plants grow in inert substrates and roots directly absorb essential nutrients from a nutrient solution (Sharma et al., 2018). Proper solution management is crucial, as pH, electrical conductivity (EC), dissolved oxygen, and temperature strongly influence nutrient availability and uptake (Fathidarehnijeh et al., 2024; Sambo et al., 2019), consequently impacting plant growth, morpho-physiological traits, and quality. Macro- and micronutrients remain soluble and readily available for uptake when the pH is maintained between 5.5 and 6.5 (Ali Al Meselmani, 2023). EC reflects the overall ionic concentration in the nutrient solution (Langenfeld et al., 2022), and optimal values typically range between 1 and 3 dS m^{-1}, although tolerance thresholds vary among crops (Shannon et al., 2019).

Compared with conventional soil cultivation, these soilless cultivation systems can reduce water consumption by 70–90% (da Silva et al., 2024), enable precise environmental and nutrient management, minimize pest and disease incidence, and support sustainable and profitable production even in resource-limited or urban contexts (Esposito et al., 2026).

Various hydroponic techniques, including NFT (Nutrient Film Technique), DWC (Deep Water Culture), ebb-and-flow, and aeroponics, have proven highly effective for leafy vegetables. In these hydroponic systems, the nutrient solution is supplied through different delivery strategies: in NFT technique, a thin flowing film of solution continuously bathes the roots; in DWC, the roots are immersed in an aerated nutrient solution; in ebb-and-flow systems, the solution is intermittently delivered and drained; and in aeroponics, the roots are misted with a finely nebulized nutrient solution (Fussy and Papenbrock, 2022; Lakhiar et al., 2018; Rajendran et al., 2024; Rani et al., 2022). Ebb-and-flow systems are ideally suited for baby leaf production in the RTE market due to their capacity to optimize root oxygenation and nutrient use efficiency (Esposito et al., 2026).

Furthermore, hydroponic systems offer a controlled environment to study salinity tolerance, enabling the precise management of saline irrigation for both moderately sensitive and salt-tolerant species (da Silva et al., 2024; Dellosa et al., 2024).

2. Aim of the work

Hydroponic cultivation represents a strategic production system for ready-to-eat (RTE) leafy vegetables, ensuring high yields, resource efficiency, and elevated hygienic standards. Nevertheless, scientific literature remains largely concentrated on a limited range of widely cultivated species (mainly commercial lettuce), while information concerning less conventional species, halophytes, and wild genotypes suitable for hydroponic environments is still scarce. This knowledge gap is particularly critical given the global interest in agricultural diversification, sustainable production models, and the development of foods with enhanced functional and nutraceutical properties. Within this framework, the present research focused on plant resources that are poorly investigated for hydroponic and fresh-cut applications: New Zealand spinach (*Tetragonia tetragonioides* (Pallas) O. Kunze), leaf celery (*Apium graveolens* L. var. *secalinum* Alef.), selected leaf lettuce genotypes (*L. sativa* TKI-122, TKI-125, and TKI-126), and wild lettuce genotypes (*L. serriola* TKI-252, and TKI-257, *L. aculeata* TKI-465, *L. dregeana* TKI-468). These species provide a unique opportunity to explore innovative leafy vegetables and broaden the species portfolio available to the RTE sector.
The overarching aim of this research was therefore to evaluate the suitability, adaptability and overall performance of these species and genotypes in controlled hydroponic environments. To achieve this, an integrated experimental approach was adopted, focusing on the following objectives:

- Establishment of cultivation protocols: Defining parameters to support efficient hydroponic growth while examining plant development, yield potential, and morphological traits relevant to baby-leaf and baby-plant production.
- Quality and postharvest characterization: Assessing consumer-oriented aspects through biochemical profiling, sensory assessment, and postharvest dynamics, with a specific emphasis on shelf life and visual quality maintenance during storage.
- Salinity tolerance assessment: Clarifying how increasing salinity influences growth performance, morpho-physiological functioning, and chemical composition, while identifying inter- and intraspecific variability in salt tolerance.
- Mitigation strategies: Exploring the use of exogenous compounds to sustain productivity, improve resilience, and preserve qualitative attributes under saline conditions.

Ultimately, this work intends to valorize neglected and wild plant resources, enhance agrobiodiversity within the RTE vegetable sector, and explore the potential for using saline or brackish water within sustainable hydroponic frameworks.

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RESEARCH ACTIVITY:
DEPARTMENT OF AGRICULTURAL, FOOD AND FOREST SCIENCES (SAAF)
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I. EFFECTS OF HYDROPONIC CULTIVATION ON BABY PLANT CHARACTERISTICS OF TETRAGONIA TETRAGONIOIDES (PALLAS) O. KUNZE AT HARVEST AND DURING STORAGE AS MINIMALLY-PROCESSED PRODUCE

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1. Introduction

In the last decades, the consumer's demand for fresh leafy vegetables and ready-to-eat products has greatly increased as they experienced a wide change in eating habits arising from the need for a healthier lifestyle (López-González et al., 2021). Fruits and vegetables, and among them leafy vegetables, are considered an important component of a healthy diet due to their content in vitamins, minerals, antioxidants, and other important phytonutrients (Kumar et al., 2020). The increasing consumer demand for diverse and nutritious fresh-cut vegetables has prompted researchers and growers to explore unconventional crops, such as underutilized vegetable crops, that can meet these needs. Underutilized vegetable crops are all of the crops which are not traded widely or farmed commercially on a large scale according to the definition gave by Jaenicke and Hoeschle (Jaenicke and Höschle-Zeledon, 2006): "species with underexploited potential for contributing to food security, health (nutritional/medicinal), income generation, and environmental services". The reasons for the low spread of underutilized vegetables, although highly appreciated in some geographic areas, can be found in the low availability of propagation material and in the lack of information on agronomic management techniques and on their nutritional and medicinal importance (Jena et al., 2018). Therefore, it would be necessary to start research activities on the management, use, and improvement of these genetic resources to offer a wider choice to farmers and consumers and, at the same time, ensure food and nutritional security.

Tetragonia tetragonioides (Pallas) O. Kunze, commonly known as New Zealand spinach, is a halophytic herbaceous plant recognized for its distinct flavor and impressive nutritional profile. Native to Australia, New Zealand, and eastern Asia, it is now widely distributed throughout the world and it is naturalized in some Mediterranean areas (Roskruge, 2011; Taylor, 1994; Wilson, 2000). Historically utilized in traditional cuisines as well as to treat many diseases, its leaves can be eaten raw as a salad vegetable or cooked in soup preparations (Egamberdieva et al., 2021). *T. tetragonoides* is valued for its nutritional profile, which includes high levels of vitamins (A, C, and E), minerals (K, P, Na, Ca, Mg, Fe, and Zn), and dietary fiber, and for its richness in other bioactive compounds (Friday and Igwe, 2021; Onoiko and Zolotareva, 2024). Furthermore, *Tetragonia* has anti-inflammatory, anti-ulcerogenic, and antioxidant activities (Choi et al., 2020). Its ability to thrive in saline and poor soil

conditions and its heat resistance make it particularly advantageous in the context of climate change, where soil salinity and heat stress are becoming increasingly pressing issues (Kovar and Olsovska, 2020). The adaptability of this species suggests its potential for cultivation in diverse agro-ecological and climatic conditions and with different cultivation techniques such as hydroponics.

Hydroponic systems consistently support strong growth, high yields, and efficient resource use for many vegetables, making them a viable and sustainable alternative to traditional soil-based farming (Sharma et al., 2018). Hydroponics is especially effective for leafy vegetables due to their fast growth cycles, high water content, and adaptability. Lettuce, for example, thrives in hydroponic systems, achieving high yields and superior quality compared to conventional methods (Çekin et al., 2024). Several studies show that hydroponic systems can match or exceed soil-based cultivation in terms of fresh weight, the number of leaves, and root development for various leafy greens. Different hydroponic methods (NFT, DWC, ebb-and-flow, and aeroponics) are all commonly used and effective for leafy vegetable production. The hydroponic cultivation systems are characterized by high resource use efficiency and sustainability. These systems may reduce the use of water by 70–90% compared to soil-based agriculture, gaining particular interest in regions with water scarcity in terms of quantity or quality (da Silva et al., 2024). They also allow for year-round production, reduced pest and disease incidence, and minimal need for weeding or spraying. Although hydroponic systems can have higher initial and operational costs, they offer advantages in urban and resource-limited settings and can be profitable with quality-based pricing or for high-value crops such as minimally processed leafy vegetables (Zha et al., 2024). Hydroponic cultivation could be highly suitable also for wild native or underutilized leafy vegetables, as found for *Amaranthus cruentus* (Croft et al., 2017), *Portulaca oleracea* (He et al., 2021), and *Urospermum picroides* (Anaclerio et al., 2021). These cultivation systems can easily enable their cultivation in controlled environments with improved yield, resource efficiency, and quality traits.

Despite the advantages and the potential of New Zealand spinach, systematic research on the agronomic practices necessary for the effective cultivation of *T. tetragonioides* remains limited, especially as regards its suitability for hydroponic cultivation and fresh-cut vegetable production and its post-harvest physiology and shelf life.

This paper aims to address this gap by evaluating the suitability of *Tetragonia tetragonioides* to hydroponic cultivation for baby plant production and its quality characteristics and shelf life as a minimally processed vegetable.

2. Materials and methods

2.1 Tetragonia baby plant production

The research was conducted in two different growing seasons (autumn–winter 2022–2023 and spring 2024) in an unheated greenhouse at the Agricultural, Food, and Forest Sciences Department (SAAF) of the University of Palermo (38°6′28″ N 13°21′3″ E; altitude 49 m). The fruits of *T. tetragonioides* (hard capsule containing about 4–6 seeds) (Franchi Sementi, Grassobbio, Italy) were sown (18 November 2022 and 1 March 2024) into polystyrene trays with different cell volumes, to set different plant densities. The trays were filled with a commercial substrate (Tappeti erbosi, Vigorplant Italia srl, Fombio, Italy), a mix of peat, bark humus, and silica sand fertilized with 850 g m^{-3} of a mineral fertilizer NPK (pH 6.0–6.8; EC 0.10–0.20 dS m^{-1}). In the first growing season, three plant densities were tested based on the expected size of Tetragonia baby plants and the range of plant density tested in other studies (Anaclerio et al., 2021): 365, 497, and 615 plants m^{-2} (trays with a cell volume of 58, 32, and 28 mL, respectively). Based on the results of the first experiment, the second growing season tested higher plant densities of 615 and 947 plants per square meter (trays with a cell volume of 28 and 18 mL, respectively). The trays were kept in a dark room at 25 °C until emergence (7 days after sowing) and were then moved on fixed benches in the greenhouse. After the emergence, the plantlets were thinned to one per cell by selecting seedlings with similar morphological traits.

The plants were cultivated in a hydroponic ebb and flow system using three concentrations of nutrient solutions (NSs) to explore the species' response to different nutrient availability: 100% (full-strength—FS), 50% (half-strength—HS), and 0% (control with only water—C). The FS NS was prepared according to Miceli et al. (Miceli et al., 2021), by adding the following to tap water (pH 7.6; electrical conductivity (EC) 500 µS cm^{-1}): 19 mM NO_3^-, 1.25 mM NH_4^+, 2 mM $H_2PO_4^-$, 11 mM K^+, 4.5 mM Ca^{2+}, 1 mM Mg^{2+}, 1.1 mM SO_4^{2-}, 40 µM Fe^{3+}, 30 µM BO_3^{3-}, 5 µM Mn^{2+}, 4 µM Zn^{2+}, 0.75 µM Cu^{2+}, and 0.50 µM Mo (Sonneveld and Voogt, 2009).

During the first cultivation period (autumn–winter 2022–2023), the average minimum and maximum temperatures inside the greenhouse were 10.0 ± 0.4 and 24.0 ± 0.5 °C and ranged from 6.0 to 29.7 °C, respectively, whereas the relative humidity was 68.5 ± 1.6% and varied from 52.0% to 100%. During spring 2024, the average air temperatures were 13.1 ± 0.4 (night) and 30.1 ± 0.5 °C (day) and varied from 8.9 to 35.6 °C, while the relative humidity was 54.6 ± 1.0% on average and ranged from 41.0% to 63.3%.

2.2 Agronomical and morpho-physiological parameters

The amount of water and NS consumed during plant growth was measured and used to calculate the water use efficiency (WUE) and the nitrogen use efficiency (NUE) as follows: WUE (g DW L^{-1} H_2O) = plant dry weight (g)/H_2O (L); NUE (g DWg^{-1} N) = plant dry weight (g)/supplied N (g) (supplied N = initial N content of the substrate + N supplied with sub fertigation) (Baligar and Fageria, 2015). The plants of *T. tetragonioides* were harvested by cutting the baby plants at the base when they had 5–7 leaves and soon before axillary bud shooting (10 January 2023 and 5 April 2024), and total baby plant yield was calculated.

Soon after harvest, 30 plants for each replicate were randomly sampled for morphological and biochemical analysis. Leaf color was measured on one expanded leaf for each plant using a colorimeter (CR-400, Minolta corporation, Ltd., Osaka, Japan) that recorded the CIELab parameters (L*, a* and b*). These parameters were used to calculate hue angle (h°) and chroma (C*) as h° = 180 + arctan(b*/a*) (McGuire, 1992) and $C^* = (a^{*2} + b^{*2})^{1/2}$. Then, each plant was divided into leaves, stem, and roots to determine the fresh weight and dry weight after drying at 65 °C for 72 h. Before drying, the leaf area was measured by scanning leaves at 300 dpi (Epson Perfection 4180 Photo, Seiko Epson Corp., Suwa, Japan) to obtain digital images that were analyzed with the ImageJ 1.52a software (National Institutes Health, Bethesda, MD, USA). The specific leaf area (SLA cm^2 g^{-1} DW) was estimated as leaf area/leaf dry weight.

2.3 Chemical determinations

The dried samples (four replicated samples for each treatment) were also used for chemical determinations. Ash content was determined through the procedure described in AOAC (Latimer, 2023) while the Kjeldahl method was used for protein determination (Palazzolo et al., 2012). The crude fiber was determined by the Weende method (Palazzolo et al., 2012). The free carbohydrate content was obtained with the anthrone method reported in Loewus (Palazzolo et al., 2012). The contents of K, Na, Ca, Mg, and Fe, were determined using atomic absorption spectroscopy following wet mineralization while P was determined using a colorimetric method (Morand and Gullo, 1970). The vitamins A and E involved a phase of extraction with an organic solvent from the food matrix, a phase of separation, and, successively, identification and quantification in the HPLC system (Palazzolo et al., 2012). Riboflavin (Vit. B2) was extracted in an autoclave with a solution of diluted H_2SO_4 and later, after enzymatic treatment, was determined through HPLC (for the fluorescent spectra) (Palazzolo et al., 2012). Niacin (Vit. B3) was extracted from the sample in an acidic solution at 12–18 °C for 30 min and measured through a microbiological method (Palazzolo et al., 2012). The thiamine content (Vit. B1) was obtained through extraction in 0.1 N HCl and oxidation by thiochromium and analysis in HPLC using fluorometric detection (Palazzolo et al., 2012). Ascorbic

acid (Vit. C) was determined according to procedures previously described by Barros et al. (Barros et al., 2007). The oxalic acid content was determined by high-performance liquid chromatography (Okutani and Sugiyama, 1994). The samples of the spring harvest were analyzed only for mineral and oxalic acid content.

2.4 Minimal processing and cold storage

Once harvested, the plants obtained with FS and HS nutrient solutions (only FS in the first cultivation season) were immediately transferred to the laboratory of Vegetable Analysis of the SAAF Department and minimally processed for fresh-cut production. The baby plants were selected, retaining only those free from defects, yellowing, or decay. Then, they were washed in tap water for 5 min two times and dried by manual centrifugation for 1 min with a handheld salad spinner. Samples of 50 g from each treatment were directly packed in polyethylene (PE) bags, sealed with a hot bar (Laica VT3112, Vicenza, Italy), and stored at 4 °C for 21 days. At the end of processing and every 7 days (0, 7, 14, and 21 days of cold storage), three replicated samples for each treatment were randomly selected and weight loss, leaf color, and overall quality were evaluated.

Weight loss (g 100 g^{-1} of initial fresh weight FW) was estimated by weighing each sample at packaging and at each sampling time.

Leaf color was assessed on the upper surface of ten leaves randomly selected from each sample. A Chroma-meter CR-400 (Minolta Corp., Ltd., Osaka, Japan) was used to measure the CIELAB color components L (lightness), a* (red-green axis), and b* (yellow-blue axis). These values were then used to calculate chroma (C*), a measure of color saturation, and hue angle (h°), a measure of color hue according to the following equations: $C^* = (a^{*2} + b^{*2})^{1/2}$ and $h° = 180° + \arctan(b^*/a^*)$. Moreover, total color difference (ΔE) was also determined at each sampling date as $\Delta E = [(L^* - L^*_0) + (a^* - a^*_0) + (b^* - b^*_0)]^{1/2}$, where L^*_0, a^*_0, and b^*_0 were the control values at the end of processing (T_0).

An informal panel of ten individuals (six men and four women, aged 28–59) assessed the overall quality (OQ) of the samples. Panelists assigned scores from 1 to 5, with 1 representing poor/unmarketable quality (off odors, extensive color changes, major defects, or decay), 3 representing the limit of marketability, and 5 representing excellent/freshly harvested quality (free from off odors, defects, and decay) (Miceli et al., 2021).

2.5 Statistics and principal component analyses

The experimental design consisted of four replicated samples of 30 plants each for every combination of plant density and nutrient solution concentration, randomly assigned in four blocks. To determine the effect of plant density and nutrient solution concentration on Tetragonia plants, a two-way analysis of variance (ANOVA) was carried out. The differences between treatments and the interactions between factors were assessed with the least significant difference (LSD) test at $p = 0.05$.

A completely randomized design with three replicates per treatment was performed for minimal processing trials. To determine the effect of storage time, plant density, and nutrient solution concentration, a three-way ANOVA was carried out. Mean values were compared by the LSD test at $p = 0.05$ to identify significant differences among treatments and significant interactions between factors.

Principal component analysis (PCA) was performed on the morpho-physiological parameters, yield, and mineral content of Tetragonia baby plants to identify the key parameters discriminating among plant density and nutrient solution concentration across two cultivation seasons. The input matrix for the PCA included the following: plant height; stem diameter; total, root, stem, and leaf fresh weight (FW); shoot/root FW ratio; total, root, stem, and leaf dry weight (DW); shoot/root DW ratio; shoot dry matter percentage; yield; water use efficiency (WUE); nutrient use efficiency (NUE); leaf number; leaf width; plant area; leaf area; specific leaf area (SLA); leaf color components L*, chroma and hue; and mineral content. Only factors with eigenvalues greater than 1.0 were retained to determine the optimal number of principal components (PCs). The relationships between input variables were further investigated by examining the PC plots, with correlated variables and examined variables reported within the subspace defined by the first and second PCs. PCA was conducted using SPSS version 13.0. (SPSS Inc., Chicago, IL, USA).

3. Results

3.1 Morphological and yield characteristics of Tetragonia tetragonioides baby plants

The suitability of *T. tetragonioides* to grow hydroponically for baby plant production was investigated during two cultivation cycles. The plants were ready for harvest after 53 days from sowing in the first period (from November 2022 to January 2023) and after 31 days from sowing in the second period (from March to April 2024).

3.1.1. First growing period (Autumn–Winter)

During the first growing cycle, the seedling height was affected only by the nutrient solution (NS) concentration; it increased with full-strength (FS) NS concentration by 37% compared to half-strength (HS) and control (0) NS (2.2 cm on average) (Table 1). The stem was thinner using 615 plants m^{-2} or control NS (1.8 and 1.4 mm, respectively) and was slightly but significantly increased by the lowest plant density, whereas it increased by 51.4% using FS nutrient solution (Table 1).

Table 1. Effects of plant density (365, 497, and 615 plants m^{-2}) and nutrient solution (NS) concentration (0%, only water—0; 50%, half strength—HS; and 100%, full strength—FS) on morphological parameters of *Tetragonia tetragonioides* baby plants grown during autumn–winter.

Source of Variance		Plant Height (cm)	Stem Diameter (mm)	Plant Fresh Weight (g FW)					Plant Dry Weight (mg DW)					Shoot Dry Matter (%)
				Total	Roots	Stem	Leaves	S/R	Total	Roots	Stem	Leaves	S/R	
Plant Density														
365		[z] 2.7	1.9a	1.31a	0.27	0.28	0.76a	3.7	97.3a	24.7a	18.7	54.0	2.9	7.2b
497		2.4	1.7ab	1.19b	0.26	0.25	0.68b	3.4	93.9a	23.1a	17.9	52.9	3.1	7.8a
615		2.4	1.8b	1.08c	0.23	0.24	0.61c	3.6	84.0b	19.6b	17.0	47.5	3.3	7.8a
Nutrient Solution (NS)														
0		2.1b	1.4c	0.54c	0.13	0.17	0.24c	3.2	43.5c	10.9c	11.7	20.9	3.1	8.0a
HS		2.3b	1.8b	1.09b	0.27	0.22	0.61b	3.1	88.4b	24.2b	15.4	48.8	2.7	7.8a
FS		3.1a	2.1a	1.95a	0.37	0.38	1.21a	4.3	143.4a	32.2a	26.5	84.7	3.5	7.1b
Density x NS														
365	0	2.2	1.5	0.58	0.14c	0.19bc	0.25	3.2b	46.3	12.7	13.0b	20.6d	2.7b	7.5
	HS	2.7	2.0	1.25	0.30bc	0.24b	0.71	3.2b	100.8	27.3	16.6b	56.9b	2.7b	7.7
	FS	3.3	2.2	2.09	0.37ab	0.40a	1.32	4.6a	144.9	34.0	26.4a	84.6a	3.3ab	6.5
497	0	2.3	1.4	0.58	0.15d	0.17c	0.25	2.8b	47.1	12.0	12.2bc	22.9d	3.0b	8.2
	HS	2.2	1.7	1.06	0.24c	0.22bc	0.6	3.4b	87.6	22.7	16.3b	48.7bc	2.9b	7.9
	FS	2.8	2.1	1.92	0.39a	0.34a	1.19	3.9ab	146.9	34.7	25.2a	87.1a	3.3ab	7.3
615	0	2.0	1.4	0.45	0.10c	0.13c	0.21	3.5b	37.0	8.0	9.7c	19.3d	3.6ab	8.4
	HS	2.1	1.8	0.96	0.26c	0.19bc	0.51	2.7b	76.7	22.7	13.1b	40.9c	2.4b	7.7
	FS	3.1	2.2	1.83	0.33b	0.39a	1.11	4.5a	138.4	28.0	28.0a	82.4a	3.9a	7.4
Significance [x]														
Density		ns	*	***	**	**	***	ns	**	**	ns	*	ns	**
NS		***	***	***	***	***	***	***	***	***	***	***	***	***
Density x NS		ns	ns	ns	*	*	ns	*	ns	ns	*	*	*	ns

[z] Each value is the mean of four replicated samples of 30 plants each. For each factor, values in a column followed by the same letter are not significantly different, according to the LSD test. [x] Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; and *** significant at $p < 0.001$.

The total plant fresh weight (FW) linearly decreased with increasing plant density and linearly increased with increasing nutrient solution concentration (Table 1). As regards the plant parts, the leaf biomass was the main part of the plant and showed a similar trend to those found for the total biomass, with no significant interaction between plant density and nutrient solution concentration, whereas the root and stem fresh weight showed different trends by increasing the nutrient solution concentration for each plant density. The root biomass was lower when using 0% NS, especially with the highest plant density (0.10 g $plant^{-1}$). The HS NS did not affect root weight with increasing plant density, whereas the FS solution determined the highest root biomass with 497 plants m^{-2} (0.39 g $plant^{-1}$) (Table 1). The highest stem fresh weight was recorded in each plant density using the FS NS (0.38 g

plant^{-1}, on average). The FS NS also determined the highest shoot/root FW ratio, especially with 365 and 615 plants m^{-2}.

The total dry biomass (Table 1) was negatively affected by the plant density only with 615 m^{-2} (down to 84.0 mg plant^{-1}) but increased linearly with increasing NS concentration (up to 143.4 mg plant^{-1}). A similar trend was recorded also for the root biomass. Increasing plant density, the stem dry weight significantly decreased with the lowest NS concentration while no significant change was recorded with HS and FS NSs. The leaf dry biomass was not affected by the 0% and FS NS when varying the plant density, whereas using the HS NS, the leaf dry weight lowered with increasing plant density. The shoot dry matter percentage was significantly reduced when growing plants with the lowest plant density or supplying the FS NS (7.2 and 7.1 on average, respectively) (Table 1).

The number of leaves and the leaf width were significantly affected only by the nutrient solution rate, showing a linear increase from 5.6 (0%) to 8.1 (FS) for the leaf number and from 7.3 mm (0%) to 15.5 mm (FS) for the leaf width (Table 2). The total leaf area lowered by increasing the plant density over 365 plants m^{-2}, with a reduction by 17% on average for the other plant densities. The nutrient solution concentration determined a greater effect; the total leaf area on control plants (0%) was 3.1 cm^2 on average and increased by 156 and 361% for HS and FS, respectively (Figure 1a). The plant density had no effect on the average leaf area, while the nutrient solution concentration increased this parameter by 93% and 213%, for HS and FS, respectively, compared with 0% (3.1 cm^2) (Table 2).

Table 2. Effects of plant density (365, 497, and 615 plants m^{-2}) and nutrient solution (NS) concentration (0%, only water—0; 50%, half strength—HS; and 100%, full strength—FS) on leaf characteristics of *Tetragonia tetragonioides* baby plants grown during autumn–winter.

Source of Variance		Number of Leaves	Leaf Width	Leaf Area (cm^2 plant^{-1})	Leaf Area (cm^2 leaf^{-1})	SLA (cm^2 g DW^{-1})	L*	Chroma	Hue°
Plant Density									
365		[z] 7.1	11.8	9.5a	1.2	169.0a	48.3	39.5a	121.4
497		6.9	11.0	7.9b	1.1	144.4b	47.4	36.8b	121.2
615		7.0	11.4	8.0b	1.1	166.7a	48.3	38.8ab	121.1
Nutrient Solution (NS)									
0		5.6c	7.3c	3.1c	0.6c	149.2b	52.1a	39.8a	117.2c
HS		7.3b	11.4b	7.9b	1.1b	162.5a	47.7b	40.3a	121.8b
FS		8.1a	15.5a	14.3a	1.8a	168.5a	44.1c	34.9b	124.8a
Density x NS									
365	0	5.4	7.2	3.1	0.6	152.2	50.8	41.0	117.9
	HS	7.8	12.0	9.7	1.2	169.7	48.3	40.8	122.3
	FS	8.2	16.1	15.7	1.9	185.1	45.8	36.6	124.1
497	0	5.4	7.2	3.0	0.6	132.0	51.4	38.6	116.9

	HS	7.0	11.1	7.3	1.0	148.8	47.9	38.8	121.3
	FS	8.3	14.7	13.3	1.6	152.4	42.9	33.1	125.4
615	0	5.9	7.7	3.1	0.5	163.2	54.1	39.8	116.7
	HS	7.1	11.0	6.9	1.0	168.9	47.0	41.5	121.8
	FS	8.0	15.6	13.9	1.7	168.0	43.6	35.0	125.0
Significance [x]									
Density		ns	ns	*	ns	***	ns	*	ns
NS		***	***	***	***	**	***	***	***
Density x NS		ns	ns	ns	ns	ns	ns	ns	ns

[z] Each value is the mean of four replicated samples of 30 plants each. For each factor, values in a column followed by the same letter are not significantly different, according to the LSD test. [x] Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; and *** significant at $p < 0.001$.

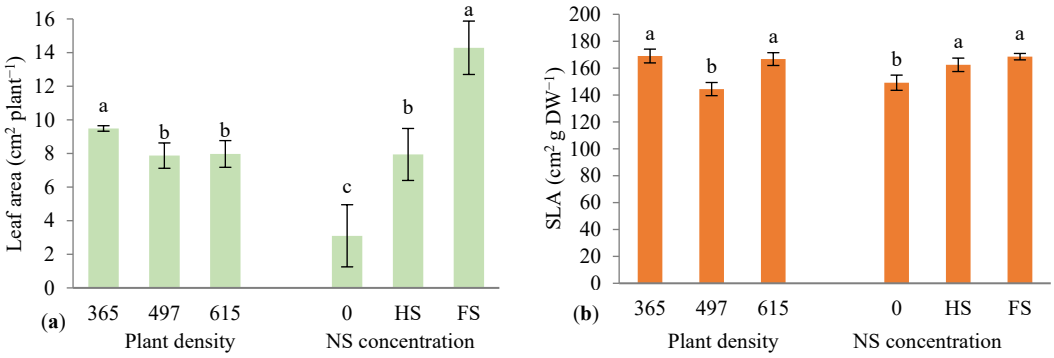


Figure 1. Effects of plant density (365, 497, and 615 plants m^{-2}) and nutrient solution (NS) concentration (0%, only water—0; 50%, half strength—HS; and 100%, full strength—FS) on (**a**) leaf area and (**b**) specific leaf area of *Tetragonia tetragonioides* baby plants grown during autumn–winter (black bars represent standard errors of the means; bars of the same color with different letters within an experimental factor are significantly different at $p \leq 0.05$ according to the LSD test).

The SLA of Tetragonia plants dropped significantly only with intermediate plant density (−14.6%). The addition of nutrients to the irrigation water significantly increased this parameter irrespective of NS concentration (+11.0%, on average, for HS and FS) (Table 2, Figure 1b). The color of Tetragonia leaves was influenced by plant growth conditions (Table 2). When increasing the NS concentration, the leaf lightness decreased and the hue angle linearly increased, whereas the color saturation was negatively affected by the highest NS concentration. The leaf color was slightly affected by the plant density, but only as regards chroma values.

The yield of baby plants was lowest using 0% nutrient solution and did not significantly increase with this NS with increasing plant density (193.4 g m^{-2} on average). The other nutrient solutions determined yield increases with increasing plant density with the highest yield recorded with FS NS and 615 plants m^{-2} (922.7 g m^{-2}) (Figure 2). The baby plants obtained using 0% nutrient solution showed a stunted growth and a yellowish leaf color irrespective of plant density so that they did not meet the commercial requirements.

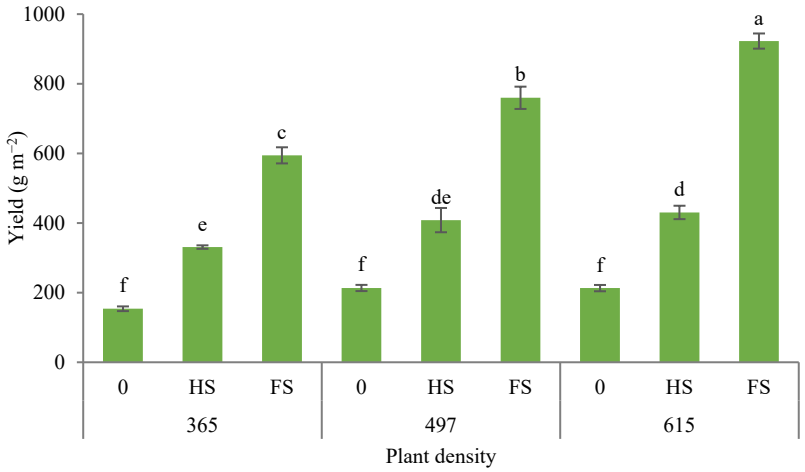


Figure 2. Effects of plant density (365, 497, and 615 plants m^{-2}) and nutrient solution (NS) concentration (0%, only water—0; 50%, half strength—HS; and 100%, full strength—FS) on the yield of *Tetragonia tetragonioides* baby plants during autumn–winter (black bars represent standard errors of the means; bars with different letters are significantly different at $p \leq 0.05$ according to the LSD test).

The lowest water use efficiency (WUE) was recorded in the plants grown at the lowest plant density supplied with 0% NS (0.5 g DW L^{-1} H_2O). The increase in NS concentration increased the WUE linearly at each plant density, with the highest WUE efficiency in the plants supplied with FS NS and grown at the two higher plant density (2.6 g DW L^{-1} H_2O, on average) (Figure 3a). The lowest nitrogen use efficiency (NUE) was recorded in the plants grown at the lowest plant density, even with increasing NS concentration (6.8 g DW g^{-1} N on average). The NUE dropped when using HS NS with 497 and 615 plants m^{-2}; the highest NUE was recorded with 497 plants m^{-2} and zero NS (13.3 g DW g^{-1} N) followed by the plants irrigated with FS NS at the two higher plant densities (12.0 g DW g^{-1} N, on average, for 497 and 615 plants m^{-2}) (Figure 3b).

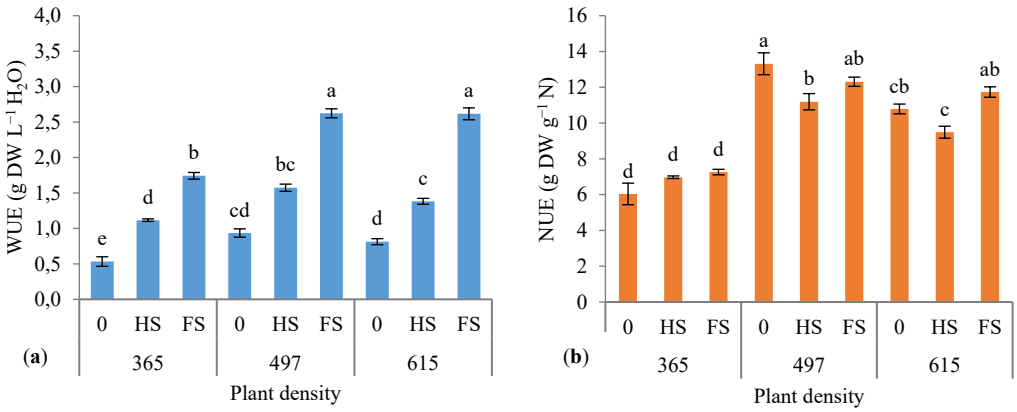


Figure 3. Effects of plant density (365, 497, and 615 plants m^{-2}) and nutrient solution (NS) concentration (0%, only water—0; 50%, half strength—HS; and 100%, full strength—FS) on the (**a**) water (WUE) and (**b**) nitrogen (NUE) use efficiency of *Tetragonia tetragonioides* baby plants during autumn–winter (black bars

represent standard errors of the means; bars with different letters are significantly different at $p \leq 0.05$ according to the LSD test).

3.1.2. Second growing period (Spring)

Based on the results of the first experiment, the second growing season tested only the highest plant densities of the first cultivation cycle (615 plants m^{-2}) and an even higher plant density (916 plants m^{-2}).

The stems of Tetragonia baby plants were a little shorter but thicker than the first cycle (Table 3). Their height was significantly higher using FS NS (+29% compared to 0% and HS NS, on average) and lower when growing plants at 947 plants m^{-2} compared to 615 plants m^{-2}. The stem diameter was negatively affected by the lowest spacing between plants but increased when adding nutrients to the irrigation water (HS and FS NSs) (Table 3).

Table 3. Effects of plant density (615 and 497 plants m^{-2}) and nutrient solution (NS) concentration (0%, only water—0; 50%, half strength—HS; and 100%, full strength—FS) on morphological parameters of *Tetragonia tetragonioides* baby plants grown during spring.

Source of Variance		Plant Height (cm)	Stem Diameter (mm)	Plant Fresh Weight (g FW)					Plant Dry Weight (mg DW)					Shoot Dry Matter (%)
				Total	Roots	Stem	Leaves	S/R	Total	Roots	Stem	Leaves	S/R	
Plant Density														
615		[z] 17.2a	2.8a	1.67	0.20a	0.31a	1.16	6.7b	119.1	16.5a	21.4	80.8	5.8	7.8
947		15.0b	2.4b	1.33	0.14b	0.28b	0.91	7.8a	89.2	12.6b	17.5	59.2	6.0	7.6
Nutrient Solution (NS)														
0		13.7b	2.2b	0.50	0.12b	0.16c	0.22	3.5c	48.0	9.6b	12.8	25.7	4.3	10.1
HS		15.7b	2.7a	1.75	0.20a	0.32b	1.23	7.7b	122.7	17.1a	21.2	84.3	6.1	6.8
FS		19.0a	2.9a	2.25	0.20a	0.41a	1.65	10.5a	141.9	17.0a	24.5	100.1	7.2	6.0
Density x NS														
615	0	14.6	2.3	0.54d	0.14	0.16	0.25d	2.8	51.5d	11.7	13.0d	26.9d	3.5c	9.9a
	HS	17.5	3.0	2.07b	0.24	0.35	1.48b	7.6	145.1a	19.2	24.7ab	101.0ab	6.5ab	6.9b
	FS	19.6	3.1	2.40a	0.23	0.41	1.77a	9.6	160.8a	18.8	26.7a	114.7a	7.3a	6.5b
947	0	12.8	2.0	0.45d	0.09	0.15	0.20d	4.2	44.5d	7.5	12.5d	24.5d	5.1b	10.3a
	HS	13.8	2.5	1.43c	0.16	0.28	0.99c	7.8	100.2c	15.0	17.8c	67.5c	5.7b	6.7b
	FS	18.4	2.7	2.10ab	0.17	0.40	1.53ab	11.3	123.0b	15.2	22.3b	85.5b	7.2a	5.6c
Significance [x]														
Density		*	***	***	***	*	***	**	***	***	***	***	ns	ns
NS		***	***	***	***	***	***	***	***	***	***	***	***	***
Density x NS		ns	ns	**	ns	ns	**	ns	**	ns	*	***	*	***

z Each value is the mean of four replicated samples of 30 plants each. For each factor, values in a column followed by the same letter are not significantly different, according to the LSD test. x Sig-nificance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; and *** significant at $p < 0.001$.

The total plant fresh weight (FW) (Table 3) linearly increased with increasing nutrient solution concentration in the plants grown at 947 plants m^{-2} ranging from 0.45 g $plant^{-1}$ (0 NS) to 2.10 g $plant^{-1}$ (FS NS), whereas at 615 plants m^{-2}, the plant fresh weight overcame 2 g $plant^{-1}$ with HS NS and was significantly higher with FS NS (2.40 g $plant^{-1}$). The same trend was found for the leaf and stem fresh weight, while root fresh biomass was lower when growing the plants at the highest plant density and for both densities when supplementing only water to the plants (0 NS).

The total dry weight (Table 3) was lowest at both plant densities in the plants supplemented only with water (48.0 mg $plant^{-1}$, on average) and increased up to the highest level when supplementing the plants grown at 615 plants m^{-2} with HS or FS NS (152.9 mg $plant^{-1}$, on average). The plants grown at 947 plants m^{-2} significantly increased dry biomass when fed with HS NS (100.2 mg $plant^{-1}$) and even more with FS NS (123.0 mg $plant^{-1}$) but did not reach the dry biomass accumulation recorded with the lower plant density. This trend was superimposable with that of shoot parts, and as found for fresh weight, the root dry biomass was higher in the plants grown at 615 plants m^{-2} and in those supplemented with HS or FS NS.

The shoot dry matter percentage was significantly reduced when increasing NS concentration, but the reduction was greater at 947 plants m^{-2} (−46.1%) compared to 615 plants m^{-2} (−34.4%) (Table 3).

During spring, the plants had less leaves than in the first growing cycle (5.1 leaves $plant^{-1}$, on average). The leaf number significantly increased up to 5.7 on average at both plant densities when the plants were supplemented with HS or FS NS (Table 4). The nutrient solution also influenced the leaf width, with higher increases in the plants grown at 615 plants m^{-2} compared to 947 plants m^{-2} (Table 4). Leaf area was strongly reduced in the plants fed only with water and had the highest expansion with FS NS at 615 plants m^{-2} (27.3 cm^2 $plant^{-1}$; 4.5 cm^2 $leaf^{-1}$). The highest plant density recorded a lower plant area, especially with HS NS (Table 4 and Figure 4a).

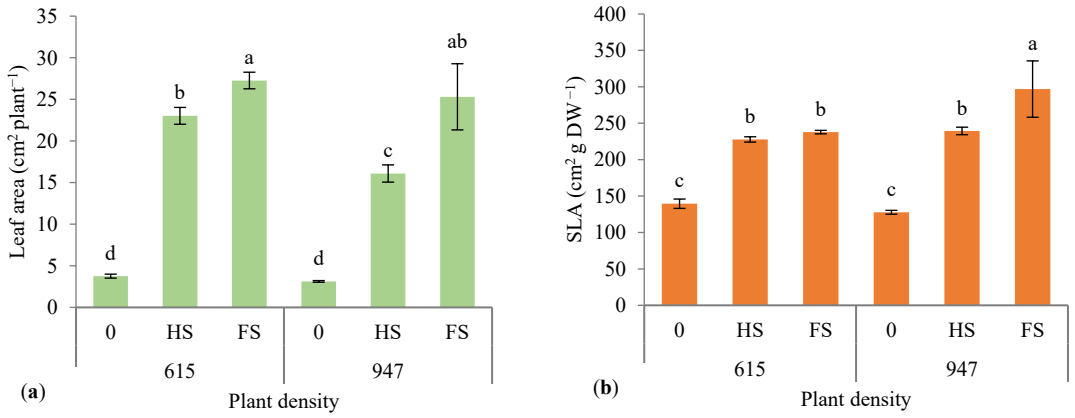


Figure 4. Effects of plant density (365, 497, and 615 plants m^{-2}) and nutrient solution (NS) concentration (0%, only water—0; 50%, half strength—HS; and 100%, full strength—FS) on (**a**) leaf area and (**b**) specific leaf area of *Tetragonia tetragonioides* baby plants grown during spring (black bars represent standard errors of the

means; bars of the same color with different letters are significantly different at $p \leq 0.05$ according to the LSD test).

Table 4. Effects of plant density (365, 497, and 615 plants m^{-2}) and nutrient solution (NS) concentration (0%, only water—0; 50%, half strength—HS; and 100%, full strength—FS) on leaf characteristics of *Tetragonia tetragonioides* baby plants grown during spring.

Source of Variance		Number of Leaves	Leaf Width	Leaf Area (cm^2 $Plant^{-1}$)	Leaf Area (cm^2 $Leaf^{-1}$)	SLA (cm^2 g DW^{-1})	L*	Chroma	Hue °
Plant Density									
615		[z] 5.2	22.2	18.0	3.2	201.7	45.3b	31.8b	125.3a
947		5.0	19.5	14.8	2.7	221.3	46.3a	33.6a	124.2b
Nutrient Solution (NS)									
0		3.9b	9.0	3.4	0.9	133.6	52.4a	42.2a	118.4b
HS		5.6a	24.3	19.6	3.5	233.6	42.3b	27.9b	127.6a
FS		5.9a	29.1	26.3	4.4	267.3	42.8b	28.0b	128.2a
Density x NS									
615	0	3.9	9.7d	3.8d	1.0d	139.5c	52.0	42.0	119.3
	HS	5.8	27.1b	23.0b	4.0b	227.7b	42.1	27.1	127.9
	FS	6.0	29.8a	27.3a	4.5a	237.8b	41.9	26.2	128.6
947	0	3.9	8.4d	3.1d	0.8d	127.7c	52.8	42.4	117.5
	HS	5.3	21.6c	16.1c	3.0c	239.4b	42.4	28.7	127.4
	FS	5.9	28.5ab	25.3ab	4.3ab	296.9a	43.8	29.8	127.7
Significance [x]									
Density		ns	***	***	***	***	**	**	**
NS		***	***	***	***	***	***	***	***
Density x NS		ns	***	**	**	***	ns	ns	ns

[z] Each value is the mean of four replicated samples of 30 plants each. For each factor, values in a column followed by the same letter are not significantly different, according to the LSD test. [x] Significance: ns = not significant; ** significant at $p < 0.01$; and *** significant at $p < 0.001$.

The two plant densities tested also showed a different trend for specific leaf area, which increased from 139.5 cm^2 g DW^{-1} (0 NS) to 232.8 cm^2 g DW^{-1} (on average for HS and FS NS) in the plants grown at 615 plants m^{-2} and reached a significantly higher SLA value using FS NS at 947 plants m^{-2} (296.9 cm^2 g DW^{-1}) (Table 4 and Figure 4b).

During spring, the leaf color was significantly affected by plant density with a lighter, more saturated, and yellowish color with 947 plants m^{-2}. Leaf color showed changes due to the nutrient solution concentration only when supplementing only water to the plants (Table 4).

The yield of baby plants was the lowest using 0% nutrient solution and did not significantly change with this NS as a function of plant density (292.8 g m^{-2} on average). These baby plants were undersized and with yellowish leaves and were not marketable as a result. The HS nutrient solutions determined a yield increase up to 1162.0 g m^{-2} on average for both plant densities, while FS NS

further increased the yield by 19% at 615 plants m^{-2} (1340.3 m^{-2}) and by 52.7% at 947 (1829.5 g m^{-2}) (Figure 5).

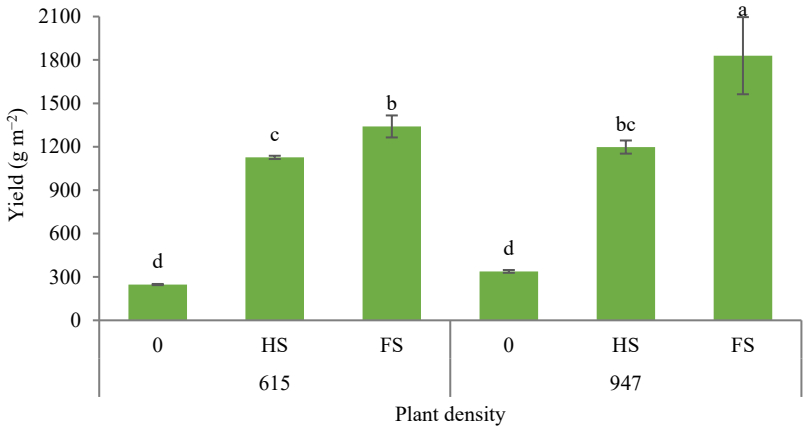


Figure 5. Effects of plant density (365, 497, and 615 plants m^{-2}) and nutrient solution (NS) concentration (0%, only water—0; 50%, half strength—HS; and 100%, full strength—FS) on the yield of *Tetragonia tetragonioides* baby plants during spring (black bars represent standard errors of the means; bars with different letters are significantly different at $p \leq 0.05$ according to the LSD test).

Water use efficiency (WUE) was mainly affected by the nutrient solution with significant increases at each increase in NS concentration (1.9 g DW L^{-1} H_2O, on average, with FS NS) (Figure 6a). The lowest nitrogen use efficiency (NUE) was recorded in the plants grown at both plant densities and supplemented with FS NS (10.8 g DW g^{-1} N on average). The NUE increased when using HS NS with 947 and 615 plants m^{-2} or 0 NS with 947 plants m^{-2} (15.2 g DW g^{-1} N, on average) but the highest NUE was recorded with 947 plants m^{-2} and zero NS (21.4 g DW g^{-1} N) (Figure 6b).

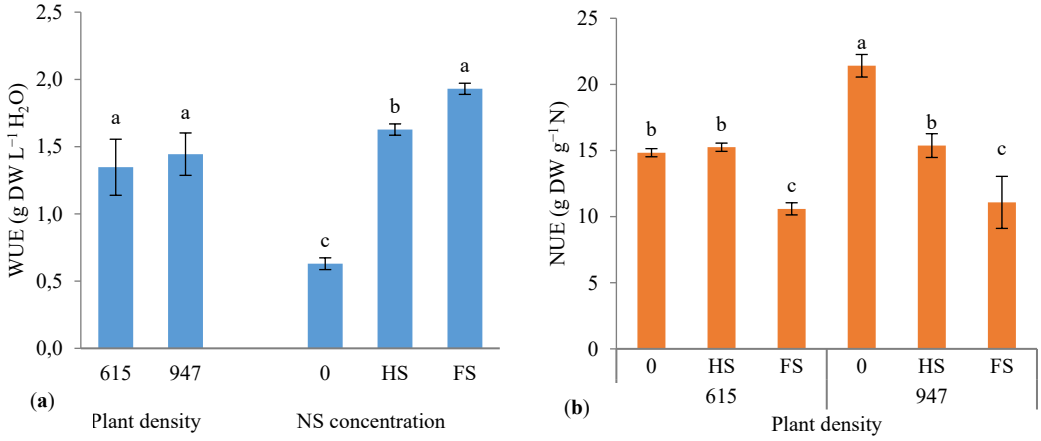


Figure 6. Effects of plant density (365, 497, and 615 plants m^{-2}) and nutrient solution (NS) concentration (0%, only water—0; 50%, half strength—HS; and 100%, full strength—FS) on (**a**) the water (WUE) and (**b**) nitrogen (NUE) use efficiency of *Tetragonia tetragonioides* baby plants during spring (black bars represent standard errors of the means; bars with different letters are significantly different at $p \leq 0.05$ according to the LSD test).

3.2. Biochemical characteristics of Tetragonia Tetragonioides baby plants

3.2.1. First growing period (Autumn–Winter)

At the end of the first cycle, the Tetragonia baby plants harvested were analyzed to determine their contents of proteins, fiber, total sugars, and minerals. The HS and FS NSs significantly boosted the protein content only when applied to 497 and 615 plants m^{-2} (+77% on average compared to 365 plants m^{-2} using HS and FS NS) (Figure 7). The fiber content increased with increasing plant density or NS concentration (Figure 7). The total sugar content slightly but not significantly changed when increasing the NS concentration with 365 and 615 plants m^{-2}, while with the intermediate plant density the HS NS determined the highest sugar content (0.43 g 100 g^{-1}), which did not statistically differ from the values recorded with HS and FS NSs at 615 plants m^{-2} (0.39 g 100 g^{-1} on average) (Figure 7).

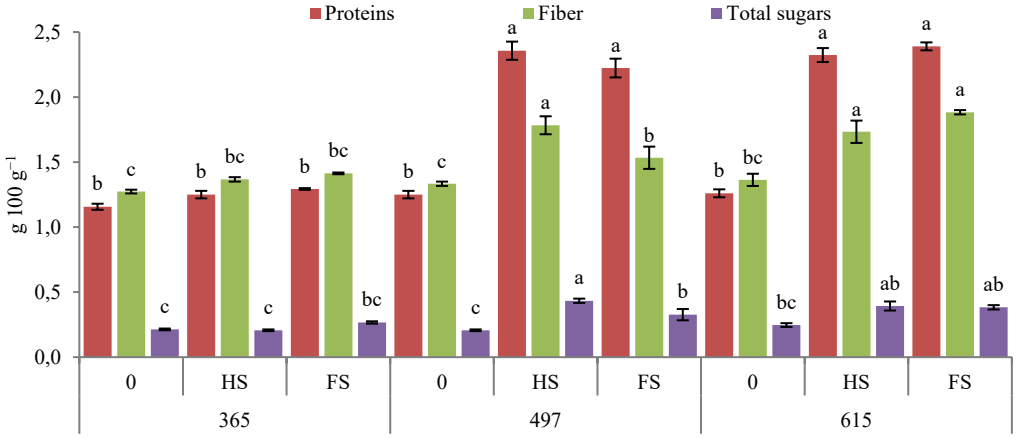


Figure 7. Effects of plant density (365, 497, and 615 plants m^{-2}) and nutrient solution (NS) concentration (0%, only water—0; 50%—HS; and 100%—FS) on protein, fiber, and total sugar content of *Tetragonia tetragonioides* baby plants grown during autumn–winter (black bars represent standard errors of the means; bars of the same color with different letters are significantly different at $p < 0.05$ according to the LSD test).

The content of K, Ca, and Mg was higher when increasing plant density or NS concentration. The Na content increased with the highest NS concentration at the lowest plant density and with both HS and FS NSs when using the other plant densities (Table 5). The P content was significantly higher using FS NS with the two higher densities (39.2 mg 100 g^{-1} on average), while the Zn content was highest in the plants grown at 365 plants m^{-2} with FS NS (2.8 mg 100 g^{-1}) and reached intermediate values with FS NS at 497 plants m^{-2} and all of the NSs at 615 plants m^{-2} (Table 5).

Table 5. Effects of plant density (365, 497, and 615 plants m^{-2}) and nutrient solution (NS) concentration (0%, only water—0; 50%—HS; and 100%—FS) on mineral element and oxalic acid content of *Tetragonia tetragonioides* baby plants grown during autumn–winter.

Source of Variance		K (mg 100 g^{-1})	Na (mg 100 g^{-1})	Ca (mg 100 g^{-1})	Mg (mg 100 g^{-1})	P (mg 100 g^{-1})	Fe (mg 100 g^{-1})	Cu (mg 100 g^{-1})	Mn (mg 100 g^{-1})	Zn (mg 100 g^{-1})	Oxalic Acid (mg 100 g^{-1})
Plant Density											
365		[z] 98.9b	89.9	41.8b	29.6b	21.2	2.9	0.15b	0.31	1.8	844.7b
497		111.5a	99.0	50.6ab	38.8a	28.4	2.8	0.16ab	0.41	1.6	933.0a
615		117.3a	99.6	60.5a	45.9a	29.1	2.7	0.24a	0.38	2.1	958.2a
NS											
0		94.6c	88.7	41.3b	29.5b	20.7	2.8	0.14b	0.29	1.6	963.8a
HS		109.2b	98.1	50.4ab	40.2a	24.1	2.8	0.17ab	0.41	1.6	918.8ab
FS		123.8a	101.7	61.3a	44.6a	33.8	2.8	0.24a	0.40	2.2	853.3b
Density x NS											
365	0	89.1	85.2c	39.2	28.7	19.7b	2.6	0.10	0.19	1.3b	872.6
	HS	93.3	87.0c	40.5	29.4	20.6b	2.8	0.13	0.33	1.4b	888.0
	FS	114.2	97.4b	45.8	30.7	23.2b	3.1	0.22	0.40	2.8a	773.4
497	0	93.3	87.0c	40.5	29.4	20.6b	2.8	0.13	0.33	1.4b	980.4
	HS	113.0	101.7a	52.7	41.3	26.3b	2.9	0.19	0.50	1.7b	940.1
	FS	128.3	108.3a	58.7	45.7	38.3a	2.7	0.17	0.41	1.8ab	878.5
615	0	101.5	93.8bc	44.2	30.5	21.9b	3.1	0.21	0.34	2.2ab	1038.5
	HS	121.3	105.7a	58.0	50.0	25.3b	2.5	0.18	0.41	1.8ab	928.2
	FS	129.0	99.3a	79.3	57.3	40.0a	2.7	0.34	0.39	2.2ab	907.9
Significance [x]											
Density		**	***	**	***	***	ns	*	ns	ns	**
NS		***	***	**	**	***	ns	*	ns	**	**
Density x NS		ns	**	ns	ns	*	ns	ns	ns	*	ns

[z] Each value is the mean of four replicated samples of 30 plants each. For each factor, values in a column followed by the same letter are not significantly different, according to the LSD test. [x] Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; and *** significant at $p < 0.001$.

The vitamin A content was significantly affected by HS NS with 497 plants m^{-2} (2368 µg 100 g^{-1}) and increased with 615 plants m^{-2} as the NS concentration increased (2373.5 µg 100 g^{-1} on average) (Figure 8a). The vitamin C content was significantly increased using the HS NS with 497 plants m^{-2} (22 mg 100 g^{-1}) (Figure 8a). The content of vitamins B1, B2, and B3 was slightly higher when increasing plant density and NS concentrations (Figure 8b). A significantly higher content of vitamin E was recorded in the plants grown at 615 plants m^{-2} supplied with HS and FS nutrient solutions or at 497 plants m^{-2} using only FS NS (1.2 mg 100 g^{-1} on average) (Figure 8c).

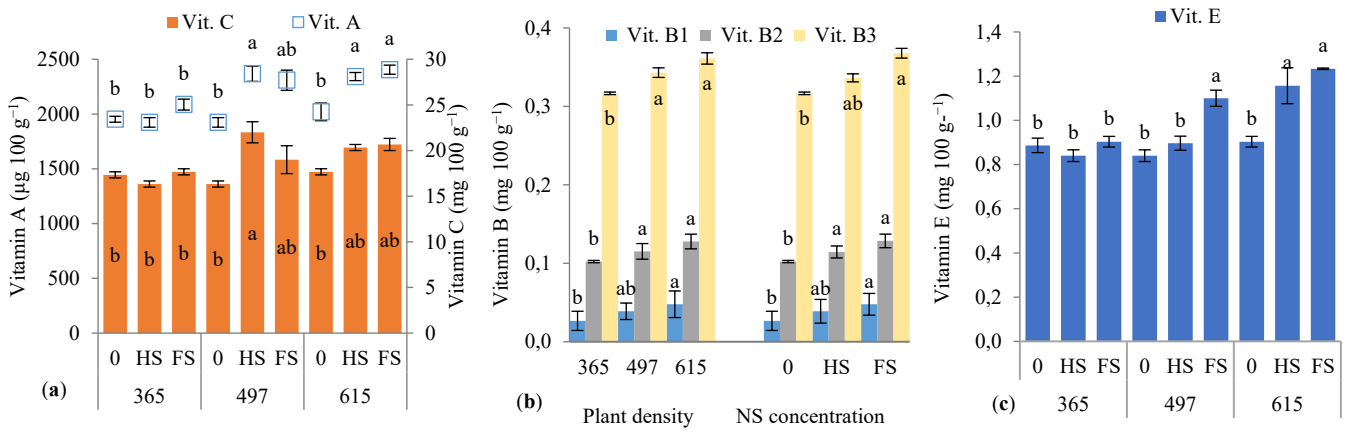


Figure 8. Effects of plant density (365, 497, and 615 plants m^{-2}) and nutrient solution (NS) concentration (0%, only water—0; 50%—HS; and 100%—FS) on (**a**) vitamin A, C, (**b**) B, and (**c**) E content of *Tetragonia tetragonioides* baby plants grown during autumn–winter (black bars represent standard errors of the means; points or bars of the same color with different letters are significantly different at $p < 0.05$ according to the LSD test).

The oxalic acid content (Table 5) was significantly reduced by the lowest plant density or increasing NS concentration. The lowest plant density showed a reduction by 10.7% on average compared to 497 and 615 plants m^{-2} (945.6 mg 100 g^{-1}, on average); the plants grown with 0 NS had an oxalic acid content of 963.8 mg 100 g^{-1}, which significantly dropped by 11.5% using FS NS (853.3 mg 100 g^{-1}).

3.2.2. Second growing period (Spring)

After the second growing period the content of K, Na, Ca, Mg, Fe, and Cu was lowered by the increase in plant density up to 947 plants m^{-2} but was highest with the highest NS concentration (Table 6). The content of P and Zn was significantly higher using FS NS with both plant densities (43.0 mg 100 g^{-1} and 1.92 mg 100 g^{-1}, on average, respectively), but using 947 plants m^{-2}, it was significantly lower with 0 and HS NS than 615 plants m^{-2}. Mn content was affected only by the nutrient solution concentration and recorded the lowest value in the plants supplemented with 0 NS.

Table 6. Effects of plant density (365, 497, and 615 plants m^{-2}) and nutrient solution (NS) concentration (0%, only water—0; 50%—HS; and 100%—FS) on mineral element and oxalic acid content of *Tetragonia tetragonioides* baby plants grown during spring.

Source of Variance	K (mg 100 g^{-1})	Na (mg 100 g^{-1})	Ca (mg 100 g^{-1})	Mg (mg 100 g^{-1})	P (mg 100 g^{-1})	Fe (mg 100 g^{-1})	Cu (mg 100 g^{-1})	Mn (mg 100 g^{-1})	Zn (mg 100 g^{-1})	Oxalic Acid (mg 100 g^{-1})
Plant Density										
616	[z] 130.5 a	111.3 a	65.1	40.4 a	33.7	4.9 a	0.26 a	0.42	1.6	1008.5 b
947	115.7 b	101.8 b	58.7	34.1 b	31.3	3.8 b	0.20 b	0.41	1.4	894.5 a
NS										

	0	120.3 b	98.6 b	41.3 b	31.8 b	25.6	3.7 b	0.2 b	0.3 b	1.3	1011.5 a
	HS	119.4 b	104.6 b	50.0 ab	31.3 b	29.0	4.4 ab	0.2 ab	0.4 a	1.3	957.1 ab
	FS	129.6 a	116.5 a	94.5 a	48.8 a	43.0	4.9 a	0.3 a	0.5 a	1.9	885.9 b
Density x NS											
615	0	128.9	104.9	46.4	35.5	27.2 bc	4.5	0.20	0.34	1.49 b	1086.5
	HS	128.4	108.0	51.5	34.5	32.1 b	4.9	0.26	0.46	1.50 b	997.2
	FS	134.2	120.9	97.5	51.3	41.8 a	5.3	0.32	0.47	1.90 a	941.9
947	0	111.7	92.2	36.1	28.2	23.9 c	2.8	0.14	0.31	1.14 c	936.5
	HS	110.3	101.1	48.5	28.0	25.9 c	3.9	0.19	0.43	1.17 c	917.1
	FS	125.1	112.0	91.5	46.2	44.2 a	4.6	0.25	0.49	1.95 a	829.9
Significance [x]											
Density		***	**	ns	***	*	*	*	ns	ns	**
NS		*	***	***	***	***	*	**	***	**	**
Density x NS		ns	ns	ns	ns	**	ns	ns	ns	*	ns

[z] Each value is the mean of four replicated samples of 30 plants each. For each factor, values in a column followed by the same letter are not significantly different, according to the LSD test. [x] Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; and *** significant at $p < 0.001$.

The oxalic acid content (Table 6) dropped significantly by increasing plant density up to 947 plants m^{-2} (894.5 mg 100 g^{-1}, on average) or increasing NS rate (885.9 mg 100 g^{-1}, on average with FS NS).

3.3. Storage of minimally processed baby plants

3.3.1. First growing period (Autumn–Winter)

After harvesting, the minimally processed baby plants fed with FS NS were stored for 21 days at 4 °C to assess their shelf life and post-harvest quality changes (Table 7).

Table 7. Effects of plant density (365, 497, and 615 plants m^{-2}) and storage (21 days at 4 °C) on minimally processed *Tetragonia tetragonioides* baby plants grown during autumn–winter.

Plant Density (Plant m^{-2})	Storage (d at 4 °C)	Weight Loss (g 100 g^{-1} FW)	L *	Chroma	Hue°	ΔE
365		2.86a	44.49	34.77	123.79	5.70
497		2.23b	45.40	35.68	124.27	5.25
615		3.59b	46.26	37.76	123.14	6.80
	0		44.10	34.91	124.82a	
	7	1.66b	44.68	35.91	124.36a	4.64b
	14	3.02ab	44.81	35.22	124.06a	4.83b
	21	3.99a	47.93	38.23	121.70b	8.27a
365	0		42.87c	33.05b	124.08	
	7	1.69	44.65bc	36.15b	125.12	5.22
	14	3.14	44.74bc	34.75b	124.55	4.96

	21	3.74	45.69bc	35.14b	121.41	6.91
497	0		45.79bc	36.64ab	125.39	
	10	1.40	43.48c	33.60b	124.22	4.41
	7	1.97	44.22bc	33.75b	124.13	4.09
	21	3.31	48.10ab	38.72ab	123.36	7.25
615	0		43.65c	35.04b	125.01	
	7	1.88	45.92b	37.98ab	123.74	4.29
	14	3.96	45.46bc	37.17ab	123.50	5.44
	21	4.92	50.01a	40.85a	120.33	10.66
Plant Density		*	*	***	ns	ns
Storage		***	***	**	***	***
Plant Density x Storage		ns	*	*	ns	ns

[z] Each value is the mean of three replicated samples of 50 g each. For each factor, values in a column followed by the same letter are not significantly different, according to the LSD test. [x] Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; and *** significant at $p < 0.001$.

The weight loss linearly increased during cold storage and was 3.99%, on average, after 21 days. Plant density also affected this parameter with an increase in weight loss with increasing plant density (from 2.23% to 3.59%, on average, for 365 and 615 plants m^{-2}, respectively). Leaf color was variously affected by plant density and storage time. The baby plants grown at 365 plants m^{-2} recorded no significant change in color lightness (L*), whereas with increasing plant density, baby plants showed an increase in leaf lightness especially at the end of storage. The color saturation (chroma) recorded a significant increase at the end of the cold storage only in the baby plants grown at the highest plant density of 615 plants m^{-2}. The hue angle tended to drop after 21 days of cold storage. The color changes were also evaluated by calculating the color difference (ΔE), which was influenced mainly by the storage time. The changes in color recorded after 7 days (4.64) increased significantly only at the end of storage (8.27).

The scores for overall quality assessed by an informal panel dropped continuously during storage. The quality loss was more severe with increasing plant density, resulting in the loss of marketability between 14 and 21 days of cold storage (Figure 9).

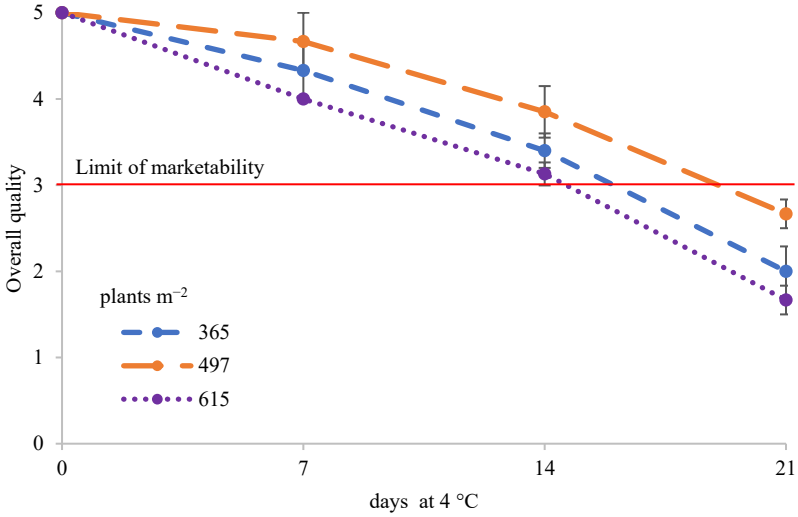


Figure 9. Influence of plant density and storage at 4 °C on the overall quality of minimally processed *Tetragonia tetragonioides* baby plants grown during autumn–winter (1: unmarketable; 3: average—limit of marketability; and 5: excellent or having a fresh appearance) (black bars represent standard errors of the means).

3.3.2. Second growing period (Spring)

After the second growing period, even the baby plants fed with HS NS were minimally processed and stored for 21 days at 4 °C.

The weight loss increased throughout the storage up to 3.78% on average after 21 days (Table 8). A higher weight loss was recorded in the baby plants supplemented with FS NS compared with HS NS and with increasing plant density. The color parameters L* and chroma did not record changes during storage, but the leaves had a lighter color in the plants grown at 947 plants m^{-2} or fed with FS NS and a more saturated color in the plants grown at the highest density (Table 8). The hue angle was higher when using the FS NS or 615 plants m^{-2} but decreased during storage from 127.9 at the beginning of cold storage to 126.7 at day 14, with no further change until the end of the storage period. The color variations expressed as ΔE showed moderate changes mainly due to the time of storage with a value of about three after 14 and 21 days of storage (Table 8).

Table 8. Effects of plant density (615 and 947 plants m^{-2}), nutrient solution (NS) concentration (50%—HS and 100%—FS), and storage (21 days at 4 °C) on minimally processed *Tetragonia tetragonioides* baby plants grown during spring.

Source of Variance			Weight Loss (%)	L *	Chroma	Hue°	ΔE
Days at 4 °C							
0				42.55	27.96	127.91a	
7			[z] 0.75c	42.96	28.25	127.11b	2.27b
14			2.38b	42.64	28.89	126.65c	2.96a
21			3.78a	42.96	28.14	126.65c	3.07a
Plant Density							
615			2.14b	42.14b	27.25b	127.37a	2.63
947			2.46a	43.41a	29.37a	126.79b	2.91
Nutrient Solution (NS)							
HS			2.12b	42.40b	28.26	126.78b	2.66
FS			2.48a	43.15a	28.35	127.37a	2.88
Day x Density x NS							
0	615	HS		42.08	27.07	127.94	
		FS		41.91	26.21	128.62	
	947	HS		42.43	28.74	127.36	
		FS		43.79	29.80	127.70	
7	615	HS	0.70	41.95	27.20	126.99	2.25
		FS	0.86	42.62	27.05	127.65	2.23
	947	HS	0.67	43.36	29.36	126.52	2.50
		FS	0.76	43.90	29.39	127.26	2.13
14	615	HS	2.01	41.08	27.19	126.70	2.49
		FS	2.50	42.37	27.59	127.31	2.76
	947	HS	2.38	42.84	30.21	126.13	3.16
		FS	2.64	44.27	30.57	126.45	3.45
21	615	HS	3.19	42.15	27.43	126.76	2.42
		FS	3.59	42.98	28.28	126.97	3.64
	947	HS	3.78	43.33	28.92	125.85	3.13
		FS	4.55	43.37	27.94	127.01	3.33
Significance [x]							
Days			***	ns	ns	***	*
Density			*	***	***	***	ns
NS			*	**	ns	***	ns
Day * Density			ns	ns	ns	ns	ns
Day * NS			ns	ns	ns	ns	ns
Density * NS			ns	ns	ns	ns	ns
Day * Density * NS			ns	ns	ns	ns	ns

[z] Each value is the mean of three replicated samples of 50 g each. For each factor, values in a column followed by the same letter are not significantly different, according to the LSD test. [x] Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; and *** significant at $p < 0.001$.

The scores for the overall quality dropped more rapidly during cold storage in the baby plants fed with HS NS and approached the limit of marketability after 2 weeks of storage, whereas the plants fed with FS NS maintained a good overall quality for the first two weeks and were near but over the limit of marketability after 21 days (Figure 10).

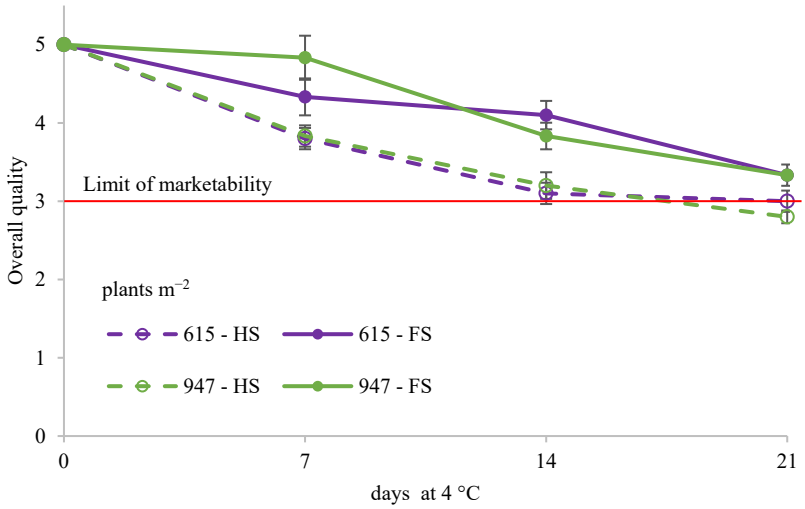


Figure 10. Influence of plant density (615 and 947 plants m^{-2}), nutrient solution (NS) concentration (50%—HS and 100%—FS), and storage at 4 °C on the overall quality of minimally processed *Tetragonia tetragonioides* baby plants grown during spring (1: unmarketable; 3: average—limit of marketability; and 5: excellent or having a fresh appearance) (black bars represent standard errors of the means).

3.4. Principal component analysis

The principal component analysis identified four principal components (PCs) with eigenvalues exceeding 1.00 (Table 9). These four PCs accounted for 91.42% of the total variance, with individual contributions of 62.98%, 17.79%, 6.54%, and 4.12%, respectively. This allowed the initial 33 variables to be effectively summarized by the four PCs.

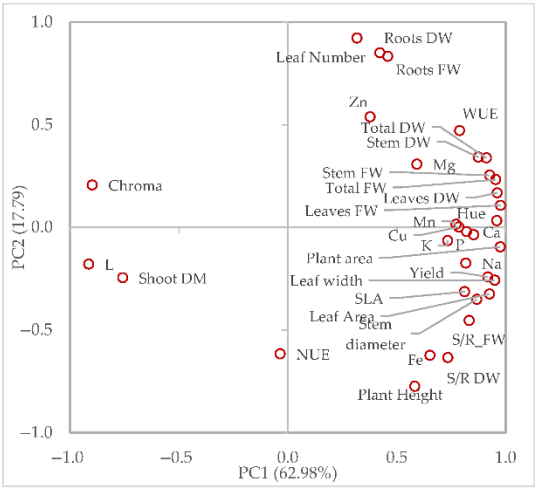
Table 9. Correlation of variables to the factors of the principal components analysis (PCA) based on the morpho-physiological parameters, yield, and mineral content of Tetragonia baby plants.

Variable	PC1	PC2	PC3	PC4
Plant height	0.583	**−0.774**	0.051	0.123
Stem diameter	**0.868**	−0.350	0.022	0.222
Total FW	**0.955**	0.233	−0.124	0.078
Roots FW	0.459	**0.833**	0.014	0.270
Stem FW	**0.927**	0.254	−0.121	0.028
Leaves FW	**0.977**	0.107	−0.140	0.048
S/R_FW	**0.833**	−0.453	−0.207	−0.156
Total DW	**0.912**	0.338	−0.056	0.193
Roots DW	0.423	**0.850**	−0.028	0.259
Stem DW	**0.875**	0.342	0.013	0.178
Leaves DW	**0.961**	0.168	−0.072	0.160

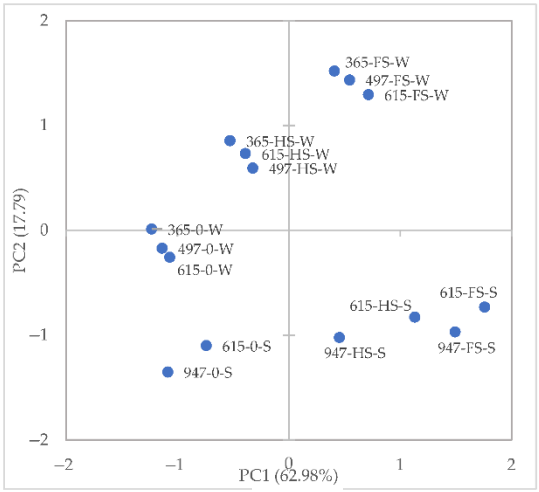
S/R DW	**0.735**	−0.634	−0.054	−0.010
Shoot DM	**−0.758**	−0.245	0.492	0.281
WUE	**0.789**	0.471	0.170	0.098
NUE	−0.035	**−0.616**	0.425	0.528
Leaf number	0.318	**0.922**	−0.091	−0.029
Leaf width	**0.950**	−0.257	−0.159	−0.008
Plant area	**0.976**	−0.095	−0.172	−0.013
Leaf area	**0.926**	−0.325	−0.178	−0.016
SLA	**0.812**	−0.313	−0.343	−0.256
L *	**−0.914**	−0.179	0.116	−0.199
Chroma	**−0.898**	0.206	0.193	−0.082
Hue	**0.960**	0.033	−0.124	0.148
Yield	**0.919**	−0.241	−0.089	−0.084
K	**0.734**	−0.064	**0.617**	0.124
Na	**0.817**	−0.174	0.387	−0.062
Ca	**0.821**	−0.021	0.280	−0.413
Mg	**0.593**	0.308	**0.596**	−0.288
P	**0.853**	−0.036	0.370	−0.118
Fe	**0.653**	**−0.623**	−0.018	−0.026
Cu	**0.785**	0.002	0.384	−0.211
Mn	**0.773**	0.015	0.197	0.010
Zn	0.378	**0.538**	0.162	−0.431

Values in bold within the same factor indicate the variable with the largest correlation.

PC1 was primarily associated with stem diameter; total, stem, and leaf fresh weight; shoot/root fresh weight; total, stem, and leaf dry weight; shoot/root dry weight; shoot dry matter; water use efficiency (WUE); leaf width; plant and leaf area; specific leaf area (SLA); leaf length (L); chroma; hue; yield; and all mineral elements except Zn. PC2 correlated with root fresh and dry weight, nutrient use efficiency (NUE), leaf number, Fe, and Zn. PC3 was related to K and Mg (Table 9). These relationships are visualized by the projection of the original variables onto the plane of the first two PCs in the loadings plot (Figure 11a). The discrimination among plant densities, nutrient solutions, and cultivation seasons is presented in the scores plot (Figure 11b), where the scores for each treatment are clearly separated into distinct clusters. Plants supplied only with water had scores located in the third quadrant. The winter harvest showed a stronger positive relationship with PC2 than the spring harvest and increasing nutrient solution concentration enhanced both PC1 and PC2 values. During spring, the differences among treatments were less pronounced, with all of their values located in the second quadrant. However, lower plant density and the full-strength nutrient solution (FS NS) appeared more related to PC1.



(**a**)



(**b**)

Figure 11. Plot of (**a**) loadings (morpho-physiological parameters, yield, and mineral content of Tetragonia baby plants) and (**b**) scores (trials) formed by the two principal components from the principal component analysis (PCA). Plant densities (365, 497, 615, and 947 plants m^{-2}); nutrient solutions (0; HS, half strength; and FS, full strength); and cultivation seasons (W, autumn–winter and S, spring).

4. Discussion

Existing literature on New Zealand spinach cultivation predominantly focuses on the utilization of mature leaves as the usable component. In contrast, the present research investigates for the first time the feasibility of employing whole baby plants (including stem and leaves) as raw material for ready-to-eat vegetable production. The growing interest in hydroponic systems to cultivate leafy vegetables for ready-to-eat leafy salads necessitates specific studies on agronomic techniques and nutrient management for new or underutilized vegetables such as New Zealand spinach (Manzocco et al., 2011).

In its zone of origin, Tetragonia plants can establish at any time of the year but they prefer warm and frost-free sites (Roskruge, 2011). The growing conditions (temperature, humidity, and light) during our trial satisfied Tetragonia's climatic needs well. However, during the second cultivation cycle

(spring), warmer temperatures and longer daylight length led to a higher growth rate, resulting in more vigorous and faster plant growth compared to the first growing cycle (winter). The first and second growing cycles (from sowing to harvest) lasted 55 and 36 days, respectively. Cultivation conditions can affect the crop duration of leafy vegetables, and a similar timeframe was also observed for *Valerianella locusta* baby plant production (Fabek et al., 2009). The climatic conditions during spring increased also the yield of baby plants as found comparing the yield recorded in the two growing seasons using 615 plant m^{-2}. At this plant density, and using the full-strength (FS) nutrient solution, the spring yield increased by 45.3% compared to the winter yield, with an increase in both fresh and dry biomass. This could be related to the higher solar radiation resulting from the high level of natural light and long photoperiod that increased photosynthesis in the spring season with respect to the winter season (Cockshull et al., 1992).

In both periods, the rise of plant density determined a significant increase in baby plant yield that was highest when the highest plant density was adopted (615 plant m^{-2} in autumn–winter and 947 plant m^{-2} in spring). This occurred despite the negative effect that a lower plant spacing had on various yield attributes such as the plant height, stem diameter, fresh and dry weight of baby plants, and leaf area. A similar plant density range (412 and 824 plants m^{-2}) was tested on other wild edible leafy plants to evaluate their domestication using a hydroponic cultivation system, yielding similar results in terms of yield and plant characteristics (Anaclerio et al., 2021). An increase in plant density was found to decrease plant height, stem and canopy width, leaf number, leaf area, and plant fresh and dry mass per plant in lettuce plants grown hydroponically (Maboko and Du Plooy, 2009). The plants of Tetragonia showed a very satisfying yield during spring, especially at the highest plant density (1.8 kg m^{-2}), that was consistent with the yield of lamb's lettuce grown hydroponically for baby plant production (Manzocco et al., 2011). Optimizing plant density is a critical factor in the hydroponic cultivation of leafy vegetables, significantly influencing both plant morphology and physiology. Achieving the ideal density maximizes yield while ensuring optimal individual plant quality and resource efficiency. Higher plant densities generally lead to a greater fresh and dry weight yield per unit area due to more plants occupying the space, as found for mustard spinach, lettuce, and basil (Jadhav et al., 2025; Maboko, 2013). However, this often comes at the expense of individual plant size, characterized by reduced fresh and dry weight per plant, shorter plant height, narrower stems, and smaller individual leaf area, as also found in our trials for Tetragonia baby plants. They showed a drop of these parameters of about 14% on average in the first trial (autumn–winter) and 20% in the second (spring). This is primarily due to intensified inter-plant competition for resources such as light and nutrients (Maboko, 2013). Leaf morphology can be also significantly affected by plant density. In dense plantings, plants often adapt by developing thinner, more elongated leaves and stems in search of light. This led to an increase in specific leaf area (SLA) in lettuce plants (Van Brenk et al.,

2024) as observed in our experiment. Plant density ranging from 365 to 615 plants m^{-2} during the winter season did not change SLA, but when increasing it from 615 up to 947 plants m^{-2} in the spring trial, SLA increased by up to 24.9% in the plants supplied with FS NS. The increase in SLA indicates a reduction in leaf thickness and potentially nutrient content per unit area, impacting overall nutritional quality.

The use of cell trays as a plant support in our ebb and flow hydroponic system resulted in a reduction in the substrate volume per plant due to increased plant density. Thus, together with limitations of space and light for the epigeal parts, plants also suffered a reduction in space for roots and a smaller stock of water and nutrients. The volume of the substrate supporting the plants in hydroponic systems plays a significant role in influencing various aspects of plant development, physiology, biomass accumulation, and leaf morphology (Hutchinson et al., 2025). A larger or more appropriate substrate volume can lead to greater fresh and dry biomass production. In contrast, limiting substrate volume or using unsuitable substrates can restrict root growth and, consequently, reduce overall fresh and dry plant mass as found in hydroponically grown arugula and lettuce plants (Ferrarezi et al., 2024; Zappelini et al., 2024). It is important to consider that while a larger substrate volume generally offers more space for root growth, the efficiency of nutrient delivery remains paramount. Reducing substrate volume in hydroponic systems can significantly lower both operational costs and environmental impact. Studies show that optimizing irrigation can reduce the amount of substrate needed without compromising plant growth or yield. This could lead to substantial savings in fertilizer costs (up to 61%) and a marked decrease in nutrient-rich effluent that can pollute the environment (Choi et al., 2015, 2016). The interaction between the growing medium and the nutrient solution, governed by factors such as porosity, water-holding capacity, and exchange capacity, is fundamental to the efficacy of the growing environment for leafy vegetables (Chhetri et al., 2022).

Plant growth and dry biomass accumulation is linked to both light interception and photosynthesis and nutrient availability. In hydroponic systems, nutrients are almost fully supplied by the nutrient solution. Thus, the optimization of nutrient solution concentration is a critical factor in hydroponic cultivation, directly influencing the growth, biomass accumulation, and morphological and biochemical characteristics of leafy vegetables. Hydroponic systems offer precise control over nutrient delivery, allowing for tailored approaches to maximize crop productivity and quality (Hazrati et al., 2024). However, determining the optimal concentration is complex, as it is highly dependent on the specific plant species, developmental stage, and environmental conditions such as plant space and access to the light (Cockshull et al., 1992). The concentration of the nutrient solution significantly impacts various growth parameters, including plant height, leaf number, and leaf area, as also observed in our experiments. While some studies suggest that within certain ranges, growth may be less affected by nutrient concentration than by environmental factors like growing season, others

demonstrate clear responses. As expected, the nutrients supplied only with the substrate in the control treatment with 0% NS were insufficient to allow correct plant growth. This resulted in unmarketable baby plants (undersized and with yellowish leaf color) especially with the reduced substrate volume at higher densities. There was an evident increasing tendency in growth and yield parameters (shoot fresh and dry biomass accumulation, leaf area, leaf number, and WUE) when increasing nutrient solution concentration up to full strength. The yield increases in Tetragonia due to the increasing NS concentration fit a linear model, although the response of most leafy vegetables to increasing nutrient concentrations in the nutrient solution generally fits a quadratic polynomial model (Yang et al., 2021). Our results indicate that the full-strength nutrient solution we used fulfilled the nutrient requirements of Tetragonia baby plants without determining nutrient imbalances or osmotic stress that could occur at higher concentrations. Various studies have proposed different optimal nitrogen ranges for hydroponically grown leafy vegetables. For instance, research on lettuce or arugula often suggests optimal N concentrations between 100 and 150 mg L^{-1} for maximizing fresh weight (Djidonou and Leskovar, 2019; Murphy and Pill, 2010). For more robust leafy greens like kale, slightly higher concentrations, possibly up to 180–200 mg L^{-1}, might be beneficial for fresh biomass yield (Maludin et al., 2020). This agrees with our results, as we found the best growth and yield response at 215 mg L^{-1} N with an electrical conductivity (EC) of the FS nutrient solution of about 2.5 $dS \cdot m^{-1}$. The EC is a measure of total ion content in the nutrient solution. Leafy vegetables, such as arugula, generally suffer with NSs with EC higher than 1.8–2.0 $dS \cdot m^{-1}$ (Yang et al., 2021); however, a salinity tolerance threshold up to an EC of 18 dS m^{-1} has been assessed for Tetragonia, which is a halophyte species (Atzori et al., 2020).

Tailoring nutrient solution concentrations can not only optimize biomass but also influence the mineral element content and phytochemical profiles of plant tissues. Higher nutrient solution concentrations increased the levels of macronutrients like nitrogen, phosphorus, potassium, and magnesium in lettuce, but also led to higher nitrate content and a slight reduction in some nutritional parameters such as carbohydrates and proteins (Fallovo et al., 2009). The higher nutrient supply determined an increase in the content of mineral elements in the baby plants of Tetragonia in both cultivation seasons without great variations due to the environmental effect. The values of mineral and vitamin content partially agree with those reported by Jaworska, and Kmiecik (Jaworska and Kmiecik, 1999) and provided by the U.S. Department of agriculture (USDA (U.S. Department of Agriculture), n.d.). A serving of 100 g of baby plants of Tetragonia produced in our trial with FS NS can, on average, satisfy the recommended daily values (DV) of β-carotene and can contribute to the intake of vitamin C (21% DV), iron (15.8% DV), Cu (23.6% DV), Mn (20.3% DV), and Zn (20.4% DV) (FDA U.S. Food and Drug Administration, 2024).

The response of plants to hydroponic cultivation may vary greatly according to species or even to varieties, to agronomic factors like spacing and nutrient solution compositions, or to environmental factors like temperature, light intensity, and duration (Sharma et al., 2018). The effects of the experimental factors (season, plant density, and nutrient solution concentration) were shown well by the PCA that summarized the different responses of New Zealand spinach to the treatments. The PCA showed that increasing plant density and nutrient solution concentration differentially affected Tetragonia baby plants depending on the cultivation season, particularly during winter and at lower plant densities.

In this work, we hypothesized that baby plants of New Zealand spinach could be a viable ready-to-eat product. Consumers' need to vary their vegetable consumption and the increasing demand for convenience vegetables could promote the spread of this species in the ready-to-eat (RTE) market. To achieve this goal, the leafy vegetable would require extended shelf life and quality maintenance (Kader, 2002; Siddiqui, 2015).

Pre-harvest environmental conditions and agronomic practices can influence product characteristics and post-harvest physiology (Miceli et al., 2021, 2019b). To investigate the effects of hydroponic agronomic management on Tetragonia baby plant shelf life, plants were minimally processed and cold-stored as a ready-to-eat (RTE) product for 21 days immediately after harvest. Minimally processed leafy vegetables are highly perishable and prone to rapid quality degradation during storage, leading to a loss of commercial value (Miceli et al., 2021; Miceli and Miceli, 2014). At harvest, vegetables are separated from the plant, severing their supply of water and nutrients. This can lead to physiological disorders, which may be exacerbated by pre-harvest environmental conditions or mineral imbalances during growth.

Deterioration during vegetable storage is often significantly influenced by weight loss, which directly affects product appearance and quality, especially in minimally processed products. This weight loss is primarily attributed to water loss through transpiration or evaporation, along with the degradation of carbohydrate reserves via respiration (Ayala-Zavala et al., 2008; Roura et al., 2000). A linear increase in weight loss was observed in Tetragonia baby plants throughout the storage period. This loss was more pronounced in plants harvested during winter compared to spring. It was also shown for lettuce and baby-leaf rocket and spinach that the shelf-life attribute can vary greatly depending on the harvesting season (Koukounaras et al., 2020). Across both harvesting seasons, elevated plant density correlated with increased weight loss. Furthermore, in the spring trial, a higher nutrient supply (comparing half-strength and full-strength nutrient solutions) resulted in increased weight loss. Weight losses exceeding 4–6% can compromise the marketability of leafy vegetables (Robinson et al., 1975). In our trials, the baby plants did not overcome this threshold except after 21 days of cold storage in the baby plant grown at higher density and supplied with the full-strength nutrient solution.

The density at which plants are cultivated in hydroponic systems can significantly affect their growth and morphology, which, in turn, may influence their post-harvest water retention. The direct influence of plant density on moisture loss in cold stored leafy vegetables is not well documented yet. Higher plant densities can lead to increased competition for light, nutrients, and space, potentially resulting in smaller, more succulent leaves with a lower thickness. Yang et al. (Yang et al., 2024) have related a lower water loss rate with the higher thickness of lettuce leaves. Leaf thickness and SLA are closely related leaf traits in leafy vegetables: as leaf thickness increases, specific leaf area decreases (Vile et al., 2005). In our experiments, especially during the spring season, an increase in the specific leaf area (SLA) occurred when plants had lower space.

The concentration of the nutrient solution is another critical pre-harvest factor in hydroponic cultivation that can affect the post-harvest quality of leafy vegetables. Studies show that while higher nutrient concentrations can boost yield and increase certain mineral contents, they may also reduce levels of carbohydrates and proteins, and can negatively impact post-harvest storability, such as increasing weight loss and reducing the shelf life of leaf lettuce (Lee et al., 2022; Lee and Chang, 2017). Additionally, the effects of nutrient concentration can vary by season, genotype, and specific quality parameters such as texture, color, and vitamin content as found in fresh-cut red and green lettuces (Luna et al., 2013). The appearance and color of minimally processed leafy vegetables are critical determinants of consumer perception and choice, influencing overall satisfaction and perceived sensory quality. Both pre-harvest (Miceli et al., 2020; Miceli and Miceli, 2014; Moncada et al., 2021) and post-harvest factors (Alfonzo et al., 2018; Miceli et al., 2019a) significantly influence these crucial visual characteristics and their post-harvest changes. During storage, winter baby plants experienced greater color changes (higher ΔE), irrespective of plant density. For spring baby plants, a lighter color was observed with increased plant density and nutrient solution concentration. Interestingly, these factors had an inverse effect on leaf greenness (hue values), which was higher with the full-strength solution but decreased at the highest plant density. Optimizing nutrient solution can delay senescence and help maintain leaf color, reduce physiological deterioration, and preserve visual quality (Vitális et al., 2023). Lower plant density may allow for better nutrient uptake and more robust tissue development, as water status and structural integrity of leaves are key indicators of freshness and color retention in leafy vegetables such as lettuce (Vitális et al., 2023). Despite seasonal variations, Tetragonia baby plants exhibited marketable quality for at least 14 days. Minimally processed leafy vegetables, while offering convenience, are inherently highly perishable due to the wounding stress of cutting, which accelerates physiological degradation, water loss, and microbial growth (Goel et al., 2025; Zdulski et al., 2024). This rapid deterioration often results in a very short shelf life, presenting a significant challenge for both producers and consumers. While various post-harvest strategies aim to extend this period, understanding the minimum achievable shelf life is

crucial for setting realistic expectations, optimizing supply chains, and ensuring product quality and safety. Generally, under optimal cold storage conditions (typically 0–5 °C and high relative humidity), the minimum shelf life for minimally processed leafy vegetables is often reported to be in the range of 4 to 7 days. Some sources indicate that even with diligent handling, certain highly perishable items, such as some types of cut lettuce, may exhibit satisfactory visual quality and freshness for as little as 5–9 days under refrigeration, with sensory changes such as browning or a loss of texture being the main limiting factors rather than microbial spoilage (Di Giuseppe et al., 2019; Jacxsens et al., 2002). Technological interventions can extend shelf life by a few days; however, most minimally processed vegetables still maintain acceptable quality for less than two weeks (Di Giuseppe et al., 2019; Jacxsens et al., 2002). This reinforces the excellent quality retention achieved with Tetragonia baby plants.

The results yielded important practical information. The obtained data on the response to climatic conditions and agronomic management can be used to establish the commercial production of New Zealand spinach baby plants and their processing as ready-to-eat (RTE) vegetables. Nevertheless, a finer tuning of the nutrient solution composition could help improve the nutritional quality.

5. Conclusions

Tetragonia plants demonstrated adaptability to hydroponic cultivation for baby plant production across the different growing periods of the two experiments. Nevertheless, climatic conditions, plant density, and nutrient supply significantly influenced plant growth, yield, nutritional quality, and post-harvest storage. The highest plant density combined with the full-strength nutrient solution resulted in the highest yield and favorable nutritional characteristics. Moreover, Tetragonia baby plants proved suitable for minimal processing, maintaining good quality retention for a minimum of 14 days. Further studies could be useful to assess the tolerance of this halophytic species to salt stress under hydroponic cultivation for baby plant production and the effects on their nutritional value.

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II. SALINITY AS AN EUSTRESSOR FOR *TETRAGONIA TETRAGONIOIDES*: IMPACT ON RESOURCE USE EFFICIENCY AND QUALITY OF HYDROPONIC MINIMALLY PROCESSED BABY PLANTS

1. Introduction

Salinity, a major abiotic stress, significantly impacts crop yield and quality worldwide. Soil salinization affects vast areas, particularly in arid and semi-arid regions, limiting agricultural productivity and posing a serious threat to global food security (Munns and Tester, 2008). This issue is expected to worsen in the coming years; by 2050, an estimated 50% of the world's arable land will be affected by salinity (Bartels and Sunkar, 2005). Salt stress negatively affects plant growth by increasing soil osmotic pressure, inducing specific-ion toxicities, and causing nutritional imbalances, often acting in concert (Läuchli and Grattan, 2011, 2007; Munns and Tester, 2008). These combined effects can lead to substantial reductions in the growth and productivity of most vegetable crops, especially of leafy vegetables. The salinity tolerance of many of these crops is low, with a typical threshold ranging from 1 to 3 dS m^{-1} depending on the species and various environmental factors (Shannon et al., 2019; Shannon and Grieve, 1998). Notably, a plant's sensitivity to salt stress is generally highest during its early developmental stages, such as the seedling and transplant establishment phases. This poses a serious risk to the cultivation of leafy vegetables for baby leaf or baby plant production. Selecting tolerant species and cultivars, alongside managing irrigation water quality, are key strategies for maintaining productivity in saline environments. To circumvent the limitations of traditional soil-based agriculture in saline regions, hydroponic systems offer a controlled environment where salinity can be precisely managed to optimize resource use efficiency. Hydroponic systems, which grow plants in nutrient-rich water solutions without soil, provide a controlled environment where salinity levels can be precisely managed (Dellosa et al., 2024). This controlled approach is particularly valuable for studying the effects of salt stress on plant growth and for implementing cultivation strategies that mitigate its negative impacts. Furthermore, hydroponics uses water more efficiently than soil-based systems, enabling the utilization of brackish or saline water where freshwater availability is limited (da Silva et al., 2024). For moderately salt-sensitive crops, such as lettuce and spinach, hydroponics allows for superior control of nutrient solutions, mitigating some negative effects of salinity and enabling production even at moderate salinity levels (Leal et al., 2020; Okasha et al., 2022). Finally, salt-tolerant crops, such as halophytes, can be successfully grown hydroponically using saline water while maintaining reasonable yields and quality (Rosales-Nieblas et al., 2025).

New Zealand spinach (*Tetragonia tetragonioides* (Pallas) O. Kunze) is a salt-tolerant halophyte with significant potential as a leafy vegetable that has garnered increasing attention in food security and

sustainable agriculture research (Atzori et al., 2020; Esposito et al., 2025). *T. tetragonioides* is an annual to short-lived perennial herbaceous plant characterized by a prostrate or sprawling growth habit, fleshy stems, and succulent, somewhat triangular-rhombic leaves. Its leaves and young shoot tips are traditionally consumed as a cooked green, often substituting for true spinach, particularly in warm-season gardening where traditional spinach quickly bolts (Dhankhar and Vilas, 2017). *T. tetragonioides* is valued not only for its mild, slightly salty flavor but also for its high nutritional value and a source of various bioactive compounds (Onoiko and Zolotareva, 2024; Roskruge, 2011). Thus, *T. tetragonioides* is positioned as a functional food or a source for novel nutraceuticals (Lee et al., 2019). Moreover, the species holds significant promise due to its robust agronomic traits. Its exceptional tolerance to environmental stresses, notably heat, drought, and salinity, establishing it as a promising crop for bio-saline agriculture in marginal or salt-affected lands. Its tolerance to saline conditions makes it a prime candidate for cultivation where water quality is a limiting factor or for use in saline hydroponic systems. However, while *T. tetragonioides* is known for its resilience, the specific effects of varying salt concentrations on its growth and yield, particularly in hydroponic setups, remain under-documented. Additionally, increasing consumer demand for fresh, ready-to-eat produce highlights the importance of understanding pre-harvest factors. The shelf life of minimally processed leafy greens is often limited by physiological and microbial degradation, which can be influenced by pre-harvest conditions, including salt stress. Because salinity-induced changes in leaf morphology and osmotic potential can alter transpiration and tissue firmness, it is crucial to evaluate the impact of salt stress on the shelf life of ready-to-eat produce. Furthermore, while salt stress can enhance nutritional quality, its influence on the post-harvest physiology of minimally processed greens remains largely unexplored. Therefore, this research aims to assess the salinity tolerance of New Zealand spinach baby plants grown hydroponically and evaluate the impact of pre-harvest salt stress on the quality and shelf life of ready-to-eat produce during cold storage.

2. Materials and methods

2.1. Experimental site and plant material

A two-year trial was carried out in a greenhouse at the Department of Agricultural, Food, and Forest Sciences (SAAF), University of Palermo, Italy (38°60′28″N, 13°21′3″E; altitude 49 m), during the autumn of 2024 and 2025. Fruits of *Tetragonia tetragonioides* (Pallas) O. Kunze (Franchi Sementi, Grassobbio, Italy) were sown on 25 October 2024 and 8 October 2025 into polystyrene trays with 160 cells (18 mL cell volume). The cells were filled with a commercial substrate (Tappeti erbosi, Vigorplant Italia srl, Fombio, Italy) pre-fertilized with 850 g m^{-3} of NPK mineral fertilizer. The trays were kept in a dark room at 25 °C until emergence (5 days after sowing). The substrate was regularly misted with tap water and covered with a plastic film to maintain high moisture levels. Following

emergence, trays were transferred to a cold greenhouse, and seedlings were thinned to one per cell, retaining only uniform individuals.

2.2. Hydroponic system and salinity treatments

Plants were cultivated in a hydroponic ebb-and-flow system with a density of 947 plants m^{-2}. The nutrient solution (NS), based on previous research (Esposito et al., 2025), was prepared using tap water (pH 7.6; electrical conductivity (EC) 750 µS cm^{-1}) supplemented with the following nutrient elements: 19 mM NO_3^-, 1.25 mM NH_4^+, 2 mM $H_2PO_4^-$, 11 mM K^+, 4.5 mM Ca^{2+}, 1 mM Mg^{2+}, 1.1 mM SO_4^{2-}, 40 µM Fe^{3+}, 30 µM BO_3^{3-}, 5 µM Mn^{2+}, 4 µM Zn^{2+}, 0.75 µM Cu^{2+}, and 0.50 µM Mo.

To evaluate the salt stress tolerance, three saline treatments were tested by varying the NaCl concentration: 0 mM (control), 50 mM, and 100 mM. The resulting nutrient solutions had pH 6.2 and EC values of 2.3, 6.4 and 10.4 mS cm^{-1} for S0, S50 and S100, respectively. Four replicate trays per treatment (160 plants for each replicate) were used each growing season. Saline treatments began when plants reached the fully expanded cotyledon stage and the first true leaf had differentiated. During the initial saline irrigation, the NaCl concentration was halved (50%) to allow gradual adaptation to salt stress.

2.3. Growth measurements and resource efficiency

Before commencing the NaCl treatments, 15 plants per treatment were randomly sampled to measure the fresh weight (FW) of the stem, leaves, roots, as well as the leaf area. Samples were dried in an oven at 65 °C for 72 h to determine their dry weight (DW). These "zero point" values were used to calculate the relative growth rate (RGR) and net assimilation rate (NAR) (Hoffmann and Poorter, 2002; Vernon and Allison, 1963). RGR was calculated with the following formulas:

$RGR\ (mg\ mg^{-1}\ d^{-1}) = (\ln(W_2) - \ln(W_1)) / (t_2 - t_1)$

$NAR\ (mg\ cm^{-2}\ d^{-1}) = (W_2 - W_1) / (LA_2 - LA_1) * (\ln(LA_2) - \ln(LA_1)) / (t_2 - t_1)$

where ln is the natural logarithm, W_2 is total dry weight at time t_2 (g), W_1 is total dry weight at time t_1 (g), LA_2 is total leaf area at time t_2 (m^2 or dm^2), LA_1 is total leaf area at time t_1 (m^2 or dm^2), $t_2 - t_1$ is the time interval (days).

Trays were weighed before and after each irrigation to estimate water and NS consumption. These data were used to calculate the water use efficiency (WUE) and nitrogen use efficiency (NUE) (Baligar and Fageria, 2015; Moncada et al., 2022). Relative water content (RWC) was determined prior to harvest using 15 plants per treatment. Baby plants were immediately weighed (FW), immersed in deionized water at 20 °C for 3 h to reach turgid weight (TW), and then dried at 80 °C to a constant weight (DW). RWC was calculated as: RWC (%) = (FW – DW)/(TW – DW) * 100 (Smart and Bingham, 1974).

2.4. Environmental monitoring

Air temperature and relative humidity were monitored hourly both inside and outside the greenhouse using a Testo 608-H1 data logger (Settimo M.se, Italy). Solar radiation was measured with an EKO Instruments MS-410 pyranometer (Tokyo, Japan). During the first trial (autumn 2024) the average temperature outside the greenhouse ranged between 16.5 ± 0.4 °C (night) and 21.3 ± 0.4 °C (day). The net solar radiation at noon averaged 437 W m^{-2}, with a day length ranging between 11 and 10 h. Inside the greenhouse, the temperature averaged 20.3 ± 0.4 °C, ranging between an average minimum of 12.8 ± 0.4 °C and maximum of 27.9± 0.6 °C; the relative humidity ranged from 49.1 ± 1.9% to 87.5 ± 0.9%; noon light intensity averaged 38,820±3,420 lux (range: 8,005–55,907 lux) with fluctuations caused by cloudiness and the average day light integral (DLI) was 18.3 M m^2 d^{-1} (Figure 1-2). In 2025 trial, the outside temperature ranged between 18.3 ± 0.5 °C and 22.5 ± 0.5 °C. The average noon net solar radiation was 492 W m^{-2} and the day length ranged from 10 to 9 h. Inside the greenhouse, the average temperature was 23.4 ± 0.5 °C and varied between an average minimum of 15.1 ± 0.4 °C and maximum 31.8± 0.5 °C; the relative humidity ranged from 44.5 ± 3.0% to 80.1 ± 1.0%; noon light intensity averaged 46,630±3,612 lux (range: 9,376–63,785 lux) with fluctuations caused by cloudiness, and the average DLI was 23.5 M m^2 d^{-1} (Figure 1-2).

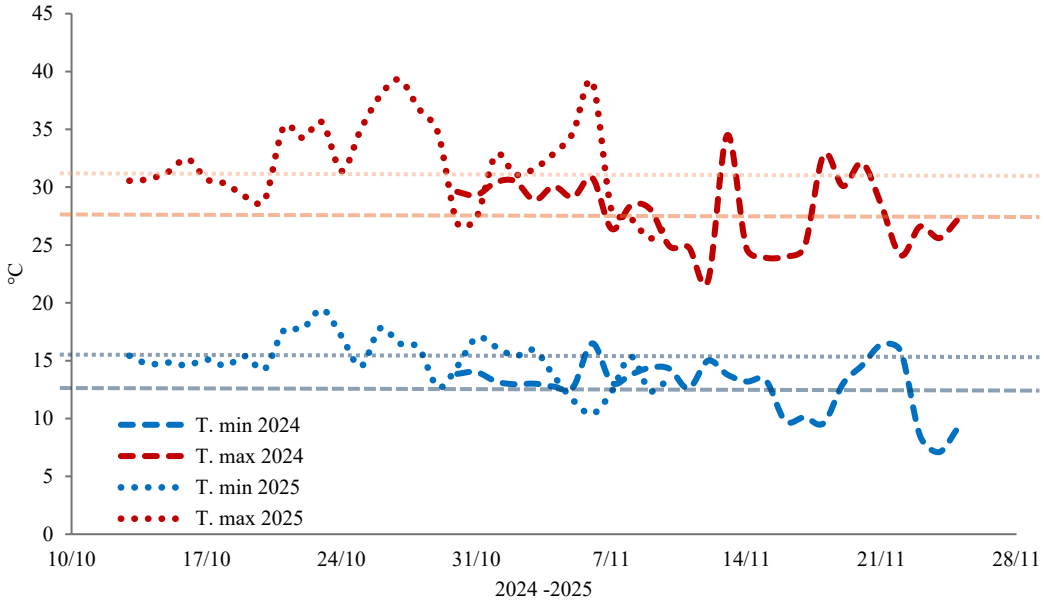


Figure 1. Daily average maximum and minimum temperatures of the air recorded inside the greenhouse with a data logger during *Tetragonia tetragonioides* cultivation in a hydroponic ebb-and-flow system (horizontal lines: blue dashed line – average minimum temperature in 2024; blue dotted line – average minimum temperature in 2025; orange dashed line – average maximum temperature in 2024; orange dotted line – average maximum temperature in 2025).

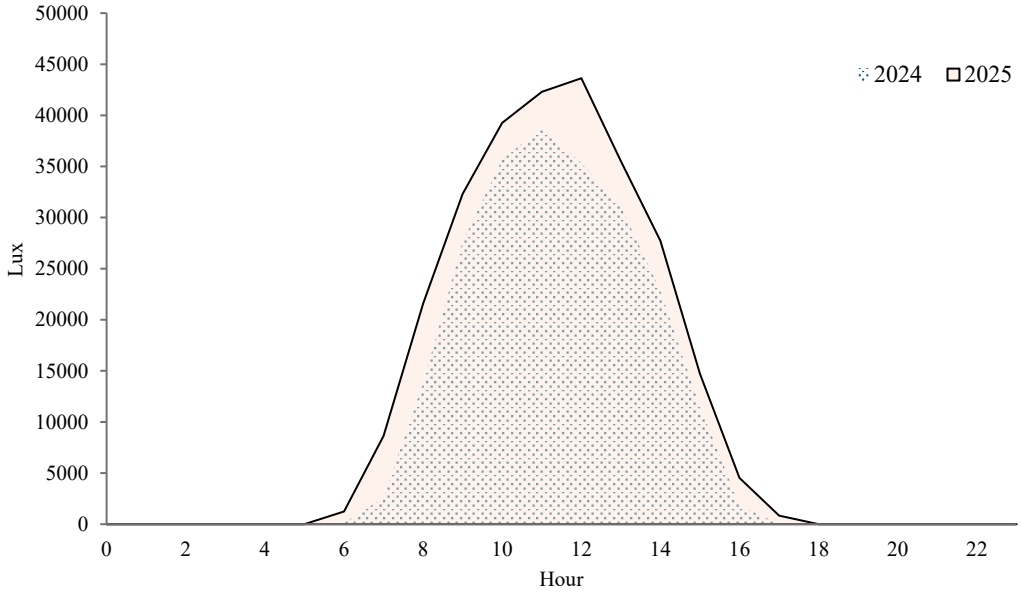


Figure 2. Hourly average light intensity recorded inside the greenhouse during *Tetragonia tetragonioides* cultivation in a hydroponic ebb-and-flow system.

2.5. Harvest and morpho-physiological analysis

Plants were harvested 32 days after sowing (DAS) (26 November) in 2024 and 33 DAS (10 November) in 2025, by cutting them at the base and the baby plant total yield was calculated.

Then, thirty plants per replicate were randomly sampled to determine morpho-physiological analysis. Leaves were separated from the stems, and leaf area was measured. Roots were washed with tap water to remove all substrate and blotted with paper. Leaves, stems, and roots were weighed to determine fresh weight (FW) and dry weight (DW) after drying at 65 °C for 72 h. Leaf color was measured on one fully expanded leaf for each plant sampled using a Chroma-meter CR-400 (Minolta Corporation, Osaka, Japan). The CIELab parameters L* (leaf lightness), a*, and b* were recorded and used to calculate color saturation (chroma $C^* = (a^2 + b^2)^{1/2}$) and hue angle ($h° = 180 + \arctan(b^*/a^*)$ (McGuire, 1992)). The stomatal conductance was measured on one fully expanded and unshaded leaf for each plant sampled using a diffusion porometer (AP4; Delta-T Devices Ltd., Cambridge, UK). The plant leaf area of both zero-point and baby plants at harvest was determined via digital image analysis using Easy Leaf Area software on leaf picture captured with a digital camera (Canon EOS 300D, Canon Inc., Tokyo, Japan) (Easlon and Bloom, 2014). Specific leaf area was calculated as: SLA ($cm^2\ g^{-1}$ DW) = leaf area (cm^2) / leaf dry weight (g DW) (Wolf et al., 1972).

2.6. Chemical determination of baby plant characteristics

Ascorbic acid content (mg 100 g^{-1} of fresh weight) was determined by extracting 10 g of a blended baby plant sample for each replicate in 100 mL metaphosphoric acid (HPO_3). The mixture was then filtered through Whatman no. 1 filter paper and a volume of 10 mL from the filtered solution was determined volumetrically with the 2-6 dichlorophenol-indophenol reagent until a slightly pink

coloration was observed and persisted for 15 s (Miceli et al., 2023). To determine soluble solids content (SSC), and nitrate (NO_3^-) content, 20 plants were randomly sampled for each replicate. A 10 g subsample from the pooled material was blended with distilled H_2O (1:5 w/v). The resulting suspensions were centrifuged at 3500 rpm for 15 min, and the supernatants were then used for subsequent determinations. SSC (°Brix) was measured using a digital refractometer (MTD-045nD, Three-In-One Enterprises Co. Ltd., New Taipei City, Taiwan). NO_3^- concentrations (expressed as mg kg^{-1} FW) were determined reflectometrically using a Merck RQflex10 reflectometer (Merck, Darmstadt, Germany) following the manufacturer's protocols (Alessandro Miceli and Miceli, 2014; Rodrigo and Ramos, 2007; Thompson et al., 2009). The total content of potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), manganese (Mn), and zinc (Zn), was determined on a dried baby plant samples for each replicate after mineralization by dry ashing procedure, modified from the standard protocol (Morand and Gullo, 1970). Briefly, 0.5 g of each sample was weighed into a porcelain crucible and placed in a muffles furnace at 550 °C for 8 hours. Subsequently, acid digestion (2% HNO_3) of the ashes was performed at 100 °C on a hotplate for 15 min. The mineral composition was determined by MP-AES (Agilent 4210 MP-AES, Milan, Italy). Phosphorus (P) was determined by first grinding the leaf samples into a fine powder. A 0.25 g portion of the material was then mineralized in porcelain crucibles using a muffle furnace at 550 °C for 8 hours. The ashes were later recovered through acid digestion using 10 mL of 1 M HCl on a hotplate at 100 °C for 15 minutes. The digested samples were recovered in 15 mL tubes and adjusted to a volume of 10 mL with MilliQ water. The amount of P was determined by the colorimetric method of Murphy and Riley (Murphy and Riley, 1962) using a spectrophotometer (UV mini-1240, Shimadzu, China).

2.7. Descriptive sensory analysis

A descriptive analysis was performed during both trials on New Zealand spinach baby plants according to Esposito et al. (Esposito et al., 2026), with some modifications. A trained panel consisting of eleven people with expertise on leafy vegetables underwent specific training for term generation and evaluation scale familiarization. Baby plants samples were evaluated in duplicate on an unstructured 120 mm line scale anchored 0–9. Two sets of the three salt stress treatments were evaluated per session, with a ten-minute break between sets. No reference sample was provided, and the presentation order was randomized among panelists within each set. The sensory attributes assessed included the overall impression assessed by sense of sight (general aspect and color), the sensations perceived by means of olfactory organ (odor), the overall impression assessed by taste, smell and tactile sense such as texture (crunchiness, juiciness, and fibrousness), basic tastes (sweet, salty, bitter) and flavor (grassy) and the overall rating. Each evaluation portion consisted of 20 g of randomly selected baby plants. The samples were maintained at room temperature and served in

plastic trays. All assessments were conducted at the laboratory of Vegetable Analysis of the SAAF Department.

2.8. Minimally processing and cold storage

Following harvest, defect-free Tetragonia baby plants (lacking lesions, oxidative damage, or yellowing) were selected for minimal processing and cold storage. The plants were washed twice in tap water (5 minutes per wash) and dried using a handheld salad spinner. Samples of 30 g (three replicates per treatment and sampling time) were packed in polyethylene (PE) bags and sealed with a hot-bar sealer (Laica VT3112, Vicenza, Italy). The bags were stored at 4 °C for 21 days.

Weight loss, overall quality (OQ), and leaf color parameters (L*, chroma, hue°, and total color difference ΔE) were evaluated at 0, 7, 14, and 21 days of storage. Weight loss (g 100 g^{-1} of initial fresh weight) was determined by the difference between the initial weight at packaging (day 0) and the weight at each sampling interval (day 7, 14, and 21).

Leaf color was measured on ten randomly selected leaves per sample using a colorimeter (CR-400, Minolta Corp., Ltd., Osaka, Japan). The chroma and hue angle were determined as described above, while the total color difference (ΔE) was calculated at each sampling date using the formula $\Delta E = [(L^*-L^*_0)^2 + (a^*-a^*_0)^2 + (b^*-b^*_0)^2]^{½}$, where L^*_0, a^*_0, and b^*_0 represent the initial values at day 0 (T_0).

The OQ was assessed by an untrained panel of eleven members (seven males and four females, aged 24–65 years) using a 5-point scale, where 5 indicated excellent, freshly harvested quality, 3 represented the limit of marketability, and 1 corresponded to poor or unmarketable quality (Miceli et al., 2021).

2.9. Statistical analysis

The cultivation experiment was arranged in a randomized block design with four replicates for each NaCl concentration. The effects of the growing period and NaCl concentration on Tetragonia baby plants were analyzed using a two-way analysis of variance (ANOVA).

For the minimal processing trials, a completely randomized design with three replicates per treatment was adopted. A three-way ANOVA was used to determine the effects of growing period, NaCl concentration and storage time. In both trials, treatment means and interactions were compared using the least significant difference (LSD) test at $p \leq 0.05$.

3. Results

3.1. Morpho-physiological and yield traits of Tetragonia tetragonioides baby plants

The height of baby plants of New Zealand spinach was significantly greater in 2025 (1.97 cm) than in 2024 (1.67 cm), while the stem diameter remained stable across both years. In both trials, the salt stress reduced baby plant height and stem diameter by an average of 16.3% and 12.3%, respectively, compared to the control (Figure 3a-3b).

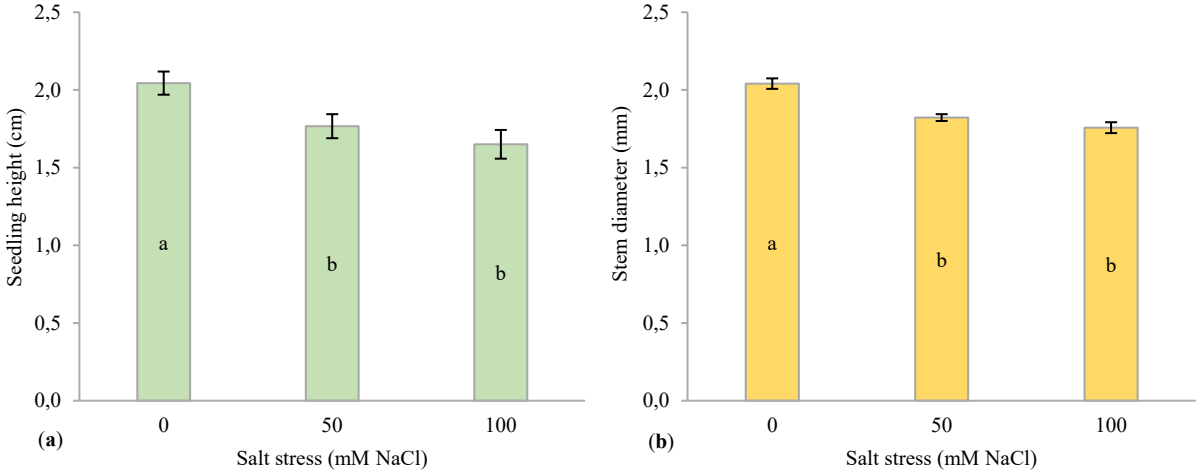


Figure 3. Effects of salt stress (0, 50, and 100 mM NaCl in the nutrient solution) on the height (**a**) and stem diameter (**b**) of *Tetragonia tetragonioides* baby plants grown in a hydroponic ebb-and-flow system during a two years experiment (black bars represent standard errors of the means; bars of the same color with different letters are significantly different at $p \leq 0.05$ according to the LSD test).

In both years, the total fresh weight (FW) of the baby plant was not significantly affected by the lower salt stress level (50 mM NaCl) (Figure 4). However, plant biomass was significantly higher in 2025 (1.97 g FW plant^{-1}, on average for 0 and 50 mM NaCl) compared to 2024 (1.64 g FW plant^{-1}, on average for 0 and 50 mM NaCl) (Figure 3). Supplementing the nutrient solution with 100 mM NaCl to tetragonia plants resulted in a significant reduction in growth, with fresh biomass decreasing to similar values in both trials (1.41 g FW plant^{-1}, on average). Leaf biomass constituted the primary portion of the total plant weight; both leaf and stem biomass followed the same trend as total biomass (Figure 4). Conversely, root fresh biomass in 2024 diverged from this trend, as salt stress led to a significant increase in root weight (Figure 4).

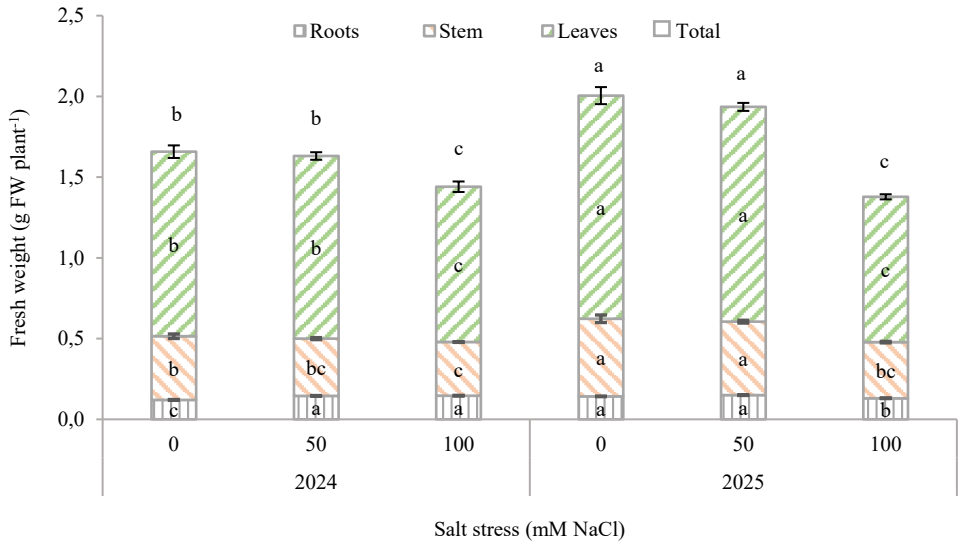


Figure 4. Effects of salt stress (0, 50, and 100 mM NaCl in the nutrient solution) and growing period (2024 and 2025) on the total, root, stem, and leaf fresh weight (FW) of *Tetragonia tetragonioides* baby plants grown in a hydroponic ebb-and-flow system (black bars represent standard errors of the means; bars of the same color with different letters are significantly different at $p \leq 0.05$ according to the LSD test).

The hypogeal (root) and epigeal (shoot) parts of the plants varied according to both the growing period and salt stress level. The shoot/root FW ratio showed a slight but significant increase in 2025 (11.5) compared to 2024 (10.6) and decreased significantly with each increase in salinity, ranging from 12.9 (0 mM NaCl) to 9.2 (0 mM NaCl).

Dry weight (DW) in control plants was significantly higher in 2025 (109.3 mg DW plant^{-1}) than in 2024 (91.9 mg DW plant^{-1}) (Figure 5). Intermediate salt stress (50 mM NaCl) induced an increase in dry biomass accumulation that was consistent across both years (+18% in 2024 and +14% in 2025). In contrast, the highest salt stress (100 mM NaCl) did not differ significantly from the control, resulting in an average of 101.4 mg DW plant^{-1}, (Figure 5). The increase in dry biomass with 50 mM NaCl was also observed in the roots and leaves during both growing seasons, and in the stem during 2025. In this trial, a negative effect on leaf dry weight was determined by the highest salt stress (Figure 5).

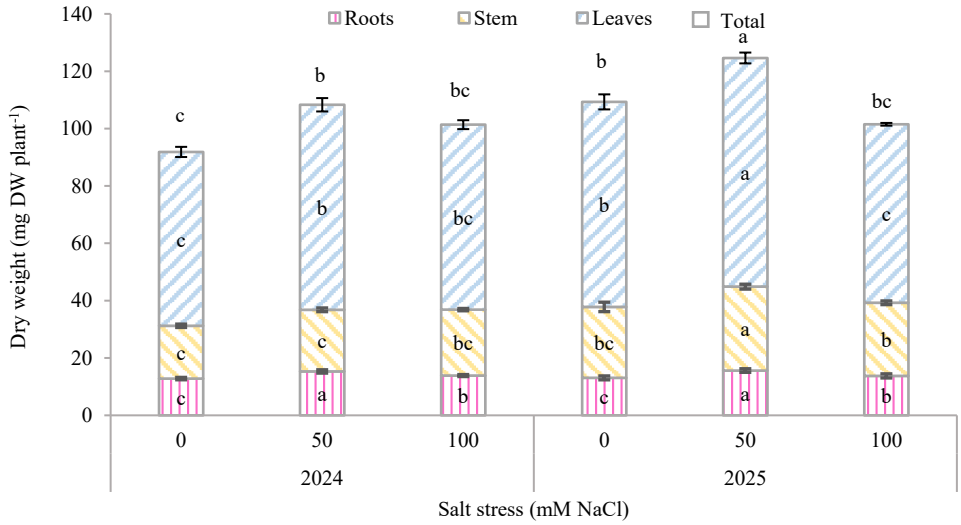


Figure 5. Effects of salt stress (0, 50, and 100 mM NaCl in the nutrient solution) and growing period (2024 and 2025) on the total, root, stem, and leaf dry weight (DW) of *Tetragonia tetragonioides* baby plants grown in a hydroponic ebb-and-flow system (black bars represent standard errors of the means; bars of the same color with different letters are significantly different at $p \leq 0.05$ according to the LSD test).

These modifications in dry biomass accumulation were consistent across plant parts, such that the shoot/root DW ratio remained unaffected by salt treatments, though it was slightly but significantly higher in 2025 (7.0) than in 2024 (6.2).

The shoot dry matter percentage was consistent across the years and increased linearly with salinity, ranging from 5.2% with 0 mM NaCl to 6.9 with 100 mM NaCl.

The number of leaves and the leaf width were significantly higher in 2025 (4.1 leaves plant^{-1} and 2.6 cm, respectively) than in 2024 (3.9 leaves plant^{-1} and 2.2 cm). Leaf width was negatively affected by salt stress, decreasing from 2.5 cm in the control to 2.3 cm in saline treatments. Total leaf area was not significantly affected by intermediate salt stress (50 mM NaCl) compared to control but was higher in 2025 (23.1 cm^2 plant^{-1}) than in 2024 (18.3 cm^2 plant^{-1}). Under 100 mM NaCl, the total leaf area decreased to an average of 15.4 cm^2 plant^{-1} across both years. Considering the average leaf area, this reduction was less pronounced in 2024 (-11.4%) than in 2025 (-32.7%). Specific leaf area (SLA) was higher in 2025 (284.3 cm^2 g DW^{-1}) than in 2024 (267.6 cm^2 g DW^{-1}). On average, SLA decreased by 15.1% and 23.1% with 50 and 100 mM NaCl, respectively, compared to control (316.2 cm^2 g DW^{-1}). The growing period and salinity also influenced leaf color. Color lightness and chroma decreased with increasing salinity, while the hue angle increased to an average of 127.2 in salt-stressed plants.

Table 1. Effects of salt stress (0, 50, and 100 mM NaCl in the nutrient solution) and growing period (2024 and 2025) on leaf characteristics of *Tetragonia tetragonioides* baby plants grown in a hydroponic ebb-and-flow system.

Source of Variance		Number of Leaves	Leaf width (cm)	Total Leaf Area (cm^2 $Plant^{-1}$)	Leaf Area (cm^2 $Leaf^{-1}$)	SLA (cm^2 g DW^{-1})	L*	Chroma	Hue°
Trial									
2024		[z] 3.9b	2.17b	17.4	4.4	267.6b	44.8b	29.1	127.1a
2025		4.1a	2.6a	20.5	5.0	284.3a	46.3a	30.9	126.4b
NaCl (mM)									
0		4.1	2.5a	21.1	5.2	316.2a	47.2	33.0	126.0b
50		4.1	2.3b	20.4	5.0	268.5b	45.2	29.4	127.2a
100		3.9	2.3b	15.4	4.0	243.1c	44.3	27.5	127.2a
Trial x NaCl									
2024	S0	4.0	2.3	18.9b	4.7b	312.7	46.3	31.3b	126.6
	S50	4.0	2.1	17.8bc	4.4bc	249.6	44.4	28.4c	127.4
	S100	3.7	2.1	15.5c	4.2bc	240.6	43.8	27.5c	127.3
2025	S0	4.2	2.7	23.3a	5.6a	319.7	48.1	34.7a	125.3
	S50	4.1	2.5	22.9a	5.6a	287.5	46.1	30.5b	126.9
	S100	4.1	2.4	15.3c	3.8c	245.6	44.8	27.6c	127.0
Significance [x]									
Trial		*	***	***	***	*	***	***	***
NaCl		ns	***	***	***	***	***	***	***
Trial x NaCl		ns	ns	***	***	ns	ns	**	ns

[z] Each value is the mean of four replicated samples of 30 plants each. For each factor, values in a column followed by the same letter are not significantly different, according to the LSD test. [x] Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; and *** significant at $p < 0.001$.

The stomatal conductance did not change across years and significantly decreased by 13.6% on average when the 100 mM of NaCl was supplied compared to S0 and S50 (0.47 mmol m^{-2} s^{-1} on average) (Figure 6).

The baby plant yield peaked in 2025 with both 0 and 50 mM NaCl (1.73 kg m^{-2}, on average), whereas the same treatments averaged 1.43 kg m^{-2} in 2024. The 100 mM NaCl treatment negatively impacted yield in both years, resulting in an average of 1.20 kg m^{-2} (Figure 7).

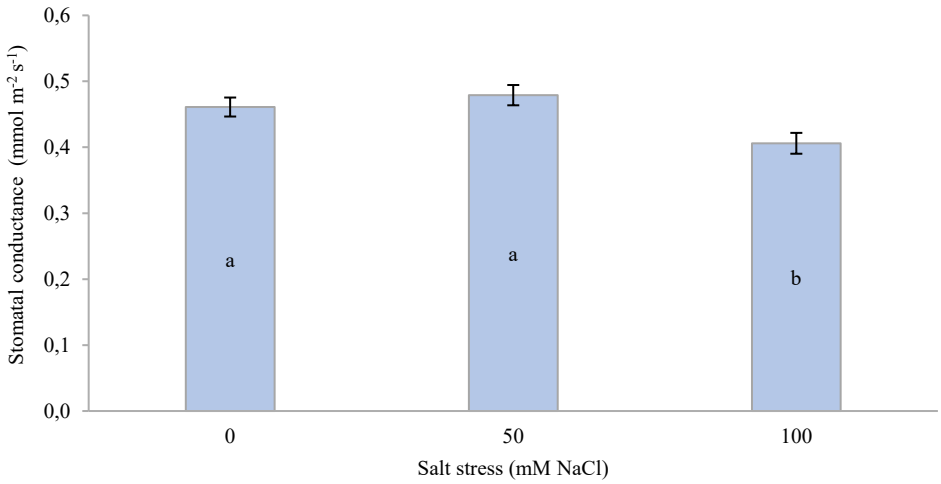


Figure 6. Effects of salt stress (0, 50, and 100 mM NaCl in the nutrient solution) on the stomatal conductance of *Tetragonia tetragonioides* baby plants grown in a hydroponic ebb-and-flow system during a two years experiment (black bars represent standard errors of the means; bars with different letters are significantly different at $p \leq 0.05$ according to the LSD test).

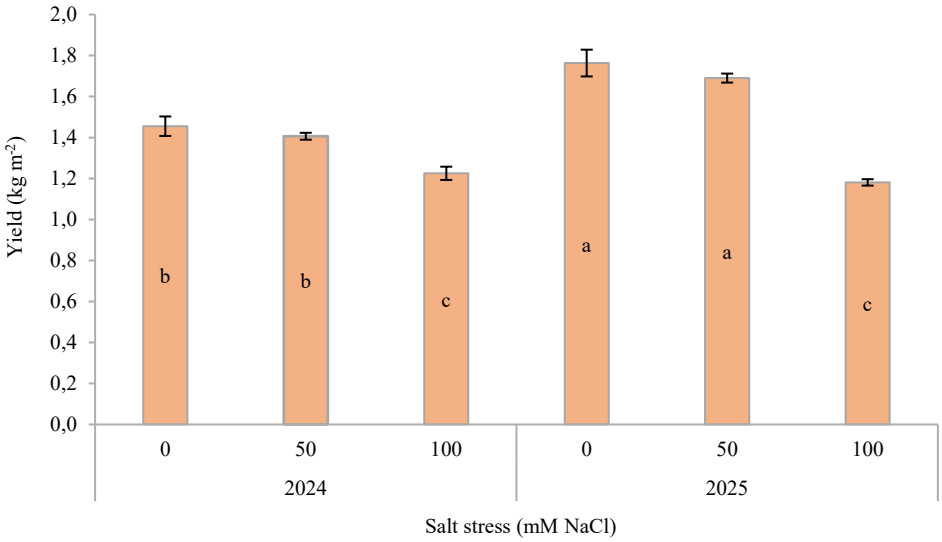


Figure 7. Effects of salt stress (0, 50, and 100 mM NaCl in the nutrient solution) and growing period (2024 and 2025) on yield of *Tetragonia tetragonioides* baby plants grown in a hydroponic ebb-and-flow system (black bars represent standard errors of the means; bars with different letters are significantly different at $p \leq 0.05$ according to the LSD test).

Water (WUE) and nitrogen (NUE) use efficiency were affected only by salinity. The WUE averaged 5.3 g DW L^{-1} H_2O in control plants and increased significantly by 19.2% under salt stress (Figure 8a). The NUE followed a quadratic trend (R^2=0.710), with the lowest value in control plants (18.4 g DW g^{-1} N on average) and the highest recorded with 50 mM NaCl (21.9 g DW g^{-1} N on average) (Figure 8b).

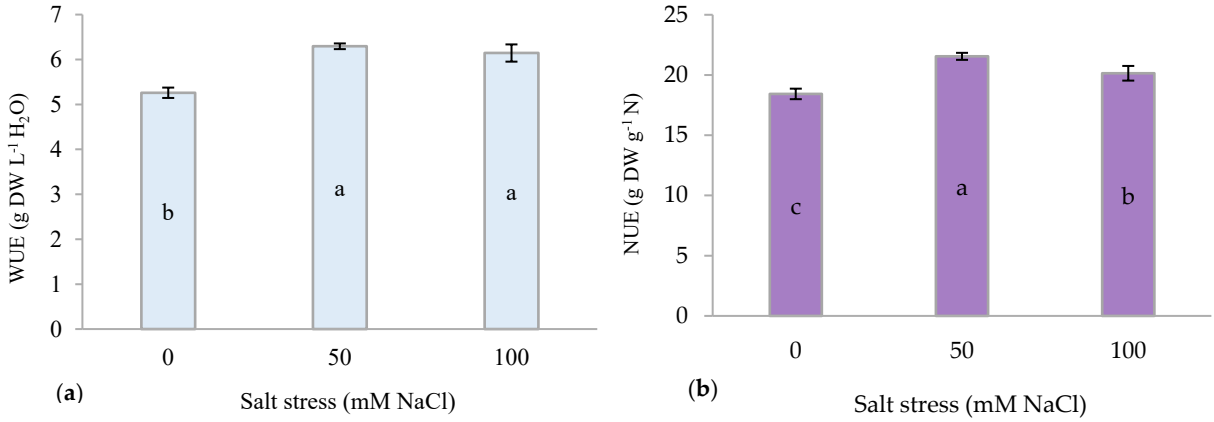


Figure 8. Effects of salt stress (0, 50, and 100 mM NaCl in the nutrient solution) on the (**a**) water (WUE) and (**b**) nitrogen (NUE) use efficiency of *Tetragonia tetragonioides* baby plants grown in a hydroponic ebb-and-flow system during a two years experiment (black bars represent standard errors of the means; bars with different letters are significantly different at $p \leq 0.05$ according to the LSD test).

Relative growth rate (RGR) and net assimilation rate (NAR) were significantly affected by the treatments (Table 2). RGR trends differed by years: it increased with salt stress in 2024 but decreased in 2025. NAR was higher in 2025 than in 2024 and generally increased from 1.09 mg cm^{-2} d^{-1} in control plants to 1.27 mg cm^{-2} d^{-1} in salt-stressed plants.

Relative water content (RWC) declined as salinity increased, with significant differences observed between the control (97.8%) and the 100 mM NaCl treatment (94.8%) (Table 2).

Table 2. Effects of salt stress (0, 50, and 100 mM NaCl in the nutrient solution) and growing period (2024 and 2025) on relative growth rate (RGR), net assimilation rate (NAR), and relative water content (RWC) of *Tetragonia tetragonioides* baby plants grown in a hydroponic ebb-and-flow system.

Source of Variance		RGR (mg mg^{-1} d^{-1})	NAR (mg cm^{-2} d^{-1})	RWC (%)
Trial				
2024		[z] 0.233	1.18b	96.4
2025		0.252	1.24a	96.1
NaCl (mM)				
0		0.245	1.09b	97.8a
50		0.248	1.24a	96.2ab
100		0.236	1.30a	94.8b
Trial x NaCl				
2024	0	0.225d	1.01	97.6
	50	0.239c	1.23	96.6
	100	0.236c	1.30	95.2
2025	0	0.264a	1.18	97.9
	50	0.257b	1.25	95.9
	100	0.237c	1.30	94.4
Significance [x]				
Trial		***	*	ns

NaCl	***	***	*
Trial x NaCl	***	ns	ns

[z] Each value is the mean of four replicated samples of 15 plants each. For each factor, values in a column followed by the same letter are not significantly different, according to the LSD test. [x] Significance: ns = not significant; * significant at $p < 0.05$; and *** significant at $p < 0.001$.

3.2. Biochemical characteristics of Tetragonia tetragonioides baby plants

The ascorbic acid content of baby plants was significantly higher in 2025 (29.1 mg 100 g^{-1} FW) than in 2024 (22.7 mg 100 g^{-1} FW). Saline treatments promoted the accumulation of ascorbic acid, increasing levels from 23.6 mg 100 g^{-1} FW in the control to an average of 27.1 mg 100 g^{-1} FW across the 50 and 100 mM NaCl treatments (Figure 9).

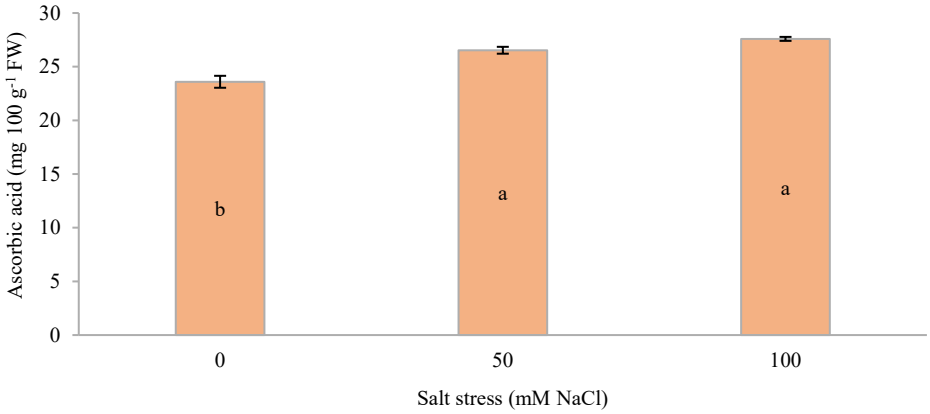


Figure 9. Effects of salt stress (0, 50, and 100 mM NaCl in the nutrient solution) on the ascorbic acid content of *Tetragonia tetragonioides* baby plants grown in a hydroponic ebb-and-flow system during a two years experiment (black bars represent standard errors of the means; bars with different letters are significantly different at $p \leq 0.05$ according to the LSD test).

The nitrate concentration in Tetragonia baby plants was significantly influenced by both the growing year and salinity levels. Plants grown in 2025 exhibited a higher nitrate content compared to those in 2024 and even if salinity prompted a significant linear accumulation of nitrates in both years (R^2=0.975 in 2024 and 0.978 in 2025) the slope of this increase was greater in 2025 (from 1346.3 to 2271.1 mg kg^{-1} FW) than in 2024 (from 863.8 to 1217.7 mg kg^{-1} FW (Figure 10).

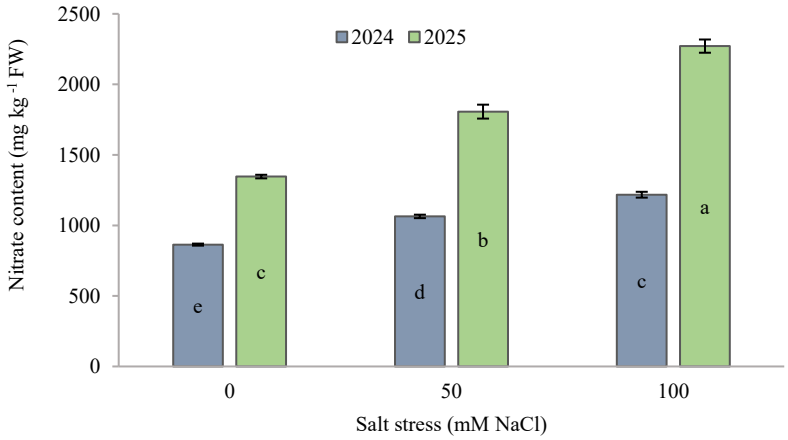


Figure 10. Effects of salt stress (0, 50, and 100 mM NaCl in the nutrient solution) and growing period (2024 and 2025) on the nitrate content of *Tetragonia tetragonioides* baby plants grown in a hydroponic ebb-and-flow system (black bars represent standard errors of the means; bars with different letters are significantly different at $p \leq 0.05$ according to the LSD test).

Similarly, salinity affected the soluble solid content (SSC) of Tetragonia baby plants. While SSC remained stable across both experimental years, it exhibited a significant linear increase (R^2=0.913) in response to salinity, rising from 2.6 °Brix in the control to 3.8 °Brix in the 100 mM NaCl treatment (Figure 11).

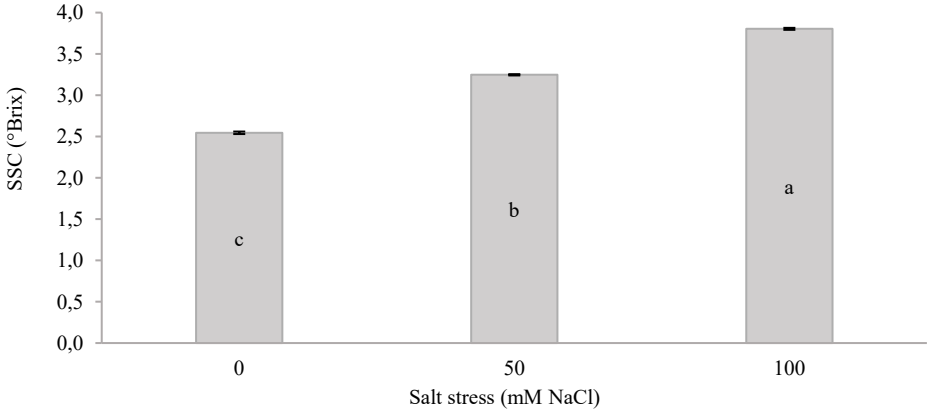


Figure 11. Effects of salt stress (0, 50, and 100 mM NaCl in the nutrient solution) on the soluble solid content (SSC) of *Tetragonia tetragonioides* baby plants grown in a hydroponic ebb-and-flow system during a two years experiment (black bars represent standard errors of the means; bars with different letters are significantly different at $p \leq 0.05$ according to the LSD test).

The mineral composition of the baby plants is reported in Table 3. Sodium (Na) content in the baby plants was significantly affected by salinity, with the impact being more pronounced in the second year of the trial. In control plants, Na content remained consistent across both years, averaging 183.6 mg 100 g^{-1} FW. Under 50 and 100 mM NaCl treatments, Na content increased by 86.1% and 149.6% in 2024, and by 119.8 and 205.6% in 2025, respectively. A slight but significant increase due to salinity was also observed in Magnesium (Mg) content, which rose by 8.4% in salt stressed plants

compared to the control (25.0 mg 100 g^{-1} FW). In contrast, Potassium (K) content in 2025 decreased by an average of 10.8% in salt-stressed plants; however, in 2024, K levels remained stable across all salinity treatments and were comparable to the 2025 control. Calcium (Ca) and Phosphorus (P) contents were higher in 2024 than in 2025. Furthermore, P increased significantly with salinity, ranging from 33.5 mg 100 g^{-1} FW (control) to 42.3 mg 100 g^{-1} FW (100 mM NaCl). No main effect of salinity was observed on the concentrations of Fe, Cu, Mn and Zn, though year-to-year variations occurred: Cu levels across all treatments and Zn levels at 50 mM NaCl were higher in 2024 than in 2025.

Table 3. Effects of salt stress (0, 50, and 100 mM NaCl in the nutrient solution) and growing period (2024 and 2025) on the mineral content (mg 100g^{-1}) of *Tetragonia tetragonioides* baby plants grown in a hydroponic ebb-and-flow system.

Source of Variance		K	Na	Ca	Mg	P	F	Cu	Mn	Zn
Trial										
2024		[z] 437.5	326.7	56.1a	26.7	43.6a	0.171	0.026a	0.119	0.032
2025		406.4	383.9	44.5b	26.0	31.5b	0.146	0.015b	0.129	0.029
NaCl (mM)										
0		439.5	183.6	49.4	25.0b	33.5c	0.169	0.020	0.113	0.030
50		415.8	372.6	49.6	27.2a	36.9b	0.170	0.021	0.125	0.031
100		410.6	509.8	52.0	26.9a	42.3a	0.137	0.020	0.134	0.030
Trial x NaCl										
2024	0	441.1a	183.0d	55.0	25.3	39.5	0.165	0.027	0.100	0.030ab
	50	433.3a	340.5c	57.2	27.7	43.0	0.188	0.024	0.129	0.036a
	100	438.1a	456.7b	56.2	27.1	48.4	0.161	0.027	0.128	0.029ab
2025	0	437.9a	184.2d	43.8	24.7	27.4	0.173	0.014	0.125	0.030ab
	50	398.2b	404.8b	42.0	26.7	30.9	0.151	0.018	0.121	0.025b
	100	383.2b	562.9a	47.7	26.7	36.1	0.113	0.014	0.140	0.031ab
Significance [x]										
Trial		***	***	***	ns	***	ns	*	ns	ns
NaCl		***	***	ns	***	***	ns	ns	ns	ns
Trial x NaCl		***	**	ns	ns	ns	ns	ns	ns	**

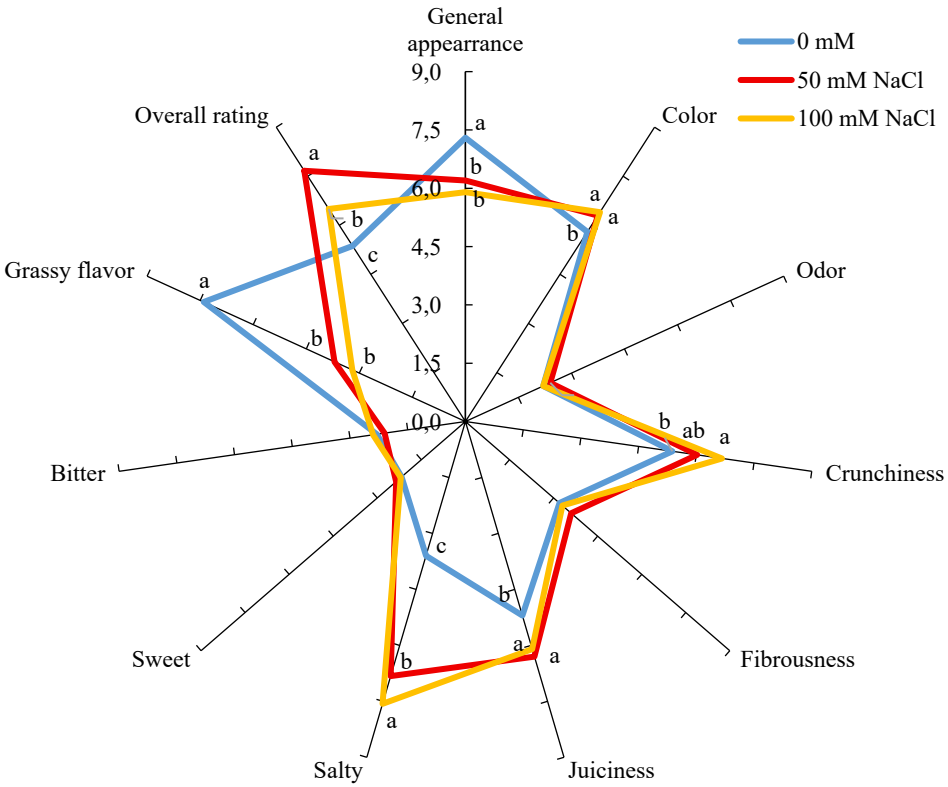
[z] Each value is the mean of four replicated samples of 15 plants each. For each factor, values in a column followed by the same letter are not significantly different, according to the LSD test. [x] Significance: ns = not significant; * significant at $p < 0.05$; and *** significant at $p < 0.001$.

3. 3. Sensory evaluation

The baby plants exhibited similar sensory scores in both years. Salt stress reduced the score for general appearance (6.1 on average) but resulted in a more intense color (6.3 on average) compared to the control (7.3 and 5.8, respectively). Crunchiness score increased from 5.4 to 6.7 as NaCl concentration rose from 0 to 100 mM. A similar upward trend was observed for salty taste, which rose from 3.6 in the control baby plants to 6.8 with 50 mM NaCl. Salinity perception further increased (7.6) when 100 mM NaCl were supplemented. Salt-stressed baby plants were characterized by higher juiciness and a significantly lower grassy flavor compared to the control. Salinity significantly

affected the overall sensory rating, which was highest with 50 mM NaCl (7.7), followed by 100 mM NaCl (6.5), and lowest in the control (5.4).

Figure 12. Effects of salt stress (0, 50, and 100 mM NaCl in the nutrient solution) on the sensory profile of



Tetragonia tetragonioides baby plants grown in a hydroponic ebb-and-flow system during a two years experiment (values within the same parameter with different letters are significantly different at $p \leq 0.05$ according to the LSD test).

3.4. Cold storage of minimally processed baby plants

Following harvest, minimally processed baby plants were stored for 21 days at 4 °C to evaluate their quality changes during cold storage as ready to eat produce. No significant effect of the growing period was observed (Table 4). Weight loss increased linearly at each sampling date, averaging 4.10% by the end of the storage period. Salinity reduced weight loss, which dropped from 4.44% in the control to an average of 2.93% in salt-treated plants (Table 4).

Leaf color was influenced by both salinity and storage time. All treatments showed an increase in color lightness (L*) after 7 days of cold storage. However, salinity significantly reduced L* values at each NaCl concentration level (Table 4). A similar salinity-induced reduction was observed for color saturation (chroma), which remained stable during storage (Table 4). The hue angle was higher in salt-stressed leaves but dropped significantly on day 7 and on day 21. Total color difference (ΔE), which was not influenced by salinity or storage time (Table 4).

Table 4. Effects of salt stress (0, 50, and 100 mM NaCl in the nutrient solution) and storage (21 days at 4 °C) on minimally processed *Tetragonia tetragonioides* baby plants grown in a hydroponic ebb-and-flow system during a two years experiment.

Source of Variance	Weight loss (g 100 g^{-1} FW)	L*	Chroma	Hue°	ΔE
NaCl (mM)					
0	[z] 4.44a	45.7a	32.1a	126.6b	3.75
50	3.16b	44.7b	30.6b	127.0a	3.50
100	2.71b	43.7c	29.3c	127.0a	3.97
Storage (d at 4°C)					
0		43.3b	30.2	128.2a	
7	2.79c	45.2a	31.0	126.6b	3.53
14	3.43b	45.1a	31.1	126.5bc	3.71
21	4.10a	45.3a	30.5	126.3c	3.98
Significance [x]					
Trial	ns	ns	ns	ns	ns
NaCl	***	***	***	***	ns
Storage	***	***	ns	***	ns
Trial x NaCl	ns	ns	ns	ns	ns
Trial x Storage	ns	ns	ns	ns	ns
NaCl x Storage	ns	ns	ns	ns	ns
Trial x NaCl x Storage	ns	ns	ns	ns	ns

[z] Each value is the mean of 4 replicated samples. For each factor, values in a column followed by the same letter are not significantly different, according to the LSD test. [x] Significance: ns = not significant; *** significant at $p < 0.001$.

Overall quality scores declined slowly during the first week of storage, averaging 4.8 across all treatments. Quality loss remained low during the second week, though scores for 0 and 100 mM NaCl treatments fell slightly below 4.5. A significant drop in overall quality was recorded at the end of cold storage, but it was more severe in control (0 mM NaCl) baby plants, which fell below the limit of marketability (2.7) (Figure 13).

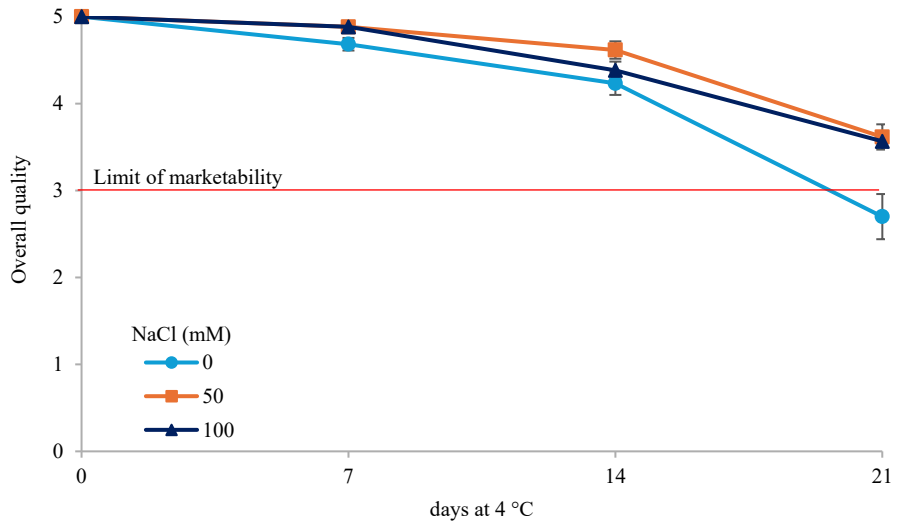


Figure 13. Influence of salt stress (0, 50, and 100 mM NaCl in the nutrient solution) and storage (21 days at 4 °C) on minimally processed *Tetragonia tetragonioides* baby plants grown in a hydroponic ebb-and-flow system during a two years experiment (1: unmarketable; 3: average-limit of marketability; and 5: excellent or having a fresh appearance) (black bars represent standard errors of the means).

4. Discussion

Tetragonia tetragonioides is a facultative halophyte able to grow under both saline and non-saline conditions. Previous studies have primarily focused on the salt tolerance of New Zealand spinach, the use of mature leaves under salt stress and the phytoremediation potential of the species (Esposito et al., 2025; Wilson, 2000). Recently, *T. tetragonioides* has proven to be suitable to hydroponic cultivation for baby plant production and minimal processing (Esposito et al., 2025). However, information on the production of baby plants under saline conditions is still limited. In this study, we conducted a two-year trial to investigate the effects of varying salinity levels on New Zealand spinach grown for baby plant production, with a specific focus on morphophysiological traits and post-harvest shelf-life. Our findings indicate that both climatic conditions and salinity levels significantly influence the morpho-physiological characteristics of Tetragonia baby plants.

In its native habitat, *T. tetragonioides* is capable of year-round establishment, though it exhibits a physiological preference for warm, frost-free environments (Roskruge, 2011). The differences observed between the 2024 and 2025 trials (higher yields, dry biomass and leaf area), can be attributed to the more favorable environmental conditions during the second trial. The 2025 trial was characterized by higher solar radiation, average Daily Light Integral (DLI) and temperature. These conditions likely determined the greater fresh biomass accumulated in 2025, given that Tetragonia is a thermophilic species (Esposito et al., 2025; Wilson, 2000). Warmer temperatures and longer photoperiods have been found to lead to higher growth rates, resulting in more vigorous and rapid development (Esposito et al., 2025). Light and temperature are the primary drivers of leaf expansion

and carbon assimilation in leafy greens. As observed in our study, higher radiation usually increases net CO_2 assimilation and the Net Assimilation Rate (NAR), which in turn promotes a higher Relative Growth Rate (RGR) and increased biomass, as seen in spinach, lettuce and other species (Proietti et al., 2023; Viršilė et al., 2019; Wilson, 1967). Previous fieldwork similarly shows net assimilation rises linearly with radiation in several crops (Wilson, 1967). Comparable seasonal variations in growth parameters have been reported in other halophytic species such as *Portulaca oleracea*, where higher light intensity, daily light integral (DLI) and optimal temperatures significantly enhanced biomass accumulation and water use efficiency (J. He et al., 2023). Furthermore, purslane has been shown to be more sensitive to salinity when grown under lower light intensity (Franco et al., 2011). A modelling study in halophytic meadows suggested that growth correlates well with temperature at low–medium salinity; however, at high salinity, soil salt level must be included to explain seasonal growth dynamics (Pis'man and Slyusar', 2012).This suggests that at 100 mM NaCl, salinity became the primary limiting factor, overriding variations in temperature and light and resulting in comparable RGR and yields across both years.

T. tetragonioides exhibits salinity tolerance at electrical conductivity (EC) values up to 10 dS m^{-1} (Neves et al., 2008). In our trials, a comparable EC value (10.4 dS m^{-1}) was reached in the nutrient solution at the highest NaCl concentration (100 mM). However, the response of New Zealand spinach in this study deviated from previous reports. In both years, fresh biomass, yield and leaf area remained stable at 50 mM NaCl compared to the control but significantly declined at 100 mM NaCl. The developmental stage of a plant is a critical factor in salinity tolerance, as young plants are generally more sensitive to salt stress than their mature counterparts (Foolad, 2004). In our study, Tetragonia baby plants were harvested just 32 days after sowing, whereas previous evaluations of salinity tolerance utilized older plants (Bekmirzaev et al., 2020; Neves et al., 2008). This difference in phenological stage likely explains the discrepancy in salt thresholds. Salt stress typically impairs photosynthesis and stomatal regulation; elevated Na^+ and Cl^- concentrations in plant tissues can induce toxicity, reducing photosynthetic activity, and disrupting stomatal function, which ultimately limits growth (Nawaz et al., 2010; Sudhir and Murthy, 2004). However, halophytes are capable of synthesizing osmoprotectants that mitigate the osmotic effects of salinity, thereby sustaining transpiration (Mishra and Tanna, 2017). Consistent with this, the salt tolerance observed at 50 mM NaCl in our study was associated with stomatal conductance levels similar to the control and significantly higher than those at 100 mM NaCl. This physiological maintenance supported higher fresh biomass accumulation and yield. The yield of Tetragonia under both 0 and 50 mM NaCl was consistent with those found in a previous study (Esposito et al., 2025) and in other baby plant crops (Manzocco et al., 2011). Nevertheless, even accounting for the reduction recorded under 100 mM

NaCl the yield obtained with the highest salinity level can be acceptable when considering the higher water and nitrogen use efficiency compared to control plants.

The accumulation of dry biomass exhibited a distinct trend compared to fresh biomass. Across both years, moderate salinity increased dry biomass accumulation in leaves, stems, and roots compared to the control, a finding consistent with observations in mature Tetragonia plants (Bekmirzaev et al., 2020; Hniličková et al., 2019; Yousif et al., 2010). Conversely, a further increase in salinity led to a decline in biomass. This pattern is common across various halophytic crops. Many halophytes are halophilic, showing optimal growth at low–to-moderate salinity levels and declining at higher concentrations. While dry biomass often peaks at moderate salinity, then plateaus or declines at higher levels, these responses remain highly species-specific. The mechanisms behind biomass increase at intermediate salinity are multifaceted. Species such as *Suaeda fruticosa* and *T. tetragonoides* require NaCl for optimal osmotic adjustment, which supports turgor and growth without suffering nutrition or photosynthesis impair due to Na^+ accumulation at moderate salinity levels. In these and other edible halophytes, increased chlorophyll content and salt-enhanced photosynthesis have been linked to biomass gains under moderate salinity. Moreover, the compartmentation and the maintenance of K^+ and other essential nutrients may support growth at intermediate salinity; however, as salinity increases, rising energetic costs for ion regulation eventually reduce biomass (Farzana et al., 2023; Gul et al., 2024; Tran and Pham, 2023). Although dry biomass declined at the highest salinity level, a near-linear increase in dry matter percentage was observed. This trend is consistent with the progressive accumulation of salts and ions in the tissues rather than a linear increase in true organic biomass (Ahmed et al., 2021; Jēkabsone et al., 2024). This suggests that while the plants were heavier in terms of dry weight, a significant portion of that weight may be attributed to inorganic ash content rather than carbon-fixed structural tissues. The mineral profile supports this interpretation. Sodium increased sharply in salt-treated plants, especially under the highest salinity level, confirming that *T. tetragonioides* is able to accumulate Na in aboveground tissues. Potassium remained relatively stable in 2024, whereas a decrease was observed in 2025 at 100 mM NaCl. This suggests that under moderate or moderately high stress *T. tetragonioides* can maintain ion balance effectively, but increased evaporative demand may imbalance Na and K uptake pathways (Barbafieri et al., 2023). The maintenance of K at moderate salinity is particularly relevant because preservation of cytosolic K is a key component of salt tolerance (Flowers and Colmer, 2008). At the same time, the partial decline observed under severe stress indicates that the capacity to maintain ionic selectivity is not unlimited, especially when salinity is combined with conditions favoring higher transpiration.

Relative water content did not show the sharp reduction that is typically observed in salt-sensitive species exposed to osmotic stress, at least under moderate salinity (Breś et al., 2022). This finding is consistent with the halophytic behavior of the species (Mann et al., 2023) and suggests that tissue

hydration was sufficiently preserved during growth. The maintenance of leaf water status under saline conditions is likely associated with osmotic adjustment (Aghaleh et al., 2011), although the specific osmolytes involved were not measured in this study.

The analysis of resource-use efficiency adds an important agronomic dimension to the interpretation of the results. Water use efficiency increased with salinity, particularly at 100 mM NaCl. This indicates that, despite the reduction in biomass at the highest salinity, plants produced more biomass per unit of water consumed. Such a response is commonly associated with lower stomatal conductance, as found in our trials, and reduced transpirational water loss under stress. Stress conditions can influence plant growth through various mechanisms, including the reduction of water and nitrogen uptake (Parihar et al., 2015). In spinach, WUE increases under moderate stress, whereas NUE decreases as EC levels rise (Ramezanifar et al., 2022). However, in more salt-tolerant species, such as certain *Amaranthus* genotypes and mature Tetragonia plants, WUE has been shown to increase consistently with rising EC levels (Atzori et al., 2020; Omamt et al., 2006). This improvement in WUE was associated with a decrease in specific leaf area (SLA), signaling the possibility of an adaptive strategy to conserve water (Ors and Suarez, 2017).

The quadratic trend observed in Nitrogen Use Efficiency (NUE), peaking at 50 mM NaCl and followed by 100 mM NaCl, suggests that moderate salinity improved the conversion of acquired nitrogen into biomass, whereas severe salinity reduced that efficiency. This response may reflect a hormetic effect, whereby low-to-moderate stress stimulates physiological performance. Similar responses have been documented in several halophytic or salt-adapted species, in which moderate salinity promotes nitrate uptake and assimilation to facilitate osmotic adjustment (R. He et al., 2023). The simultaneous increase in nitrate concentration with increasing salinity is particularly informative. It indicates that nitrogen continued to accumulate in leaf tissues even when NUE decreased. A plausible interpretation is that at moderate salinity nitrogen metabolism remained sufficiently efficient to support growth, while at 100 mM NaCl a larger share of nitrate was retained in tissues without being translated proportionally into biomass. In halophytes like *T. tetragonioides*, nitrates are not merely a nutrient but a crucial osmoticum. It can be hypothesized that as external salinity increases to 100 mM, the plant continues to accumulate nitrates to maintain a favorable osmotic gradient. The decline in NUE at 100 mM, despite higher nitrate levels, could be explained by a shift from assimilation to sequestration. At this higher stress level, the metabolic cost of maintaining ion homeostasis and the potential competitive inhibition between Cl^- and NO_3^- for enzymes like nitrate reductase, could have slowed the conversion of stored nitrates into organic matter (Li and Yang, 2023).

Phosphorus also increased under salinity, while magnesium remained relatively stable and showed a slight increase in salt-treated plants. These responses indicate that salinity did not impair mineral

accumulation in a generalized manner. On the contrary, some nutrients were maintained or even enriched in the edible tissues. This is agronomically relevant because it suggests that moderate salinity can modify the mineral composition of the product without necessarily compromising plant performance.

Net assimilation rate increased in the saline treatments compared with the control. In the present case, the increase in NAR suggests that *T. tetragonioides* maintained a favorable balance between photosynthetic activity and leaf area development under moderate salt stress. This is coherent with the general behavior of halophytes (Cao et al., 2023; de Vos et al., 2013), which are often able to sustain physiological activity at salinity levels that would markedly depress the performance of salt-sensitive species. Even so, the increase in NAR should not be interpreted as evidence that salinity was broadly beneficial under all conditions, because at 100 mM NaCl the reduction in yield and fresh biomass clearly indicates that the cost of stress became substantial.

Plant and leaf morphology are significantly influenced by salt stress, although the specific effects vary across species. In our trials, NaCl in the nutrient solution led to a significant decrease in plant height, stem diameter and leaf width even in the absence of a biomass reduction at 50 mM. Consequently, salt stressed Tetragonia baby plants appeared more compact, with thinner stems and narrower leaves. These traits may be advantageous for the ready-to-eat (RTE) market and visually appealing in salad mixes. Height reduction under salinity is a well-documented response in Tetragonia and other species, primarily associated with osmotic stress and ion toxicity that inhibit longitudinal growth (Sogoni et al., 2021). Comparable results have been reported in both halophytic and glycophytic species, including *Sesuvium portulacastrum*, lettuce, and small-leaf basil (Attia et al., 2011; Cao et al., 2023; Garrido et al., 2014). While salt-sensitive species and some halophytes restrict leaf expansion under saline conditions to limit transpiration (Acosta-Motos et al., 2017), other halophytes increase leaf area to dilute internal salts or maximize light capture (Kaleem et al., 2024). These different strategies depend on the life form, habitat, and salinity level. Our results align with these adaptive trends; total leaf area remained stable under moderate salinity but decreased at 100 mM NaCl, supporting the classification of *T. tetragonioides* as a salt-tolerant species. This morphological shift was further evidenced by a linear decrease in Specific Leaf Area (SLA), indicating an increase in leaf thickness or mesophyll density. This adaptation is common in halophytes as it increases the cellular volume available for the vacuolar sequestration of Na^+ and Cl^- ions, thereby protecting the photosynthetic apparatus (Flowers and Colmer, 2015). This protection is further supported by the observed increase in NAR under salt stress. While salinity typically inhibits photosynthesis in glycophytes, moderate halophytes can leverage the accumulation of inorganic ions and organic osmolytes to reduce the osmotic potential without impairing CO_2 fixation (Parida and Das, 2005). Increased succulence and leaf thickening are core halophyte traits and involve enlarged

mesophyll cells and vacuoles, alongside an increased number of chloroplasts per cell (Wungrampha et al., 2020). These morphological adjustments often coincide with physiological changes and with shifts in visual quality. While salinity stress generally reduces chlorophyll biosynthesis and promote pigment degradation, thereby altering leaf coloration and its visual perception (EL-Bauome et al., 2024; Simko, 2020), Tran et al. (Tran and Pham, 2023) reported that chlorophyll content actually increases in Tetragonia under salt stress, resulting in a darker green appearance. A similar pattern was observed in our study: 50 and 100 mM NaCl treatments reduced leaf lightness (L*) and saturation (Chroma) while increasing the hue angle indicating a shift toward a deeper, more saturated green. Similar shifts in leaf coloration have been reported in other leafy vegetables such as lettuce, rocket, and spinach (Deveci et al., 2019; Vetrano et al., 2020).

The increase in ascorbic acid and soluble solid content (SSC) observed in our study confirms that moderate salinity acts as a positive eustressor for the secondary metabolism of *T. tetragonioides*. The significant rise in ascorbic acid (Vitamin C) in salt-stressed plants is a typical defensive response to oxidative stress. Under saline conditions, plants increase the production of antioxidant compounds to scavenge reactive oxygen species (ROS) generated by osmotic and ionic stress (Hasanuzzaman et al., 2021). This enrichment not only enhances the plant's biological resilience but also significantly boosts the nutritional value for the consumer.

The nitrate content in New Zealand spinach is a critical quality parameter, as high nitrate intake is associated with health risks (Brkić et al., 2017). In our trials, while salinity prompted a significant linear accumulation of nitrates, the levels remained well within the safety limits established by European regulations (n. 1258/2011) for similar leafy vegetables like spinach (European Union, 2011). Interestingly, the nitrate accumulation was more pronounced in the 2025 trial. This can be attributed to the higher light intensity and temperature recorded during the second year, which generally promotes higher transpiration rates and, consequently, greater nitrate transport from the roots to the leaves. While chloride and nitrate often compete for the same anion transporters, leading to decreased nitrate in many salt-sensitive species, our results showed a linear increase in nitrates under salinity. It can be hypothesized that in *T. tetragonioides* the need for nitrates as an osmoticum may override the competitive inhibition of chloride at the concentrations tested (50-100 mM) (Landorfa-Svalbe et al., 2022), although the underlying transport and assimilation processes were not examined directly in our study. This specific halophytic trait allows the plant to maintain high turgor and metabolic activity even in challenging environments, though it requires careful monitoring to ensure the final product remains a safe functional food.

Sensory evaluation revealed that salinity significantly improved the organoleptic profile of the baby plants. The perceived increase in crunchiness and saltiness could be ascribed to the accumulation of minerals and the concurrent reduction in leaf water content (lower RWC). The highest overall sensory

rating at 50 mM NaCl further suggests that moderate salinity may function as an eustressor, yielding a more flavorful and high-quality product. This organoleptic improvement is chemically rooted in the altered mineral profile. The significant accumulation of Na and the maintenance of essential macronutrients like Ca and P contribute to the salty and savory perceptions reported by the panel. Moreover, the lack of significant changes in micronutrients (Fe, Cu, Mn, and Zn) in response to salinity ensures that the eustress approach enhances flavor and nutritional value without compromising the safety or the metallic-off-notes often associated with microelement imbalances. Furthermore, the reduction in grassy flavor and the intensification of leaf color make the product more appealing. Similar improvements in the sensory quality of saline-irrigated crops have been documented in species like *Salicornia* and *Salsola*, where salt-induced accumulation of sugars and acids balances the increased saltiness to create a complex and desirable flavor profile (Ventura and Sagi, 2013).

Regarding consumer safety and nutritional guidelines, the sodium (Na) accumulation in Tetragonia baby plants warrants consideration. The World Health Organization (WHO) recommends a daily sodium intake of less than 2,000 mg for adults to reduce the risk of cardiovascular diseases (Jaques et al., 2021). In our study, the Na content of control plants represented approximately 9.2% of the recommended daily limit per 100 g serving. Under the 100 mM NaCl treatment in 2025, Na content peaked and a 100 g portion would provide 28.1% of the daily allowance. While this value is higher than traditional glycophytic leafy greens like common spinach (*Spinacia oleracea*), it remains manageable within a balanced diet, especially considering that the intensified salty taste of the baby plants (as noted in the sensory evaluation) may naturally reduce the consumer's need to add supplemental table salt. Thus, Tetragonia baby plants grown under moderate salinity can be marketed as a naturally pre-seasoned functional food, though clear labeling of sodium content would be advisable for individuals on salt-restricted diets.

Pre-harvest environmental conditions and agronomic management significantly influence product quality at harvest and post-harvest physiology during storage. To assess the influence of the NS salinity on the shelf-life of Tetragonia baby plant shelf life, samples were minimally processed immediately after harvest and stored at 4 °C for 21 days as a ready-to-eat (RTE) product. Minimally processed leafy vegetables typically deteriorate rapidly during storage, leading to substantial commercial losses (Miceli et al., 2021). Harvest-induced separation from the parent plant severs the supply of water and nutrients, triggering physiological disorders often exacerbated by pre-harvest environmental factors or mineral imbalances. The loss of marketability is primarily driven by weight loss, resulting from water loss through transpiration or evaporation along with the respiration and the depletion of carbohydrate reserves (Ayala-Zavala et al., 2008; Roura et al., 2000). In our post-harvest trials, the weight loss of baby plants increased across all treatments over the cold storage period, but

a notable result was the reduction in weight loss for salt-stressed plants. The exact mechanisms responsible for this response were not examined directly, but the data clearly show a beneficial effect of salinity on weight-loss control during storage. Plants exposed to high salinity often develop anatomical and physiological adaptations that mitigate water loss, such as increased cuticle thickness and altered stomatal traits (Agüero et al., 2008). For instance, the halophyte *Salvadora persica* exhibits increased leaf succulence and thicker epidermis under saline conditions, coupled with reduced stomatal density and pore area, which contribute to improve WUE by limiting transpiration (Parida et al., 2016). This is consistent with the increased WUE recorded in our salt-stressed treatments. However, the relationship between stomatal density, cuticle properties, and salt tolerance can be complex and species-specific, with some reports showing variable responses depending on the plant type and salinity level (Boughalleb et al., 2009; Hasanuzzaman et al., 2023). Post-harvest water loss is influenced by these traits, as more efficient stomatal control and thicker cuticles reduce transpiration after harvest, thereby maintaining tissue hydration and quality (Othman et al., 2024). Overall, plants adapt to salinity by modifying leaf anatomy and stomatal behavior to conserve water, which can reduce water loss both during growth and postharvest stages (Othman et al., 2024; Parida et al., 2016).

Leaf color is a critical quality attribute and a primary driver of consumer preference, with bright and saturated green tones being particularly valued (Barrett et al., 2010; Spinardi et al., 2010). During cold storage, color lightness increased while hue angle decreased. Nevertheless, salinity level contributed to maintain a darker, more saturated and greener color compared to the control. Pre-harvest salinity can influence color retention and postharvest quality of leafy vegetables in several ways. Research on *Amaranthus* and lettuce indicates that moderate salinity levels (e.g., 50–100 mM NaCl) increase the concentration of leaf pigments such as chlorophyll, carotenoids, as well as bioactive compounds like betalains, polyphenols, and flavonoids. These compounds enhance antioxidant capacity and may assist in stabilizing leaf color during storage (Hossain et al., 2022; Sarker and Oba, 2019). Overall, controlled preharvest salinity can enhance antioxidant compounds and pigment stability, which supports better leaf color retention during postharvest storage of leafy vegetables, though effects vary by species and salinity level.

Tetragonia baby plant retained good quality for over 14 days under cold storage, confirming the findings of Esposito et al. (Esposito et al., 2025). The maintenance of overall quality (OQ) was superior in salt-stressed plants compared to the control, particularly at the end of the 21-day storage period. The control plants lost marketability primarily due to faster wilting and loss of turgor. This suggests that preharvest exposure to moderate salinity may improve the shelf-life of the minimally processed product.

Overall, the findings of this study present a synergy between environmental conditions and salt-induced eustress. While favorable climatic conditions (higher radiation and temperature) significantly boosted yield under low-to-moderate salinity, the imposition of 100 mM NaCl acted as a physiological bottleneck, negating seasonal advantages. From a practical perspective, the results indicate that salinity management can influence both agronomic performance and product quality and post-harvest performance of this underutilized crop.

5. Conclusions

This study shows that *Tetragonia tetragonioides* is a suitable candidate for hydroponic baby leaf production, exhibiting a robust ability to balance yield with enhanced quality under moderate saline conditions. The two-year trial revealed that while favorable climatic conditions significantly boost growth at low-to-moderate salinity, a concentration of 50 mM NaCl acts as a beneficial eustressor. At this level, the species maximizes both water and nitrogen use efficiency without sacrificing biomass, while simultaneously developing a more compact morphology and intense coloration desirable for the ready-to-eat market.

Furthermore, the preharvest exposure of the baby plants to moderate salinity improved post-harvest performance with reduced weight loss, superior color retention, and extended their shelf-life to over 21 days. While higher salinity (100 mM NaCl) eventually limits growth regardless of favorable weather, the use of moderate salt stress emerges as a strategic tool for sustainable agriculture. This approach could help growers to conserve freshwater resources while delivering a sensory-rich, resilient product that meets both consumer preferences and commercial standards for longevity in the cold chain. Further research is needed to evaluate the bioavailability of the accumulated minerals and to determine if dynamic salinity management could further refine the balance between yield and the nutritional profile of the final product.

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III. SUITABILITY OF *APIUM GRAVEOLENS* L. VAR. *SECALINUM* ALEF. TO HYDROPONIC CULTIVATION FOR BABY LEAF PRODUCTION

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1. Introduction

In the food sector, a growing health-oriented trend has led to increased consumer demand for simple and innovative dietary solutions, with a preference for fresh foods rich in nutraceutical compounds such as vitamins, essential nutrients, dietary fibers, carotenoids, and flavonoids (Aschemann-Witzel et al., 2013a; Giménez et al., 2020; Jiménez-Monreal et al., 2009; Olaimat and Holley, 2012; Vecchio and Cavallo, 2019). This trend is further supported by the growing consumer interest in organic and functional food products, which are increasingly recognized for their potential role in the prevention of health disorders (Aschemann-Witzel et al., 2013b). Minimally processed products, such as fresh-cut leafy vegetables, are functional foods which offer a practical solution to meet consumer demand for healthier diets, providing convenient, time-saving options for meal preparation (Aschemann-Witzel et al., 2013a; Nassivera and Sillani, 2015; Ricci et al., 2018). To further expand the fresh-cut sector and meet consumer demands, it is essential to diversify the product offering by introducing new vegetable types, particularly lesser-known crops with high nutritional value. Among these crops, leaf celery (*Apium graveolens* L. var. *secalinum* Alef.), also known as smallage, is a biennial herbaceous plant characterized by smaller size, shorter leaves, thinner and shorter petiole, higher aromatic flavor, and regrowth capacity after mowing compared to stalk celery (*Apium graveolens* L. var. *dulce* (Mill.) Pers.*)* (Román and Hensel, 2011; Rożek et al., 2016). The characteristic aroma of leaf celery is ascribed to sedanolide, a volatile compound commonly found in celery (Marongiu et al., 2013). Their leaves can be consumed raw in salad mixes or cooked. They can also be dried or frozen with minimal loss of aromatic quality and used as a spice (Rożek, 2007). Several studies have highlighted the medicinal potential and nutritional value of *Apium graveolens* botanical varieties (ribbed celery, leaf celery, and celeriac). These include analgesic, antibacterial, anti-inflammatory, antioxidant, antirheumatic, and cardio-, neuro-, and gastroprotective properties, as well as a low caloric content and a notable content of mineral elements (K, P, Na, Ca, Mg, Mn, Fe, and Zn), vitamins, proteins and phenols (Al-Howiriny et al., 2010; Genatrika et al., 2019; Jittiwat et al., 2021; Khairullah et al., 2021; Ramadhan, 2020; Salehi et al., 2019; Sowbhagya, 2014; Stephen et al., 2020). Moreover, leaf celery is characterized by a higher content of vitamin C compared to the other botanical varieties of *Apium graveolens* (Rozek et al., 2013).

Global interest in leaf celery has accelerated significantly, largely driven by its high nutraceutical profile and rich endowment of bioactive compounds, particularly flavonoids (such as apigenin and luteolin) and phthalides, which possess established antioxidant and anti-inflammatory effects (Kholieqoh et al., 2022; Li et al., 2020). Despite the historical dominance of stalk (*A. graveolens* var. *dulce*) and root varieties (*A. graveolens* var. *rapaceum*), international agronomic research is now focusing on optimized cultivation techniques for leaf celery, owing to its multiple harvesting potential and rapid leaf regrowth capability. Recent studies have investigated the impact of variables such as irrigation management and harvest frequency on overall yield and essential oil content of open field grown leaf celery, demonstrating its high-efficiency production potential (Lakitan et al., 2020; Rożek, 2013; Rożek et al., 2016). Leaf celery is particularly valued across Asian markets, where it is traditionally utilized as a fresh herb and flavoring agent. This demand has spurred focused research on its qualitative and aromatic characteristics, with studies comparing the volatile compositions of different ecotypes for their commercial valorization and application in the food and fragrance industries (Reale et al., 2021; Turner et al., 2021).

To meet the growing demand for high-quality fresh-cut produce, the cultivation of leafy vegetables for ready-to-eat products increasingly relies on soilless cultivation systems, where plants are grown in inert substrates and provided with a complete nutrient solution. (Sharma et al., 2018). Hydroponics is a method of growing plants using mineral nutrient solutions without soil encompasses diverse systems such as Nutrient Film Technique (NFT), Deep Water Culture (DWC), ebb-and-flow and aeroponics. These soilless methods present significant advantages to produce leafy vegetables destined for fresh-cut markets. Notably, they enable accelerated growth rates, higher yields within smaller footprints, precise control over environmental factors, enhanced resource use efficiency, precise nutrient delivery, and reduced contamination risks leading to enhanced product quality and consistency regardless of external climate conditions (Kumar, Vikanksha and Singh, 2023; Tüzel et al., 2019). The ebb and flow (flood and drain) hydroponic system is a widely used soilless cultivation method, particularly suited for leafy vegetables and baby leaf crops intended for ready-to-eat products. Its main advantages include improved root oxygenation, efficient water and nutrient use, and the potential for high-quality, minimally processed produce. Hydroponic systems significantly enhance agricultural water-use efficiency, typically achieving reductions of 70–90% compared to conventional soil-based methods (da Silva et al., 2024). This substantial conservation capability has garnered considerable attention, particularly in regions facing quantitative or qualitative water scarcity. Beyond water savings, these controlled environment systems facilitate year-round crop production, minimize the incidence of pests and diseases, and substantially reduce the necessity for weeding or pesticide applications. While initial capital expenditure and subsequent operational costs can be elevated, hydroponics offers compelling advantages in urban and resource-constrained

settings. Profitability is often realized through premium pricing or by cultivating high-value specialty crops, such as minimally processed leafy vegetables (Zha et al., 2024).

To fully realize the advantages of hydroponic cultivation, the agronomic management of each crop requires fine-tuning. Extensive information exists for the hydroponic cultivation of common leafy vegetables (e.g., lettuce, rocket, and basil). However, integrating novel or underutilized species into soilless systems requires specialized guidelines for plant management and nutrient provision.

Based on its morphological leaf characteristics, we hypothesize that leaf celery is a suitable crop for hydroponic baby leaf production and minimal processing. However, while this crop is presently cultivated in soil in restricted regions, significant knowledge gaps exist regarding its optimal agronomic management for hydroponic baby leaf production and post-harvest cold storage as a minimally processed product. Therefore, this paper aims to address these gap areas by evaluating for the first time the suitability and sustainability of *Apium graveolens* L. var. *secalinum* Alef. to hydroponic cultivation for baby leaf production and its quality characteristics and shelf life as a minimally processed vegetable. Ultimately, the practical aim of this study is to provide a commercially viable production blueprint for multi-cut hydroponic baby leaf celery.

2. Materials and methods

2.1. Leaf celery baby leaf production

The trials were carried out in a cold greenhouse located at the Department of Agricultural, Food, and Forest Sciences (SAAF) of the University of Palermo, Italy (38°60′28″N 13°21′3″E; altitude 49 m) during two different growing seasons (S1 – winter-spring, S2 – spring-summer) in 2023. The seeds of a Sicilian landrace of *Apium graveolens* L. var. *secalinum* Alef. were sown (January 2023 and April 2023) into polystyrene trays filled with a commercial substrate (Tappeti erbosi, Vigorplant Italia srl, Fombio, Italy, a peat based mix fertilized with 850 g m^{-3} of a mineral fertilizer NPK). The trays were placed for both trials in a germination room at 25 °C until emergence (10 days after sowing). Then, the seedlings were thinned to one per cell keeping those with uniform morphological characteristics.

Leaf celery plants were grown at two plant densities (615 and 947 plants m^{-2}) in polystyrene trays with 104 or 160 cell with a volume of 28 and 18 ml, respectively, using a hydroponic ebb and flow system that supplemented three concentrations of nutrient solutions (NS) for each plant density: 0% (control with only water—0), 50% (half-strength—HS), and 100% (full-strength—FS). Four replicated trays for each treatment (150 plants for each replicate) were used in each growing season. The full-strength (FS) nutrient solution was prepared by mixing tap water (pH 7.6, and electrical conductivity (EC) 500 μS cm^{-1}) with the following mineral elements: 19 mM NO_3^-, 1.25 mM NH_4^+,

2 mM $H_2PO_4^-$, 11 mM K^+, 4.5 mM Ca^{2+}, 1 mM Mg^{2+}, 1.1 mM SO_4^{2-}, 40 µM Fe^{3+}, 30 µM BO_3^{3-}, 5 µM Mn^{2+}, 4 µM Zn^{2+}, 0.75 µM Cu^{2+}, and 0.50 µM Mo (Sonneveld and Voogt, 2009).

During each growing season, air temperature and relative humidity outside and inside the greenhouse were recorded every hour using a data logger (mod. 608-H1, Testo s.p.a., Settimo M.se, Italy). A pyranometer (MS-410, EKO Instruments Co. Ltd, Tokyo, Japan) was used for solar radiation measurements. During S1, the average temperature outside the greenhouse ranged between 11.7 ± 0.3 °C (night) and 16.0 ± 0.4 °C (day). The average net solar radiation at noon was 579 W m^{-2}, with a day length ranging between 10 and 13 h. In S2, the temperature ranged between 20.2 ± 0.4 °C and 24.7 ± 0.5 °C, with an average noon net solar radiation of 712 W m^{-2} and a day length from 13 to 15 h. The average hourly air temperature inside the greenhouse was 15.3 ± 1.0 °C during the first growing cycle and 22.8 ± 0.8 °C during the second. The temperature ranged between an average minimum of 9.6 ± 0.3 °C and maximum of 25.3 ± 0.5 °C in the first cycle, and between an average minimum of 18.2 ± 0.3 °C and maximum 29.9 ± 0.5 °C in the second (Figure 1). The relative humidity was 75.0 ± 4.0% and 82.3 ± 2.6% on average during the first and second cycle, respectively. Inside the greenhouse, noon light intensity averaged 44,080±2,845 lux (range: 3,410–70,740 lux) in S1 and 54,933±3,789 lux (range: 5,805–74,021 lux) in S2, with fluctuations driven by cloudiness.

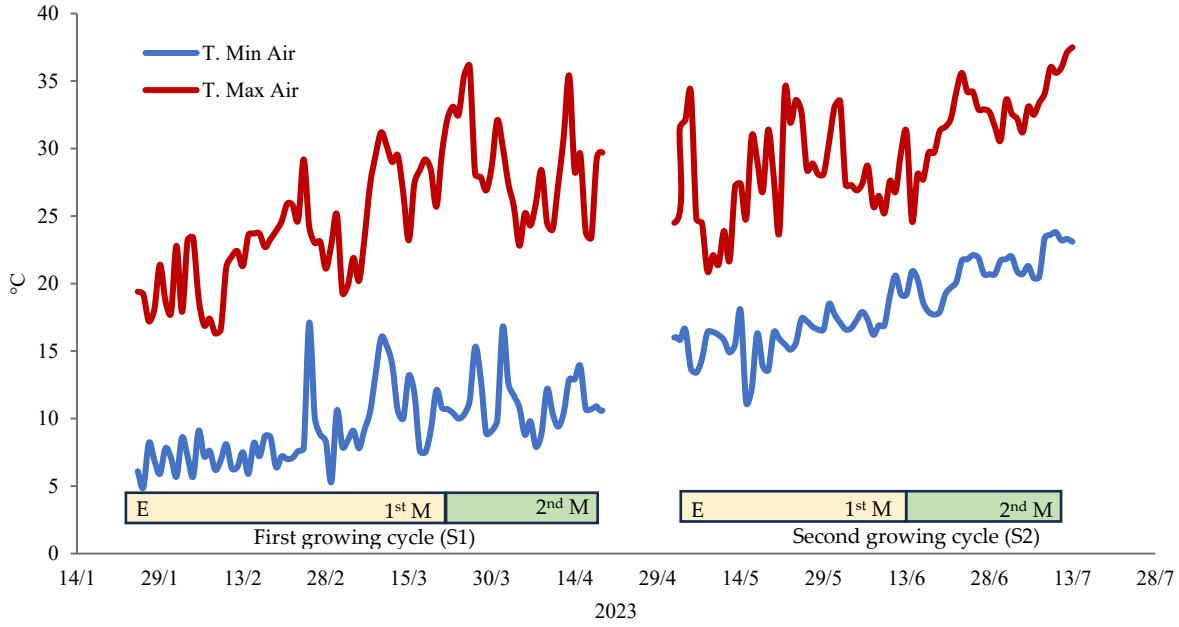

Figure 1. Daily average maximum and minimum temperatures of the air recorded inside the greenhouse with a data logger during leaf celery cultivation with a hydroponic ebb and flow system The two growing cycles (S1 – winter-spring, S2 – spring-summer) are reported in the horizontal bars (E = emergence; M = mowing).

The ebb and flow system was configured to deliver a 15-minute irrigation cycle once every 24 hours. At each sub-irrigation event, the polystyrene trays were weighed before irrigation and after the drainage of the excess nutrient solution. The weight differences were used to determine the volume of water and nutrient solutions consumed by the plants during the growth period. The values obtained

were used to calculate water use efficiency as WUE (g DW L^{-1} H_2O) = plant dry weight (g)/H_2O (L), and nitrogen use efficiency as NUE (g DWg^{-1} N) = plant dry weight (g)/supplied N (g) (supplied N = initial N content of the substrate + N supplied with sub fertigation) (Baligar and Fageria, 2015).
Two harvests were performed to assess the regrowth and yield capacity of leaf celery plants (n = 30 plants per replicate). Leaves were mowed at a height of 3–4 cm above the plant collar when the plants possessed approximately five true leaves, thereby allowing for subsequent regrowth (Figure 2).

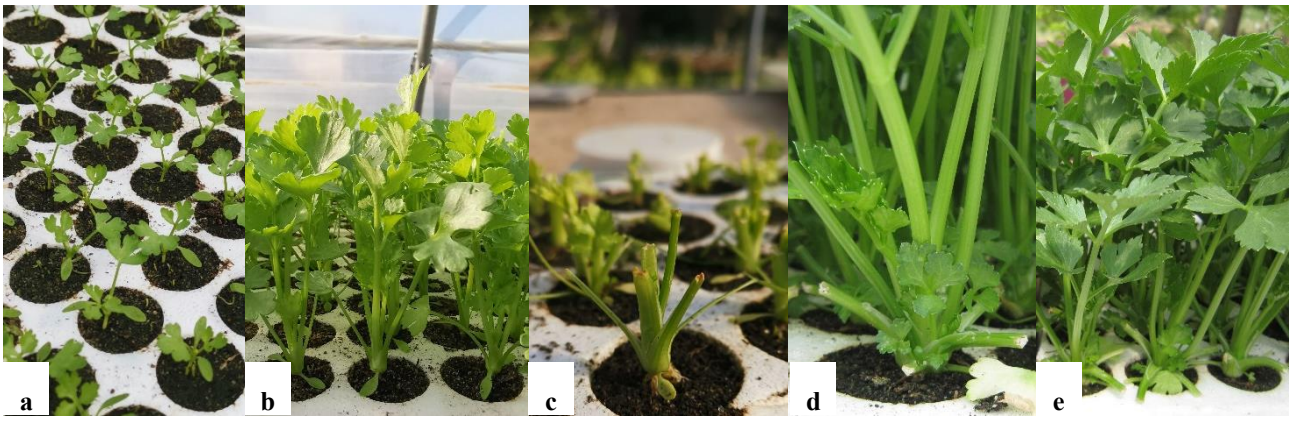

Figure 2. Leaf celery plants grown on polystyrene trays in an ebb-and-flow hydroponic system at different growth stages: a) plants at the two-leaf stage; b) plants before the first mowing; c) plants soon after the first mowing; d) plant regrowth; e) plants ready for the second mowing.

Before each mowing, another sample of 30 plants for each replicate and each treatment was collected to evaluate the following parameters: plant height, stem diameter, number of leaves, leaf area, leaf color, and fresh biomass accumulation. Subsequently, the leaves were separated from the stem and dried in an oven at 65 °C for 72 h to determine the dry biomass. Leaf color was measured using a colorimeter (CR-400, Minolta corporation, Ltd., Osaka, Japan), which recorded the CIELab parameters (L*, a*, and b*) for the calculation of chroma (C*) and hue angle (h°) as $C^* = (a^{*2} + b^{*2})^{1/2}$ and $h° = 180 + \arctan(b^*/a^*)$ (McGuire, 1992).
Leaf area was determined for each sample by taking digital images of the leaves on a black background with a red tile (2 cm x 2 cm) used as area reference. The digital images were taken using a digital camera (Canon EOS 300D, Canon Inc. Tokio, Japan) with a fixed source of light that were then processed with the Easy Leaf Area software to calculate the total leaf area (Easlon and Bloom, 2014). Specific leaf area (SLA, cm^2 g^{-1} DW) was calculated as the ratio of leaf area to leaf dry weight.

2.2. Chemical determination of baby leaf characteristics

Dried leaf samples (four replicated samples for each treatment) were used for the determination of biochemical compounds. Chemical determinations were restricted to HS and FS treatments, as plants receiving only water (0 NS) exhibited stunted growth and chlorosis, making them commercially unacceptable. The quantification of protein and crude fibre content in the samples was conducted in

accordance with the Kjeldahl and Weende methods (Palazzolo et al., 2012). Total sugar content was determined using the anthrone method as modified by Loewus (Loewus, 1952). Briefly, carbohydrate content was determined colorimetrically after reaction with anthrone reagent in concentrated sulfuric acid, and absorbance was measured at 630 nm with a spectrophotometer (UV mini-1240, Shimadzu, China) using glucose as the standard. The total content of potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), manganese (Mn), and zinc (Zn), was determined after mineralization by dry ashing procedure, modified from the standard protocol (Morand and Gullo, 1970). Briefly, 0.5 g of each sample was weighed into a porcelain crucible and placed in a muffles furnace at 550 °C for 8 hours. Subsequently, acid digestion (2% HNO_3) of the ashes was performed at 100 °C on a hotplate for 15 min. The content in macronutrients and heavy metals was determined by MP-AES (Agilent 4210 MP-AES, Milan, Italy). Phosphorus (P) was determined by first grinding the leaf samples into a fine powder. A 0.25 g portion of the material was then mineralized in porcelain crucibles using a muffle furnace at 550 °C for 8 hours. The ashes were later recovered through acid digestion using 10 mL of 1 M HCl on a hotplate at 100 °C for 15 minutes. The digested samples were recovered in 15 mL tubes and adjusted to a volume of 10 mL with MilliQ water. The amount of P was determined by the colorimetric method of Murphy and Riley (Murphy and Riley, 1962) using a spectrophotometer (UV mini-1240, Shimadzu, China). Vitamin A was extracted from the food matrix using an organic solvent, followed by chromatographic separation, identification, and quantification by high-performance liquid chromatography (HPLC) (Palazzolo et al., 2012). Thiamine (vitamin B1) was extracted using 0.1 N HCl, followed by oxidation to thiochrome, and subsequently quantified by HPLC with fluorometric detection (Palazzolo et al., 2012). Riboflavin (vitamin B2) was extracted in an autoclave with a solution of diluted H_2SO_4. After enzymatic treatment, the samples were analyzed by HPLC with fluorescence detection (AOAC, 1985). Niacin (vitamin B3) was extracted in an acidic solution at 121°C for 30 min and measured through a microbiological method (Palazzolo et al., 2012). The titrator strain was *Lactobacillus plantatarum* ATCC8014. The assay was performed in a liquid culture medium containing all essential growth factors except niacin. The presence of niacin in the sample caused a proportional growth of *L. plantarum* after 24 h of incubation at 37°C. The growth was evaluated using a turbidimeter (HI 98713, Hanna Instruments, Woonsocket, RI, USA) and was then compared with the values of a standard curve prepared in parallel to the test. Ascorbic acid (vitamin C) was removed before the measurement by incubation for 15 min with a special 'spatula ascorbate-oxidase' (Barros et al., 2007). The HPLC analyses were performed with a HPLC Ultimate 3000 (Thermo Scientific, Germany) system comprising UltiMate 3000 Series SD, BM and RS Pump Series, UltiMate 3000 Series ACC-3000 Autosampler Column Compartment and UltiMate 3000 Series VWD-3100 and VWD-3400 RS Variable Wavelength Detectors. Integration, data storage and processing were performed by Thermo Scientific Dionex Chromleon Chromatography Data System

Version 7.2.7. The determinations were made in isocratic conditions, at 25°C, using a mobile phase made of sulfuric acid 0.005 N. The flow rate of the mobile phase was 0.6 ml/min for all the chromatographic separations. The volume injected was 5 µl for either prepared sample or standard solution.

2.3. Sensory evaluation

A descriptive analysis was carried out during both growing seasons on the leaves from the first mowing of the two plant densities and HS and FS nutrient solutions according to Raffo et al. (Raffo et al., 2006) with some modifications. A trained panel of twelve people with expertise on vegetables received specific training on the products for terms generation and training on the evaluation scale. Leaf celery samples were evaluated in duplicate on an unstructured 120 mm line scale anchored 0–9. Two sets of the four treatments (615-HS; 615-FS; 947-HS; 947-FS) were evaluated in one session, with a ten-minute break between the sets. No reference sample was provided to the panelists. The order of presentation was randomized among panelists within each set. The sensory attributes taken into account considered the overall impression assessed by sense of sight (general aspect and color), the sensations perceived by means of olfactory organ (odor), the overall impression assessed by taste, smell and tactile sense such as texture (crunchiness, juiciness, and fibrousness), basic tastes (sweet, salty, bitter) and flavor (aromaticity, flavor persistency and astringency) and the overall rating. Individual portions for evaluations consisted in a sample of 20 g of leaves randomly selected from the leaves harvested. The samples were held at room temperature before evaluation and served in plastic trays. All assessments were conducted at the laboratory of Vegetable Analysis of the SAAF Department.

2.4. Minimally processing and cold storage of leaf celery baby leaves

Soon after the first mowing of both growing cycles, only the leaves from HS and FS treatments (Figure 3) were minimally processed as the water-only supplemented plants displayed stunted growth and chlorosis, thus precluding their commercial viability. Leaves were washed in tap water for 5 minutes two times and dried by manual centrifugation for 1 min with a handheld salad spinner. Twelve samples of approximately 50 grams for each treatment were packed in polyethylene (PE) bags, closed with a hot bar (Laica VT3112, Vicenza, Italy), and stored at 4 °C for 21 days. The overall quality, weight loss, and leaf color were evaluated soon after packaging and after 7, 14 and 21 days on three randomly selected samples for each treatment. An untrained panel consisting of twelve panelists (five males and seven females, aged 26 to 58) evaluated the overall quality (OQ) of the samples. Each individual assigned a score on a 5-point scale, where 5 corresponded to excellent freshly harvested quality (absence of off odors, defects, and decay), 3 represented the limit of marketability, and 1 indicated poor or unmarketable quality (presence of off odors, pronounced

discoloration, major defects, or decay) (Esposito et al., 2025). Weight loss (g 100 g^{-1} of initial fresh weight, FW) was determined by measuring the weight of each sample at packaging (day 0) and at each subsequent sampling point (days 7, 14, and 21). Leaf color was evaluated on ten leaves randomly selected for each sample using a Chroma Meter CR-400 (Minolta Corp., Ltd., Osaka, Japan) as reported above.

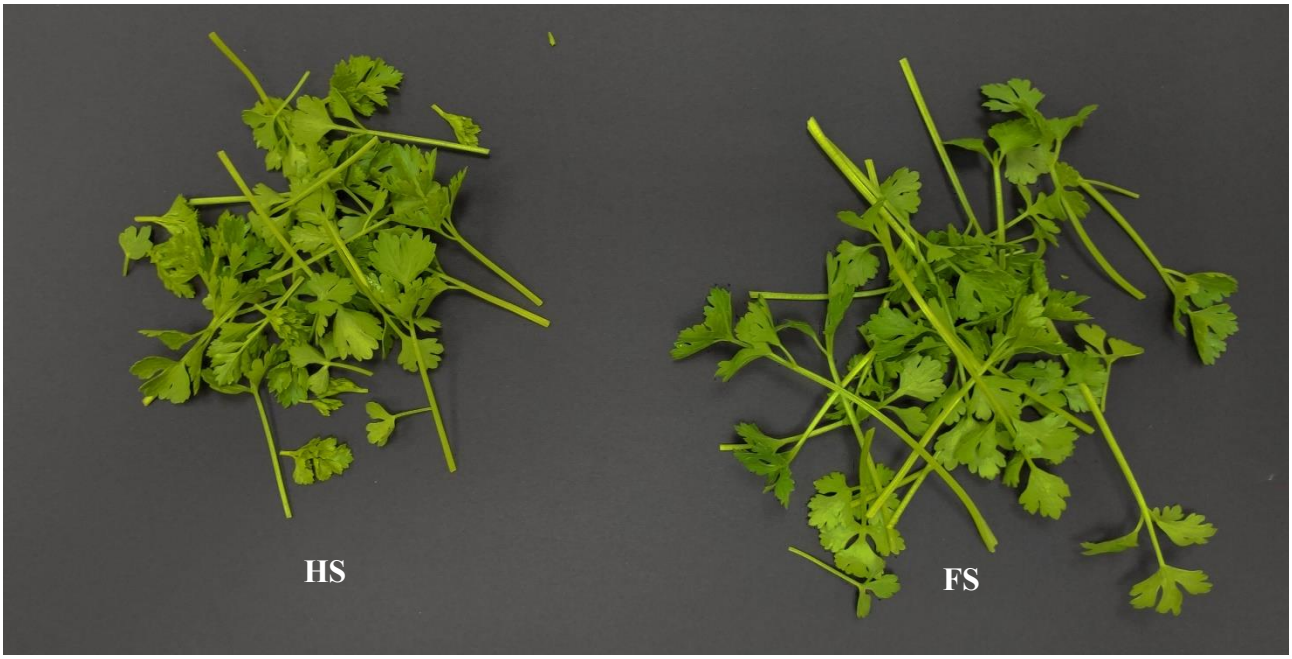

Figure 3. Leaves collected from the hydroponic ebb-and-flow cultivation system using half strength (HS) or full strength (FS) nutrient solutions. The leaves are shown soon after minimal processing, immediately before cold storage began in sealed polyethylene (PE) bags at 4 °C for 21 days.

2.5. Statistics

The experimental design consisted of four replicates of 30 plants each for every combination of plant density and nutrient solution concentration, arranged in a randomized complete block design. A three-way analysis of variance (ANOVA) was performed to evaluate the effects of growing period, plant density, and nutrient solution concentration on leaf celery at both the first and second mowing. Treatment differences and factor interaction were assessed using the least significant difference (LSD) test at $p \leq 0.05$.

A two-way ANOVA was carried out to evaluate the effects of growing periods and plant treatments on the sensory traits.

For the minimal processing trials, we used a completely randomized design with three replicates per treatment. A four-way ANOVA was then performed for each growing season to determine the effects of storage time, plant density, and nutrient solution concentration. Mean values were compared using the LSD test at $p \leq 0.05$ to identify significant differences and interactions among treatments.

3. Results

3.1. Yield and morpho-physiological characteristics of leaf celery plants

To take advantage of leaf celery's regrowth capacity after leaf harvesting, two mowings were carried out for each growing cycle. The first mowing occurred 63 and 54 days after sowing for the first and second trials, respectively. The control plants (irrigated with only water) exhibited stunted growth in both growing seasons, precluding a second harvest (Figure 4). Therefore, the second mowing was conducted only for plants receiving the HS and FS nutrient solutions. This took place 25 days and 29 days after the first mowing in the first and second cycles, respectively. The experimental factors exerted diverse effects on the morpho-physiological and leaf characteristics of leaf celery plants at the two harvests.

Figure 4. Differences in size and canopy structure of plants grown under varying plant densities (low density: 615 plants m^{-2}; high density: 947 plants m^{-2}) and nutrient solution strengths (0: water only; HS: half strength; FS: full strength). Pictures were taken before the first mowing of the winter-spring growing season. Plants were grown in an ebb-and-flow hydroponic system.

3.1.1 First mowing

Total plant fresh weight (FW) generally increased with increasing nutrient solution (NS) concentration, but the trend was modified by the growing season and plant density (Table 1). Leaf celery plants consistently exhibited lower fresh weight in the second growing cycle compared to the first. The lowest total biomass was recorded using 0 NS irrespective of plant density and growing season (0.22 g plant^{-1} on average in the first cycle and 0.05 g plant^{-1} on average in the second with no significant difference) (Table 1). The increase in NS concentration resulted in an increase in plant fresh biomass. This increase showed no significant difference due to plant density when using HS NS (1,70 g plant^{-1} on average) during the first season, and when using both HS (1,00 g plant^{-1} on average) and FS NS (1,86 g plant^{-1} on average) in the second growing cycle. The highest total biomass accumulation (3.46 g plant^{-1}) was observed with the combination of FS NS, a density of 615 plants m^{-2}, and the first growing season (Table 1). The stem fresh weight was affected only by plant density and NS concentration. It was higher at the lower plant density or with increasing the NS concentration (Table 1). Similar to the total fresh weight, leaf fresh biomass increased with increasing NS concentration but displayed different trends as a function of both the growing season and plant density. Overall, leaf fresh weight was lower during the second trial, when no difference was observed between the two plant densities tested for each NS concentration, with values ranging from 0.03 g plant^{-1} on average with 0 NS to 1.56 g plant^{-1} on average with FS NS. In the first season, a similar leaf fresh weight was recorded in plants of both plant densities fed with HS NS (1.49 g plant^{-1} on average). However, FS NS significantly increased this parameter, reaching 2.31 g plant^{-1} with 947 plant m^{-2} and even higher with 615 plant m^{-2} (3.12 g plant^{-1}) (Table 1).

Dry biomass accumulation was also variously affected by the combination of factors. Similar to fresh biomass results, total and leaf dry weight were lower in the second growing season (S2) compared to the first (S1). In S2, both parameters increased significantly with every step of NS concentration increase, showing no significant effect of plant density (total dry weight 164.2 mg plant^{-1} on average with FS NS). During S1, total and leaf dry biomass followed different accumulation trends as a function of plant density. A more marked increase was observed when plants were grown at the lower density (615 plant m^{-2}) (total dry weight: 323.3 mg plant^{-1} with FS NS and 214.7 mg plant^{-1} with HS NS) compared to the higher density (947 plant m^{-2}) (total dry weight: 323,3 mg plant^{-1} with FS NS and 169.0 mg plant^{-1} with HS NS) (Table 1). The dry biomass accumulated in the stem was higher during S1 and when adopting the lowest plant density and linearly increased up to an average of 24.0 mg plant^{-1} (FS NS) with increasing NS concentration.

The leaf dry matter percentage was highest in the plants grown at 615 plant m^{-2} (12.0%) and was lowest in both growing seasons using the FS nutrient solution (9.2%). Reducing the NS concentration,

leaf dry matter percentage increased with both HS and 0 NS (up to 19.3%) in the first cycle and only with 0 NS in the second cycle (11.3%) (Table 1).

Table 1. Effects of growing season (S1 – winter-spring, S2 – spring-summer), plant density (615, 947 plants m^{-2}) and nutrient solution (NS) concentration (0%, only water - 0; 50%, half strength - HS; 100%, full strength - FS) on biomass accumulation at the first mowing of *Apium graveolens L.* var. *secalinum* Alef. plants grown using an ebb-and-flow hydroponic system.

Source of Variance (First mowing)		Plant fresh weight (g FW)			Plant dry weight (mg DW)			Leaf dry matter (%)
		Total	Stem	Leaves	Total	Stem	Leaves	
Growing season (S)								
S1 – winter-spring		[z] 1.64	0.19	1.45	169.1	17.1a	151.9	13.4
S2 – spring-summer		0.97	0.16	0.81	87.6	12.7b	74.9	10.0
Plant density (PD)								
615 plants m^{-2}		1.43	0.20a	1.23	142.3	16.7a	125.6	12.0a
947 plants m^{-2}		1.18	0.15b	1.03	114.3	13.1b	101.3	11.4b
Nutrient solution (NS)								
0		0.13	0.04c	0.09	21.4	4.6c	16.8	15.3
HS		1.35	0.19b	1.16	142.5	16.1b	126.4	10.6
FS		2.44	0.30a	2.14	221.0	24.0a	197.0	9.2
S x PD								
S1	615	1.86	0.21	1.64	192.6	19.7	172.9	13.5
	947	1.43	0.16	1.27	145.6	14.6	131.0	13.3
S2	615	1.00	0.18	0.82	92.0	13.8	78.2	10.4
	947	0.94	0.14	0.79	83.1	11.6	71.6	9.6
S x NS								
S1	0	0.22	0.06	0.15	37.5	7.7	29.8	19.3a
	HS	1.70	0.20	1.49	191.8	19.3	172.5	11.5b
	FS	3.01	0.30	2.72	277.8	24.3	253.5	9.3c
S2	0	0.05	0.02	0.03	5.3	1.5	3.8	11.3b
	HS	1.00	0.17	0.84	93.2	12.8	80.3	9.7c
	FS	1.86	0.30	1.56	164.2	23.7	140.5	9.0c
PD x NS								
615	0	0.14	0.04	0.10	22.5	4.8	17.7	15.8
	HS	1.48	0.22	1.26	157.3	18.7	138.7	10.7
	FS	2.67	0.33	2.34	247.0	26.7	220.3	9.4
947	0	0.13	0.04	0.09	20.3	4.3	16.0	14.8
	HS	1.22	0.16	1.07	127.7	13.5	114.2	10.5
	FS	2.20	0.27	1.94	195.0	21.3	173.7	8.9
S x PD x NS								
S1	615 0	0.23e	0.07	0.16e	39.7e	8.3	31.3e	19.3
	615 HS	1.87c	0.23	1.64c	214.7b	22.3	192.3b	11.7
	615 FS	3.46a	0.34	3.12a	323.3a	28.3	295.0a	9.5
	947 0	0.21e	0.06	0.14e	35.3e	7.0	28.3e	19.4
	947 HS	1.52c	0.17	1.35c	169.0c	16.3	152.7c	11.3
	947 FS	2.57b	0.26	2.31b	232.3b	20.3	212.0b	9.2
S2	615 0	0.05e	0.02	0.03e	5.3f	1.3	4.0e	12.2
	615 HS	1.08d	0.20	0.89d	100.0d	15.0	85.0d	9.6
	615 FS	1.87c	0.32	1.55c	170.7c	25.0	145.7c	9.4
	947 0	0.05e	0.01	0.03e	5.3f	1.7	3.7e	10.3

947	HS	0.92d	0.14	0.78d	86.3d	10.7	75.7d	9.7
	FS	1.84c	0.28	1.57c	157.7c	22.3	135.3c	8.7
Significance [x]								
Growing season		***	ns	***	***	***	***	***
Plant density		***	**	**	***	**	***	*
Nutrient solution		***	***	***	***	***	***	***
S x PD		**	ns	**	***	ns	***	ns
S x NS		**	ns	***	***	ns	***	***
PD x NS		*	ns	*	***	ns	**	ns
S x PD x NS		*	ns	**	*	ns	**	ns

[z] Each value is the mean of 4 replicated samples of 30 plants each. For each factor, values in a column followed by the same letter are not significantly different, according to the LSD test. [x] Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$.

Leaf celery plants from the first growing period (S1) had significantly more leaves than those from the second (S2) (an average of 6.2 and 4.4 leaves, respectively). Additionally, using the lower plant density resulted in a slightly, but significantly, higher leaf count compared to the higher plant density. Furthermore, leaf number increased quadratically with rising nutrient solution concentration, ranging from 3.94 (0 NS) to 6.23 (FS NS) (Table 2).

The length of leaf and petiole was differently affected by nutrient solution concentration across the two growing seasons. Both parameters increased with increasing the NS concentration in both growing seasons, but with a steeper increasing trend in S2 compared to S1. Consequently, the highest leaf and petiole lengths were recorded in S2 (17.3 cm and 12.0 cm, respectively), surpassing the maximum values in S1 (16.1 cm and 10.8 cm, respectively) (Table 2).

Total and average leaf area showed no difference between plant densities when using 0 NS or HS NS. However, when plants were supplied with FS NS, the increase in leaf area was significantly greater when using 615 plant m^{-2} (64.9 cm^2 $plant^{-1}$ and 10.5 cm^2 $leaf^{-1}$ for total and average leaf area, respectively) compared to 947 plant m^{-2} (53.8 cm^2 $plant^{-1}$ and 9.0 cm^2 $leaf^{-1}$ for total and average leaf area, respectively) (Table 2). Moreover, a decreasing trend in average leaf area was observed in both seasons with increasing plant density, but this reduction was greater in S1 (-13.5%) than in S2 (-9.5%).

The specific leaf area (SLA), which is inversely related to leaf thickness, recorded lower values in S1 compared to S2, with no significant effect of NS (an average of 204.7 cm^2 g DW^{-1}). The highest SLA value was observed using HS NS in S2 (Table 2).

The different environmental conditions resulting from growing seasons and plant densities did not strongly influence the color characteristics of the leaf celery leaves (Table 2). The color lightness (L*) showed a slight decrease in S1 with increasing NS concentration (down to 47.9 with FS NS) and recorded the highest value using HS NS in S2 (52.3). The lowest chroma (color intensity/saturation) values were calculated in both seasons in the leaves of plants fed with FS NS (34.1 on average). During S1, chroma gradually increased up to 42.2 (0 NS) whereas it reached 39.0 with HS NS in S2, with no further significant reduction when supplementing 0 NS (Table 2). Hue angle was significantly

affected solely by nutrient availability. The 0 NS determined the lowest hue value (115.2°, on average) indicating a more yellowish-green color. Plants nourished with FS NS had leaves that recorded the highest hue values (121.9°, on average), reflecting a greener color (Table 2).

Table 2. Effects of growing season (S1 – winter-spring, S2 – spring-summer), plant density (615, 947 plants m^{-2}) and nutrient solution (NS) concentration (0%, only water - 0; 50%, half strength - HS; 100%, full strength - FS) on leaf characteristics at the first mowing of *Apium graveolens* L. var. *secalinum* Alef. plants grown using an ebb-and-flow hydroponic system.

Source of Variance (First mowing)		Leaf number	Leaf length (cm)	Petiole length (cm)	Leaf area (cm^2 $plant^{-1}$)	Leaf area (cm^2 $leaf^{-1}$)	SLA (cm^2 g DW^{-1})	L*	Chroma	Hue°
Growing season (S)										
S1 – winter-spring		[z] 6.2a	10.2	6.5	32.5	4.8	204.7	50.1	37.5	119.3
S2 – spring-summer		4.4b	11.2	8.0	33.3	6.6	430.5	49.7	37.6	118.8
Plant density (PD)										
615 plants m^{-2}		5.4a	10.6	7.1	35.6	6.0	312.6	50.2	37.6	118.8
947 plants m^{-2}		5.2b	10.9	7.4	30.2	5.4	322.5	49.6	37.5	119.3
Nutrient solution (NS)										
0		3.9c	3.0	1.5	3.5	0.8	284.1	49.3	40.5	115.1c
HS		5.7b	12.4	8.8	35.8	6.5	337.1	51.8	38.0	120.1b
FS		6.2a	16.7	11.4	59.4	9.8	331.5	48.5	34.1	121.9a
S x PD										
S1	615	6.4	10.2	6.5	35.9	5.1b	201.4	50.1	36.9b	119.1
	947	6.0	10.3	6.5	29.1	4.4b	207.9	50.0	38.0a	119.5
S2	615	4.4	10.9	7.7	35.3	7.0a	423.9	50.2	38.3a	118.6
	947	4.3	11.4	8.3	31.3	6.3a	437.2	49.1	36.9b	119.0
S x NS										
S1	0	4.8	3.3e	1.1d	5.6	1.2	186.6c	51.0ab	42.2a	114.9
	HS	6.7	11.2d	7.6c	33.6	5.0	195.6c	51.3ab	37.0bc	120.6
	FS	7.2	16.1b	10.8b	58.3	8.1	231.8c	47.9b	33.2c	122.3
S2	0	3.1	2.6e	1.9d	1.4	0.5	381.7b	47.7b	38.9b	115.4
	HS	4.7	13.7c	10.1b	38.0	8.0	478.5a	52.3a	39.0b	119.6
	FS	5.3	17.3a	12.0a	60.4	11.4	431.3ab	49.1b	35.0c	121.4
PD x NS										
615	0	3.9	2.9	1.5	3.7d	0.9d	267.8	49.8	40.3	114.5
	HS	5.9	12.4	8.6	38.2c	6.7c	336.8	51.7	38.1	120.2
	FS	6.3	16.4	11.2	64.9a	10.5a	333.3	49.0	34.5	121.8
947	0	4.0	3.0	1.6	3.3d	0.8d	300.5	48.8	40.8	115.8
	HS	5.5	12.5	9.0	33.4c	6.3c	337.3	51.9	37.8	120.0
	FS	6.1	17.1	11.7	53.8b	9.0b	329.7	48.0	33.8	121.9
Significance [x]										
Growing season		***	***	***	ns	***	***	ns	ns	ns
Plant density		*	ns	ns	***	***	ns	ns	ns	ns
Nutrient solution		***	***	***	***	**	**	***	***	***
S x PD		ns	ns	ns	ns	***	ns	ns	*	ns
S x NS		ns	***	**	ns	ns	*	***	***	ns
PD x NS		ns	ns	ns	**	*	ns	ns	ns	ns

S x PD x NS	ns	ns	ns	ns	ns	ns	ns	ns	ns

[z] Each value is the mean of 4 replicated samples of 30 plants each. For each factor, values in a column followed by the same letter are not significantly different, according to the LSD test. [x] Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$.

3.1.2 Second mowing

Total plant fresh weight was significantly higher in S1 than in S2 (Table 3). During both seasons, the plant biomass decreased with increasing plant density, showing a similar average reduction (37.9%) for both densities. NS concentration significantly affected total FW biomass accumulation, with variations depending on both the growing season and plant density (Table 3). The effect of increasing nutrient content in the NS was greater during S1 (+49.3%) or when using 615 plant m^{-2} (+46.6%) than during S2 (+33.0%) or when using 947 plant m^{-2} (+39.4%). Despite these different response rates, no significant differences were observed between the 615-FS and 947-FS treatments (3.31 g $plant^{-1}$ on average).

Stem FW was higher in the first cycle compared to the second (1.09 and 0.45 g $plant^{-1}$ on average, respectively). In S1, it increased with increasing NS concentration and decreased with increasing plant density. Conversely, in S2, NS concentration had no significant effect, and stem fresh weight was only lowered using the higher density.

Leaf fresh weight constituted 78.5% of the total plant fresh biomass and thus showed trends superimposable to those observed for total fresh weight with respect to the experimental factors.

The same trends were found in leaf and total dry weight accumulation. This consistency was confirmed by the non-significant variations in the leaf dry matter percentage, which showed only a slight but significant increase from 9.7% (S1) to 10.4% (S2) (Table 3).

Table 3. Effects of growing season (S1 – winter-spring, S2 – spring-summer), plant density (615, 947 plants m^{-2}) and nutrient solution (NS) concentration (0%, only water - 0; 50%, half strength - HS; 100%, full strength - FS) on biomass accumulation at the second mowing of *Apium graveolens L.* var. *secalinum* Alef. plants grown using an ebb-and-flow hydroponic system.

Source of Variance	Plant fresh weight (g FW)			Plant dry weight (mg DW)			Leaf dry matter (%)
(Second mowing)	Total	Stem	Leaves	Total	Stem	Leaves	
Growing season (S)							
S1 – winter-spring	[z] 4.73	1.09	3.64	437.3	86.5	350.8	9.7b
S2 – spring-summer	2.27	0.45	1.82	232.0	44.6	187.4	10.4a
Plant density (PD)							
615 plants m^{-2}	4.32	0.94	3.38	406.3	78.5	327.8	9.8
947 plants m^{-2}	2.68	0.61	2.08	263.0	52.6	210.4	10.3
Nutrient solution (NS)							
HS	2.87	0.62	2.25	280.3	53.8	226.5	10.2
FS	4.13	0.93	3.20	389.0	77.3	311.7	9.9
S x PD							

S1	615		5.83a	1.32	4.52a	533.8a	103.8	430.0a	9.6
	947		3.63b	0.87	2.76b	340.7b	69.2	271.5b	9.9
S2	615		2.80c	0.56	2.24c	278.7c	53.2	225.5c	10.1
	947		1.73d	0.34	1.40d	185.4d	36.1	149.3d	10.7
S x NS									
S1		HS	3.80b	0.84	2.96b	361.0b	68.0	293.0b	10.0
		FS	5.67a	1.35	4.31a	513.5a	105.0	408.5a	9.5
S2		HS	1.95d	0.40	1.55d	199.7d	39.7	160.0d	10.4
		FS	2.59c	0.50	2.09c	264.4c	49.6	214.8c	10.4
PD x NS									
615		HS	3.50b	0.75	2.76b	337.0b	64.5	272.5b	10.0
		FS	5.13a	1.13	4.00a	475.5a	92.5	383.0a	9.7
947		HS	2.24c	0.49	1.75c	223.7c	43.2	180.5d	10.4
		FS	3.12b	0.72	2.40b	302.4b	62.1	240.3c	10.2
S x PD x NS									
S1	615	HS	4.64	0.99b	3.65	436.0	80.7b	355.3	9.8
		FS	7.03	1.65a	5.38	631.7	127.0a	504.7	9.4
	947	HS	2.95	0.68c	2.27	286.0	55.3c	230.7	10.2
		FS	4.31	1.06b	3.25	395.3	83.0b	312.3	9.6
S2	615	HS	2.36	0.51d	1.86	238.0	48.3cd	189.7	10.2
		FS	3.24	0.62cd	2.62	319.3	58.0c	261.3	10.0
	947	HS	1.53	0.30e	1.23	161.3	31.0d	130.3	10.6
		FS	1.94	0.38de	1.56	209.5	41.2d	168.3	10.8
Significance [x]									
Growing season			***	***	***	***	***	***	*
Plant density			***	***	***	***	***	***	ns
Nutrient solution			***	***	***	***	***	***	ns
S x PD			***	**	***	***	**	***	ns
S x NS			***	***	***	***	***	**	ns
PD x NS			**	*	**	**	ns	**	ns
S x PD x NS			ns	*	ns	ns	*	ns	ns

[z] Each value is the mean of 4 replicated samples of 30 plants each. For each factor, values in a column followed by the same letter are not significantly different, according to the LSD test. [x] Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$.

The lower plant density (615 plants m^{-2}) slightly increased the leaf number compared to the higher plant density (947 plants m^{-2}), ranging from 4.4 to 5.1 leaves per plant, respectively. (Table 4).

The leaf and petiole length were significantly affected by the experimental factors. The highest values were recorded during the first growing season (17.1 cm and 10.3 cm, respectively), with the lowest plant density (17.7 cm and 10.6 cm, respectively), and when supplying plants with FS nutrient solution (17.8 cm and 10.8 cm, respectively).

The total leaf area decreased during S2 compared to S1 and with increasing plant density (Table 4). Increased density reduced total leaf area by 28.8% in S1 and 27.0% in S2. The increase in NS

concentration led to a significant increase in total leaf area by 31.3% in S1 (74.1 cm^2 $plant^{-1}$) or by 34.0% when using 615 plants m^{-2} (73.7 cm^2 $plant^{-1}$). However, the NS effect was less pronounced and non-significant in S2 (45.3 cm^2 $plant^{-1}$ on average for HS NS and FS NS) and with 947 plants m^{-2} (46.3 cm^2 $plant^{-1}$ on average for HS NS and FS NS). A similar effect of NS concentration was found on the average leaf area, which significantly increased with the supply of FS NS in S1 (15.7 cm^2 $leaf^{-1}$ on average) and with the lower plant density (14.7 cm^2 $plant^{-1}$ on average). Furthermore, in S1, the leaves were generally more expanded compared to S2 (+39.9% on average) (Table 4).

The specific leaf area (SLA) was significantly affected by the experimental factors (Table 4). It increased by 23.4% in S2 compared to S1 (189.8 cm^2 g DW^{-1}), by 12.3% at the highest density compared to the lowest density (206.1 cm^2 g DW^{-1}), and by 11.4% with the supply of HS NS compared to FS NS (207.0 cm^2 g DW^{-1}).

The increase of plant density determined a significantly higher L* value only in S2 (48.2) and when combined with HS NS (49.2) (Table 4). The other combinations of plant densities and NS showed no significant differences, with an average L* value of 46.0. A chroma value of 30.6 on average was obtained in S2 growing plants at 947 plants m^{-2}. This was significantly higher than the other combinations of growing seasons and plant densities (27.8 on average). The lowest leaf chroma values were recorded in plants supplied with FS NS irrespective of plant density (26.9 on average). Reducing nutrient availability (HS NS) significantly increased chroma, and this effect was significantly higher at the greater plant density (28.7 and 31.6 on average with 615 and 947 plants m^{-2}, respectively). Plant density affected the hue angle of the leaves in different ways depending on the growing season and nutrient solution concentration. A higher hue angle was recorded in S2 with 615 plants m^{-2} (127.8° on average) compared to the other season and plant density combinations (124.1° on average) (Table 4). Conversely, supplying plants grown at 947 plants m^{-2} with HS NS significantly reduced the leaf hue angle (122.9° on average) compared to the other plant density and NS combinations (125.8° on average).

Table 4. Effects of growing season (S1 – winter-spring, S2 – spring-summer), plant density (615, 947 plants m^{-2}) and nutrient solution (NS) concentration (0%, only water - 0; 50%, half strength - HS; 100%, full strength - FS) on leaf characteristics at the second mowing of *Apium graveolens L.* var. *secalinum* Alef. plants grown using an ebb-and-flow hydroponic system.

Source of Variance (Second mowing)	Leaf number	Leaf length (cm)	Petiole length (cm)	Leaf area (cm^2 $plant^{-1}$)	Leaf area (cm^2 $leaf^{-1}$)	SLA (cm^2 g DW^{-1})	L*	Chroma	Hue°
Growing season (S)									
S1 – winter-spring	[z] 4.9	17.1a	10.3a	65.3	13.5	189.8b	46.5	27.9	124.0
S2 – spring-summer	4.7	15.4b	9.6b	45.3	9.6	247.8a	47.0	29.2	126.1
Plant density (PD)									
615 plants m^{-2}	5.1a	17.7a	10.6a	64.3	12.6	206.1b	46.0	27.6	126.1
947 plants m^{-2}	4.4b	14.9b	9.3b	46.3	10.4	231.5a	47.6	29.5	124.0
Nutrient solution (NS)									
HS	4.8	14.8b	9.1b	49.1	10.2	230.5a	47.8	30.2	124.4
FS	4.7	17.8a	10.8a	61.5	12.9	207.0b	45.7	26.9	125.8
S x PD									
S1 615	5.1	18.6	11.0	76.3a	15.0	177.7	46.2b	27.4b	124.4b
S1 947	4.6	15.6	9.6	54.3b	11.9	201.8	46.9ab	28.3b	123.7b
S2 615	5.1	16.8	10.2	52.4b	10.3	234.4	45.8b	27.7b	127.8a
S2 947	4.3	14.1	9.0	38.3c	9.0	261.2	48.2a	30.6a	124.3b
S x NS									
S1 HS	5.0	15.3	9.2	56.5b	11.2b	196.4	47.6	29.6	123.6
S1 FS	4.7	19.0	11.3	74.1a	15.7a	183.1	45.4	26.2	124.5
S2 HS	4.6	14.3	8.9	41.8c	9.1c	264.7	48.0	30.7	125.1
S2 FS	4.8	16.6	10.3	48.8c	10.1bc	230.9	46.0	27.6	127.0
PD x NS									
615 HS	5.2	15.9	9.6	55.0b	10.6b	212.3	46.5b	28.7b	125.8a
615 FS	5.1	19.5	11.6	73.7a	14.7a	199.9	45.5b	26.4c	126.4a
947 HS	4.5	13.7	8.5	43.3c	9.7b	248.8	49.1a	31.6a	122.9b
947 FS	4.4	16.1	10.0	49.3bc	11.2b	214.2	46.0b	27.3c	125.1a
Significance [x]									
Growing season	ns	**	*	***	***	***	ns	***	***
Plant density	***	***	***	***	***	**	***	***	***
Nutrient solution	ns	***	***	***	***	**	***	***	***
S x PD	ns	ns	ns	*	ns	ns	*	**	***
S x NS	ns	ns	ns	**	**	ns	ns	ns	ns
PD x NS	ns	ns	ns	**	*	ns	**	**	*
S x PD x NS	ns	ns	ns	ns	ns	ns	ns	ns	ns

[z] Each value is the mean of 4 replicated samples of 30 plants each. For each factor, values in a column followed by the same letter are not significantly different, according to the LSD test. [x] Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$.

3.1.3 Baby leaf yield

The yield of baby leaves obtained from the first mowing was significantly affected by the growing season, plant density and the nutrient solution (NS) concentration (Table 5). Yield was highest during the first growing season (S1) and at the highest plant density, but the response varied across growing seasons and plant densities relative to the nutrient solution treatments supplemented. The lowest yield was recorded when only water (control-0 NS) was supplied, irrespective of growing season and plant density (0.07 kg m^{-2} on average). Overall, increasing the NS concentration linearly increased yield, although the slope of the increase varied by season and plant density. The highest yield in the first mowing was recorded during S1 using the FS NS (2.05 kg m^{-2} on average), which was 68.6% higher than the yield from the same NS in S2 (1.22 kg m^{-2} on average) (Figure 5). This latter value did not significantly differ from the yield obtained with the HS NS in S1 (1.14 kg m^{-2} on average). An ascending trend due to the NS concentration was also observed for both plant densities, but the yield increase was greater in plants grown at the highest plant density (+27.7% with FS and +29.9% with HS compared to the yield recorded using 615 plants m^{-2}, 0.78 and 1.44 kg m^{-2}, respectively) (Table 5). The baby leaf yield from the second mowing was higher than the first, and it was significantly affected only by the growing season and nutrient solution concentration. As observed for the first mowing, the highest yield was recorded during the first growing cycle when plants were supplemented with FS NS (3.19 kg m^{-2}) (Figure 5). In the same growing cycle, the use of HS NS led to a 31.2% yield reduction. Compared to the first cycle, yields obtained with the second mowing of the second cycle were almost halved, showing drops of 51.6% and 47.5% for FS and HS NS, respectively (Figure 5).

The total baby leaf yield mirrored the trends observed in the first and second mowings (Figure 5). A slight but significant increase in yield was found with increasing plant density (from 2.14 kg m^{-2} at 615 plants m^{-2} to 2.29 kg m^{-2} at 947 plants m^{-2}, on average). Increased nutrient supply also had a positive effect, though yield responses varied across growing seasons (Figure 5). The highest yield (5.25 kg m^{-2}) was recorded in the first growing cycle when the full-strength nutrient solution (NS) was applied (Figure 5). However, the total yield dropped by 36.3% with the half-strength (HS) NS in the first cycle. This reduction was even more pronounced during the second trial, which saw a yield decrease of 47.3% with full-strength (FS) NS and 65.7% with HS NS when compared to the highest recorded yield.

Table 5. Effects of growing season (S1 – winter-spring, S2 – spring-summer) plant density (615, 947 plants m^{-2}) and nutrient solution (NS) concentration (0%, only water - 0; 50%, half strength - HS; 100%, full strength - FS) on baby leaf yield of *Apium graveolens* L. var. *secalinum* Alef. grown using an ebb-and-flow hydroponic system.

Source of Variance		Baby leaf yield (kg m^{-2})		
		1st mowing	2nd mowing	Total
Growing season (S)				
S1 – winter-spring		[z] 1.11	2.70	2.90
S2 – spring-summer		0.63	1.35	1.53
Plant density (PD)				
615 plants m^{-2}		0.76	2.08	2.14b
947 plants m^{-2}		0.98	1.97	2.29a
Nutrient solution (NS)				
0		0.07		0.07
HS		0.89	1.68	2.57
FS		1.64	2.37	4.00
S x NS				
	0	0.12d		0.12e
S1	HS	1.14b	2.20b	3.34b
	FS	2.05a	3.19a	5.25a
	0	0.03d		0.03e
S2	HS	0.64c	1.16d	1.80d
	FS	1.22b	1.55c	2.76c
PD x NS				
	0	0.06e		0.06
615	HS	0.78d	1.70	2.47
	FS	1.44b	2.46	3.90
	0	0.09e		0.09
947	HS	1.01c	1.66	2.67
	FS	1.84a	2.28	4.11
Significance [x]				
Growing season		***	***	***
Plant density		***	ns	*
Nutrient solution		***	***	***
S x PD		ns	ns	ns
S x NS		***	***	***
PD x NS		***	ns	ns
S x PD x NS		ns	ns	ns

[z] Each value is the mean of 4 replicated samples of 30 plants each. For each factor, values in a column followed by the same letter are not significantly different, according to the LSD test. [x] Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$.

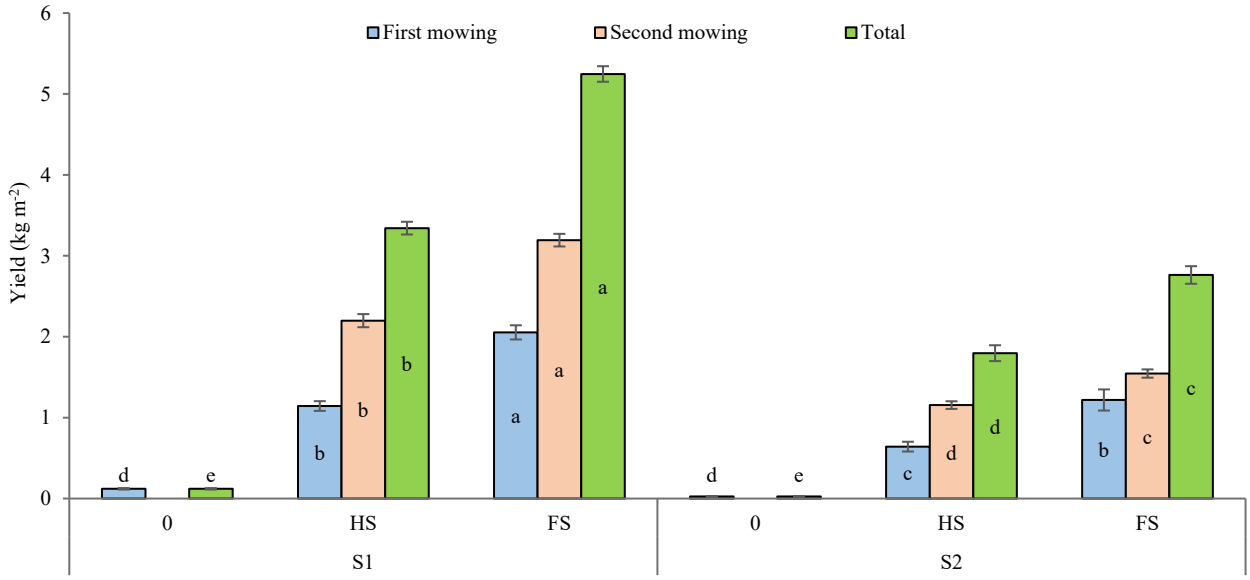

Figure 5. Effects of growing season (S1 – winter-spring, S2 – spring-summer) and nutrient solution concentration (0%, only water - 0; 50%, half strength - HS; 100%, full strength - FS) on the baby leaves yield of *Apium graveolens* L. var. *secalinum* Alef. grown using an ebb-and-flow hydroponic system (black bars represent standard errors of the means; bars of the same color with different letters are significantly different at $p \leq 0.05$ according to the LSD test).

3.1.4 Water and nitrogen use efficiency

The water use efficiency (WUE) for the first mowing, second mowing, and total harvest was mainly influenced by the growing season and NS concentration (Table 6). As expected, WUE was higher during the winter-spring period (S1) than the spring-summer period (S2). The average WUE in S1 was 1.5 g DW L^{-1} H_2O for the first mowing and 5.9 g DW L^{-1} H_2O for the second mowing. These values represent increases of 38.5% and 77.1%, respectively, compared to S2. The highest WUE was calculated in plants supplied with FS NS, averaging 2.1 g DW L^{-1} H_2O for the first mowing and 5.3 g DW L^{-1} H_2O for the second mowing. Conversely, the application of HS NS reduced WUE by 31.6% in the first mowing and 24.3% in the second mowing. Furthermore, the WUE of the first mowing also increased with higher plant density (from 1.2 to 1.4 g DW L^{-1} H_2O). Considering both mowings, the WUE in S2 increased significantly with increasing NS concentration, rising from 0.1 g DW L^{-1} H_2O (0 NS) to 2.7 g DW L^{-1} H_2O (FS NS). This latter value was similar to the WUE recorded with HS NS in S1. In S1, the use of FS NS was even more effective, further increasing WUE up to 3.9 g DW g^{-1} N (Figure 6).

Table 6. Effects of growing season (S1 – winter-spring, S2 – spring-summer), plant density (615, 947 plants m^{-2}) and nutrient solution (NS) concentration (0%, only water - 0; 50%, half strength - HS; 100%, full strength - FS) on water use efficiency (WUE) of *Apium graveolens* L. var. *secalinum* Alef. plants grown using an ebb-and-flow hydroponic system.

Source of Variance	WUE (g DW L^{-1} H_2O)		
	1st mowing	2nd mowing	Total
Growing season (S)			
S1 – winter-spring	[z] 1.5a	5.9a	2.4
S2 – spring-summer	1.1b	3.3b	1.6
Plant density (PD)			
615 plants m^{-2}	1.2b	4.7	2.0
947 plants m^{-2}	1.4a	4.6	2.0
Nutrient solution (NS)			
0	0.2c		0.2
HS	1.5b	4.0b	2.5
FS	2.1a	5.3a	3.3
Significance [x]			
Growing season	***	***	***
Plant density	***	ns	ns
Nutrient solution	***	***	***
S x PD	ns	ns	ns
S x NS	ns	ns	***
PD x NS	ns	ns	ns
S x PD x NS	ns	ns	ns

[z] Each value is the mean of 4 replicated samples of 30 plants each. For each factor, values in a column followed by the same letter are not significantly different, according to the LSD test. [x] Significance: ns = not significant; *** significant at $p < 0.001$.

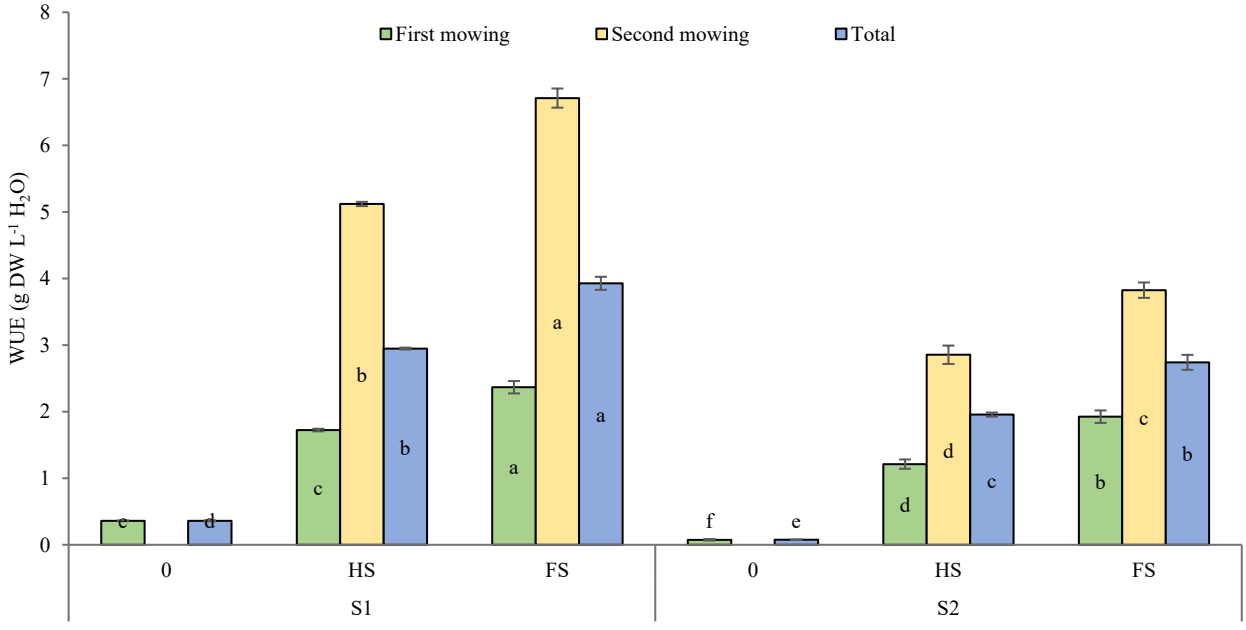

Figure 6. Effects of growing season (S1 – winter-spring, S2 – spring-summer) and nutrient solution concentration (0%, only water - 0; 50%, half strength - HS; 100%, full strength - FS) on the water use efficiency (WUE) of *Apium graveolens* L. var. *secalinum* Alef. plants grown using an ebb-and-flow hydroponic system (black bars represent standard errors of the means; bars of the same color with different letters are significantly different at $p \leq 0.05$ according to the LSD test).

In the first mowing, the Nitrogen Use efficiency (NUE) of leaf celery was significantly affected by growing season, plant density and nutrient solution concentration. In S1, NUE was higher at greater plant density and followed a quadratic trend with increasing nutrient solution concentration. The highest NUE value (24.8 g DW g^{-1} N) was achieved using HS NS combined with the 947 plants m^{-2} density. In S2, the lowest NUE was recorded with the 0 NS treatment (1.8 g DW g^{-1} N, on average across both plant densities). Increasing the NS concentration to half strength NS significantly improved NUE, with no further significant increase observed when using FS NS (Figure 7). Overall, for S2, the NUE averaged 12.2 g DW g^{-1} N using 615 plants m^{-2} and was significantly higher using 947 plants m^{-2} (15.9 g DW g^{-1} N). The second mowing recorded a greater NUE in S1 with HS NS (51.7 g DW g^{-1} N) compared to FS NS (42.8 g DW g^{-1} N), while there was no significant effect of NS concentration in S2 (29.9 g DW g^{-1} N, on average) (Figure 7). Considering both mowings, the total NUE was generally lower in S2 and showed no difference due to plant density. The lowest total NUE was found with 0 NS (1.8 g DW g^{-1} N, on average) and showed similar values with HS NS or FS NS (21.1 g DW g^{-1} N, on average) in S2. In S1, supplying 0 NS resulted in a NUE of 11.7 g DW g^{-1} N using 615 plants m^{-2}, which increased up to 16.4 using 947 plants m^{-2}. In this growing season (S1), NUE increased with HS NS up to the highest values (35.6 g DW g^{-1} N, on average) then significantly declined with FS NS (29.2 g DW g^{-1} N, on average) with no significant difference ascribed to plant density (Figure 7).

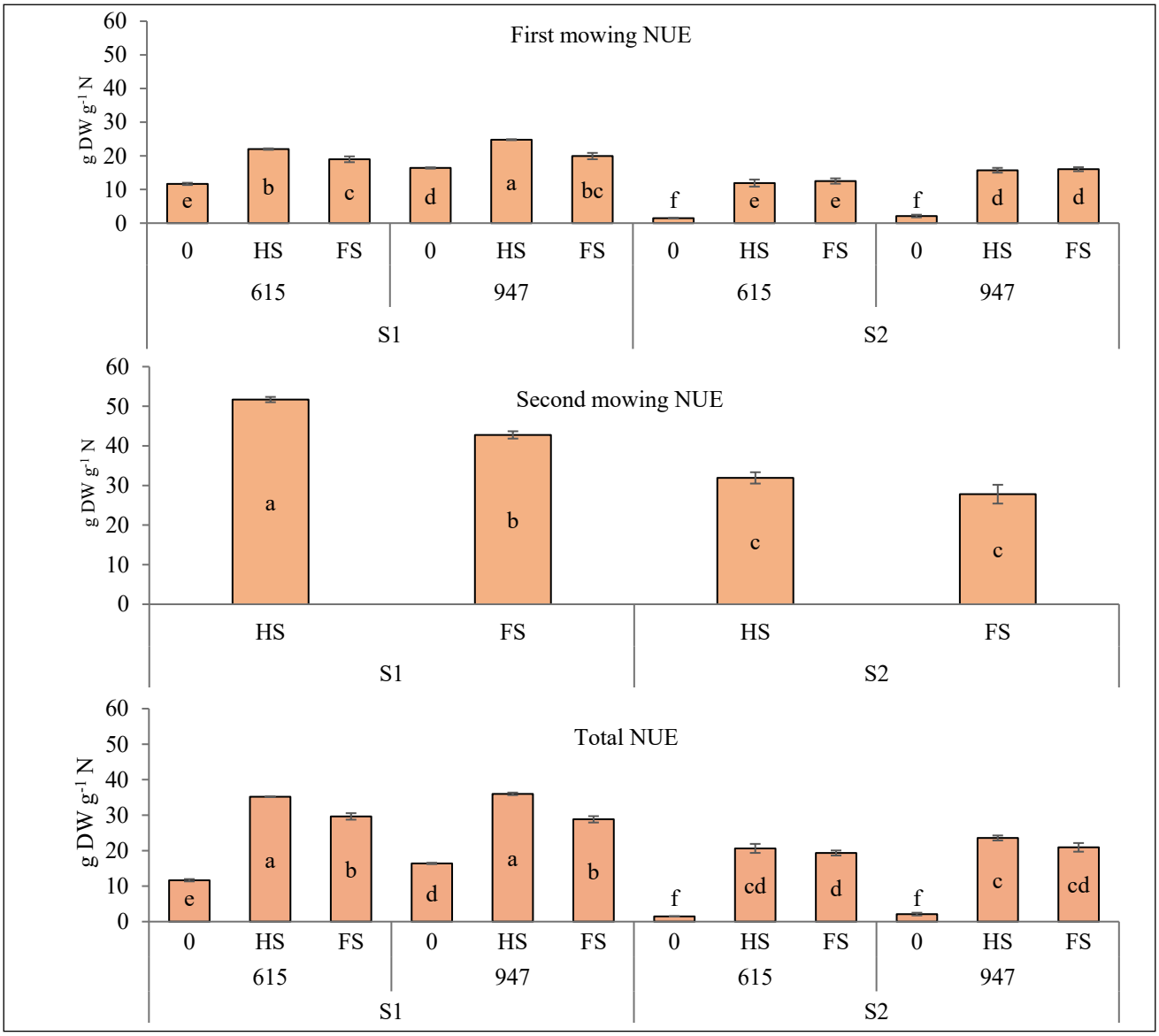

Figure 7. Effects of growing season (S1 – winter-spring, S2 – spring-summer) and nutrient solution concentration (0%, only water - 0; 50%, half strength - HS; 100%, full strength - FS) on the nitrogen use efficiency (NUE) of *Apium graveolens* L. var. *secalinum* Alef. plants grown using an ebb-and-flow hydroponic system (black bars represent standard errors of the means; bars with different letters are significantly different at $p \leq 0.05$ according to the LSD test).

3.2. Biochemical and sensorial characteristics of leaf celery baby leaves

3.2.1. Biochemical characteristics

The experimental factors affected protein content in both mowings (data not shown). In the first mowing, it was significantly lower (-28.6%) in the leaves of plants grown in S2 at 615 plants m^{-2} and supplied with HS NS compared to the other treatments (2.82 g 100 g^{-1} FW on average).

In the second mowing, the NS concentration variously affected the protein content according to growing season and plant density. The highest protein content (2.84 g 100 g^{-1} FW on average) was recorded in S1 in plants grown at 947 plants m^{-2} supplemented with both HS and FS NS and in S2 in

the plants grown at 615 plants m^{-2} and supplemented with FS NS. The lowest protein content (2.01 g 100 g^{-1} FW on average) was recorded in S1 using 615 plants m^{-2} and HS NS. Increasing NS concentration, the protein content significantly increased in both growing seasons in the plants grown at 615 plants m^{-2}, but to a greater extent in S2 compared to S1. Conversely, no significant change was observed with increasing the nutrient availability with 947 plants m^{-2}.

The fiber content was not significantly affected by the experimental factors in the first mowing (1.41 g 100 g^{-1} FW on average) (data not shown). In the second mowing, the average fiber content remained similar but exhibited some slight but significant effects due to factor interactions. It ranged from a maximum of 1.51 g 100 g^{-1} FW in S1 combined with 615 plants m^{-2} to a minimum of 1.35 g 100 g^{-1} FW in S2 combined with 947 plants m^{-2}. The HS NS treatment showed contrasting effects: it determined the lowest fiber content (1.34 g 100 g^{-1} FW) when applied in S2 or at the higher plant density (947 plants m^{-2}). Conversely, it resulted in the highest fiber content (1.50 g 100 g^{-1} FW) when applied in S1 or at the lower plant density (615 plants m^{-2}).

Nutrient availability modestly influenced the soluble sugar content of the leaves from first mowing in both growing seasons (data not shown). The soluble sugar content ranged from 2.07 g 100 g^{-1} FW with HS NS in S1 to 2.19 g 100 g^{-1} FW with the same NS in S2. The leaves from the second mowing also showed no significant change in soluble sugar content with increasing NS concentration. The highest soluble sugar values (2.30 g 100 g^{-1} on average) were recorded in S1 with 615 plants m^{-2} and FS NS and in S2 with 947 plants m^{-2} and FS NS. Conversely, the lowest values (2.13 g 100 g^{-1} on average) were observed in S1 with 947 plants m^{-2} and FS NS and in S2 with 615 plants m^{-2}, and FS NS. The treatments supplied with HS NS did not show significant differences across seasons or densities, resulting in an average value of 2.19 g 100 g^{-1}.

The vitamin content of leaf celery baby leaves showed small variation in both mowings (Table 7). The vitamin A content averaged 238.3 μg 100 g^{-1} FW in the first mowing and 223.7 μg 100 g^{-1} FW in the second mowing. The highest values in the first mowing (250.1 μg 100 g^{-1} FW on average) were recorded with FS NS in S1 and with HS in S2. In this growing season, the increase in NS concentration significantly lowered the vitamin A content down to 218.2 μg 100 g^{-1} FW on average. The second mowing confirmed a lower content (-3.6%) of vitamin A in S2 (219.6 μg 100 g^{-1} FW on average) compared to S1 (227.8 μg 100 g^{-1} FW on average) and a reduction of this parameter in both seasons with increasing the NS concentration (-6.2%). The niacin content (vitamin B3) averaged 0.20 mg 100 g^{-1} FW and was not affected by the treatments (data not shown). The riboflavin content (vitamin B2) showed similar values in both mowings (0.20 mg 100 g^{-1} FW on average). It was lower in S2 compared to S1 in the first mowing. In the second mowing, riboflavin content was negatively affected by the higher nutrient content (FS NS) when combined with 615 plants m^{-2}. The vitamin C averaged 32.3 mg 100 g^{-1} FW on average in the first mowing. In S1, vitamin C was higher and not

affected by the NS (33.6 mg 100 g^{-1} FW on average). In S2, it showed a significant drop using FS NS (29.5 mg 100 g^{-1} FW on average). In the second mowing, the vitamin C content recorded the lowest values with the highest plant density (28.2 mg 100 g^{-1} FW on average) and had a significant increase when supplying HS NS to the plants grown at 615 plants m^{-2}.

Table 7. Effects of growing season (S1 – winter-spring, S2 – spring-summer), plant density (615, 947 plants m^{-2}) and nutrient solution (NS) concentration (0%, only water - 0; 50%, half strength - HS; 100%, full strength - FS) on the vitamin content (mg 100 g^{-1} FW) of *Apium graveolens* L. var. *secalinum* Alef. baby leaves obtained using an ebb-and-flow hydroponic system.

Source of Variance		First Mowing				Second Mowing			
		A	B2	B1	C	A	B2	B1	C
Growing season (S)									
S1 – winter-spring		[z] 244.7a	0.21a	0.06	33.6a	227.8a	0.19	0.06	29.3
S2 – spring-summer		231.9b	0.19b	0.06	31.0b	219.6b	0.19	0.06	29.2
Plant density (D)									
615 plants m^{-2}		238.8	0.21	0.06	33.1	223.8	0.20a	0.06	30.3a
947 plants m^{-2}		237.8	0.20	0.06	31.5	223.5	0.18b	0.06	28.2b
Nutrient solution (NS)									
HS		240.3	0.21	0.06	32.9	230.8a	0.20	0.06	30.2a
FS		236.3	0.20	0.06	31.7	216.5b	0.19	0.06	28.3b
S x NS									
S1	HS	234.8ab	0.22	0.06	33.3a	236.8	0.19	0.06	30.0
	FS	254.5a	0.21	0.07	33.8a	218.7	0.19	0.06	28.5
S2	HS	245.7a	0.20	0.06	32.5ab	224.8	0.20	0.06	30.3
	FS	218.2b	0.19	0.06	29.5b	214.3	0.19	0.06	28.0
D x NS									
615	HS	239.8	0.21	0.06	33.5	233.3	0.22a	0.07	32.5a
	FS	237.7	0.21	0.06	32.7	214.3	0.19b	0.06	28.0b
947	HS	240.7	0.20	0.06	32.3	228.3	0.17b	0.06	27.8b
	FS	235.0	0.20	0.06	30.7	218.7	0.19ab	0.06	28.5b
Significance [x]									
Growing season		*	*	ns	*	**	ns	ns	ns
Plant density		ns	ns	ns	ns	ns	**	ns	*
Nutrient solution		ns	ns	ns	ns	***	ns	ns	*
S x D		ns	ns	ns	ns	ns	ns	ns	ns
S x NS		***	ns	ns	*	ns	ns	ns	ns
D x NS		ns	ns	ns	ns	ns	***	ns	**
S x D x NS		ns	ns	ns	ns	ns	ns	ns	ns

[z] Each value is the mean of 3 replicated samples. For each factor, values in a column followed by the same letter are not significantly different, according to the LSD test. [x] Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$.

Table 8 reports the mineral content of the baby leaves collected with the first mowing. The K content did not vary when supplying HS NS. However, it significantly increased in S1 with FS NS (281.3 mg 100 g^{-1} FW on average) and decreased in S2 with the same NS (272.2 mg 100 g^{-1} FW on average). The Ca content was highest in S1 combined with 947 plants m^{-2} (34.8 mg 100 g^{-1} FW on average),

while it averaged 29.8 mg 100 g^{-1} FW in all the other treatments. The Mg content was positively affected only by the increase of NS concentration (15.4 mg 100 g^{-1} FW on average). The baby leaves recorded a Fe content of 0.36 mg 100 g^{-1} FW on average in S1, with a similar value recorded in S2 with HS NS. In S2, the increase of nutrient availability had a negative effect on Fe content, which dropped down to 0.32 mg 100 g^{-1} FW on average. The Zn content averaged 1.30 mg 100 g^{-1} FW in all treatments except when supplying FS NS with 947 plants m^{-2} in S2, which recorded 1.25 mg 100 g^{-1} FW. Na, P, and Cu were not affected by the treatments and their content averaged 140.8, 40.0, and 0.11 mg 100 g^{-1} FW, respectively.

The baby leaves collected with the second mowing showed variations due to the treatments only as regards Fe content (data not shown). It was lowest in S2 with 615 plants m^{-2} (0.32 mg 100 g^{-1} FW on average) and significantly increased with 947 plants m^{-2} up to 0.37 mg 100 g^{-1} FW on average. Similar values were recorded in S1 irrespective of plant density. The other mineral elements averaged the following values: 274.5 mg 100 g^{-1} FW of K, 139.7 mg 100 g^{-1} FW of Na 31.3 mg 100 g^{-1} FW of Ca, 14.8 mg 100 g^{-1} FW of Mg, 47.2 mg 100 g^{-1} FW of P, 0.11 mg 100 g^{-1} FW of Cu, and 1.30 mg 100 g^{-1} FW of Zn (data not shown).

Table 8. Effects of growing season (S1 – winter-spring, S2 – spring-summer), plant density (615, 947 plants m^{-2}) and nutrient solution (NS) concentration (0%, only water - 0; 50%, half strength - HS; 100%, full strength - FS) on the mineral content of *Apium graveolens* L. var. *secalinum* Alef. baby leaves (first mowing) obtained using an ebb-and-flow hydroponic system.

Source of Variance		First Mowing							
		K (mg 100g^{-1} FW)	Na (mg 100g^{-1} FW)	Ca (mg 100g^{-1} FW)	Mg (mg 100g^{-1} FW)	P (mg 100g^{-1} FW)	Fe (mg 100g^{-1} FW)	Cu (mg 100g^{-1} FW)	Zn (mg 100g^{-1} FW)
Growing season (S)									
S1 – winter-spring		[z] 280.3	142.6	32.3	15.2	49.1	0.36	0.11	1.30
S2 – spring-summer		275.7	139.1	29.8	15.1	47.0	0.34	0.11	1.29
Plant density (PD)									
615 plants m^{-2}		278.6	138.8	29.8	14.9	48.8	0.35	0.11	1.30
947 plants m^{-2}		277.4	142.8	32.3	15.3	47.3	0.34	0.11	1.29
Nutrient solution (NS)									
HS		279.2	140.8	30.5	14.8b	48.2	0.35	0.11	1.30
FS		276.8	140.8	31.7	15.4a	47.9	0.34	0.11	1.29
S x PD									
S1	615	279.7	139.2	29.8b	14.8	48.8	0.36	0.11	1.30
	947	281.0	146.0	34.8a	15.5	49.3	0.36	0.11	1.30
S2	615	277.5	138.5	29.8b	15.0	48.8	0.35	0.11	1.30
	947	273.8	139.7	29.8b	15.2	45.2	0.32	0.11	1.28
S x NS									

S1	HS	279.3ab	142.5	31.5	14.8	48.8	0.36a	0.11	1.30
	FS	281.3a	142.7	33.2	15.5	49.3	0.36a	0.11	1.30
S2	HS	279.2ab	139.2	29.5	14.8	47.5	0.35a	0.11	1.30
	FS	272.2b	139.0	30.2	15.3	46.5	0.32b	0.11	1.28
S x PD x NS									
S1	615 HS	278.3	141.3	30.3	14.7	49.0	0.35	0.11	1.31a
	615 FS	281.0	137.0	29.3	15.0	48.7	0.36	0.11	1.29ab
	947 HS	280.3	143.7	32.7	15.0	48.7	0.36	0.11	1.29ab
	947 FS	281.7	148.3	37.0	16.0	50.0	0.35	0.11	1.31a
S2	615 HS	281.0	137.0	29.3	15.0	48.7	0.36	0.11	1.29ab
	615 FS	274.0	140.0	30.3	15.0	49.0	0.34	0.11	1.31a
	947 HS	277.3	141.3	29.7	14.7	46.3	0.35	0.11	1.30a
	947 FS	270.3	138.0	30.0	15.7	44.0	0.29	0.11	1.25b
Significance [x]									
Growing season		*	ns	*	ns	ns	*	ns	ns
Plant density		ns	ns	*	ns	ns	ns	ns	ns
Nutrient solution		ns	ns	ns	*	ns	*	ns	ns
S x PD		ns	ns	*	ns	ns	ns	ns	ns
S x NS		*	ns	ns	ns	ns	*	ns	ns
PD x NS		ns	ns	ns	ns	ns	ns	ns	ns
S x PD x NS		ns	ns	ns	ns	ns	ns	ns	**

[z] Each value is the mean of 3 replicated samples. For each factor, values in a column followed by the same letter are not significantly different, according to the LSD test. [x] Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$.

3.3. Sensory evaluation

The baby leaves exhibited similar sensory parameter scores in both seasons, with the exception of general appearance, which received a significantly higher score in S1 (7.1 on average) compared to S2 (6.4 on average) (Figure 8). The different combinations of plant density and nutrient solution concentrations had a small effect on almost all the sensory parameter scores (Figure 9). The 947-HS treatment negatively affected general appearance with an average score of 5.6. The FS nutrient solution resulted in a higher color score (6.35 score on average) compared to the HS NS, which reduced color scores at both plant densities, particularly in combination with 947 plants m^{-2} (4.1 on average). NS concentration did not affect the odor score of baby leaves grown at 615 plants m^{-2} (3.8 on average). However, at 947 plants m^{-2} the odor score was higher with FS NS (4.1 on average) compared to HS NS (2.9 on average). Conversely, the juiciness of baby leaves grown at 615 plants m^{-2} recorded the lowest score with HS NS (4.1 on average) and the highest with FS NS (5.1 on average). Juiciness did not vary at 947 plants m^{-2}, scoring intermediate scores (4.6 on average). The average overall rating score was 6.3.

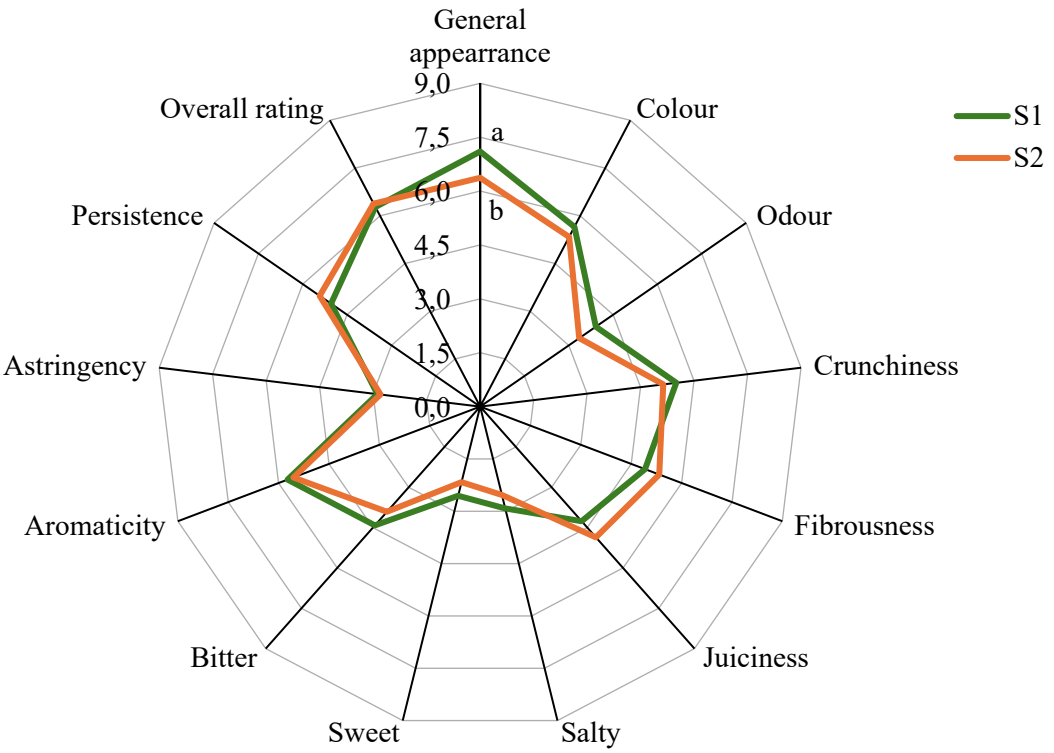

Figure 8. Effects of growing season (S1 – winter-spring, S2 – spring-summer) on the baby leaf sensory profile of *Apium graveolens* L. var. *secalinum* Alef. plants grown using an ebb-and-flow hydroponic system (values within the same parameter with different letters are significantly different at $p \leq 0.05$ according to the LSD test).

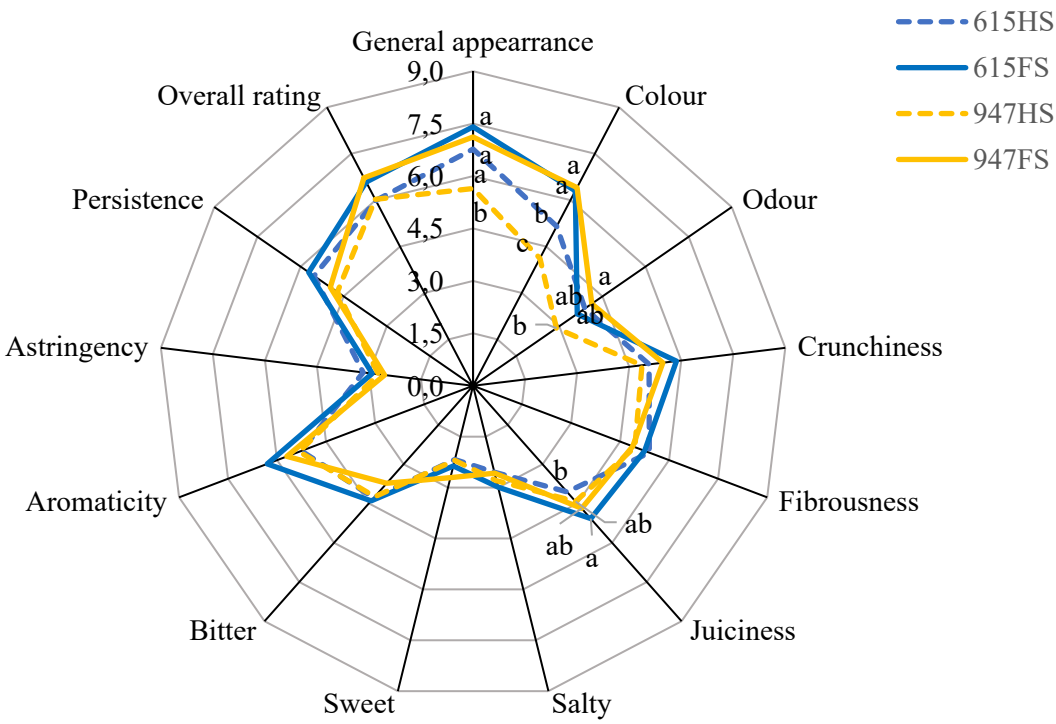

Figure 9. Effects of plant density (615 and 947 plants m^{-2}) and nutrient solution (NS) concentration (50%, half strength - HS; 100%, full strength - FS) on the baby leaf sensory profile of *Apium graveolens* L. var. *secalinum*

Alef. plants grown using an ebb-and-flow hydroponic system (values within the same parameter with different letters are significantly different at $p \leq 0.05$ according to the LSD test).

3.4. Cold storage of minimally processed baby leaf

Immediately after harvest, the quality retention and overall rating of minimally processed leaf celery were assessed over a 21-day cold storage period (4 °C). This assessment included baby leaves from the first mowing of both growing seasons. Only the leaves collected from plants supplied with HS and FS nutrient solution were processed, as those obtained with 0 NS were not marketable.

Weight loss increased throughout the storage period. Similar weight loss values were recorded in both growing seasons until day 14 (an average of 3.06%). In the final week of storage, weight loss increased further, with the highest loss (5.46%) recorded in S2. This loss was 27.5% higher than that observed in S1. Moreover, baby leaves from HS NS recorded a higher weight loss during storage, especially when combined with a density of 615 plants m^{-2} (Table 9).

The overall quality of the leaf celery baby leaves decreased during storage in both seasons, but with different trends. In S2, the score dropped more slowly during the first week; however, the overall quality was similar after 14 days of storage in both seasons (4.12 on average). The baby leaves lost marketability (corresponding to a score threshold of 3) in both seasons during the last week of cold storage. The final score after 21 days of storage was significantly lower in the first season (2.54) compared to second (2.96). The overall quality showed a different trend across the storage also as a function of NS or plant density. The highest plant density determined a higher quality loss at 7 and 14 days of storage, whereas the HS NS scored lower values of overall quality throughout the storage period (Table 9).

Leaf color is a critical factor affecting the perceived quality of minimally processed leafy vegetables. The color lightness (L*) increased in S1 from 49.6 to 54.5 during the cold storage period, indicating lighter color (green fading), while it did not change significantly in S2 (50.0 on average). Furthermore, baby leaves grown with FS NS were darker (lower L* values) than those from HS NS. A different trend according to the growing season was observed for chroma. In S1, it had a lower initial value (35.1 on average) and further decreased until day 14 (27.5 on average), while it remained almost constant in S2 in the same period (36.5 on average). At the end of the storage period, chroma significantly increased up to an average of 37.9 for both seasons (Table 9). An increase in chroma values (+15.1% on average) was also observed with a reduced nutrient solution concentration, but with generally higher chroma values in S2 compared to S1. During the cold storage period, the hue angle dropped toward a less greenish (more yellowish) color from 121.9 (day 0) to 120.1 (day 21) on average. A negative effect on hue angle was also found when using HS NS. The hue angle averaged

121.1 in both seasons with FS NS, while it dropped down to 120.8 in S2 and even more (119.4) in S1 when supplying the plants with HS NS.

Table 9. Effect of growing season (S1 – winter-spring, S2 – spring-summer), plant density (615 and 947 plants m^{-2}), nutrient solution (NS) concentration (50% - HS; 100% - FS) and storage at 4 °C on the quality parameters of minimally processed *Apium graveolens* L. var. *secalinum* Alef. baby leaves obtained using an ebb-and-flow hydroponic system (Overall quality scores: 1 - unmarketable; 3 - average—limit of marketability; 5 - excellent or having a fresh appearance).

Source of Variance		Weight loss (%)	Overall Quality	L*	Chroma	Hue°
Growing season (S)						
S1 – winter-spring		[z] 3.12	3.93	51.4	33.2	120.7
S2 – spring-summer		3.20	4.22	50.0	36.9	121.4
Days at 4 °C (D)						
0			5.00	50.1	36.0	121.9a
7		1.54	4.43	49.9	34.5	121.0b
14		3.06	4.12	50.3	31.8	121.4ab
21		4.87	2.75	52.5	37.9	120.1c
Plant density (PD)						
615		3.52	4.23	50.7	35.1	121.2
947		2.80	4.03	50.5	35.0	121.2
Nutrient Solution (NS)						
HS		3.48	3.92	52.8	37.5	120.1
FS		2.84	4.23	48.6	32.6	122.1
S x D						
S1	0		5.00a	49.6c	35.1c	121.5
	7	1.78d	4.15c	50.6bc	32.5d	120.7
	14	3.30c	4.03c	51.0b	27.5e	121.2
	21	4.28b	2.54e	54.5a	37.8ab	119.6
S2	0		5.00a	50.7bc	37.0b	122.2
	7	1.30d	4.71b	49.3c	36.5b	121.2
	14	2.82c	4.22c	49.6c	36.0bc	121.6
	21	5.46a	2.96d	50.5bc	38.0a	120.6
S x NS						
S1	HS	3.44	3.74	53.7	35.4b	119.4c
	FS	2.81	4.12	49.2	31.0d	122.1a
S2	HS	3.51	4.10	52.0	39.6a	120.8b
	FS	2.88	4.34	48.0	34.2c	122.0a
D x PD						
0	615		5.00a	49.6	34.9	121.5
	947		5.00a	49.6	35.3	121.4
7	615	1.75	4.30b	50.8	31.9	120.1
	947	1.82	4.00c	50.4	33.0	121.4
14	615	3.68	4.26bc	51.6	27.6	120.4
	947	2.93	3.80d	50.3	27.4	122.0
21	615	4.67	2.58e	53.8	37.3	120.0
	947	3.90	2.50e	55.2	38.3	119.2
D x NS						

0	HS		5.00a	51.8	38.0	120.9
	FS		5.00a	48.5	34.1	122.8
7	HS	1.68	4.17c	52.4	37.5	119.6
	FS	1.40	4.69b	47.5	31.5	122.3
14	HS	3.54	3.93d	52.2	34.1	120.7
	FS	2.59	4.31bc	48.3	29.4	122.1
21	HS	5.20	2.59f	55.0	40.5	119.1
	FS	4.54	2.91e	50.0	35.3	121.0
PD x NS						
615	HS	4.07a	4.01	52.9	37.5	120.0
	FS	2.96b	4.36	48.7	32.5	122.1
947	HS	2.88b	3.84	52.8	37.5	120.2
	FS	2.72b	4.10	48.5	32.7	122.0
Significance [x]						
Growing season		ns	***	***	***	*
Day (D)		***	***	***	***	***
Plant density (PD)		***	***	ns	ns	ns
Nutrient solution (NS)		***	***	***	***	***
S x D		***	***	***	***	ns
S x PD		ns	ns	ns	ns	ns
S x NS		ns	ns	ns	*	**
D x PD		ns	***	ns	ns	ns
D x NS		ns	***	ns	ns	ns
PD x NS		**	ns	ns	ns	ns
S x D x PD		ns	ns	ns	ns	ns
S x D x NS		ns	ns	ns	ns	ns
S x PD x NS		ns	ns	ns	ns	ns
D x PD x NS		ns	ns	ns	ns	ns
S X D x PD x NS		ns	ns	ns	ns	ns

[z] Each value is the mean of three replicated samples of 50 g each. For each factor, values in a column followed by the same letter are not significantly different, according to the LSD test. [x] Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; and *** significant at $p < 0.001$.

4. Discussion

Investigations on leaf celery (*Apium graveolens* L. var. *secalinum* Alef.) have primarily focused on its cultivation in soil or on the chemical composition of its essential oil. However, the growing interest in novel leafy vegetables for fresh-cut salads necessitates the adaptation of agronomic techniques for hydroponic cultivation of new species. Therefore, this study, for the first time, investigates the suitability of leaf celery for baby leaf production in a hydroponic system and assesses the shelf life of the minimally processed product. Crucially, this study is the first to quantitatively assess the combined impact of plant density and nutrient solution concentration across different climatic conditions on yield and quality of hydroponically grown baby leaf, both at harvest and during cold storage as a ready-to-eat product.

4.1. Agronomic performance and production cycles

A. graveolens generally grows better in areas with cool seasons with monthly mean temperatures ranging from 15 to 21 °C and good soil moisture (Salehi et al., 2019). While the S1 mean temperature

of 17.5±0.4 °C aligned with these optimal conditions, the S2 mean temperature of 24.1±0.4 °C slightly exceeded this range. Nevertheless, even if this experiment demonstrated that leaf celery can be successfully cultivated in our conditions, the variation in temperatures and photoperiod differently affected yield, morpho-physiological traits, chemical composition, sensory profile, and post-harvest quality. Baby leaf vegetables typically require 20–40 days from sowing to harvest, depending on the species and growing conditions (Di Gioia et al., 2017). Some species allow for repeated cuts, with regrowth harvested after an additional 15–20 days, though regrowth speed depends on temperature and cultivar (Groenbaek et al., 2019). Leaf celery showed a slower growth rate for the first harvest compared to other baby leaf vegetables, whereas the interval from first to second mowing was aligned to other species. The environmental conditions did not affect the length of growing cycle which lasted 88 and 81 days overall in S1 and S2 respectively.

Despite the similar timeframe in the two growing seasons, the yield, biomass accumulation and leaf morphology showed significant variations. Total fresh weight (FW), stem FW, and total baby leaf yield were consistently higher in S1. This is likely due to the more favorable growing conditions during the winter-spring period, characterized by lower temperatures and more optimal light intensity, compared to the higher heat stress and light intensity of the spring-summer period (S2). The larger leaf and petiole lengths and overall greater leaf expansion in S1 support this interpretation, as optimal environmental conditions promote cell expansion and overall vegetative growth. The yield of S1 was higher than S2 by 90% and 86% with FS and HS NS, respectively. The yield obtained with HS NS in S1 was even higher than the maximum yield recorded in S2. These differences could be ascribed to the higher maximum temperatures and light intensity recorded in S2. Increased air temperature is strongly correlated with an increase in vapor pressure deficit (VPD) (Grossiord et al., 2020), which likely induced heat and water stress in the leaf celery plants. Although VPD was not directly measured, the higher temperatures in S2 likely exceeded the 1.5 kPa threshold, above which leafy vegetables typically experience midday stomatal closure regardless of substrate moisture (Amitrano et al., 2021a). This mechanistic response, characterized by reduced stomatal and mesophyll conductance, limits net CO_2 assimilation and water use efficiency (Yu et al., 2023; Zhang et al., 2017). Furthermore, the longer photoperiod and the higher light intensity in S2 (13 to 15 h of light; 54,933 lux) compared to S1 (10 to 13 h of light; 44,080 lux) may have further inhibited biomass accumulation. While a 12 h light/12 h dark photoperiod has been shown to increase celery biomass and yield (Chu et al., 2023), excessive light intensity can be inhibitory: supra-optimal light can shift metabolism toward stress-related compounds (flavonoids) at the expense of primary vegetative growth (Qin et al., 2024). Leafy celery is considered sensitive to heat and water stress (Malhotra, 2006), and all cultivated botanical varieties of *A. graveolens*, have high water requirements (Breschini and Hartz, 2002). Although our ebb-and-flow system aimed to fulfill these requirements, the high

temperatures recorded in S2, and the low volume of substrate of the hydroponic system might have led to transient periods where water supply could not meet the evapotranspiration demands, thus limiting plant growth and yield. This issue could potentially be avoided in future spring-summer cultivations by using an alternative hydroponic system, such as a floating system.

The complete nutrient supply of the full-strength nutrient solution allowed to maximize yield in both seasons, confirming that the species is demanding in terms of nutrient requirements (Salehi et al., 2019). Using a half-strength solution decreased total yield by 35.6% on average, also affecting leaf biomass and morphology. Nevertheless, no symptoms of nutrient deficiency were recorded during plant growth. Conversely, the nutrients supplied by the commercial substrate alone in the control treatment (0 NS) failed to support proper plant growth, leading to a very low yield of unmarketable baby leaves that were undersized and yellowish.

Plant density has various effects on plant growth. Although a greater number of plants per unit area can increase yield, competition for limited light and nutrient resources may decrease biomass accumulation. In our study, the total baby leaf yield increased by 7% on average using the higher plant density. However, a different effect of plant density was observed in each mowing. The first mowing yielded 28.9% more baby leaves with 947 plants m^{-2} compared to 615 plants m^{-2}, whereas no significant effect of plant density on yield was observed with the second mowing. This outcome could be related to biomass accumulation trends, following the "constant final yield" law, where yield per area plateaus as individual plant size shrinks due to competition (Friedman, 2024; Postma et al., 2021). In leafy vegetables and crops like cucumber, lower density improves per-plant yield and quality, but total yield per area remains stable (Feng et al., 2020). Exceptions can occur if densities are extremely low (yield drops due to too few plants) or extremely high (competition reduces both per-plant and total yield) (Cavalieri et al., 2022). The increase in plant density from 615 to 947 plants m^{-2} negatively affected both fresh and dry biomass accumulation per plant. Specifically, the fresh weight per plant of the leaves dropped by 17.4% on average for both HS and FS NS in the first mowing. The reduction was even more marked at the second mowing, accounting for a 36.0% drop with HS NS and a 39.2% drop with FS NS, which influenced the overall yield trend of the baby leaf. These findings align with previous studies that have demonstrated how factors such as plant density and nitrogen content in the nutrient solution can affect yield and biomass accumulation in various leafy crops, including celery, lettuce, new Zealand spinach, spinach, and rocket plants (Esposito et al., 2025; Gonnella et al., 2003; Jadhav et al., 2025; Öztekin et al., 2018; Petropoulos et al., 2016; Rożek, 2007). This reduction was also observed in the dry biomass, which increased with increasing nutrient solution concentration but decreased with increasing plant density up to 947 plants m^{-2}, showing a greater drop when using FS NS.

The regrowth capacity of leaf celery enables multiple mowings. Although the time to the first mowing was longer than to the second mowing, when using the FS NS, the yield of baby leaves was significantly higher by 44% on average in the second harvest compared to the first. A similar trend was found by Rożek (Rożek, 2007) on leaf celery grown in soil for mature leaves harvest, which recorded yield increases by 40% on average comparing the first and second harvest. In both seasons, the biomass at the second mowing was higher compared to the first mowing even if the number of leaves per plant dropped from the first to the second mowing. The average increase in leaf weight per plant from the first to the second harvest was 94.0% with HS NS and 49.5% with FS NS in terms of fresh biomass and 79.2% and 60.9% in terms of dry biomass. Some studies on baby leaf crops show a substantial increase in both fresh and dry biomass from the first to the second harvest when crops are managed with successive mowing or cutting (Formisano et al., 2021). Similar trends are also observed in vertical farming systems regardless of photoperiod (Ciriello et al., 2023), confirming the positive effect of successive harvests on yield even under different temperatures and photoperiods as found in our study. The increase in biomass after the first cut is linked to physiological changes in the plants such as improved photosynthetic activity and reduced stomatal resistance that result in higher net CO_2 assimilation, and greater transpiration rates, which stimulate faster regrowth efficiency. The cut may also determine hormonal response that stimulate cell division and a higher rate of new leaf formation, and encourage root growth improving nutrient and water uptake for subsequent above-ground growth (Ciriello et al., 2023; Formisano et al., 2021). Furthermore, mowing can stimulate nutrient uptake and transport (nitrate, phosphate, sugars) in roots, and trigger the mobilization of stored carbohydrates in roots and crowns to rebuild leaves, providing energy and building blocks for rapid new growth (Li et al., 2023).

The greater yield increase observed with HS NS compared to FS NS may be attributed to nutrient accumulation within the substrate resulting from repeated ebb-and-flow cycles. In subirrigation/ebb–flow systems, salts are transported upward via capillary flow and are only partially taken up by the plants; consequently, substrate electrical conductivity (EC) tends to accumulate over time (Méndez-Cifuentes et al., 2023). Because of this accumulation, using full-strength nutrient solution can push root-zone EC into a range where osmotic stress and ion imbalance reduce growth, even though nutrient availability is high (Samarakoon et al., 2006). Therefore, a lower-strength nutrient solution can be more effective during the regrowth phase, as the excessive substrate EC associated with FS NS likely led to salinity buildup, becoming a limiting factor for growth.

4.1. Physiological responses and resource use efficiency

The varying growth rates observed during the initial and regrowth periods, influenced by environmental and agronomic factors, also affected the water and nitrogen use efficiency by

impacting biomass accumulation. Regarding water use efficiency (WUE), leaf celery plants exhibited higher efficiency during the first growing season, particularly with an increased nutrient supply.

The significantly higher WUE recorded in S1 compared to S2 indicates that plants were more efficient at converting water into biomass during the cooler season. This aligns with findings in other leafy vegetables, where high temperatures and high vapor pressure deficits, typical of summer, lead to increased transpiration and reduced instantaneous WUE (Amitrano et al., 2021b; Grossiord et al., 2020). Lower temperatures and greater soil moisture retention may reduce evapotranspiration and water loss, thereby promoting water use efficiency (Hatfield and Dold, 2019), as shown in S1. Conversely, higher temperatures and longer daylengths have been shown to increase evapotranspiration and possibly reduce stomatal aperture and CO_2 fixation, which in turn reduces biomass accumulation and water use efficiency. Higher nutrient levels can support plant growth and enhance biomass production resulting in WUE increases as found in many other leafy vegetables grown hydroponically (Sharma et al., 2018). Increased nutrient supply improves photosynthetic capacity, leaf area, and overall plant vigor, which in turn can enhance WUE (Chrysargyris and Tzortzakis, 2023; Esposito et al., 2025). Plant density only affected WUE until the first mowing, with plants grown at 947 plant m^{-2} showing higher water use efficiency. Higher density generally increases leaf area index (LAI) and intercepted photosynthetically active radiation (PAR), especially early in the season, by accelerating canopy closure (Mattera et al., 2013). This rapid closure also reduces soil evaporation by shading the substrate and shifting the total water loss toward transpiration. Shading cuts radiation at the soil surface, lowering soil temperature and potential soil evaporation; as ground cover and LAI increase, a larger fraction of evapotranspiration is transpiration rather than soil evaporation (Pereira et al., 2020; Wang and Liu, 2007). Furthermore, denser canopies reduce within-canopy vapor pressure deficit (VPD), thereby lowering the evaporative demand near the soil surface (Amitrano et al., 2021b).

The climatic conditions during the first growing season increased the total yield of baby leaf and led to different nutrient efficiency. The highest total NUE was recorded with the HS NS in S1, followed by a decline with FS NS. This quadratic trend is common in horticulture: nutrient-deficient conditions (HS NS) force the plant to utilize absorbed N more efficiently for biomass production, whereas supra-optimal conditions (FS NS) allow for luxury N consumption, resulting in a higher N concentration in the tissue and a drop in the NUE metric (Yang et al., 2021). As observed in our experiment, plant nitrogen demand can vary with environmental conditions (Govindasamy et al., 2023). High temperatures decrease nitrogen uptake and assimilation (Qaderi et al., 2025), limiting biomass accumulation and nitrogen use efficiency, as shown by the results from the first and second mowings during S2. Lower nutrient availability often results in higher NUE, whereas excessive nitrogen (N) applications tend to decrease NUE (Santamaria et al., 2002). As observed for WUE, the plant density

of 947 plants m^{-2} showed a higher NUE only at the first mowing. Root architecture changes under high planting density, where competition can lead to reduced nitrogen loss and improved nitrogen use efficiency (Govindasamy et al., 2023).

4.3. Quality traits

Baby leaves for commercial production require stable characteristics across mowings and growing seasons.

A high-quality baby leaf product must meet specific characteristics, which vary among species. These typically include a leaf length of 8–10 cm and a petiole that is less than 35% of the leaf length (Gil and Garrido, 2020). However, this applies only partially to leaf celery, which has a unique leaf morphology with long petioles and a reduced leaf lamina compared to other leafy vegetable crops. Considering only marketable leaves from HS and FS NS, leaf length showed the highest differences in S1 ranging from 13.7 to 17.1 cm for the first and second mowing, respectively, whereas it remained stable during S2 (15.5 cm on average for first and second mowing). As expected, increased nutrient supply increased leaf length in both mowings and both growing seasons, whereas increased plant density reduced leaf length only at the second mowing. Hence, the highest leaf length difference was about 5 cm comparing the leaves of the first mowing and the second mowing from plants grown at 615 plants m^{-2}. The environmental and agronomic factors also influenced the leaf area which increased from S1 to S2 and from the first to the second mowing and was reduced by increasing plant density or reducing nutrient supply. The different environmental conditions recorded during the first and second mowing affected leaf morphology. Lower temperatures and light intensity can limit leaf expansion and result in thinner leaves and smaller leaf development (Rodríguez et al., 2015; Shafiq et al., 2021; Wingler, 2015), as observed in the first mowing of S1. In contrast, higher temperatures and excessive light intensity can damage the photosystems and reduce photosynthetic efficiency, transpiration, CO_2 fixation, and chlorophyll biosynthesis, thereby limiting plant growth (Lu et al., 2017; Mathur et al., 2014), as found in the second mowing of S2. The leaf length, petiole length, and leaf area were positively affected during S1-second mowing and S2-first mowing probably due to similar climate conditions recorded in these periods. Some studies on lettuce and pak choy shows how an increase in temperature, up to certain levels, can positively affect these parameters (Kong et al., 2023; Ouyang et al., 2020). Mowings affected not only the total leaf biomass but also the leaf morphology. The second mowing resulted in plants with greater leaf length, petiole length, and average leaf area. The physiological changes in the plants after the first harvest stimulate a faster regrowth and hormonal changes that stimulate cell division and expansion resulting in increased leaf dimension (Ciriello et al., 2023; Formisano et al., 2021). The effect of the experimental parameters on leaf morphology was complex as each factor may have contrasting effects compared to others and according to the maturity stage of plants as found for plant density which had a role in controlling

leaf and petiole length only in mature plants after the first harvest. Similarly, leaf thickness, which is related to the specific leaf area (SLA), was lower in S2 than in S1 and responded to plant density and nutrient solution concentrations only at the second mowing. SLA and leaf thickness are two closely related leaf traits. Usually, to an increase of leaf thickness correspond a decrease of the specific leaf area (Vile et al., 2005). In dense plantings, plants are characterized by the development of thinner, elongated stems and leaves in response to reduced light availability. This adaptation, which has been shown to increase specific leaf area (SLA) in other leafy vegetables (Van Brenk et al., 2024), was also observed in leaf celery in our experiment.

As well as leaf morphology, leaf color can greatly affect the quality of baby leaf products perceived by consumers. Both genotype and environmental conditions (e.g., temperature, light, and season) significantly influence the color of baby leaf greens. Color variation is closely tied to the accumulation of pigments like anthocyanins, chlorophylls, and carotenoids, which are themselves influenced by weather and nutrient availability (Marin et al., 2015; Simko, 2020). In leafy vegetables, leaf color changes as plants progress through different growth stages, primarily due to shifts in pigment concentrations (chlorophylls, carotenoids, anthocyanins) and underlying physiological processes (Kong and Nemali, 2021). This shift was also recorded in the present study, where leaf celery had a more vivid and less green leaf color at the first mowing, while leaves from the second mowing had a greener, less saturated color. This could be related to a more efficient nutrient uptake in mature plants, which supports the synthesis of chlorophyll and β-carotene and leads to a more intense green color (Hashmi et al., 2019). Plant stage also modified the response of leaf color to the experimental factor. During the initial growth period (until the first mowing), leaf color was only affected by nutrient availability. In contrast, during the subsequent regrowth period, plant density and the growing season also influenced leaf color. Nutrient solution concentration can also affect chlorophyll content and, consequently, leaf color (Di Mola et al., 2020). The lack of nutrient supply significantly reduced chlorophyll synthesis, resulting in small and yellowish leaves. In both mowings, leaves were lighter when HS NS was supplied. The leaves became less saturated and shifted toward greener hues when the full-strength nutrient solution was provided. Plant density only affected leaf color in the second mowing. Increasing plant density up to 947 plants m^{-2} resulted in an increase in L* and chroma, and a decrease in hue angle. At higher plant densities, chlorophyll biosynthesis may decrease due to reduced photosynthetic activity caused by shading among plants (Shafiq et al., 2021). The biochemical composition of leafy vegetables, such as their content of vitamins, minerals, antioxidants, and other compounds, is strongly influenced by agronomic factors, though climate (temperature, light, photoperiod, humidity) often has a larger effect (Poiroux-Gonord et al., 2010; Rempelos et al., 2023). Also the stage of harvesting (baby or adult plants) influences the concentration of vitamins and other biochemical components, with significant variability among species and stages

(Mallor et al., 2023). In our trials, leaf celery showed limited variations in leaf composition due to the growing season or the time of harvest, with sufficient stability across the growing seasons and mowings. However small but significant variations have been recorded in response to the interactions between climatic conditions (growing season) and plant management (plant density and nutrient availability) only in protein, vitamin A and C, and some mineral elements. Protein content was generally increased by FS NS, particularly in S2 at low density. However, Vitamin A content was reduced by increasing NS concentration in the second mowing, which suggests an inverse relationship between vegetative growth rate (driven by high N in FS NS) and the accumulation of certain secondary metabolites. This supports findings in other leafy greens where excessive N can dilute or inhibit the synthesis of certain vitamins or health-related compounds (Mozafar, 1993). Mg content was positively correlated with increasing NS concentration, indicating that supply was limiting at the HS level. The values of mineral and vitamin content partially agree with those provided by the U.S. Department of agriculture (USDA, 2019) or reported for *Apium graveolens* (Salehi et al., 2019). Compared to other celery varieties, the baby leaves from our trials were distinguished by a higher content of Na, Mg, P, Fe, and Zn, as well as vitamins A, B1, B2, and C. A serving of 100 g of baby leaves of leaf celery produced in our trial can, on average, significantly contribute to the intake of vitamin A (34% DV), and vitamin C (34% DV), and supply high levels of P, K, Na, Zn, and Cu (FDA U.S. Food and Drug Administration, 2024).

Regarding the sensory profile, leaf celery showed only minor differences between the first and second growing cycle, generally maintaining a good overall evaluation. However, some sensory attributes, such as general appearance and color, were negatively affected by the highest plant density when combined with HS NS. When high plant density is paired with suboptimal (half-strength) nutrient solutions, the compounded stress leads to lower pigment synthesis and poor appearance. The stress from competition is not fully alleviated by reduced nutrient supply, and pigment levels (chlorophyll, carotenoids, anthocyanins) are consistently lower than in plants grown with full-strength nutrients (Majidi et al., 2025; Song et al., 2020). Growing practices and fertilization are likely to have a significant influence on celery's sensory quality. In particular, the optimization of irrigation and nitrogen rates have been deeply investigated to improve crop quality (Raffo et al., 2006). Consumer perception is highly influenced by leaf size and color, with a preference for a bright green color (Barrett et al., 2010). The negative impact of high plant density and low nutrient availability recorded on these characteristics of the baby leaves could explain the changes recorded for some parameter of the sensory evaluation.

4.4. Post-harvest quality and commercial shelf life

The consumer demand for diverse and novel ready-to-eat leafy vegetables drove our experiment to further evaluate the quality of minimally processed leaf celery baby leaves during cold storage. Fresh-

cut leafy vegetables are a highly perishable product. Their storability can be negatively affected by both pre-harvest and post-harvest factors (Miceli et al., 2021). After harvest, leaves can suffer from physiological disorders due to the cessation of water and nutrient supply from the plant. While these alterations are unavoidable, their onset and intensity can be controlled or mitigated through proper pre-harvest and post-harvest management, thereby extending shelf life. Weight loss is one of the main causes of vegetable deterioration. A linear increase in weight loss was observed in leaf celery baby leaves during the first two weeks of storage. This loss was more pronounced at the end of the storage period in the baby leaf harvested during the second cycle compared to the first. A greater weight loss was also recorded when using a lower plant density with limited nutrient supply. The harvesting time and the growing season may also influence the storability of other leafy vegetables (Koukounaras et al., 2020). Moreover, in a hydroponic system, plant density and nutrient availability can play a complex role in modifying tissue characteristics, thus affecting water loss and leaf decay. The response to nutrient concentration is not linear but can vary according to other growing factors such as plant density, genotype and climatic conditions (Luna et al., 2013), as also found in our trial. The weight losses recorded for leaf celery baby leaves were approximately 3% on average during the initial 14 days of storage. This value remained below the 4–6% threshold for marketability loss in leafy vegetables (Robinson et al., 1975) for most of the storage period, only reaching that range at day 21. The greater weight loss recorded in S2 and under low nutrient/low density conditions is likely linked to differences in tissue thickness and cell wall rigidity (previously discussed in relation to SLA), with weaker cell walls or cuticles, leading to greater water loss and higher respiration rates during storage (Luna et al., 2013; Miceli et al., 2021). Together with weight loss, the fresh-cut baby leaves showed color changes during storage. These changes were moderate until day 14 and became more pronounced at the end of the storage period, especially in S1. Leaf color is a strong visual indicator of freshness and quality in fresh-cut produce. Studies on leafy vegetable species show that greener leaves are consistently rated as fresher by sensory panels and consumers (Cho et al., 2008; Koyama et al., 2021; Løkke et al., 2012). Loss of green color, yellowing, increased lightness and more saturated color are all clear signs of freshness loss and product deterioration, associated with chlorophyll breakdown, enzymatic browning, and water loss (Cho et al., 2008; Løkke et al., 2012; Toivonen and Brummell, 2008). Freshness perception is also affected by leaf turgor and glossiness, both of which decline as water is lost. This loss of water leads to a duller, less vibrant color and increased wrinkling (Luo et al., 2021; Toivonen and Brummell, 2008). In our experiment, the most significant color changes, in terms of chroma and hue, were only observed at the end of the storage period when also the water loss was highest. This was particularly true for the baby leaves grown with the half-strength nutrient solution. These factors resulted in a more saturated and less greenish color. The variations in weight and color were also correlated with the overall quality, as rated by an

informal panel that assessed marketability during cold storage. Fresh-cut leaf celery maintained acceptable overall quality up to day 14 of cold storage, with better scores recorded on plants grown with a full-strength nutrient solution and a lower plant density. These findings suggests that while HS nutrient solutions may increase nutrient use efficiency by reducing luxury consumption, the lower ion availability likely limits the synthesis of structural cell-wall components and osmoprotectants necessary for prolonged post-harvest survival (Hazrati et al., 2024; Ogden et al., 2018). Mechanistically, the FS treatment provides a more robust supply of precursors for secondary metabolism, as evidenced by the superior color retention (lower L* and higher hue angle). The 947−HS treatment combination resulted in the poorest appearance score, indicating a combined negative effect of high competition and nutrient stress on quality retention. Ready-to-eat leafy vegetables are highly perishable due to their morphological and physiological characteristics. Most minimally processed vegetables show sensory changes in color or texture that negatively affect their marketability within 5-9 days of cold storage, even with diligent handling (Di Giuseppe et al., 2019; Jacxsens et al., 2002). Thus, the two-week shelf life recorded for the minimally processed leaf celery is a notable achievement, offering a substantial commercial advantage for both producers and consumers.

5. Conclusions

This study successfully investigated the suitability of leaf celery (*Apium graveolens* L. var. *secalinum* Alef.) for hydroponic baby leaf production under varying agronomic and environmental conditions, providing the first quantitative assessment of this system and delivering management insights into plant density and nutrient solution concentration. Our work established a robust cultivation protocol and identified key factors regulating yield, nutritional quality, resource use efficiency, and post-harvest performance. This comprehensive study is the first to demonstrate the viability of leaf celery as a multi-cut, fresh-cut baby leaf vegetable in a hydroponic system, expanding the repertoire of leafy greens for ready-to-eat salads.

The Full-Strength Nutrient Solution (FS NS) consistently maximized total yield, confirming leaf celery's substantial nutrient demand for vegetative growth. Total yield and biomass were significantly higher in the winter-spring season (up to 90% higher than in the spring-summer with FS NS), demonstrating that optimal climatic conditions (cooler temperatures) are the dominant factor governing productivity over high nutrient supply or density. The second mowing yielded significantly higher biomass than the first (44% higher, on average), confirming the species' strong regrowth capacity, which is critical for maximizing output per unit of area and time. The higher plant density (947 plants m^{-2}) increased total yield per unit area and improved both NUE and WUE in the first mowing, suggesting it is the optimal density for maximizing resource capture. The Half-Strength

Nutrient Solution (HS NS) maximized Nitrogen Use Efficiency (NUE) in the winter-spring season, confirming the expected trade-off between maximizing resource efficiency (achieved at slight nutrient deficit) and maximizing total biomass (achieved with full supply). The leaf celery provided beneficial compounds, including vitamins A and C as well as essential mineral elements. Increasing NS concentration (FS NS) increased total biomass but was associated with a reduction in Vitamin A content in the second mowing, highlighting a dilution effect or an inverse relationship between growth rate and accumulation of certain health-promoting compounds. Its sensory profile and overall acceptability highlighted its potential use in ready-to-eat salad mixes, where it can contribute a distinctive aromatic flavor. Minimally processed baby leaves maintained acceptable overall quality for up to 14 days under cold storage. Quality retention was best for leaves grown with FS NS, while the combination of high density and nutrient stress (the 947−HS treatment) resulted in the poorest general appearance and shelf stability, indicating compromised tissue quality.

Overall, hydroponic leaf celery cultivation is most productive during the cooler winter-spring season and should utilize the Full-Strength nutrient solution to maximize yield and post-harvest quality. However, for growers prioritizing sustainability and N efficiency, the Half-Strength NS offers significantly better NUE. Future work should focus on optimizing the hydroponic system design (e.g., floating system) to mitigate heat stress in the warmer season and achieve more consistent year-round production.

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IV. SALINITY STRESS EFFECTS ON BABY LEAF PRODUCTION OF LEAF CELERY (*APIUM GRAVEOLENS* L. VAR. *SECALINUM*) AND CITRULLINE-MEDIATED MITIGATION

1. Introduction

Salinity is a primary abiotic stressor limiting global agricultural productivity, particularly in the Mediterranean basin where the use of marginal-quality water for irrigation is increasing (Munns and Tester, 2008). Leaf celery (*Apium graveolens* L. var. *secalinum*) has emerged as a high-value crop in the "baby leaf" industry due to its aromatic profile and health-promoting compounds. While celery is classified as moderately salt-sensitive (Maksimovic and Ilin, 2012), its production in closed hydroponic systems often leads to substrate "salt-loading," which compromises regrowth vigor and cumulative yield across successive harvests. Innovative agronomic strategies, such as the application of exogenous compounds, are essential for maintaining productivity under saline conditions. Among these protective agents, amino acids are particularly significant; however, plant responses vary considerably depending on the specific compound, species, dosage, and application method. Their exogenous use can elicit various beneficial effects, ranging from enhanced growth and productivity to improved nutritional or commercial quality in several leafy vegetable crops (Bulgari et al., 2019; Haghighi et al., 2022). Moreover, these applications improve abiotic stress tolerance by modulating osmotic and ionic balance, activating antioxidant defense systems, and preserving photosynthetic capacity (Ahmad et al., 2025; Peña Calzada et al., 2022). Given the various beneficial effects associated with amino acid application, numerous studies have evaluated the efficacy of several compounds, primarily proline, but also glutathione, melatonin, arginine and glycine (Ali et al., 2019; Khalid et al., 2022; Ma et al., 2026). Nevertheless, many other compounds, including citrulline, require further investigation to evaluate their potential as growth promoters and stress mitigants. Citrulline is a non-proteinogenic amino acid and a key intermediate in the arginine biosynthetic pathway (Song et al., 2020). This amino acid accumulates in the tissues of various species in response to abiotic stress, helping to mitigate oxidative damage (Akashi et al., 2001). Furthermore, it may act as a precursor for nitric oxide (NO) signaling, a molecule recognized for its role in modulating various morphological processes and defense mechanisms (Crawford, 2006; Kolupaev et al., 2023). Despite its potential role in mitigating oxidative damage, the efficacy of exogenous citrulline in alleviating salinity stress in leaf celery remains unexplored. Therefore, the primary objective of this study was to evaluate the response of leaf celery to varying saline conditions within a closed-loop hydroponic cultivation system. Specifically, we investigated the capacity of citrulline to preserve biomass accumulation, resource use efficiency (WUE and NUE) across two successive mowings, simulating commercial "cut-and-come-again" production. Furthermore, the study sought to determine the carry-

over effects of citrulline and salinity on the post-harvest shelf-life and aesthetic quality of the harvested material as a minimally processed "baby leaf" product. By linking metabolic biostimulation to both yield and storage performance, this research aims to provide a sustainable solution for saline hydroponic systems.

2. Materials and methods

2.1. Leaf celery baby leaf production

The trial was carried out during the 2024-2025 winter seasons in an unheated greenhouse at the Department of Agricultural, Food, and Forest Sciences (SAAF), University of Palermo, Italy (38°06'28″ N, 13°21′03″ E; 49 m a.s.l.). Seeds of a Sicilian landrace of *Apium graveolens* L. var. *secalinum* Alef. were sown in November 2024 in polystyrene trays filled with a commercial substrate (Tappeti erbosi, Vigorplant Italia srl, Fombio, Italy, which was enriched with 850 g m^{-3} of a mineral fertilizer NPK). Seed germination took place in a growth chamber at 25 °C in complete darkness, with constant humidity maintained until seedling emergence (10 days after sowing). Following emergence, the trays were moved to the greenhouse, and seedlings were thinned to one uniform plant per cell.

Based on previous research on leaf celery (Esposito et al., 2026), plants were grown at a density of 947 plants m^{-2} (160 cells per tray; 18 mL cell volume) using a hydroponic ebb-and-flow system supplied with a full-strength nutrient solution (NS). The NS was prepared using tap water (pH 7.6; EC = 0.9 mS cm^{-1}) supplemented with the following macronutrients: 19 mM NO_3^-, 1.25 mM NH_4^+, 2 mM $H_2PO_4^-$, 11 mM K^+, 4.5 mM Ca^{2+}, 1 mM Mg^{2+}, and 1.1 mM SO_4^{2-}. Micronutrients were provided at concentrations of 40 µM Fe^{3+}, 30 µM BO_3^{3-}, 5 µM Mn^{2+}, 4 µM Zn^{2+}, 0.75 µM Cu^{2+}, and 0.50 µM Mo (Sonneveld and Voogt, 2009).

Three NaCl concentrations were established in the NS: 0 mM (control; S0), 50 mM (S50), and 100 mM (S100), resulting in electrical conductivity (EC) values of 2.3, 6.4, and 10.4 mS cm^{-1}, respectively. To evaluate the mitigation of salinity stress, L-Citrulline (Anastore Bio, S.L. – Abs, Boulogne-Billancourt, France) was foliar-applied via nebulization at three concentrations: 0 mM (plants sprayed with only water; Cit0), 1 mM (Cit1), and 10 mM (Cit10). The experimental design consisted of a factorial combination of salinity and citrulline treatments arranged in a randomized complete block design with four replicates of 160 plants each. Treatments commenced when the first true leaf was fully expanded, and citrulline applications were repeated at 7-day intervals.

Environmental parameters inside the greenhouse, including air temperature and relative humidity, were continuously monitored using a data logger (mod. 608-H1, Testo S.p.A., Settimo Milanese, Italy). During the first mowing, average minimum and maximum air temperatures were 15.0 ± 0.3°C and 22.6 ± 0.5°C, respectively, with an overall mean temperature of 18.8 ± 0.4°C and an average

relative humidity of 65.8 ± 1.5%. Throughout the second mowing (the regrowth phase), air temperatures reached an average minimum of 11.8 ± 0.3 °C and an average maximum of 19.4 ± 0.4 °C, with an average relative humidity of 69.2 ± 2.2%.

The ebb and flow system was configured to deliver a 15-minute irrigation cycle once every 24 hours. Water and nutrient solution consumption was determined gravimetrically by weighing the trays immediately before and after each irrigation event. Water use efficiency (WUE) was calculated as the ratio of plant dry biomass (g) to total water consumption (L) (Jones, 2004). Nitrogen use efficiency (NUE) was calculated as the ratio of plant dry biomass (g) to the total nitrogen supplied (N in the substrate + N provided through sub-fertigation) (Congreves et al., 2021).

To evaluate the effects of salinity and citrulline on yield and regrowth capacity, two sequential harvests (first and second mowings) were performed. At each mowing, 30 plants per replicate were collected by harvesting leaves 3–4 cm above the plant collar, when plants had approximately six fully expanded leaves. The first mowing was conducted 69 DAS (days after sowing). The second mowing was performed 43 days after the first.

Morpho-physiological parameters, including plant height, stem diameter, fresh and dry biomass accumulation, number of leaves, leaf area, and leaf color, were assessed on a separate set of 30 plants per replicate prior to each mowing. Leaf color parameters (L*, a*, and b*) were measured using a CR-400 portable colorimeter (Konica Minolta Inc., Osaka, Japan). Leaf chroma (C*) and hue angle (h°) were calculated according to standard colorimetric equations (McGuire, 1992).

For dry biomass determination, leaves were separated from stems and dried separately in a forced-air oven at 65 °C for 72 h until a constant weight was reached. Leaf area was determined using Easy Leaf Area software (Easlon and Bloom, 2014) via digital image analysis of individual leaves captured with a digital camera (Canon EOS 300D, Canon Inc., Tokyo, Japan). Specific leaf area (SLA) was calculated using the following formula: SLA ($cm^2\ g^{-1}$ DW)= leaf area (cm^2)/leaf dry weight (g DW) (Garnier et al., 2001).

2.2. Chemical determination

The concentrations of potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), manganese (Mn), and zinc (Zn) were quantified following the dry ashing of oven-dried leaf material (four replicates per treatment), according to the method described by Morand and Gullo (Morand and Gullo, 1970) with minor modifications. Briefly, 0.5 g of finely ground dry tissue was weighed into porcelain crucibles and mineralized in a muffle furnace at 550 °C for 8 h. The resulting ash was solubilized via acid extraction with 2% (v/v) HNO_3 on a hot plate at 100 °C for 15 min. After cooling, the extracts were filtered and brought to volume with deionized water. Elemental concentrations were determined using microwave plasma–atomic emission spectrometry (MP-AES; Agilent 4210 MP-AES, Agilent Technologies, Milan, Italy).

Phosphorus (P) concentration was determined separately using a colorimetric procedure. Finely powdered dry leaf samples (0.25 g) were subjected to dry mineralization in a muffle furnace at 550 °C for 8 h. The ash was subsequently dissolved in 10 mL of 1 M HCl by heating at 100 °C for 15 min on a hot plate. The solution was then transferred to 15 mL tubes, and the final volume was adjusted to 10 mL with Milli-Q water. Phosphorus concentration was quantified spectrophotometrically following the molybdenum-blue method (Murphy and Riley, 1962) using a UV–visible spectrophotometer (UV Mini-1240, Shimadzu, Kyoto, Japan).

2.3. Cold storage and shelf-life evaluation

The shelf life of minimally processed baby leaf celery from the first mowing was evaluated for each treatment. For the assessment of weight loss, overall quality, and leaf color, twelve samples (approximately 50 g each) per treatment were packaged in polyethylene (PE) bags and heat-sealed (Laica VT3112, Vicenza, Italy). Only undamaged leaves, free from yellowing or necrosis, were selected for minimal processing.

Prior to packaging, leaves were washed twice in tap water for 5 min, manually centrifuged for 1 min using a handheld salad spinner. Samples were stored at 4 °C and evaluated at the time of packaging (day 0), and after 7, 14, and 21 days, using three randomly selected replicates per treatment at each sampling point.

Weight loss (g 100 g^{-1} fresh weight, FW) was calculated as the difference between the initial sample weight (day 0) and the weight at each sampling interval (Javanmardi and Kubota, 2006). Overall quality was evaluated by an untrained panel of twelve members using a 5-point hedonic scale (5 = excellent, freshly harvested quality; 3 = limit of marketability; 1 = poor/unmarketable), as described by Esposito et al. (Esposito et al., 2026).

2.4. Experimental design and statistical analysis

The experiment followed a randomized complete block design with four replicates of 30 plants for each combination of salinity level and citrulline concentration. Data from the first and second mowings were analyzed separately using a two-way analysis of variance (ANOVA) to evaluate the effects of NaCl and citrulline on leaf celery plants.

For the shelf-life experiment, a completely randomized design was employed. Data were analyzed via a three-way ANOVA to assess the effects of NaCl, citrulline, and storage time on leaf celery baby leaf from the first mowing.

When the ANOVA indicated significant differences, mean separation was performed using the Least Significant Difference (LSD) test at $p \leq 0.05$.

3. Results

3.1. First mowing

The fresh weight (FW) of leaf celery was significantly influenced by both NaCl (S) and citrulline (Cit) treatments. Shoot fresh biomass significantly decreased with increasing salinity, ranging from 1.8 to 1.5 g plant^{-1} (Figure 1a). Conversely, citrulline application significantly increased shoot FW, reaching the highest value at 10 mM (2.0 g plant^{-1}, a 55.2% increase compared to the control) (Figure 1b). Salinity negatively affected stem FW: the highest value (0.27 g plant^{-1}) was obtained under control conditions (S0) and the lowest at S100 (0.24 g plant^{-1}). At S50, an intermediate stem fresh biomass value of 0.26 g plant^{-1} was observed (Figure 1a). Citrulline significantly increased stem FW in a dose-dependent manner, with values ranging from 0.20 to 0.31 g plant^{-1} (Figure 1b). Under S0, plants exhibited the highest leaf FW (1.5 g plant^{-1}). Salinity reduced leaf FW by an average of 16.2% compared to S0, with no significant differences observed between S50 and S100 (Figure 1a). Citrulline application significantly enhanced leaf FW; specifically, the 10 mM and 1 mM concentration resulted in increases of 54.8% and 20.2%, respectively, compared to the control.

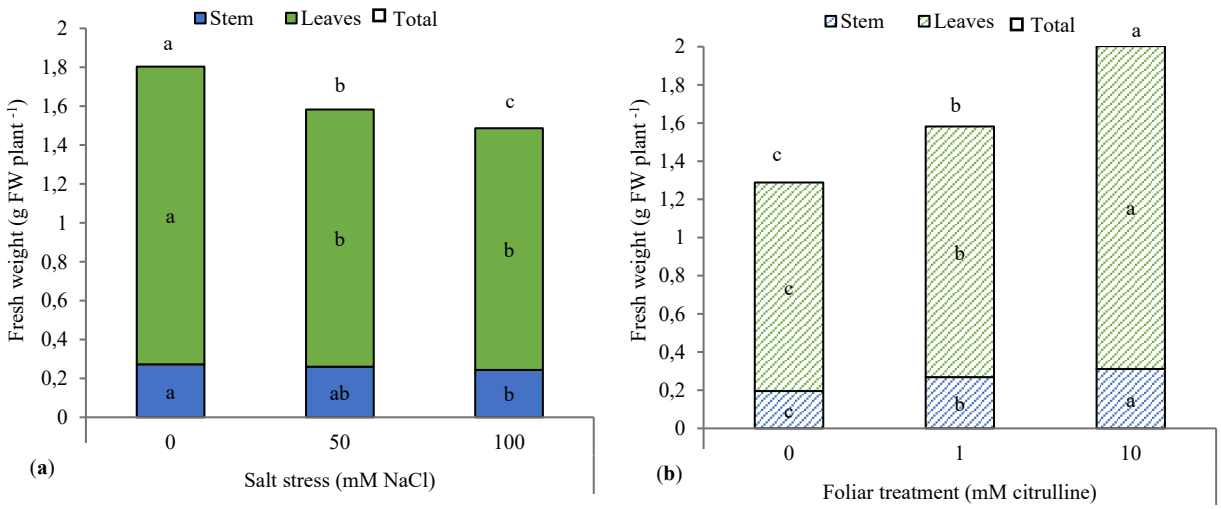

Figure 1. Effects of (**a**) salt stress (0, 50, and 100 mM NaCl in the nutrient solution) and (**b**) foliar treatment (0, 1 and 10 mM citrulline) on the shoot, stem, and leaves fresh weight (FW) at the first mowing of *Apium graveolens* L. var. *secalinum* Alef. plants grown using an ebb-and-flow hydroponic system (bars of the same color with different letters are significantly different at $p \leq 0.05$ according to the LSD test).

Total and leaf dry biomass were significantly affected only by the citrulline treatment, showing significant increases at each concentration level, reaching 165.4 and 145.8 mg plant^{-1}, respectively, at 10 mM (Table 1). In the absence of citrulline, stem dry weight (DW) did not differ statistically across NaCl levels, resulting in the lowest overall values (15.0 mg plant^{-1} on average). Under S0 conditions, citrulline had no significant effect on stem DW (17.4 mg plant^{-1} on average); however, under severe salinity (S100), the Cit10 treatment resulted in the highest stem DW (23.3 mg plant^{-1})

(Table 1). Leaf dry matter percentage ranged between 8.0% and 9.6% but was not significantly affected by the experimental factors.

Table 1. Effects of salt stress (0, 50, and 100 mM NaCl in the nutrient solution) and citrulline foliar treatment (0, 1 and 10 mM) on the dry biomass accumulation at the first mowing of *Apium graveolens* L. var. *secalinum* Alef. plants grown using an ebb-and-flow hydroponic system.

Source of Variance (1st mowing)		Plant dry weight (mg DW)			Leaf dry matter (DM%)
		Shoot	Stem	Leaves	
NaCl (mM)					
0		[z] 142.0	17.4	124.6	8.1
50		132.6	17.7	114.9	8.7
100		130.0	16.8	113.2	9.1
Citrulline (mM)					
0		109.7c	15.0	94.7c	8.7
1		129.4b	17.2	112.2b	8.6
10		165.4a	19.7	145.8a	8.7
NaCl x Citrulline					
S0	Cit0	113.7	16.7bc	97.0	8.0
	Cit1	149.7	18.3bc	131.3	8.4
	Cit10	162.7	17.3bc	145.3	8.0
S50	Cit0	106.7	15.3c	91.3	8.6
	Cit1	128.7	19.3b	109.3	9.2
	Cit10	162.3	18.3bc	144.0	8.4
S100	Cit0	108.7	13.0c	95.7	9.5
	Cit1	110.0	14.0c	96.0	8.1
	Cit10	171.3	23.3a	148.0	9.6
Significance [x]					
NaCl		ns	ns	ns	ns
Citrulline		***	***	***	ns
NaCl x Citrulline		ns	***	ns	ns

[z] Each value is the mean of 4 replicated samples of 30 plants each. For each factor, values in a column followed by the same letter are not significantly different, according to the LSD test. [x] Significance: ns = not significant; *** significant at $p < 0.001$.

Leaf number was significantly influenced only by the citrulline treatment, increasing from 5.7 leaves plant^{-1} (0 mM) to 6.3 leaves plant^{-1} (10 mM) (Table 2). Leaf and petiole length followed similar trends, characterized by a significant NaCl × Citrulline interaction. In the absence of citrulline, celery plants grown at S0 had leaves and petioles lengths of 15.8 and 11.1 cm, respectively. Increasing salt stress to S100 markedly reduced these parameters to 13.9 and 10.1 cm, respectively, while S50 showed intermediates values (Table 2). Under non-saline conditions (S0), both parameters increased with increasing citrulline concentration, peaking at 18.9 cm and 13.3 cm, respectively, at 10 mM citrulline (Table 2). At S50, 10 mM citrulline increased leaf and petiole length by an average of 18.0% and 15.7%, respectively, compared to both Cit0 and Cit1; notably, these values were comparable to

those of the S0-Cit10 treatment. Furthermore, the S100-Cit1 and S100-Cit10 treatments restored leaf and petiole lengths to levels statistically comparable to the non-saline control (Table 2).

Total and average leaf area were negatively affected by salinity. Under non-saline growing conditions (S0) and in the absence of citrulline, total and average leaf area was 34.8 cm^2 $plant^{-1}$ and 6.0 cm^2 $leaf^{-1}$, respectively; these values decreased equally at 50 and 100 mM NaCl (averaging 30.4 cm^2 $plant^{-1}$ and 5.3 cm^2 $leaf^{-1}$). The highest total and average leaf areas were observed in S0-Cit10 treatment (47.1 cm^2 $plant^{-1}$ and 7.5 cm^2 $leaf^{-1}$, respectively) (Table 2). Citrulline exerted a clear mitigating effect on these parameters; at both S50 and S100, the application of 1 mM citrulline resulted in leaf areas comparable to non-saline untreated plants (S0-Cit0). The 10 mM citrulline treatment further increased leaf area above non-saline control levels, with the most pronounced mitigating effect observed in the S50-Cit10 treatment (46.5 cm^2 $plant^{-1}$ and 7.4 cm^2 $leaf^{-1}$, respectively).

Specific leaf area (SLA) was not significantly affected by any experimental factor, averaging 331.7 cm^2 g^{-1} DW (Table 2).

Leaf color parameters (lightness, chroma, and hue angle) were significantly influenced by both NaCl and citrulline treatments. The highest lightness (L*) was recorded at S0 (48.7), and decreased significantly with each increase in salinity, down to 45.9 at S100. L* also decreased at the highest citrulline concentration (10 mM) (Table 2). Chroma followed a trend similar to L* for both salinity and citrulline treatments (Table 2). Hue angle (hue°) significantly increased under salt stress (123.6° on average for S50 and S100). Citrulline positively affected hue angle, which ranged from 122.9° (Cit0) to 123.8° (Cit10) (Table 2).

Table 2. Effects of salt stress (0, 50, and 100 mM NaCl in the nutrient solution) and foliar treatment (0, 1 and 10 mM citrulline) on the leaf characteristics at the first mowing of *Apium graveolens* L. var. *secalinum* Alef. plants grown using an ebb-and-flow hydroponic system.

Source of Variance (1st mowing)		Number of Leaves	Leaf length (cm)	Petiole length (cm)	Leaf Area (cm^2 $Plant^{-1}$)	Leaf Area (cm^2 $Leaf^{-1}$)	SLA (cm^2 g DW^{-1})	L*	Chroma	Hue°
NaCl (mM)										
0		[z] 6.0	17.3	12.2	41.5	6.8	336.6	48.7a	34.8a	122.6b
50		6.0	16.1	11.5	37.6	6.2	329.0	47.4b	33.0b	123.4a
100		6.0	15.8	11.3	36.0	6.0	329.4	45.9c	30.5c	123.8a
Citrulline (mM)										
0		5.7c	14.9	10.7	31.9	5.6	344.3	47.7a	33.3a	122.9b
1		6.0b	16.4	11.7	37.6	6.3	337.8	47.6a	33.2a	123.1ab
10		6.3a	17.9	12.6	45.6	7.3	312.9	46.7b	31.8b	123.8a
NaCl x Citrulline										
S0	Cit0	5.8	15.8c	11.1c	34.8c	6.0c	360.1	49.2	34.4	121.8
	Cit1	6.0	17.3b	12.1b	42.7b	7.1b	325.5	48.4	35.6	123.0
	Cit10	6.3	18.9a	13.3a	47.1a	7.5a	324.1	48.3	34.3	123.0

S50	Cit0	5.8	15.2cd	10.8cd	31.3d	5.4d	342.8	47.5	33.9	123.2
	Cit1	6.0	15.3c	11.0c	35.1c	5.9c	321.4	47.7	32.9	123.2
	Cit10	6.3	18.0ab	12.6ab	46.5ab	7.4ab	322.9	47.0	32.1	123.7
S100	Cit0	5.7	13.9d	10.1d	29.6d	5.2d	330.0	46.4	31.5	123.6
	Cit1	6.0	16.6bc	11.8bc	35.2c	5.9c	366.6	46.6	31.1	123.2
	Cit10	6.2	16.8bc	12.0bc	43.1b	7.0b	291.7	44.8	29.0	124.6
Significance [x]										
NaCl		ns	***	***	***	***	ns	***	***	**
Citrulline		***	***	***	***	***	ns	**	***	*
NaCl x Citrulline		ns	**	**	**	**	ns	ns	ns	ns

[z] Each value is the mean of 4 replicated samples of 30 plants each. For each factor, values in a column followed by the same letter are not significantly different, according to the LSD test. [x] Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$.

3.2 Second mowing

Total and leaf fresh weight (FW) followed similar trends. In the absence of citrulline, fresh biomass was severely reduced at both S50 (total FW -31.2%; leaf FW -34.8%) and S100 (total FW-52.3%; leaf FW -59.2%) compared to the control (2.89 and 2.25 g plant^{-1}, respectively) (Table 3).

Citrulline application had varying effects depending on the level of salt stress. Under control conditions (S0), citrulline exerted a growth-promoting effect, with biomass increasing by an average of 14.2% with Cit1 and 32.2% with Cit10 for total and leaf FW (Table 3). At S50, Cit10 mitigated the negative impact of salinity, resulting in a smaller reduction in fresh biomass (-18.1% for total FW and -22.6% for leaf FW compared to S0-Cit0). Conversely, at S100, citrulline had no significant effect on fresh biomass (Table 3). Stem FW was significantly reduced by salinity, decreasing by 18.2% under S50 and 32.0% under S100 compared to S0 (0.70 g plant^{-1}). In contrast, both citrulline concentrations significantly increased stem biomass, reaching a maximum of 0.65 g plant^{-1} with the Cit10 treatment (Table 3).

Table 3. Effects of salt stress (0, 50, and 100 mM NaCl in the nutrient solution) and foliar treatment (0, 1 and 10 mM citrulline) on the fresh biomass accumulation at the second mowing of *Apium graveolens* L. var. *secalinum* Alef. plants grown using an ebb-and-flow hydroponic system.

Source of Variance (2st mowing)		**Plant fresh weight (g FW)**		
		Total	**Stem**	**Leaves**
NaCl (mM)				
0		[z] 3.30	0.70a	2.60
50		2.16	0.57b	1.59
100		1.47	0.48c	0.99
Citrulline (mM)				
0		2.09	0.52c	1.55
1		2.28	0.58b	1.70
10		2.56	0.65a	1.93
NaCl x Citrulline				
S0	Cit0	2.89c	0.65	2.25c
	Cit1	3.26b	0.69	2.57b

	Cit10	3.75a	0.77	2.98a
S50	Cit0	1.99e	0.51	1.47e
	Cit1	2.12de	0.57	1.55de
	Cit10	2.37d	0.64	1.74d
S100	Cit0	1.38f	0.41	0.92f
	Cit1	1.46f	0.47	0.99f
	Cit10	1.55f	0.55	1.06f
Significance [x]				
NaCl		***	***	***
Citrulline		***	***	***
NaCl x Citrulline		**	ns	**

[z] Each value is the mean of 4 replicated samples of 30 plants each. For each factor, values in a column followed by the same letter are not significantly different, according to the LSD test. [x] Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$.

Regarding dry biomass accumulation, there was no significant interaction between salt stress and citrulline concentration. The total and leaf dry weight (DW) reached their highest values at S0 (316.1 and 267.0 mg plant^{-1}, respectively). Increasing salinity resulted in a linear decrease in dry biomass accumulation, culminating in reductions of -32.0% and -43.9%, respectively at S100 (Figure 2a). Conversely, stem dry biomass was positively affected by salinity, showing a significant increase under both salt stress levels (+34.3% on average compared to S0) (Figure 2a). Citrulline application significantly increased dry biomass accumulation in both leaves and stems. This increase was significant for leaf DW with both Cit1 (+8.9%) and Cit10 (+18.6%), while for stem DW, the increase was significant only with Cit10 (+15.4%) (Figure 2b). Leaf dry matter content (DM%) was significantly affected only by the salinity treatment. Salt stress determined a significant linear increase in this parameter, which ranged from 10.3% (S0) to 15.2% (S100).

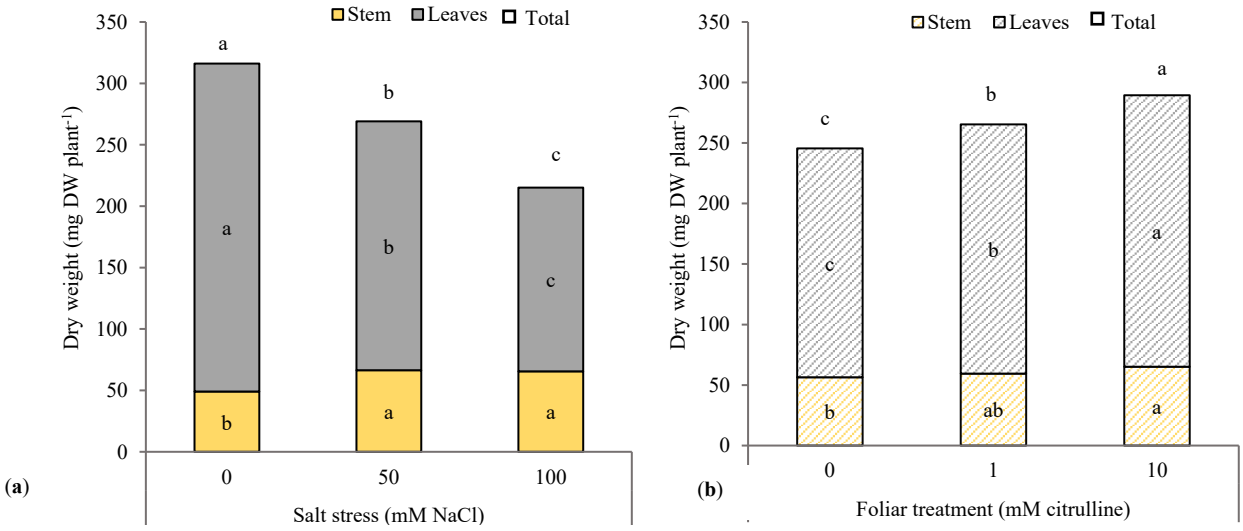

Figure 2. Effects of (**a**) salt stress (0, 50, and 100 mM NaCl in the nutrient solution) and (**b**) foliar treatment (0, 1 and 10 mM citrulline) on the shoot, stem, and leaves dry weight (DW) at the second mowing of *Apium graveolens* L. var. *secalinum* Alef. plants grown using an ebb-and-flow hydroponic system (bars of the same color with different letters are significantly different at $p \leq 0.05$ according to the LSD test).

Growing leaf celery plants under S0 produced 5.8 leaves plant^{-1} on average, while salinity significantly decreased leaf number at both S50 (5.6 leaves plant^{-1}) and S100 (5.0 leaves plant^{-1}) (Table 4). Citrulline application significantly increased leaf number with increasing concentration, ranging from 5.2 (Cit0) to 5.8 (Cit10) leaves plant^{-1} (Table 4). Leaf and petiole lengths were significantly influenced by the NaCl × Citrulline interaction, with similar trends observed for both parameters. At S0, total leaf length increased with citrulline concentration, from 17.4 cm (Cit0) to 19.8 cm at Cit10. At S50 an without citrulline application, leaf length decreased by 33.2% to 11.6 cm. However, 10 mM citrulline significantly mitigated the salinity effect, increasing leaf length by 8.7% compared to S50-Cit0. The most severe inhibition was recorded at S100, which resulted in the shortest leaf and petiole lengths (averaging 9.7 and 5.3 cm, respectively); at this salinity level, citrulline application did not exert a significant effect (Table 4).

The total and average leaf area at S0 averaged 60.2 cm² plant^{-1} and 10.4 cm² leaf^{-1}, respectively. These values significantly declined with increasing salinity to S50 (-34.3% and -32.1% compared to S0) and especially at S100 (-61.2% and -54.8% compared to S0) (Table 4). Leaf area increased with increasing citrulline concentrations, showing the lowest values at Cit0 (37.2 cm² plant^{-1} and 7.1 cm² leaf^{-1}, on average) and the highest at Cit10 (45.2 cm² plant^{-1} and 7.7 cm² leaf^{-1}, on average) (Table 4).

Specific leaf area (SLA) was affected only by NaCl treatments, significantly decreasing by 13.5% at S50 and by 30.9% at S100 compared to the control (226.0 cm^2 g DW^{-1}) (Table 4).

Leaf color parameters were significantly affected only by NaCl treatment. Leaf lightness (L*) was higher at S0 (45.3) and significantly decreased under salt stress (43.5 on average at S50 and S100) (Table 4). Leaf celery showed greater color saturation (chroma) at S0, which decreased with increasing salinity, ranging from 26.0 to 22.7 (Table 4). Hue angle showed a significant reduction only at S100 (124.4°; Table 4).

Table 4. Effects of salt stress (0, 50, and 100 mM NaCl in the nutrient solution) and foliar treatment (0, 1 and 10 mM citrulline) on the leaf characteristics at the second mowing of *Apium graveolens* L. var. *secalinum* Alef. plants grown using an ebb-and-flow hydroponic system.

Source of Variance (2st mowing)	Number of Leaves	Leaf length (cm)	Petiole length (cm)	Leaf Area (cm^2 Plant^{-1})	Leaf Area (cm^2 Leaf^{-1})	SLA (cm^2 g DW^{-1})	L*	Chroma	Hue°
NaCl (mM)									
0	[z] 5.8a	18.5	11.3	60.2a	10.4a	226.0a	45.3a	26.0a	126.3a
50	5.6b	12.1	6.9	39.6b	7.1b	195.4b	43.9b	23.8b	126.6a
100	5.0c	9.7	5.3	23.4c	4.7c	156.2c	43.1b	22.7c	124.4b
Citrulline (mM)									
0	5.2c	12.7	7.2	37.2c	7.1c	190.0	44.5	23.6	125.3
1	5.4b	13.4	7.7	40.8b	7.4b	192.2	44.1	24.7	124.7
10	5.8a	14.2	8.4	45.2a	7.7a	195.4	43.7	24.2	127.4

NaCl x Citrulline										
S0	Cit0	5.5	17.4c	10.3c	54.4	9.8	231.0	45.8	25.2	126.3
	Cit1	5.7	18.3b	11.0b	59.5	10.4	224.1	45.2	26.8	126.4
	Cit10	6.1	19.8a	12.5a	66.8	10.9	222.7	44.9	26.1	126.3
S50	Cit0	5.3	11.6e	6.5e	36.3	6.8	191.8	44.1	22.6	124.9
	Cit1	5.6	12.0de	6.9de	39.2	7.0	195.6	43.9	24.6	124.1
	Cit10	5.8	12.6d	7.3d	43.2	7.4	198.7	43.6	24.3	130.8
S100	Cit0	4.6	9.2f	5.0f	21.0	4.6	147.0	43.6	23.1	124.7
	Cit1	5.0	9.7f	5.3f	23.7	4.8	156.8	43.3	22.6	123.5
	Cit10	5.3	10.1f	5.5f	25.4	4.8	164.7	42.4	22.3	125.0
Significance [x]										
NaCl		***	***	***	***	***	***	***	***	***
Citrulline		***	***	***	***	***	ns	ns	ns	ns
NaCl x Citrulline		ns	***	***	ns	ns	ns	ns	ns	ns

[z] Each value is the mean of 4 replicated samples of 30 plants each. For each factor, values in a column followed by the same letter are not significantly different, according to the LSD test. [x] Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$.

3.3 Baby leaf yield and resource use efficiency

The first mowing occurred 69 DAS. Baby leaf yield was significantly affected by both NaCl levels and citrulline concentration. Salinity negatively impacted yield, with the highest values recorded under non-saline conditions (1.45 kg m^{-2}; Table 5). In contrast, the S50 and S100 treatments did not differ significantly from each other, both resulting in an average yield reduction of 16.2% compared to S0. Citrulline application significantly enhanced yield in a dose-dependent manner, ranging from 1.03 kg m^{-2} (Cit0) to 1.60 kg m^{-2} (Cit10) (Table 5).

The second mowing was carried out 43 days after the first. In absence of citrulline, baby leaf yield markedly declined with increasing NaCl levels, falling from 2.13 kg m^{-2} (S0-Cit0) to 0.87 kg m^{-2} (S100-Cit0) (Table 5). Under non-saline conditions (S0), both Cit1 and Cit10 treatments significantly enhanced baby leaf yield, with the greatest effect observed at Cit10 (2.8 kg m^{-2}, +32.6% compared to S0–Cit0; Table 5). At moderate salinity (S50), Cit10 increased baby leaf yield to 1.65 kg m^{-2} (+18.7% compared with S50–Cit0), while Cit1 resulted in intermediate values. Under severe salinity (S100), the lowest baby leaf yield was recorded (0.9 kg m^{-2} on average), and citrulline application exerted no significant effect (Table 5).

Total baby leaf yield (the cumulative of both harvests) was significantly influenced by the NaCl × Citrulline interaction. Under non-saline conditions (S0), total yield increased with increasing citrulline concentration, peaking 4.55 kg m^{-2} with Cit10 (+38.7% compared to the untreated control; 3.28 kg m^{-2}) (Table 5). At S50, while no differences were observed between Cit0 and Cit1 (2.50 kg m^{-2} on average), Cit10 significantly increased total yield to 3.27 kg m^{-2} (+30.7% compared to the average of S50-Cit0 and S50-Cit1) (Table 5). Notably, the application of 10 mM citrulline at S50 restored the total yield to levels comparable to the untreated non-saline control (S0-Cit0). At S100, a

similar trend was observed: 1 mM citrulline had no effect, whereas 10 mM citrulline significantly increased yield to levels comparable to the S50-Cit0 and S50-Cit1 treatments.

Table 5. Effects of salt stress (0, 50, and 100 mM NaCl in the nutrient solution) and foliar treatment (0, 1 and 10 mM citrulline) on the baby leaf yield of *Apium graveolens* L. var. *secalinum* Alef. plants grown using an ebb-and-flow hydroponic system.

Source of Variance		Baby leaf yield (kg m^{-2})		
		1st mowing	2st mowing	Total
NaCl (mM)				
0		[z] 1.45a	2.46	3.91
50		1.25b	1.50	2.75
100		1.18b	0.93	2.11
Citrulline (mM)				
0		1.03c	1.46	2.50
1		1.24b	1.61	2.86
10		1.60a	1.82	3.43
NaCl x Citrulline				
S0	Cit0	1.14	2.13c	3.28c
	Cit1	1.48	2.44b	3.92b
	Cit10	1.72	2.82a	4.55a
S50	Cit0	1.01	1.39e	2.40de
	Cit1	1.13	1.47de	2.60d
	Cit10	1.62	1.65d	3.27c
S100	Cit0	0.95	0.87f	1.82e
	Cit1	1.12	0.93f	2.05e
	Cit10	1.46	1.00f	2.47d
Significance [x]				
NaCl		***	***	***
Citrulline		***	***	***
NaCl x Citrulline		ns	**	*

[z] Each value is the mean of 4 replicated samples of 30 plants each. For each factor, values in a column followed by the same letter are not significantly different, according to the LSD test. [x] Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$.

Water use efficiency (WUE) and nitrogen use efficiency (NUE) calculated for the first mowing were significantly affected by the citrulline application. WUE was lowest in the absence of citrulline (3.98 g DW L^{-1} H_2O) and increased significantly with the application of 10 mM citrulline (5.48 g DW L^{-1} H_2O) (Figure 3). Similarly, NUE increased significantly with increasing citrulline concentrations, ranging from 25.56 to 38.39 g DW g^{-1} N, with significant improvements observed at every citrulline level (Figure 4).

In the second mowing, WUE and NUE exhibited similar trends. No significant differences were detected between S0 and S50, which yielded the highest WUE (2.6 g DW L^{-1} H_2O on average) and NUE (19.1 g DW g^{-1} N on average). In contrast, the S100 treatment significantly reduced both WUE and NUE. Citrulline application significantly enhanced both parameters; both Cit1 and Cit10

treatments resulted in significantly higher values than the control, averaging 2.6 g DW L^{-1} H_2O for WUE and 18.9 g DW g^{-1} N for NUE (Figure 3 and 4). Considering the entire growing cycle, total WUE was primarily affected by citrulline application, which induced an increasing trend from 2.6 g DW L^{-1} H_2O at Cit0 to 3.4 g DW L^{-1} H_2O at Cit10. Total NUE was 22.4 g DW g^{-1} N at S0 and did not change significantly at S50, whereas the highest salt stress (S100) resulted in a significant reduction of this parameter (19.6 g DW g^{-1} N). Regarding total NUE, citrulline application followed the same trend as WUE, with significant increases at each concentration level, rising from 18.2 g DW g^{-1} N at Cit0 to 24.3 g DW g^{-1} N at Cit10 (Figures 3 and 4).

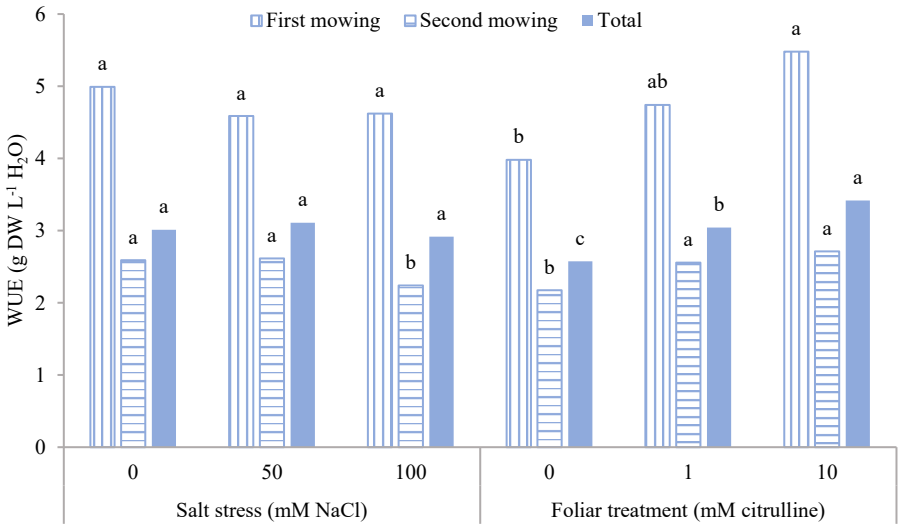

Figure 3. Effects of salt stress (0, 50, and 100 mM NaCl in the nutrient solution) and foliar treatment (0, 1 and 10 mM citrulline) on the water use efficiency (WUE) of *Apium graveolens* L. var. *secalinum* Alef. plants grown using an ebb-and-flow hydroponic system (bars of the same color with different letters are significantly different at $p \leq 0.05$ according to the LSD test).

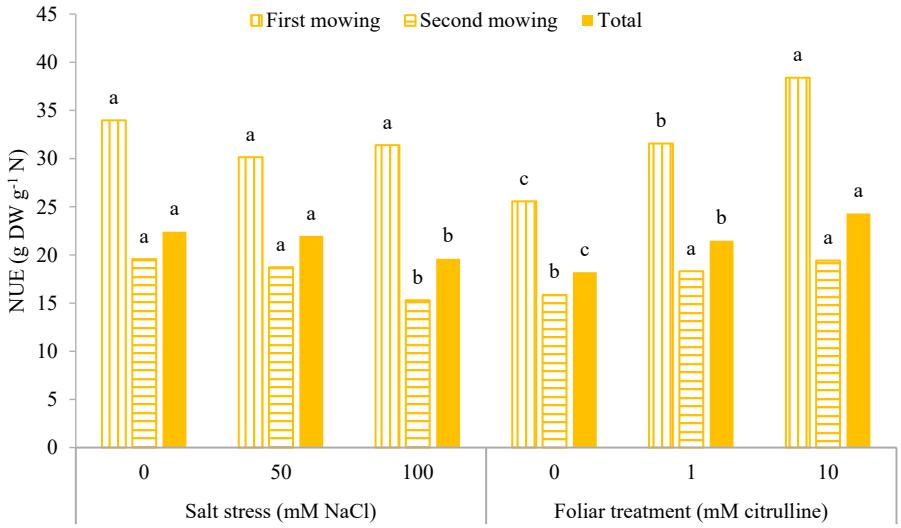

Figure 4. Effects of salt stress (0, 50, and 100 mM NaCl in the nutrient solution) and foliar treatment (0, 1 and 10 mM citrulline) on the nitrogen use efficiency (NUE) of *Apium graveolens* L. var. *secalinum* Alef. plants

grown using an ebb-and-flow hydroponic system (bars of the same color with different letters are significantly different at $p \leq 0.05$ according to the LSD test).

3.4 Elemental composition

At the first mowing, the concentrations of Ca, K, Mg, and Na were significantly affected only by NaCl treatments. Ca content decreased with increasing salinity, ranging from 191.4 to 149.9 mg 100 g^{-1} FW (Table 5). K content was 442.6 mg 100 g^{-1} FW at S0 and was significantly reduced only at S100, while Mg was reduced equally by S50 and S100 (30.6 mg 100 g^{-1} FW, on average) (Table 5). In contrast, sodium (Na) content increased by 230.2% at S50 and 291.2% at S100 compared to the S0 control (98.5 mg 100 g^{-1} FW; Table 6). Fe content was influenced by both salinity and citrulline treatments, significantly increasing with the highest NaCl levels (up to 5.8 mg 100 g^{-1} FW at S100) and the highest citrulline concentrations (up to 5.7 mg 100 g^{-1} FW at 10 mM) (Table 5). Cu content was not affected by any experimental factors. P and Zn contents remained stable across the treatments, except for a significant increase under sever salt stress (S100) in the plants treated with 10 mM citrulline. Mn content did not change as a function of citrulline under S0 whereas it was higher with Cit1 under S50 and with Cit10 under S100.

Table 6. Effects of salt stress (0, 50, and 100 mM NaCl in the nutrient solution) and foliar treatment (0, 1 and 10 mM citrulline) on the mineral content of *Apium graveolens* L. var. *secalinum* Alef. baby leaves (first mowing) obtained using an ebb-and-flow hydroponic system.

		First mowing								
Source of Variance (1st mowing)		**Ca (mg 100 g^{-1} FW)**	**K (mg 100 g^{-1} FW)**	**Mg (mg 100 g^{-1} FW)**	**Na (mg 100 g^{-1} FW)**	**P (mg 100 g^{-1} FW)**	**Zn (mg 100 g^{-1} FW)**	**Fe (mg 100 g^{-1} FW)**	**Cu (mg 100 g^{-1} FW)**	**Mn (mg 100 g^{-1} FW)**
NaCl (mM)										
0		[z] 191.4a	442.6a	40.2a	98.5c	34.9	0.58	4.2b	0.14	1.08
50		168.0b	418.7ab	32.0b	325.4b	37.9	0.62	5.0ab	0.18	1.16
100		149.9c	388.2b	29.3b	385.5a	40.3	0.64	5.8a	0.15	1.12
Citrulline (mM)										
0		169.0	425.1	33.8	253.4	36.4	0.57	4.5b	0.15	1.09
1		169.0	410.0	33.5	272.9	37.5	0.62	4.8ab	0.14	1.13
10		171.4	414.3	34.0	283.0	39.2	0.64	5.7a	0.17	1.14
NaCl x Citrulline										
S0	Cit0	187.9	465.4	39.6	97.5	37.1b	0.58b	3.9	0.11	1.08ab
	Cit1	192.7	440.4	40.4	102.9	34.7b	0.55b	4.0	0.15	1.04b
	Cit10	193.6	421.9	40.5	95.1	32.9b	0.59b	4.5	0.15	1.12ab
S50	Cit0	167.2	442.8	32.0	315.8	36.8b	0.59b	5.1	0.19	1.13ab
	Cit1	175.8	421.5	33.4	333.3	38.7b	0.68ab	5.1	0.15	1.31a
	Cit10	160.9	391.7	30.6	326.9	38.1b	0.58b	4.9	0.20	1.04b
S100	Cit0	151.8	367.2	30.0	346.9	35.4b	0.55b	4.4	0.16	1.06b
	Cit1	138.4	368.2	26.9	382.5	39.1b	0.62ab	5.2	0.12	1.03b
	Cit10	159.6	429.4	30.9	427.0	46.6a	0.76a	7.8	0.16	1.26a
Significance [x]										

NaCl	***	*	***	***	**	ns	*	ns	ns
Citrulline	ns	ns	ns	ns	ns	ns	*	ns	ns
NaCl x Citrulline	ns	ns	ns	ns	**	*	ns	ns	**

[z] Each value is the mean of 3 replicated samples. For each factor, values in a column followed by the same letter are not significantly different, according to the LSD test. x Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$.

The leaves from the second mowing (Table 7) differed in mineral content compared to the first mowing, especially regarding certain cations, and were affected only by salinity. Ca content was lower than in the first mowing and decreased significantly only under S50. K content under S0 was similar to the first mowing but increased with salinity (516.5 mg 100 g^{-1} FW on average for S50 and S100). Salinity also greatly influenced Na content in the second mowing, with Na levels being higher than those recorded in the first mowing; a similar trend was observed for Fe levels. While P, Zn and Mn contents under S0 were similar to those of the first mowing, the increase in their concentration due salt stress was more pronounced in the second mowing.

Table 7. Effects of salt stress (0, 50, and 100 mM NaCl in the nutrient solution) and foliar treatment (0, 1 and 10 mM citrulline) on the mineral content of *Apium graveolens* L. var. *secalinum* Alef. baby leaves (second mowing) obtained using an ebb-and-flow hydroponic system.

Source of Variance	Second mowing								
	Ca (mg 100 g^{-1} FW)	K (mg 100 g^{-1} FW)	Mg (mg 100 g^{-1} FW)	Na (mg 100 g^{-1} FW)	P (mg 100 g^{-1} FW)	Zn (mg 100 g^{-1} FW)	Fe (mg 100 g^{-1} FW)	Cu (mg 100 g^{-1} FW)	Mn (mg 100 g^{-1} FW)
NaCl (mM)									
0	[z] 150.8a	414.8b	44.0a	102.7c	34.6c	0.63c	7.1	0.10b	1.02c
50	136.0b	502.1a	36.0b	409.0b	41.9b	0.99b	8.4	0.19a	1.26b
100	148.2a	521.0a	40.1ab	596.2a	62.3a	1.56a	9.5	0.17a	1.58a
Significance [x]									
NaCl	**	**	**	***	***	***	ns	**	***
Citrulline	ns	ns	ns	ns	ns	ns	ns	ns	ns
NaCl x Citrulline	ns	ns	ns	ns	ns	ns	ns	ns	ns

[z] Each value is the mean of 3 replicated samples. For each factor, values in a column followed by the same letter are not significantly different, according to the LSD test. x Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$.

3.5 Cold storage of minimally processed baby leaf

Immediately after harvest, the quality retention and overall rating of minimally processed leaf celery from the first mowing were assessed over a 21-day cold storage period at 4 °C. Both weight loss (%) and overall quality (OQ) scores were significantly influenced by the experimental treatments (Table 8).

Weight loss increased progressively throughout the storage period. This increase was similar under S0 and S50 (up to 4.01% after 21 days), whereas it was significantly higher at each sampling date under S100 (up to 6.66% after 21 days). Weight loss was also affected by citrulline treatments, though this effect was only evident at the end of cold storage period; at day 21, samples treated with citrulline

(both Cit1 and Cit10) recorded a higher weight loss (5.21%, on average) compared to the control (4.26% on average).

The OQ score reached its highest values under S0 in Cit10 samples (4.5). Increasing salinity up to 100 mM NaCl in the absence of citrulline reduced the OQ score to its minimum value (3.8). Under these saline conditions, the application of citrulline restored the OQ score to control values (4.2 on average). The overall quality of the baby leaves decreased during storage, but at different rates depending on the salinity or citrulline treatment. Quality retention was not significantly affected by intermediate salt stress (S50), with scores remaining above the marketability limit after 21 days in both S0 and S50. However, under S100, the OQ score decreased significantly compared to other treatments by day 14 and fell below the marketability limit by the end of cold storage (2.8). In the absence of citrulline (Cit0), the OQ score began to decline after 14 days of storage (3.9) and fell below the limit of marketability at the end of storage period (2.8). Citrulline treatments improved quality retention at day 14 (4.3 on average for Cit1 and Cit10), and the highest dose of (Cit10) significantly improved the OQ score compared to the control at the end of the 21-day storage period (3.2).

Table 8. Effects of salt stress (0, 50, and 100 mM NaCl in the nutrient solution), foliar treatment (0, 1 and 10 mM citrulline) and storage at 4 ◦C on the quality parameters of minimally processed *Apium graveolens* L. var. *secalinum* Alef. baby leaves obtained using an ebb-and- flow hydroponic system (Overall quality scores: 1 - unmarketable; 3 - average—limit of marketability; 5 - excellent or having a fresh appearance).

Source of Variance		Weight loss (%)	Overall Quality
NaCl (mM)			
S0		[z] 2.10	4.4
S50		2.32	4.4
S100		4.17	4.1
Citrulline (mM)			
0		2.52	4.2
1		3.02	4.3
10		3.06	4.3
Days at 4°C (D)			
0			5.0
7		0.98	4.9
14		2.72	4.1
21		4.89	3.0
NaCl x Citrulline			
S0	Cit0	1.70	4.3b
	Cit1	2.26	4.4ab
	Cit10	2.34	4.5a
S50	Cit0	2.05	4.4ab
	Cit1	2.54	4.3ab
	Cit10	2.36	4.4ab
S100	Cit0	3.79	3.8c
	Cit1	4.25	4.3ab
	Cit10	4.47	4.1b
NaCl x D			
S0	0		5.0a
	7	0.30d	5.0a
	14	2.07c	4.3b

	21	3.94b	3.2d
S50	0		5.0a
	7	0.66d	5.0a
	14	2.22c	4.4b
	21	4.07b	3.0de
S100	0		5.0a
	7	1.98c	4.8a
	14	3.87b	3.7c
	21	6.66a	2.8e
Citrulline x D			
Cit0	0		5.0a
	7	0.91d	4.9a
	14	2.38c	3.9c
	21	4.26b	2.8e
	0		5.0a
Cit1	7	1.07d	5.0a
	14	2.86c	4.3b
	21	5.12a	3.1de
Cit10	0		5.0a
	7	0.96d	4.9a
	14	2.92c	4.3b
	21	5.30a	3.2d
Significance [x]			
NaCl		***	***
Citrulline		***	***
Days (D)		***	***
NaCl x Citrulline		ns	***
NaCl x D		**	***
Citrulline x D		*	*
NaCl x Citrulline x D		ns	ns

[z] Each value is the mean of three replicated samples of 50 g each. For each factor, values in a column followed by the same letter are not significantly different, according to the LSD test. x Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; and *** significant at $p < 0.001$.

4. Discussion

This study investigated the salt tolerance of leaf celery for baby leaf production and, for the first time, the efficacy of exogenous citrulline in mitigating salinity-induced stress. Our findings confirm that salinity impairs growth by reducing fresh biomass accumulation and yield, consistent with the classification of *Apium graveolens* L. as a moderately salt-sensitive species (Maksimovic and Ilin, 2012). The sensitivity to saline stress can be evaluated through various metrics, most notably the percentage loss of biomass or yield (Zörb et al., 2019). While previous studies reported that 100 mM NaCl significantly reduces shoot fresh weight (FW) in various types of celery (Gao et al., 2023; Song et al., 2024), our results demonstrate that this response is heavily influenced by mowing and regrowth cycles. Leaf celery is known for its ability to enhance biomass accumulation and yield following successive mowings (Esposito et al., 2026), a trait clearly observed in our trial. Under non-saline conditions (S0), the plants exhibited vigorous regrowth, accumulating 83.4% more biomass in the second mowing than the first. However, this "regrowth vigor" was severely compromised by salinity. During the first mowing, S50 and S100 treatments induced a comparable yield reduction (averaging 16.2%) regardless of the salt concentration. In contrast, the second mowing saw a dramatic intensification of stress, with yield losses averaging 50.5%. This divergent trend suggests that while

leaf celery employs effective osmotic tolerance strategies during initial growth, these mechanisms may become overwhelmed during subsequent regrowth cycles. A common strategy in glycophytes under saline conditions is the reduction of leaf size and lamina expansion to minimize transpiration and water loss (He et al., 2022; Moncada et al., 2020). This phenomenon was evident in our trial: despite salinity, the plants produced an identical number of leaves with stable thickness, but with a slight reduction in leaf area. This morphological plasticity suggests that during the first mowing, the plant prioritizes maintaining leaf production over expansion to mitigate water deficit.

The intensification of biomass and yield loss during the second mowing suggests a physiological shift from transient osmotic stress to cumulative ionic toxicity, likely driven by the progressive accumulation of sodium (Na^+) in plant tissues (Neumann, 1997). To initiate regrowth after the first mowing, the plant must mobilize internal reserves to produce new foliage *de novo*. This regenerative process triggers a surge in metabolic activity and transpiration, which inadvertently accelerates the translocation of Na^+ into the newly developing, highly sensitive sink tissues. This was supported by our data on Na^+ content of the leaves that was increased by salt stress to different extent due to temporal accumulation. During the initial growth cycle Na^+ content increased 2.6 and 2.9-fold in S50 and S100 treatments, respectively, yet yield remained relatively stable between the two. However, by the second mowing, Na^+ levels in S100 plants spiked to 4.8-fold over control, corresponding with the dramatic yield collapse. These trend is likely exacerbated by "salt-loading" of the substrate, a phenomenon where frequent saline fertigation combined with high evaporative demand leads to an exponential increase in root-zone salinity over time (Feng et al., 2025; Liu et al., 2021).

The elevated Na^+ content in salt-stressed plants presents a potential nutritional concern, as excessive sodium intake is directly linked to the prevalence of cardiovascular diseases (Jaques et al., 2021). Based on a standard 100 g serving of fresh baby leaves, the contribution to the adult Recommended Daily Intake (RDI) of 2 g day^{-1} (Breś et al., 2022) averaged 5% under control conditions (S0) across both mowings. However, salinity significantly shifted this profile. By the first mowing, the RDI contribution rose to 16.3% (S50) and 19.3% (S100). This trend intensified by the second mowing, where S50 plants reached levels (20.4%) comparable to the S100 plants of the first mowing. Most notably, S100 plants in the second mowing reached 29.8% of the RDI. While these values remain within safety thresholds, the inclusion of S100-stressed regrowth in the diet should be carefully managed within the context of total daily sodium consumption.

The "salt-loading" of the substrate appears to have directly undermined both Water Use Efficiency (WUE) and Nitrogen Use Efficiency (NUE). During the second mowing under S100, both parameters were approximately halved (-47.6% and -51.3 %, respectively) compared to the first mowing. This suggest that the energetic cost of maintaining water uptake against a mounting osmotic gradient eventually outweighed the plant's carbon fixation capacity (Langenfeld et al., 2022) and significantly

hindered nitrogen metabolism (Phan et al., 2023). Interestingly, salinity did not negatively impact WUE or NUE during the initial growth phase. This indicates that leaf celery is initially capable of maintaining its photosynthetic and metabolic integrity through morphological plasticity. Specifically, the reduction in leaf area and petiole length served to minimize the total transpiring surface, allowing the plant to balance its water budget during the first cycle. However, the cumulative ion toxicity and substrate saturation during regrowth likely rendered these morphological adaptations insufficient,

The exogenous application of amino acids can bolster plant metabolism by promoting nitrogen uptake and priming defence responses (Abdelkader et al., 2021; Colla et al., 2015), thereby enhancing biomass accumulation in a dose-dependent manner (Haghighi et al., 2022; Iqbal et al., 2021). In our study, the efficacy of citrulline, a non-proteinogenic amino acid and a key precursor in the arginine biosynthetic pathway (Song et al., 2020), was particularly evident. Citrulline is synthesized from ornithine via the action of ornithine carbamoyltransferase (OTC) (Majumdar et al., 2015). In previous studies, exogenous ornithine has been shown to stimulate arginine and proline biosynthesis, both of which are critical for osmotic adjustment and protein stability under stress (da Rocha et al., 2012; Winter et al., 2015).

We hypothesize that exogenous citrulline acts analogously to ornithine and arginine by fueling the arginine–proline–Nitric Oxide (NO) pathway. This is a multifaceted defense: while proline provides osmotic protection, NO acts as a potent signaling molecule that regulates N-metabolism and antioxidant activity (Bajguz, 2014; Hasanuzzaman et al., 2018; Matysiak et al., 2020). This is particularly significant for oxidative stress management; while salinity triggers the production of Reactive Oxygen Species (ROS) that damage lipids and proteins (Akyol et al., 2020), citrulline likely bolsters the plant's enzymatic machinery (e.g., superoxide dismutase and catalase), creating a biochemical 'shield' that preserves cellular integrity during the more intense regrowth phase. Analysis of baby leaf yield revealed that both citrulline doses (1 and 10 mM) increased productivity across both mowings; however, the efficacy of citrulline was notably attenuated during the regrowth phase. When excluding saline treatments, the growth-promoting effect of 1 and 10 mM citrulline was approximately halved during the second mowing compared to the first. For the 1 mM dose, the yield increase dropped from +20.3% to +10.2% while the 10 mM dose saw a decreased from +55.0% to +24.7% relative to the untreated control. This discrepancy likely stems from several factors influencing foliar citrulline absorption, most notably ambient temperature. Favorable climatic conditions are known to enhance foliar uptake (Pecha et al., 2012). In our trial, the first mowing occurred at an average daily temperature of 18.8 °C (the optimal range for celery), likely facilitating peak physiological activity and foliar penetration. Conversely, the 16.9% temperature drop during the second mowing, likely coupled with reduced stomatal conductance (Agurla et al., 2018), may have limited absorption, thereby diminishing the citrulline effect during the final harvest.

Ultimately, citrulline demonstrated a remarkable capacity to bridge the "economic gap" created by saline irrigation. Its potential as a biostimulant is most evident in its ability to effectively shift the plant's salt-tolerance threshold: the application of 10 mM citrulline under moderate salinity (S50) resulted in a cumulative baby leaf yield comparable to the non-saline, untreated control (S0-Cit0). Even under severe stress (S100), the 10 mM treatment enabled plants to achieve a total yield equivalent to untreated plants grown under only moderate salinity (S50-Cit0). This "compensatory effect" suggests that citrulline can effectively mitigate the productivity losses of an entire salinity tier, allowing growers to maintain standard yields despite using lower-quality irrigation water. Furthermore, the enhancement of NUE indicates that citrulline acts as a highly bioavailable nitrogen source (Song et al., 2020), facilitating a more efficient 'nitrogen shunt' that maintains metabolic momentum.

Beyond biomass, citrulline acted as a morphological rescuer. While salinity typically reduces leaf and petiole length in *Apium graveolens* L. (Kaya, 2023), 10 mM citrulline increased leaf area and petiole length across all treatments during the first mowing, a response consistent with exogenous arginine applications in lettuce and pea (Abdelkader et al., 2023; El-Beltagi et al., 2024). Even under severe salinity (S100), citrulline-treated plants produced leaves with lengths comparable to untreated non-saline controls. Although cumulative stress and lower temperatures attenuated reduced this efficacy by the second mowing, the resulting compact leaf profile and stress-hardened characteristics may remain highly desirable for the specific aesthetic and quality standards of the baby leaf market.

It is well established that saline water irrigation can negatively impact leaf quality by promoting the onset of necrosis, yellowing, and senescence (Allu et al., 2014; Ondrasek et al., 2021). In the baby leaf industry, visual quality is the primary determinant of marketability (Ciriello et al., 2021). Salinity typically reduces leaf lightness (L*) and saturation (Chroma) (Aydın, 2025), a trend also observed across both mowings in our trial. Interestingly, the hue angle increased under saline stress, a phenomenon similarly documented in basil (Ciriello et al., 2023). The increase in hue angle under salinity is an interesting nuance. In some species, this can indicate a shift toward a "darker green" or even a "bluish-green, which consumers often associate with freshness. The application of 10 mM citrulline effectively stabilized these chromatic parameters, preserving the visual integrity of the foliage. This improvement is likely tied to the stimulation of the arginine-proline-NO pathway. Nitric oxide (NO) has been shown to enhance the synthesis and stability of photosynthetic pigments by mitigating oxidative damages to chloroplasts, thereby maintaining leaf color intensity and delaying the onset of salt-induced yellowing (Mirakhorli et al., 2022).

Regarding post-harvest performance, leaf celery proved suitable to minimal processing across most salinity levels. Control (S0) and moderate salinity (S50) samples maintained marketable quality for up to 21 days; however, S100 samples exhibited significant weight loss and became unmarketable

after 14 days of cold storage. The exogenous application of citrulline exerted a protective effect on visual parameters, maintaining Overall Quality (OQ) above the marketability threshold throughout the 21-day storage period, even in salt-stressed samples. While pre-harvest amino acid application effects on post-harvest longevity are rarely studied, previous research on green leafy vegetables have shown that post-harvest arginine dips can reduce enzymatic browning and ethylene synthesis, thereby delaying senescence (Sohail et al., 2021; Wills and Li, 2016). This preservation of visual integrity is likely mediated by Nitric Oxide (NO) produced via the citrulline-arginine pathway. NO is a well-documented antagonist of ethylene, capable of downregulating ethylene biosynthesis and receptor expression to delay senescence and retain chlorophyll (Ahlawat and Liu, 2021; Liu et al., 2023; Zhu et al., 2022). Conversely, citrulline-treated plants showed increased weight loss by day 21 compared to the control. This likely reflects a carry-over of the enhanced metabolic rate and physiological vigor observed during the growth phase, which persist post-harvest, resulting in accelerated transpiration and respiratory water loss during storage (Shezi et al., 2024). Consequently, while citrulline significantly improves visual shelf-life by delaying senescence, its implementation in the baby leaf industry may require optimized post-harvest strategies, such as the use of Modified Atmosphere Packaging (MAP) or high-barrier films, to mitigate the secondary effect of increased weight loss and preserve the physical turgor of the product.

5. Conclusions

This study demonstrates that the exogenous application of citrulline is a potent strategy to mitigate salinity-induced stress in leaf celery, effectively bridging the economic gap caused by the use of marginal-quality water. A primary finding of this research is the divergent salt-tolerance capacity of *Apium graveolens* L. var. *secalinum* between successive mowings. The species exhibited robust adaptive capacity during the first growth cycle, maintaining physiological and metabolic integrity through morphological plasticity. However, this tolerance was significantly diminished during the regrowth phase after the first mowing, as the transition from transient osmotic stress to cumulative ionic toxicity surpassed the plant's natural recovery mechanisms and impaired both water and nitrogen use efficiency. In this context, 10 mM citrulline was identified as the optimal concentration for "yield rescue." Remarkably, this treatment enabled plants under moderate salinity (S50) to match the productivity of non-saline controls (S0) and allowed plants under severe stress (S100) to match the productivity of untreated S50 plants. The mitigative effect of citrulline is likely mediated through the arginine-proline-NO pathway, which bolsters nitrogen use efficiency (NUE) and preserves photosynthetic pigments, as evidenced by stabilized L*, chroma, and hue angle values. While the efficacy of the citrulline was partially influenced by seasonal temperature variations and cumulative sodium toxicity in the regrowth phase, it successfully maintained overall product quality above the marketability threshold throughout 21 days of cold storage. While leaf celery possesses innate

strategies to cope with initial salinity, citrulline acts as a decisive metabolic buffer and morphological rescuer, offering the baby leaf industry a highly effective tool to maintain yield and visual standards in salt-affected environments across multiple mowings.

From a practical agronomic perspective, these findings suggest a dual-salinity management strategy: employing 100 mM NaCl during the first mowing, where baby leaf yield is largely preserved, and reducing the concentration to 50 mM NaCl for the second mowing. This approach could effectively optimize consumer sodium intake while simultaneously mitigating the excessive yield decline associated with cumulative ionic stress.

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RESEARCH ACTIVITY:
DEPARTMENT OF FOOD SCIENCE
AARHUS UNIVERSITY (DENMARK)

V. EVALUATION OF SALT STRESS TOLERANCE IN *LACTUCA* GERMPLASM AND THE EFFICACY OF ROOT-APPLIED EXOGENOUS MITIGANTS IN A HYDROPONIC SYSTEM

1. Introduction

Lettuce (Lactuca spp.) represents one of the most widely consumed leafy vegetables globally (Yang et al., 2022), with increasing relevance in fresh-cut and ready-to-eat (RTE) markets. Its rapid growth (Q. Li et al., 2022), high nutritional value (Shatilov et al., 2019), and versatility make it particularly attractive for convenience-oriented diets. Contemporary consumers are increasingly seeking food products that combine healthfulness, convenience, and sustainability, driving substantial growth in the global convenience foods market (Stranieri et al., 2017; van Bussel et al., 2022), including plant-based and fresh vegetable offerings. Ready-to-eat products are gaining particular traction as busy lifestyles, dual-income households, and health-conscious diets fuel demand for nutritious yet convenient meal options (Jackson and Viehoff, 2016).

Despite its commercial potential, lettuce is generally regarded as a salt-sensitive crop (Vetrano et al., 2020), and salt stress adversely affects water uptake, nutrient balance, photosynthetic efficiency, and biomass accumulation. Responses to salinity vary widely among genotypes, with some cultivated and wild lettuce accessions exhibiting greater resilience than others (Xu and Mou, 2015). Identifying genotypes that maintain productivity and desirable morphological traits under saline conditions is thus critical for expanding lettuce production into marginal environments and ensuring consistent quality for fresh and RTE markets.

Soil and water salinity represent significant bottlenecks for global vegetable production, particularly for salt-sensitive species like lettuce (*Lactuca sativa* L.). Salinity triggers a biphasic stress response: an immediate osmotic phase that limits water uptake and a slower ionic phase characterized by the toxic accumulation of and in leaf tissues (Choudhary et al., 2023). These conditions result in the degradation of proteins, lipids, and photosynthetic pigments due to the overproduction of reactive oxygen species (ROS) (Franzoni et al., 2022). To mitigate these adverse effects, the application of exogenous protective compounds has emerged as a promising strategy. Among these, the antioxidants Glutathione (GSH) and Ascorbic Acid (AsA) play a pivotal role in the Asada-Halliwell cycle, directly neutralizing ROS and maintaining cellular redox homeostasis (Hasanuzzaman et al., 2020). Similarly, signaling molecules such as Nitric Oxide (NO), often supplied via the donor Sodium Nitroprusside (SNP), have been shown to regulate ion transporters, helping plants maintain a favorable ratio under stress (Pandey et al., 2023).

Phytohormones and their derivatives, including Salicylic Acid (SA) and Methyl Jasmonate (MeJA), act as systemic signals that prime the plant's endogenous defense mechanisms, enhancing stomatal

control and the expression of stress-responsive genes (R. Sultana et al., 2025). Furthermore, metabolic precursors such as L-Alanine and L-Tryptophan can serve as compatible solutes or precursors for essential growth regulators; specifically, L-Tryptophan is the primary biosynthetic precursor for Indole-3-Acetic Acid (IAA) (Tang et al., 2023), which is critical for maintaining root system architecture under salinity-induced inhibition.

Although foliar spraying is the most common method for exogenous protective compounds application, as it enhances penetration into plant tissues (Kaya, 2025; Pecha et al., 2012), root application represents a valid alternative. The mode of application of exogenous compounds can markedly influence plant metabolism and growth responses (Puglisi et al., 2022). Several studies have demonstrated that direct root or substrate application of exogenous compounds can effectively stimulate root system development, leading to enhanced shoot biomass accumulation and increased yield (Campobenedetto et al., 2021; J. Li et al., 2022; Lucini et al., 2018). While many studies have explored the foliar application of these compounds, the efficacy of root-applied mitigants in hydroponic systems remains less characterized. Applying these substances directly to the nutrient solution allows for immediate contact with the primary site of salt perception—the roots—potentially offering a more rapid and uniform defense response. In closed-loop hydroponic systems, such as Deep-Water System (DWS), this approach ensures high bioavailability and precise control over the concentration delivered to the rhizosphere, bypassing the physical barriers of the leaf cuticle that often limit the efficiency of foliar sprays.

Given the genetic diversity within the *Lactuca* genus, identifying genotypes with contrasting salt tolerance is a prerequisite for developing effective mitigation strategies. While wild lettuce relatives often harbor robust stress-tolerance traits, commercial cultivars frequently exhibit high sensitivity, making them ideal candidates for physiological "rescue" via exogenous treatments.

Therefore, the present study was divided into two sequential phases. First, Trial 1 aimed to screen seven lettuce genotypes—comprising both *L. sativa* and its wild relatives (*L. serriola*, *L. aculeata*, and *L. dregeana*)—to characterize their morphological and physiological responses to salinity and identify the most salt-sensitive genotype. Subsequently, Trial 2 was designed to evaluate the effectiveness of several exogenous compounds, applied via the root zone, in mitigating the deleterious effects of salt stress on the sensitive genotype identified in the first trial. By assessing a wide range of antioxidants, signaling molecules, and metabolic precursors, this study seeks to identify the most effective chemical interventions for maintaining lettuce productivity in saline hydroponic environments.

2. Material and methods

2.1 Plant material and experimental design

Two trials were conducted in 2024 in a controlled growth chamber at the Department of Food Science, Agro Food Park, Aarhus University, Denmark. In Trial 1 (T1), leaf lettuce (*L. sativa* L.) and wild lettuce (*L. serriola*, *L. aculeata*, and *L. dregeana*) genotypes were screened for their responses to salt stress, suitability for hydroponic cultivation, and potential for ready-to-eat vegetable production. In Trial 2 (T2), based on T1 results, various exogenous compounds were applied to the nutrient solution (NS) to evaluate their capacity to mitigate salt stress in the most salt-sensitive leaf lettuce genotype (TKI-122). Both experiments used seeds from the collection maintained by the Department of Food Science, Aarhus University, and adopted identical growing conditions and sterilization procedures.

2.2 Seed sowing, sterilization and germination

In T1, seeds were sown on 6 March 2024; in T2, seeds of the genotype TKI-122 were sown on 21 May 2024. Seeds were surface-sterilized in 2.5% (v/v) sodium hypochlorite and vortexed for 10 min, followed by five 3-minute rinses with distilled water. Sterilized seeds were placed in Petri dishes on a double-layer filter paper moistened with 0.5 mM $Ca(NO_3)_2$ to stimulate germination. Following cold stratification at 4 °C for 48 h (ending 8 March for T1 and 23 May for T2), seeds were transferred to a growth chamber at 21/18 °C (day/night) with a 12 h photoperiod and low light intensity (100–120 µmol m^{-2} s^{-1}). At radicle emergence (3 days after sowing), seedlings were transferred to a growth system described by Conn et al. (Conn et al., 2013) and covered with a transparent plastic box to maintain high humidity. Upon cotyledon emergence, the covers were removed and light intensity was increased to 250 µmol m^{-2} s^{-1}.

2.3. Hydroponic growth conditions

Fourteen days after sowing (20 March for T1 and 4 June for T2), uniform seedlings were transferred to a deep-water system (DWS) consisting of 2.5 L polyethylene (PE) containers filled with nutrient solution (NS). The NS was prepared with deionized water containing: 1.2 mM $Ca(NO_3)_2$, 4.5 mM KNO_3, 1.5 mM $MgSO_4$, 0.75 mM KH_2PO_4, 20 µM Fe, 1.25 µM Mn, 1.5 µM Zn, 25 µM H_3BO_3, 0.5 µM Cu, 0.175 µM $Na_3MoO_4 \cdot 2H_2O$, and 50 µM KCl. During plant growth, the NS was continuously aerated to avoid oxygen depletion. Each container was fitted with a lid perforated to accommodate four plants. To prevent algal growth, both containers and lids were covered with black plastic film. Climatic conditions in the growth chamber were maintained 21/18 °C (day/night) with a 12 h photoperiod (of 250 µmol m^{-2} s^{-1}).

In both trials, plants were subjected to two salt treatments by adding to the NS: 0 mM NaCl (control, S0) and 60 mM NaCl (S60). The NS was maintained at pH 6.0, with electrical conductivity (EC) of 2.0 mS cm^{-1} (S0) and 6.5 mS cm^{-1} (S60) and was fully renewed every six days until the end of the experiments.

2.4. Zero-Point sampling and growth rate calculation

After a 2-day acclimatization period in the DWS (16 days after sowing, DAS), ten seedlings per treatment were randomly harvested to determine initial fresh weight (FW), dry weight (DW), and leaf area. DW was determined after oven-drying the plants at 65 °C for 72 h. These "zero-point" values were used to calculate the relative growth rate (RGR) according to the following equation (Williams, 1946):

$RGR\ (g\ g^{-1}\ d^{-1}) = (\ln DW_2 - \ln DW_1) / (t_2 - t_1)$

where DW_1 and DW_2 represent the plant dry weight at the initial ("zero-point") sampling (t_1) and at the final harvest (t_2), respectively.

2.5. Salt treatments

Salinity treatment (S60) was initiated immediately after zero-point sampling. To avoid osmotic shock, NaCl was added to the NS in two steps: 30 mM initially and the remaining 30 mM after 24 h.

In T1, salt tolerance was evaluated in seven genotypes: three leaf lettuce (*L. sativa* L.: TKI-122, TKI-125, TKI-126) and four wild lettuce (*L. serriola*: TKI-252, TKI-257; *L. aculeata*: TKI-465; *L. dregeana*: TKI-468).

Based on T1 results, in T2, the salt-sensitive genotype TKI-122 was treated with various exogenous compounds added directly to the NS (root application) to evaluate salt stress mitigation. The compounds and concentrations, selected based on previously reported efficacy in leafy vegetables, included: Glutathione (GSH), L-Alanine (Ala), L-Tryptophan (Trp), Sodium Nitroprusside (SNP), Ascorbic Acid (AsA), Methyl Jasmonate (MeJA), and Salicylic Acid (SA). These were applied exclusively under 60 mM NaCl conditions and were supplied in the NS in two steps following the same protocol used for NaCl addition to minimize precipitation and degradation. Because MeJA and SA are poorly soluble in water, they were first dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 1 mL L^{-1} DMSO in the NS. A "NaCl + DMSO" control was included to account for potential solvent effects. A total of 17 treatments were evaluated (Table 1).

Table 1. Exogenous compounds at low (L), medium (M), and high (H) concentrations added to the nutrient solution under salinity stress.

Treatment	Code Treatment	Exogenous compound concentration
Control (0 mM NaCl)	C	
NaCl (60 mM)	NaCl	
NaCl + Dimethyl Sulfoxide	NaCl + DMSO	1 ml L^{-1} DMSO
NaCl + Glutathione	GSH	1 mM
NaCl + L-Alanine	Ala	1 mM
NaCl + L-Trypthophan	Trp	1 mM
NaCl + Sodium Nitroprusside	SNP-L	0.02 mM

NaCl + Sodium Nitroprusside	SNP-M	0.1 mM
NaCl + Sodium Nitroprusside	SNP-H	0.5 mM
NaCl + Ascorbic Acid	AsA-L	0.1 mM
NaCl + Ascorbic Acid	AsA-H	1 mM
NaCl + Methyl Jasmonate	MeJA-L	0.01 µM + 1 ml L^{-1} DMSO
NaCl + Methyl Jasmonate	MeJA-M	0.1 µM + 1 ml L^{-1} DMSO
NaCl + Methyl Jasmonate	MeJA-H	1 µM + 1 ml L^{-1} DMSO
NaCl + Salicylic Acid	SA-L	0.01 µM + 1 ml L^{-1} DMSO
NaCl + Salicylic Acid	SA-M	0.1 µM + 1 ml L^{-1} DMSO
NaCl + Salicylic Acid	SA-H	1 µM + 1 ml L^{-1} DMSO

2.6. Harvest, morphological, and physiological measurements

In both trials, lettuce plants were harvested 30 DAS (5 April 2024 for T1 and 20 June 2024 for T2) because after this period in T1 visible salt stress symptoms were observed in at least one genotype. Twenty plants per replicate were collected. Plants were separated into leaves, stems, and roots to determine leaf number and fresh weight (FW) and then oven-dried at 65 °C for 72 h for DW determination.

Leaf area of both zero-point and lettuce plants at harvest was measured using a leaf area meter (LI-3100C, LI-COR, Lincoln, NE, USA). The specific leaf area (SLA) was calculated as the ratio of leaf area to leaf DW (VILE et al., 2005).

Non-destructive measurements including chlorophyll index (Chl), epidermal flavonoid content (Flav), anthocyanin index (Anth), and nitrogen balance index (NBI) were recorded for ten leaves per replicate using a Dualex Scientific+ sensor (Force-A, France) immediately prior to harvest.

2.7. Statistical analysis

Trials followed a randomized complete block design with four replicates per treatment (n = 80 plants total per treatment).

T1 data were analyzed via two-way ANOVA (Genotype × Salinity). T2 data were analyzed via one-way ANOVA. Means were compared using the least significant difference (LSD) test at $p \leq 0.05$.

3. Results

3.1 Experiment T1: Phenotypic and physiological variation in cultivated and wild lettuce under salt stress

The lettuce genotypes exhibited distinct morpho-physiological characteristics and responses to salt stress.

3.1.1. Fresh biomass accumulation and partitioning

Under control conditions (S0), the highest total fresh biomass was recorded in TKI-126 (10.6 g $plant^{-1}$) followed closely by TKI-465 and TKI-468 with no significant difference. Conversely, the

lowest total fresh biomass was observed in TKI-257 (4.3 g plant^{-1}), while the remaining genotypes ranged between 9 and 6 g plant-1 (Figure 1).

Salinity (S60) negatively affected the fresh biomass accumulation in all the genotypes except TKI-257, which showed no significant variation compared to the control. TKI-122 exhibited the greatest reduction in total fresh weight (FW) among leaf lettuce genotypes (−43.5%). However, the wild genotype TKI-252 was identified as the most salt-sensitive overall, with a fresh biomass loss of 53.8% (Figure 1). In contrast, TKI-126 was the most resilient to salt stress, experiencing only a 12.4% reduction in FW.

Root fresh biomass varied markedly across genotypes. TKI-465 produced the highest root FW at both salinity levels (3.46 g plant^{-1} for S0 and 3.28 g plant^{-1} for S60), despite a slight but significant reduction of 5.3% under salt stress (Figure 1). The lowest root fresh biomass was recorded in TKI-122 and TKI-125. While root FW remained stable under salinity in TKI-257 (1.50 g plant^{-1}, on average) and TKI-468 (1.93 g plant^{-1}, on average), it significantly decreased in all other genotypes (Figure 1).

Regarding leaf fresh biomass, TKI-126 accumulated the highest fresh weight under S0 (8.2 g plant^{-1}), followed by TKI-468 (7.4 g plant^{-1}), whereas TKI-257 (2.6 g plant^{-1}) had the lowest. Salinity significantly reduced leaf FW in all genotypes except TKI-257 (Figure 1). The most pronounced leaf FW reductions were observed in TKI-252 (-58.8%) and TKI-122 (-45.5%). The genotypes showing the highest tolerance in terms of leaf biomass were TKI-257 (-10.2%), TKI-126 (-12.7) and TKI-125 (-19.5).

Stem FW contributed minimally to total biomass. TKI-126 reached the highest value at S0 (0.43 g plant^{-1}), while TKI-122 and TKI-252 showed the most significant reductions under salinity. Again, TKI-257 remained unaffected by salt treatment (averaging 0.15 g) (Figure 1).

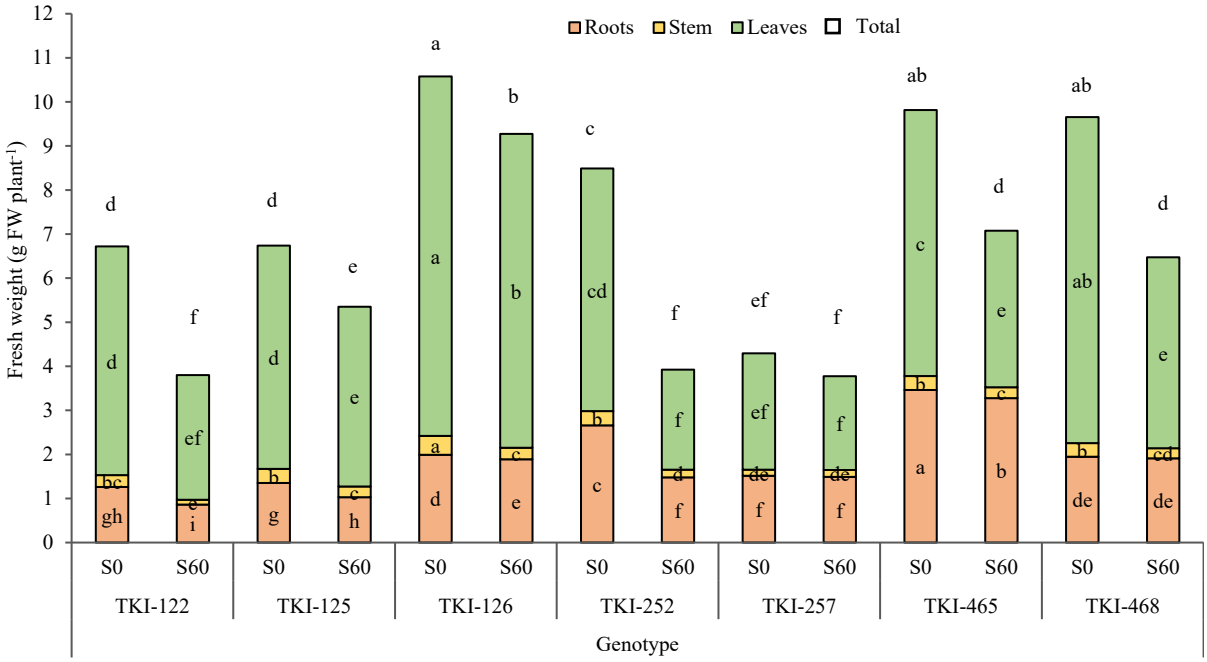

Figure 1. Effects of salt stress (0, and 60 mM NaCl in the nutrient solution) on total, root, stem, and leaf fresh weight (FW) of leaf lettuce (TKI-122, 125, and 126) and wild lettuce (TKI-252, 257, 465, and 468) genotypes grown using a hydroponic system (bars of the same color with different letters are significantly different at $p \leq 0.05$ according to the LSD test).

In non-stressed plants, the shoot/root FW ratio averaged 4.2 in leaf lettuce genotypes and 4.0 in the wild genotype TKI-468 (4.0), whereas other wild lettuce genotypes averaged 2.0. Salinity generally altered biomass partitioning by reducing the shoot/root FW ratio; however, this reduction was statistically significant only in TKI-122 (reduced to 3.4), TKI-468 (2.8) and TKI-465 (1.2), indicating a shift toward root maintenance at the expense of shoot growth in these genotypes.

3.1.2. Dry biomass accumulation and partitioning

Total dry weight (DW) was significantly influenced by the interaction between genotype and salinity, with a general significant decline observed as NaCl concentration increased, except for TKI-126 and TKI-257. Under S0, TKI-252 and TKI-468 exhibited the highest total DW (654.2 mg plant^{-1}, on average), while the lowest values were recorded in TKI-122 and TKI-257 (351.5 mg plant^{-1}, on average) (Figure 2). Salinity-induced DW reduction was most severe in the wild lettuce genotype TKI-252 (-53.6%), followed by the leaf lettuce genotype TKI-122 (-34.4%).

Roots dry biomass patterns differed from shoot patterns. Under control conditions, TKI-252 produced the highest root DW (144.2 mg plant^{-1}), followed by the other wild lettuce genotypes; leaf lettuce genotypes showed lower root development, with TKI-122 recording the minimum (52.3 mg plant^{-1}) (Figure 2). Under salt stress, TKI-122, TKI-125, TKI-252 suffered significant root DW losses. In contrast, TKI-465exhibited a unique stress-adaptive response, significantly increasing its root DW up

to 143.5 mg plant^{-1} under S60. The remaining genotypes maintained almost unvaried the root DW under salinity condition compared to control.

TKI-252 showed the greatest dry biomass accumulation in control conditions also in the stem (35.7 mg plant^{-1}). Together with TKI-125, TKI 252 suffered the greatest biomass reduction under salinity (−42.8% and -41.2%, respectively). A lower but significant negative effect of salinity was recorded also in TKI 122 and TKI 126, while no significant effect was observed in the other genotypes (Figure 2).

TKI-468 and TKI-126 accumulated the most leaf dry biomass under control conditions, while TKI-126 and TKI-257 stood out for maintaining their leaf DW under saline conditions. All other genotypes experienced significant leaf DW reductions, ranging from −27.7% (TKI-125) to −57.0% (TKI-252) Figure 2).

Consistent with fresh weight observations, the shoot/root DW ratio was higher in leaf lettuce genotypes and TKI-468 (6.2, on average) than in other wild genotypes (3.1, on average). Under salt stress, only TKI-122, TKI-465 and TKI-468 showed a significant drop in this ratio, indicating altered dry matter partitioning.

Dry matter percentage varied across the genotypes but with no significant change due to salinity excluding TKI-122 that ranged from 5.3 (S0) to 6.2% (S60). In the other genotypes averaged from 8.2% of TKI-257 to 5.9 of TKI-465.

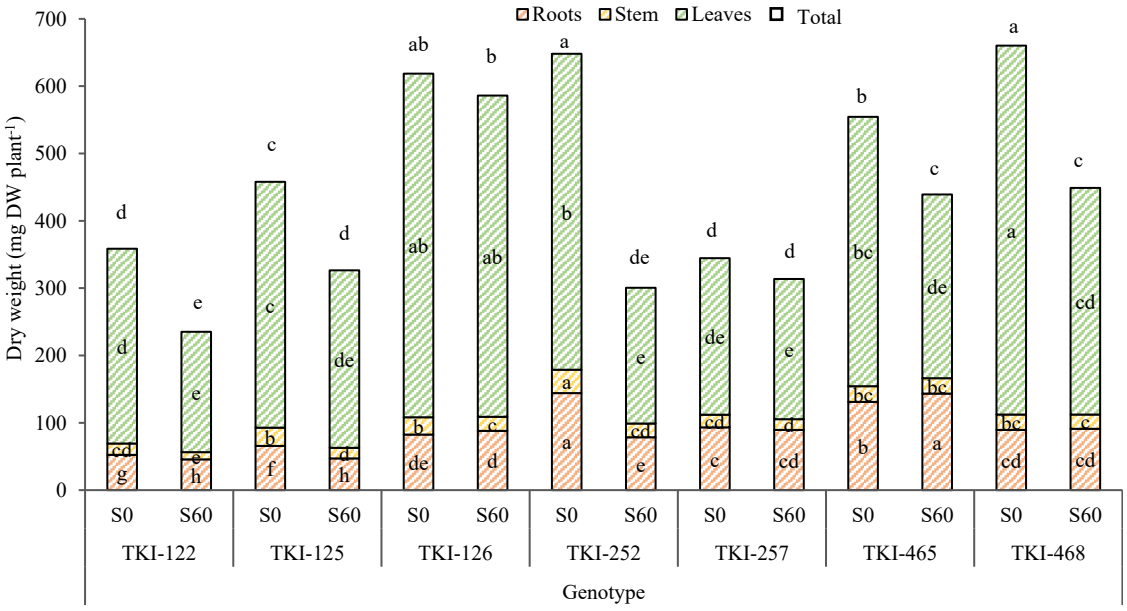

Figure 2. Effects of salt stress (0, and 60 mM NaCl in the nutrient solution) on total, root, stem, and leaf dry weight (DW) of leaf (TKI-122, 125, and 126) and wild (TKI-252, 257, 465, and 468) lettuce genotypes grown using a hydroponic system (bars of the same color with different letters are significantly different at $p \leq 0.05$ according to the LSD test).

3.1.3. Leaf morphology and growth analysis

Leaf morphology and expansion were significantly hampered by salinity (Table 2). Wild genotypes generally produced more leaves (from 12.7 leaves plant^{-1} in TKI-468 to 22.0 leaves plant^{-1} in TKI-465) than leaf lettuce genotypes (8.5 leaves plant^{-1} on average in TKI-122 and TKI-126, and 12.0 leaves plant^{-1} in TKI-125) (Table 2). However, salinity significantly reduced leaf number only in the leafiest genotypes, ranging from a minimum of −13.2% (TKI-467) to a maximum of −28.8% (TKI-465) (Table 2).

Total leaf area of control plants was highest in TKI-468 and TKI-126 (240.1 cm^2 plant^{-1}, on average) and the lowest in TKI-257 (77.8 cm^2 plant^{-1}) (Table 2). Salinity caused a drastic reduction in total leaf area for all genotypes except TKI-125, with TKI-252 suffering the most substantial loss (−61.2%) followed by TKI-122, TKI-465 and TKI-468 which recorded a reduction by 40.1%, on average (Table 2). This reduction was driven by a decrease in individual leaf size; specifically, salinity "shrank" leaf size by approximately 33.0% in TKI-122 and TKI-468 and by 47.8% in TKI-252 (Table 2). Interestingly, the Specific leaf area (SLA) remained stable within each genotype despite salinity, indicating that while leaves became smaller, their relative thickness did not change. TKI-122 produced the thinnest leaves (highest SLA, 560.5 cm^2 g DW^{-1}, on average), whereas TKI-257 produced the thickest (lowest SLA, 318.5 cm^2 g DW^{-1}, on average).

Table 2. Effects of salt stress (0, and 60 mM NaCl in the nutrient solution) on leaf characteristics of leaf lettuce (TKI-122, 125, and 126) and wild lettuce (TKI-252, 257, 465, and 468) genotypes grown in a hydroponic system.

Source of Variance		Number of Leaves	Leaf Area (cm^2 Plant^{-1})	Leaf Area (cm^2 Leaf^{-1})	SLA (cm^2 g DW^{-1})
Genotype					
TKI-122		[z] 8.0	131.5	16.3	560.5
TKI-125		11.7	141.3	12.1	457.5
TKI-126		8.3	209.1	25.1	423.6
TKI-252		12.8	130.5	9.8	381.2
TKI-257		13.0	70.2	5.5	318.5
TKI-465		18.8	135.6	7.1	398.5
TKI-468		11.8	201.7	16.8	455.1
NaCl (mM)					
0		13.3	175.9	14.9	436.3
60		10.8	115.5	11.6	419.5
Genotype x NaCl					
TKI-122	S0	8.3d	164.4bc	19.7b	568.9a
	S60	7.7d	98.6d	12.9c	552.2a
TKI-125	S0	12.0c	151.6c	12.6c	416.1bc
	S60	11.3c	131.1cd	11.6c	498.9ab
TKI-126	S0	8.7d	227.3a	26.3a	446.8b

	S60	8.0d	190.8b	23.8a	400.3bc
TKI-252	S0	14.7b	188.0b	12.8c	400.5bc
	S60	11.0c	72.9de	6.7d	361.8cd
TKI-257	S0	15.0b	77.8de	5.2d	335.5cd
	S60	11.0c	62.6e	5.7d	301.5d
TKI-465	S0	22.0a	169.3bc	7.7d	423.4bc
	S60	15.7b	101.8d	6.5d	373.6c
TKI-468	S0	12.7c	252.9a	19.9b	462.5b
	S60	11.0c	150.5c	13.7c	447.8b
Significance [x]					
Genotype		***	***	***	***
NaCl		***	***	***	ns
Genotype x NaCl		*	***	***	*

[z] Each value is the mean of 4 replicated samples of 20 plants each. For each factor, values in a column followed by the same letter are not significantly different, according to the LSD test. x Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$.

The Relative Growth Rate (RGR) under optimal conditions was highest in TKI-468 (0.346 g g^{-1} d^{-1}), followed by TKI-126 (0.340 g g^{-1} d^{-1}); the lowest RGR was calculated for TKI-257 (0.272 g g^{-1} d^{-1}). Although RGR generally declined with salinity (Figure 3), significant drops were restricted to TKI-126, TKI-252 and TKI-468, with TKI-252 showing the most pronounced decline (−18.3%).

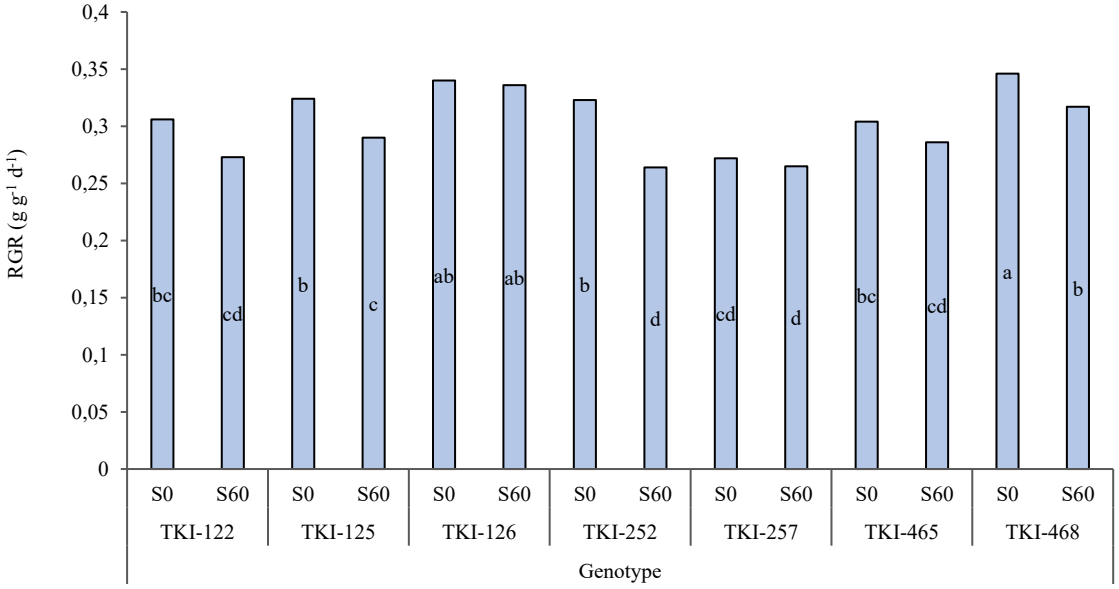

Figure 3. Effects of salt stress (0, and 60 mM NaCl in the nutrient solution) on relative growth rate (RGR) of leaf (TKI-122, 125, and 126) and wild (TKI-252, 257, 465, and 468) lettuce genotypes grown using a hydroponic system (bars with different letters are significantly different at $p \leq 0.05$ according to the LSD test).

3.1.4. Non-destructive physiological measurements (Dualex)

The Chlorophyll index (Chl) varied nearly threefold across genotypes. Under control conditions, TKI-122 had the lowest Chl (6.67); it almost doubled in TKI-125, TKI-126 and TKI-468 and was about three times in the other genotypes. Salinity triggered a significant increase in Chl inTKI-125

(+30.3%), TKI-252 (+20.3%), TKI-465 (+34.2%) and TKI-468 (+27.9%), likely due to a "concentration effect" in smaller, more compact leaves.

Epidermal flavonoids (Flav) and anthocyanins (Anth) showed genotype-specific patterns. TKI-122 exhibited the highest Anth index (0.49), while TKI-126 had the lowest flavonoid content. Salinity effects on these secondary metabolites were limited, except for TKI-252 (where Flav decreased) and TKI-465 (where Flav increased). (Table 3).

The nitrogen balance index (NBI), which represents the ratio of Chl to Flav, was highest in TKI-126 and lowest in TKI-122, with salinity causing a significant increase only in TKI-252. (Table 3).

Table 3. Effects of salt stress (0, and 60 mM NaCl in the nutrient solution) on chlorophyll index (Chl), epidermal flavonoid content (Flav), anthocyanin index (Anth), and nitrogen status of the crop (NBI) of leaf (TKI-122, 125, and 126) and wild (TKI-252, 257, 465, and 468) lettuce genotypes grown using a hydroponic system.

Source of Variance		Chl	Flav	Anth	NBI
Genotype					
TKI-122		[z] 6.83	0.53	0.49a	13.07
TKI-125		18.16	0.56	0.31b	34.06
TKI-126		14.53	0.33	0.17cd	45.34
TKI-252		19.71	0.46	0.15d	47.07
TKI-257		19.57	0.50	0.14d	39.56
TKI-465		19.60	0.55	0.15d	35.64
TKI-468		16.58	0.49	0.21c	34.76
NaCl (mM)					
0		14.84	0.48	0.24	32.44
60		18.01	0.49	0.22	38.84
Genotype x NaCl					
TKI-122	S0	6.67d	0.55ab	0.52	12.67c
	S60	6.99d	0.52ab	0.47	13.46c
TKI-125	S0	15.77c	0.55ab	0.30	28.89bc
	S60	20.55ab	0.57ab	0.33	39.22b
TKI-126	S0	13.99c	0.29d	0.17	49.16ab
	S60	15.07c	0.37cd	0.17	41.53b
TKI-252	S0	17.89bc	0.51b	0.17	35.79b
	S60	21.53a	0.41c	0.13	58.36a
TKI-257	S0	18.29bc	0.51c	0.15	36.65b
	S60	20.86ab	0.49bc	0.12	42.48b
TKI-465	S0	16.74bc	0.50bc	0.16	33.83b
	S60	22.46a	0.61a	0.14	37.45b
TKI-468	S0	14.55c	0.49bc	0.23	30.12b
	S60	18.61b	0.49bc	0.20	39.40b
Significance [x]					
Genotype		***	***	***	***

NaCl	***	ns	ns	**
Genotype x NaCl	**	**	ns	*

z Each value is the mean of 4 replicated samples of 10 leaves each. For each factor, values in a column followed by the same letter are not significantly different, according to the LSD test. x Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$.

3.1.5. Summary of Trial 1 and selection of genotype for Trial 2

The screening in Trial 1 revealed a wide spectrum of salt tolerance among the evaluated genotypes. The leaf lettuceTKI-126 and the wild lettuce TKI-257 emerged as the most salt-tolerant, maintaining biomass and growth rates under saline conditions. In contrast, the wild genotype TKI-252 and the leaf lettuce cultivar TKI-122 were identified as highly salt-sensitive.

Specifically, TKI-122 exhibited the most severe reduction in fresh weight among the cultivated leaf lettuce types (−43.5%) and displayed the lowest chlorophyll index and nitrogen balance index (NBI) under control conditions. Furthermore, it produced the thinnest leaves (highest SLA) and the lowest root dry weight, suggesting a limited structural and physiological buffer against osmotic stress. Because TKI-122 represents a commercially relevant leaf lettuce type with high sensitivity to salinity, it was selected as the model genotype for Trial 2 to evaluate the effectiveness of root-applied exogenous compounds in mitigating salt-induced growth inhibition.

3.2. Experiment T2: Evaluation of exogenous organic compounds for salt stress mitigation in the sensitive genotype TKI-122

The inclusion of dimethyl sulfoxide (DMSO) in the saline nutrient solution (NaCl + DMSO) did not induce significant changes in any evaluated morpho-physiological traits compared to NaCl alone. Consequently, these two treatments were pooled and are hereafter reported as a single NaCl reference.

3.2.1. Fresh biomass accumulation and partitioning

The growth of TKI-122 under salinity was consistent with Trial 1 results, with salt stress reducing total fresh weight (FW) by 43.2% (from 6.90 g plant^{-1} in S0 to 3.92 g plant^{-1}in NaCl). Root-applied compounds significantly influenced this response (Figure 4). Total fresh weight (FW) generally decreased as concentrations of Sodium Nitroprusside (SNP), Ascorbic Acid (AsA), and Methyl Jasmonate (MeJA) increased. Specifically, MeJA showed a potent inhibitory effect, reducing total FW to as low as 0.2 g in the MeJA-H treatment. In contrast, Salicylic Acid (SA) exhibited a quadratic response; while the lowest concentration (SA-L, 0.01 μM) significantly enhanced FW compared to NaCl, the intermediate dose (SA-M, 0.1 μM) fully restored total FW (6.50 g) to control levels. Other treatments also provided significant mitigation: AsA-L (0.1 mM), Ala, and Trp increased total fresh biomass by 41.2%, 26.7%, and 16.5%, respectively, relative to the NaCl treatment.

Salinity significantly reduced root FW from 1,37 to 0.89 g plant^{-1}. Root development was positively influenced by Ala, Trp, and SA-L (0.01 μM), which significantly increased root FW compared to

NaCl (1.1 g plant^{-1}, on average). Remarkably, AsA-L and SA-M restored root development to levels comparable to the non-saline control (Figure 4). The root FW decreased as increasing SNP and MeJA concentrations; however, only SNP-M and SNP-L showed values comparable to NaCl, while SNP-H and all MeJA treatments caused a significant reduction in root FW. Similar trends were observed for Glutathione (GSH) and SA-H (Figure 4).

While no treatment fully restored stem biomass to control levels, SA-M and Ala provided the most significant improvements in stem FW compared to the NaCl-only plants (Figure 4). The leaf FW followed a pattern similar to that observed for the total FW. GSH, SNP, MeJa AsA-H and SA-H variously reduced leaf biomass accumulation even more than the sole saline stress. Ala, AsA-L and SA-L were effective in mitigating salt stress and improved leaf FW compared to NaCl, whereas SA-M fully counteracted salt stress resulting in a leaves FW of 4.9 g plant^{-1}, that did not differ from control (5.2 g plant^{-1}) (Figure 4).

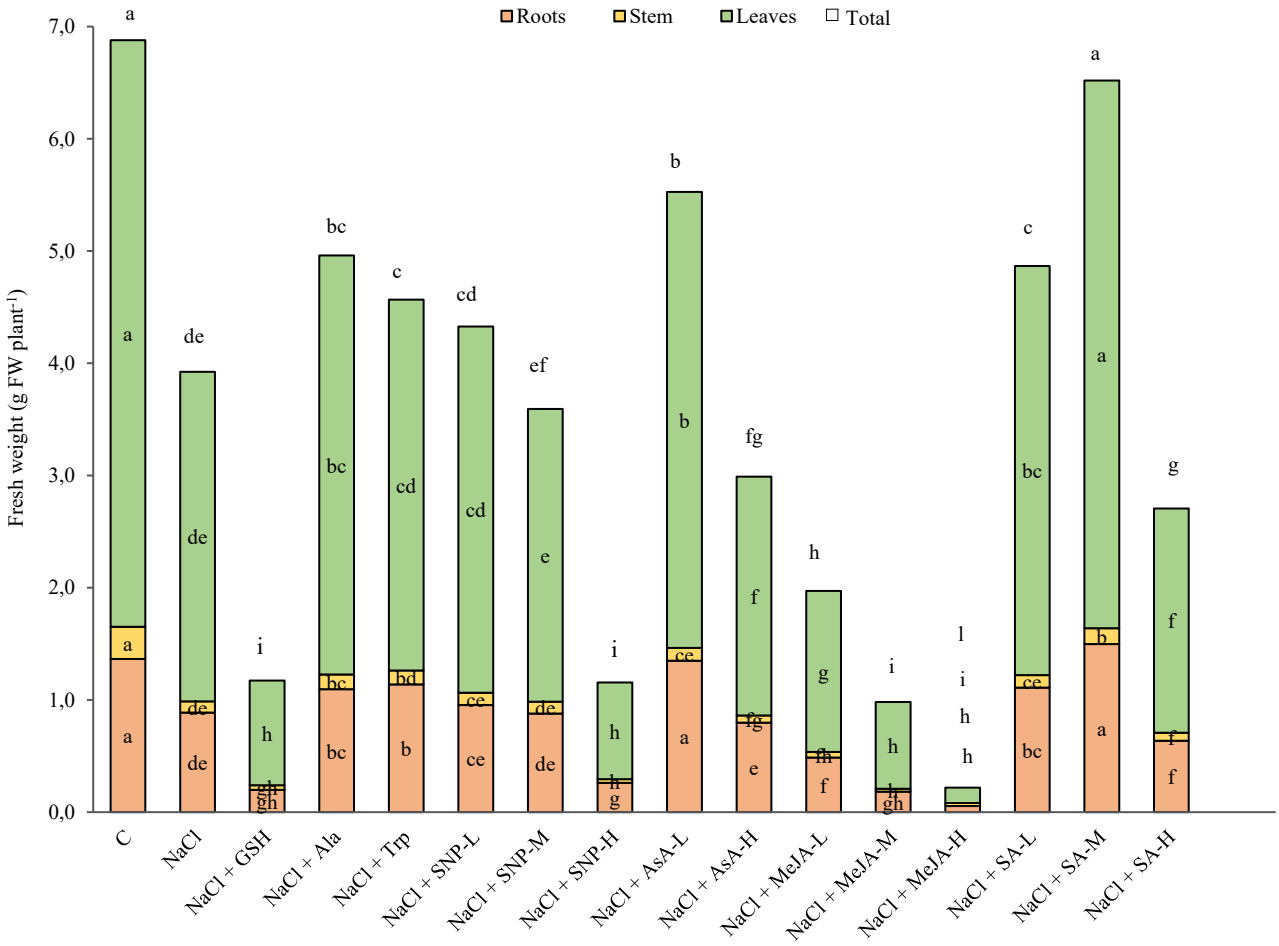

Figure 4. Effects of salt stress (0 and 60 mM NaCl in the nutrient solution) and root-applied treatments at low (L), medium (M), and high (H) concentrations (control, NaCl, Glutathione (GSH), L-Alanine (Ala), L-Tryptophan (Trp), Sodium Nitroprusside (SNP), Ascorbic Acid (AsA), Methyl Jasmonate (MeJA), and Salicylic Acid (SA)) on total, roots, stem, and leaf fresh weight (FW) of leaf lettuce genotype TKI-122 grown using a hydroponic system (bars of the same color with different letters are significantly different at $p \leq 0.05$ according to the LSD test).

3.2.2. Dry biomass accumulation and partitioning

Dry weight (DW) trends mirrored those of fresh biomass (Figure 5). Salinity reduced total DW by 32.6% (249.8 mg plant^{-1}) compared to the control (370.5 mg plant^{-1}). SA-M was the only treatment to fully restore total DW to control levels (377.9 mg plant^{-1}). Ala and AsA-L also significantly enhanced DW by 16.3% and 33.3% over NaCl, respectively. MeJA induced the most severe reductions in DW, ranging from −54.6% (MeJA-L) to −91.0% (MeJA-H) compared to NaCl.

The root DW increased significantly compared to NaCl with Ala, Trp, and SA-L (Figure 5). Remarkably, AsA-L and SA-M not only restored root development but, in the case of dry weight, caused root biomass to exceed that of the non-saline control (Figure 5). Conversely, high concentrations of SNP, MeJA, and GSH further inhibited dry biomass accumulation in the roots.

While no treatment fully restored stem biomass to control levels, SA-M and Ala provided the most significant improvements in stem FW compared to the NaCl-only plants. (Figure 5).

Leaf DW closely mirrored total DW trends. SA-M was the only treatment that fully avoided the negative salinity effect on leaf dry weight (287.0 mg plant^{-1}), while AsA-L increased salt tolerance resulting in a leaf DW higher by 31.7% compared to NaCl (189.5 mg plant^{-1}). Conversely, GSH, SNP-H, all MeJA concentrations, and SA-H significantly reduced leaves DW compared to NaCl, with the strongest effect found with MeJA-H (10.6 mg plant^{-1}) (Figure 5).

Salinity increased shoot dry matter percentage from 5.7% in control plants to 6.6%. Most of the treatments did not modify this parameter compared to NaCl. Ala, MeJa, and SA-L had a dry matter percentage similar to control plants. On the contrary, an increase was found in MeJa-H (+8.0%), SNP-H (+8.9%) and GSH (+11.1%).

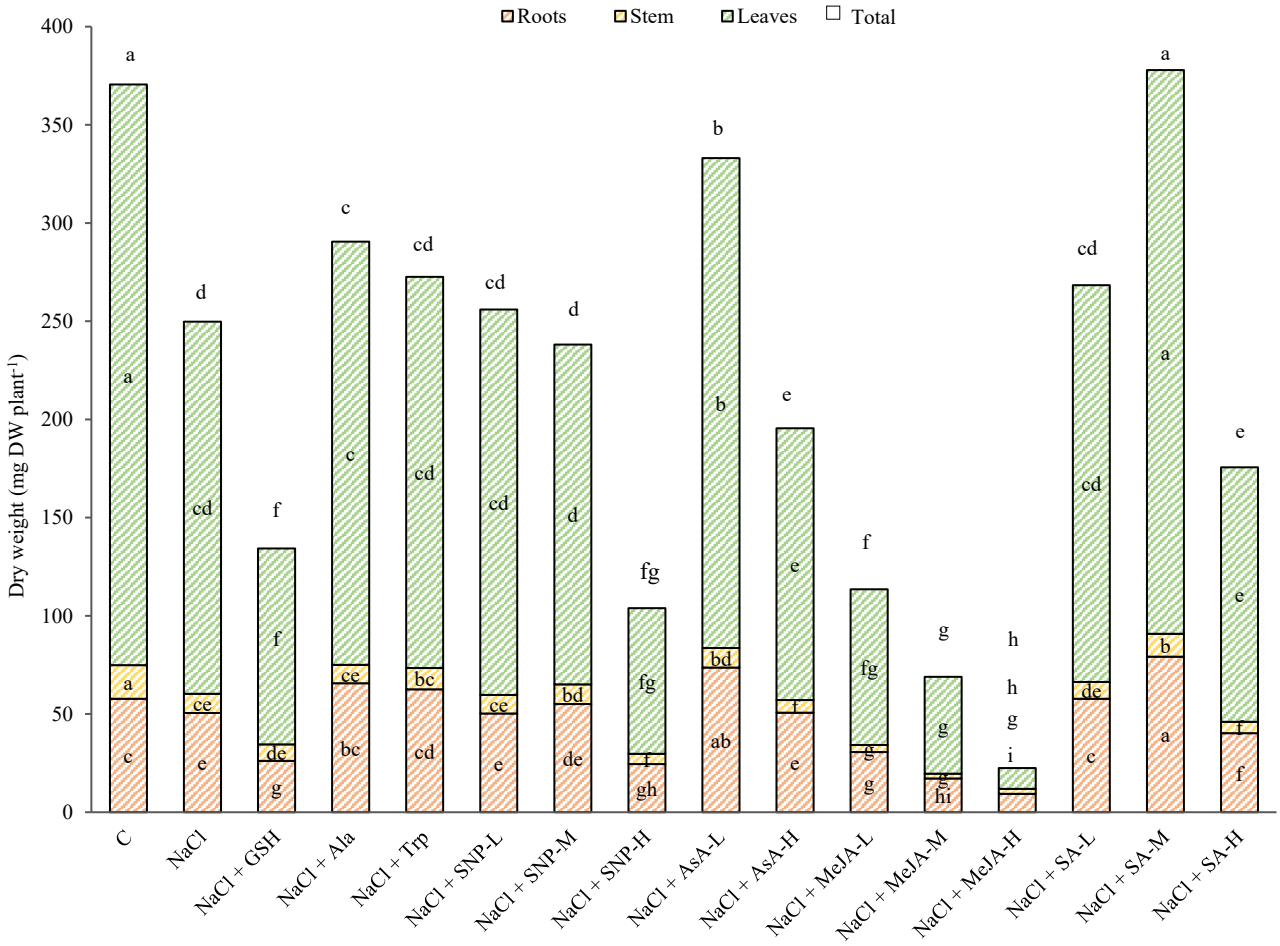

Figure 5. Effects of salt stress (0 and 60 mM NaCl in the nutrient solution) and root-applied treatments at low (L), medium (M), and high (H) concentrations (control, NaCl, Glutathione (GSH), L-Alanine (Ala), L-Tryptophan (Trp), Sodium Nitroprusside (SNP), Ascorbic Acid (AsA), Methyl Jasmonate (MeJA), and Salicylic Acid (SA)) on total, roots, stem, and leaf dry weight (DW) of leaf lettuce genotype TKI-122 grown using a hydroponic system (bars of the same color with different letters are significantly different at $p \leq 0.05$ according to the LSD test).

3.2.3. Leaf morphology and Relative Growth Rate (RGR)

Salinity significantly reduced the leaf number, which declined from 8.2 leaves plant^{-1} in the control to 7.3 leaves plant^{-1} in the NaCl-only plants (Table 4). Ala, AsA-L, and SA-M were effective in maintaining leaf numbers comparable to the control (8.0 leaves plant^{-1} on average). In contrast, GSH, SNP-H, SA-H and all MeJa concentrations caused a reduction in leaf number, with the most severe decrease in MeJa-H (3.5 leaves plant^{-1}) (Table 4).

The total leaf area (cm^2 plant^{-1}) and the average leaf area (cm^2 leaf^{-1}) were markedly reduced by salinity, showing decreases by 43.1% (95.1 cm^2 plant^{-1}) and 36.3% (13.0 cm^2 leaf^{-1}), respectively (Table 4). Although no treatment fully restored total leaf area, SA-M (147.8 cm^2 plant^{-1}, 18.5 cm^2 leaf^{-1}), Asa-L (128.5 cm^2 plant^{-1}, 16.1 cm^2 leaf^{-1}) and Ala (119.5 cm^2 plant^{-1}, 14.4 cm^2 leaf^{-1})

significantly mitigated leaf area reduction compared to NaCl (Table 4). On the contrary, GSH, SNP-H, AsA-H, SA-H and all MeJa concentrations depressed leaf expansion compared to NaCl.

Specific Leaf Area (SLA) was notably affected by the exogenous treatments. While salinity alone reduced SLA, treatments such as Ala, AsA, and SA maintained intermediate values between the control (565.7 $cm^2\ g^{-1}$ DW) and NaCl (501.7 $cm^2\ g^{-1}$ DW) (Table 4). SLA was further reduced by GSH, SNP-H and MeJa-M application (Table 4).

Table 4. Effects of salt stress (0 and 60 mM NaCl in the nutrient solution) and root-applied treatments at low (L), medium (M), and high (H) concentrations (control, NaCl, Glutathione (GSH), L-Alanine (Ala), L-Tryptophan (Trp), Sodium Nitroprusside (SNP), Ascorbic Acid (AsA), Methyl Jasmonate (MeJA), and Salicylic Acid (SA)) on leaf characteristics of leaf lettuce genotype TKI-122 grown using a hydroponic system.

Source of Variance	Number of Leaves	Leaf Area ($cm^2\ Plant^{-1}$)	Leaf Area ($cm^2\ Leaf^{-1}$)	SLA ($cm^2\ g\ DW^{-1}$)
Treatment				
C	[z] 8.2a	167.2a	20.4a	565.7a
NaCl	7.3bd	95.1ef	13.0eg	501.7bc
NaCl + GSH	5.3f	33.9il	6.4l	339.2f
NaCl + Ala	8.0ab	119.5cd	14.9cd	555.8ab
NaCl + Trp	7.3bd	99.2ef	13.5df	500.2bc
NaCl + SNP-L	7.2cd	103.3df	14.4ce	526.4ab
NaCl + SNP-M	7.0ce	86.8fg	12.4fh	504.4bc
NaCl + SNP-H	5.3f	29.5il	5.6lm	402.8de
NaCl + AsA-L	8.0ab	128.5c	16.1c	516.9ab
NaCl + AsA-H	6.7de	75.3gh	11.3gh	545.6ab
NaCl + MeJA-L	5.0f	41.0i	8.2i	516.8ab
NaCl + MeJA-M	4.2g	18.0lm	4.3m	366.8ef
NaCl + MeJA-H	3.5h	4.8m	1.4n	454.5cd
NaCl + SA-L	7.0ce	105.7de	15.1cd	525.1ab
NaCl + SA-M	8.0ab	147.8b	18.5b	514.2ab
NaCl + SA-H	6.3e	68.4h	10.8h	526.6ab
Significance [x]				
Treatment	***	***	***	***

[z] Each value is the mean of 4 replicated samples of 20 plants each. For each factor, values in a column followed by the same letter are not significantly different, according to the LSD test. x Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$.

The Relative Growth Rate (RGR), which declined by 10.1% under salinity compared to the control (0.317 $g\ g^{-1}\ d^{-1}$), was successfully maintained at control levels by Ala, AsA-L, and SA-M (Figure 6).

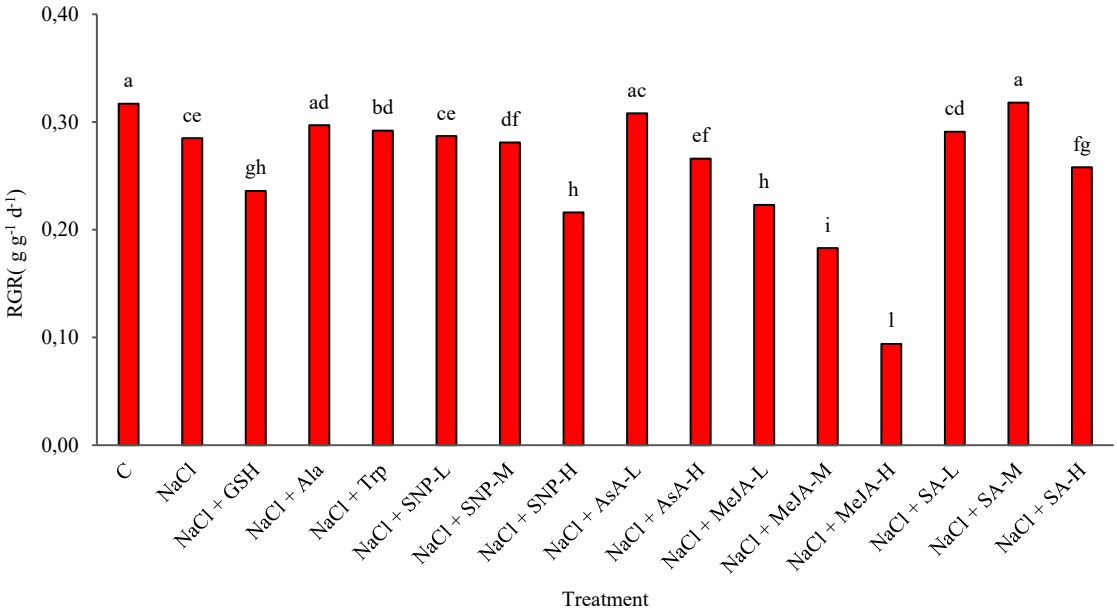

Figure 6. Effects of salt stress (0 and 60 mM NaCl in the nutrient solution) and root-applied treatments at low (L), medium (M), and high (H) concentrations (control, NaCl, Glutathione (GSH), L-Alanine (Ala), L-Tryptophan (Trp), Sodium Nitroprusside (SNP), Ascorbic Acid (AsA), Methyl Jasmonate (MeJA), and Salicylic Acid (SA)) on relative growth rate (RGR) of leaf lettuce genotype TKI-122 grown using a hydroponic system (bars with different letters are significantly different at $p \leq 0.05$ according to the LSD test).

3.2.4. Non-destructive physiological measurements (Dualex)

Due to severe growth inhibition, plants treated with MeJA, GSH, and SNP-H were excluded from Dualex analysis (Table 5). NaCl significantly increased the chlorophyll index (Chl) by 19.4% over the control (6.7). The highest Chl was recorded in plants supplied with Trp (9.1). Ala, SNP-L, AsA, SA-H and SA-L showed intermediate Chl values, averaging 7.3. Epidermal flavonoid content (Flav) showed no significant difference between NaCl and control. The control treatment exhibited the highest Flav (0.55), whereas significantly lower Flav values were recorded with Ala, AsA, SA-H and SA-M treatments (0.47 on average) (Table 5). Similarly, the anthocyanin index (Anth) was highest in the control (0.52). Among the treatments, only Trp maintained Anth comparable to the control (0.47), while all the other had lower values that did not statistically differed from NaCl (0.44) (Table 5). Interestingly, while salinity slightly increased the Nitrogen Balance Index (NBI), the application of SA-M, Ala, and Trp significantly boosted NBI further (+33.4% higher than control, on average), with SA-M reaching the highest value (17.8), suggesting improved nitrogen utilization efficiency under stress (Table 5).

Table 5. Effects of salt stress (0 and 60 mM NaCl in the nutrient solution) and root-applied treatments at low (L), medium (M), and high (H) concentrations (control, NaCl, Glutathione (GSH), L-Alanine (Ala), L-Tryptophan (Trp), Sodium Nitroprusside (SNP), Ascorbic Acid (AsA), Methyl Jasmonate (MeJA), and Salicylic Acid (SA)) on chlorophyll content (Chl), epidermal flavonoid content (Flav), anthocyanin index

(Anth), and nitrogen status of the crop (NBI) of leaf lettuce genotype TKI-122 grown using a hydroponic system.

Source of Variance	Chl	Flav	Anth	NBI
Treatment				
C	[z] 6.7c	0.55a	0.52a	12.7c
NaCl	8.0ab	0.52ab	0.44bc	15.5ac
NaCl + Ala	7.5bc	0.48bc	0.44bc	16.1ab
NaCl + Trp	9.1a	0.53ab	0.47ab	17.5ab
NaCl + SNP-L	7.2bc	0.50ac	0.45bc	14.9ac
NaCl + SNP-M	8.2ab	0.52ab	0.44bc	16.3ab
NaCl + AsA-L	7.5bc	0.45c	0.40bc	16.8ab
NaCl + AsA-H	7.4bc	0.46c	0.38c	16.8ab
NaCl + SA-L	7.2bc	0.49ac	0.46b	14.7bc
NaCl + SA-M	8.1ab	0.46c	0.41bc	17.8a
NaCl + SA-H	7.2bc	0.48bc	0.43bc	15.2ac
Significance [x]				
Treatment	**	**	**	*

[z] Each value is the mean of 4 replicated samples of 20 plants each. For each factor, values in a column followed by the same letter are not significantly different, according to the LSD test. x Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$.

4. Discussion

4.1. Genotypic variation in growth and biomass partitioning under salinity

Lettuce is well suited for various hydroponic systems (Ferguson et al., 2014; Q. Li et al., 2018; Petropoulos et al., 2016). However, optimal growth under hydroponic conditions requires careful management of nutrient solution parameters, especially pH and electrical conductivity (EC). Trejo-Téllez et al. (Trejo-Téllez and Gómez-Merino, 2012) reported optimal ranges of 5.5–6.5 for pH and 1.5–2.5 dS cm^{-1} for EC. EC provides an indirect measure of dissolved salts and nutrients in the solution (Hershey and Sand, 1993). Under saline conditions, increased salt concentrations can limit water and nutrient uptake, impair photosynthetic efficiency, transpiration, and stomatal conductance, ultimately reducing biomass accumulation and yield, and potentially causing ionic toxicity (Atta et al., 2023; Balasubramaniam et al., 2023; Parida and Das, 2005; Sudhir and Murthy, 2004). This study evaluated the effects of salinity on several lettuce genotypes (leaf lettuce and wild lettuce) grown in a hydroponic system designed for ready-to-eat (RTE) vegetable production.

Lettuce is generally considered a salt-sensitive crop, exhibiting variable morphological and physiological responses depending on the severity of salinity stress (Moncada et al., 2020; Vetrano et al., 2020). Previous studies have highlighted genotype-dependent responses, with some genotypes showing increases or decreases in fresh and dry biomass under salt stress (Adhikari et al., 2021; Xu and Mou, 2015). In our first experiment, all genotypes experienced reductions in total biomass under

salt stress, but the magnitude and the consequences on different morpho-physiological parameters varied. Among the leaf lettuce genotypes, TKI-126 was the most salt-tolerant, with only a 12.3% reduction in total fresh weight, while TKI-122 was the most salt-sensitive (−43.5%). Within the wild genotypes, TKI-257 showed the smallest decrease in fresh biomass (−12.1%), whereas TKI-252 exhibited the largest reduction (−53.8%) compared to the control.

Genotypes TKI-126 and TKI-468 were the most productive under optimal conditions, exhibiting high leaf fresh and dry biomass. This productivity was closely linked to greater total leaf area, likely enhancing light interception and promoting dry matter accumulation (Kong and Nemali, 2021). Their higher relative growth rate (RGR) further supported biomass accumulation, reflecting superior intrinsic growth potential. Notably, the wild lettuce genotype TKI-468 also demonstrated favorable morphological traits for the RTE market, with highly expanded and moderately thick leaves, reflecting intermediate characteristics between *L. sativa* and *L. serriola* (Koopman et al., 2001).

Reductions in RGR under salinity are commonly associated with impaired photosynthetic capacity, as decreased CO_2 assimilation limits carbohydrate synthesis and constrains dry biomass accumulation (Hniličková et al., 2017; S. Yousif et al., 2010; Thompson et al., 2017). Consistent with this, TKI-126 maintained high performance under salinity, with only a 1.2% reduction in RGR and a 5.3% decrease in dry biomass, making it the most stress-resilient genotype. TKI-257 displayed moderate declines (−2.6% RGR; −8.9% dry biomass), suggesting an effective balance between growth maintenance and stress adaptation. In contrast, TKI-122 and TKI-252 showed the largest reductions in both RGR and biomass, indicating higher salt sensitivity, likely due to limitations in carbon assimilation and reduced capacity to sustain canopy function.

Salinity also induced morphological adaptations affecting leaf development, with reductions in leaf number and expansion representing a common strategy to minimize water loss and preserve water balance under osmotic stress (Acosta-Motos et al., 2017; He et al., 2022; Moncada et al., 2020).

In this study, leaf number decreased in all genotypes; however, reductions were more pronounced in wild genotypes (−24.4% on average) than in cultivated leaf lettuce genotypes (−7.0% on average). TKI-257 mirrored previous observations in *L. serriola* (Chen et al., 2025), showing lower leaf area and dry biomass than cultivated lettuce under optimal conditions. In contrast, the wild genotype TKI-252 (*L. serriola*) acted markedly different; it was more productive under optimal conditions, with 108.6% higher leaf fresh biomass and 141.7% greater total leaf area compared to TKI-257. However, this higher growth potential was associated with reduced salinity tolerance, illustrating a trade-off between growth potential and stress resilience in wild *L. serriola* genotypes.

Specific leaf area (SLA) typically decreases under salinity, corresponding to thicker leaves with higher dry matter content (Xu and Mou, 2016), a pattern also observed in our trial. Interestingly, TKI-125 exhibited an atypical increase in SLA under salinity, paired with higher chlorophyll per unit leaf

area and an 11.1% reduction in dry matter. This suggests that, in this genotype, salinity primarily exerted an osmotic rather than ionic stress, favouring water and solute accumulation rather than structural biomass production. The increase in chlorophyll density may represent a compensatory mechanism to maintain photosynthetic capacity and support carbon assimilation under growth-limiting conditions (Adhikari et al., 2019). Chlorophyll responses varied among genotypes, either increasing or remaining unchanged, consistent with reports in lettuce and Chinese cabbage (Šamec et al., 2021; Xu and Mou, 2015). Salinity often reduces pigment content in sensitive species (Chen et al., 2024) but responses depend on species, genotype and nutritional status, which can improve ion balance and enhance stress defences (Ferreira et al., 2018; Nawaz et al., 2020).

Salinity also triggered divergent metabolic responses. Plants often accumulate flavonoids and anthocyanins to mitigate oxidative stress and protect photosynthetic machinery (Li and Ahammed, 2023). In our experiment, however, no significant effect of salinity was observed on the anthocyanin index. As reported by Gazula et al. (Gazula et al., 2007) higher anthocyanin concentrations are associated with red leaf coloration and lower hue angle values. Consistent with this pattern, genotype TKI-122, characterized by red leaves, exhibited the highest anthocyanin index and lowest chlorophyll concentration. The flavonoid response was more dynamic and varied among genotypes; the increase in TKI-465 under salt stress suggests a robust activation of antioxidant defence mechanisms, whereas the sharp decrease in TKI-252 likely reflects a breakdown of the antioxidant system, contributing to its sever biomass loss.

The Nitrogen Balance Index (NBI) mirrored these genotype-specific trends, emphasizing that the interaction between nitrogen status and antioxidant capacity is a key determinant of salt tolerance (Adhikari et al., 2021; Ferreira et al., 2018; Nawaz et al., 2020). These results highlight the need for targeted mitigation strategies, particularly for high-value sensitive genotypes like TKI-122, which was unable to activate sufficient endogenous defenses to overcome the salinity levels tested.

4.2. Efficacy of root-applied compounds in mitigating salt stress

Based on the results obtained in the T1 trial, several exogenous compounds were subsequently tested via root application to mitigate salt stress in the most sensitive leaf lettuce genotype, TKI-122.

Trial 2 demonstrated that the exogenous application of specific compounds to the nutrient solution can significantly alter the salt stress response of the sensitive genotype TKI-122. The most striking result was the fully restorative effect of intermediate-dose Salicylic Acid (SA-M), which stood in contrast to the varying degrees of success achieved with Ascorbic Acid (AsA-L) and L-Alanine (Ala), and the severe phytotoxicity induced by Methyl Jasmonate (MeJA).

Enhanced root growth often improves shoot biomass accumulation in leafy vegetables (Gallegos et al., 2020; Liu et al., 2024). In our study, the substances that promoted greater root development compared to the saline control also positively influenced shoot growth, confirming a functional link

between belowground and aboveground biomass. This relationship was not strictly linear. Salinity can negatively affect water and mineral nutrient uptake, with direct consequences for biomass accumulation (Acosta-Motos et al., 2017). However, the capacity to mitigate salt stress was not solely dependent on the chemical nature of the substance applied but was fundamentally governed by concentration (Yuan and Dickinson, 2024).

Salicylic acid (SA) is widely recognized for its role in alleviating salt stress by regulating ionic homeostasis and key metabolic pathways (Arif et al., 2020). Our results demonstrate that SA alleviates salinity in a clear dose-dependent quadratic manner. While the highest concentration (SA-H) was inhibitory and the lowest (SA-L) was insufficient to elicit a full response, the intermediate concentration (SA-M, 0.1 μM) fully restored fresh and dry biomass, relative growth rate (RGR), and leaf number to control levels. This aligns with previous reports of root-applied SA success in salt-stressed tomato (Souri and Tohidloo, 2019; S. Sultana et al., 2025). Notably, SA-M treated plants reached control-level biomass despite having smaller leaf areas and thinner laminae, suggesting that biomass recovery was achieved through compensatory growth and improved metabolic efficiency rather than a full restoration of original leaf morphology. The success of SA-M can be attributed to enhanced osmotic adjustment and membrane stability. By reducing the Nitrogen Balance Index (NBI) and maintaining chlorophyll levels, SA likely improved nitrogen utilization and photosynthetic efficiency under stress. Furthermore, the significant increase in root dry weight observed with SA-M suggests that this treatment promoted a more robust root system, allowing for better water and nutrient uptake despite the high osmotic pressure of the saline nutrient solution. Interestingly, the decrease in flavonoid content in SA-treated plants relative to the NaCl control may indicate that SA effectively reduced the primary stress level, thereby lowering the plant's requirement for energy-expensive endogenous antioxidant production.

While SA-M fully rescued TKI-122 from salt stress, Ascorbic Acid, L-Alanine, and L-Tryptophan also provided significant mitigation, albeit to a lesser extent.

Ascorbic acid (AsA) serves as a key antioxidant involved in photosynthetic metabolism and enzyme synthesis (Khan et al., 2011; Smirnoff and Wheeler, 2000). Together with Glutathione (GSH), it operates within the ascorbate–glutathione (AsA–GSH) cycle, a central redox pathway responsible for the detoxification of reactive oxygen species (ROS) and the maintenance of cellular redox homeostasis (Ahmad et al., 2022; Hasanuzzaman et al., 2019). Exogenous foliar application of AsA has been shown to improve growth and stress tolerance in several crops, including tomato, sweet pepper, and wheat, resulting in enhanced biomass accumulation, yield, and resilience under adverse conditions (Chen et al., 2024; El-Beltagi et al., 2022; El-Hawary et al., 2023). Beyond foliar treatments, exogenous root application of AsA has been reported to influence root system architecture. In *Arabidopsis thaliana*, low AsA concentrations stimulated root expansive growth,

whereas higher concentrations inhibited root development (X. Li et al., 2018). In our trial, root-applied AsA showed a concentration-dependent effect on root architecture similar to that observed in *Arabidopsis thaliana*. The lower AsA concentration (0.1 mM; AsA-L) was highly effective, promoting 69.2% more root fresh biomass than the higher dose and increasing total fresh biomass by 41.2% relative to NaCl. These effects may be ascribed to its action as a direct antioxidant to neutralize reactive oxygen species (ROS) produced at the root-zone interface.

L-Alanine (Ala) also provided significant protection against salinity. The external supply of amino acids, applied either as individual compounds or in tailored mixtures, is increasingly explored as a practical strategy to enhance plant tolerance to abiotic stresses. Rather than acting through a single mechanism, exogenous amino acids influence multiple physiological processes, including osmotic adjustment, redox homeostasis, and nutrient utilization, thereby supporting photosynthetic performance under saline conditions (Ahmad et al., 2025; Colla et al., 2015; Peña Calzada et al., 2022). L-Alanine and L-Tryptophan likely functioned as compatible osmolytes or precursors to growth-promoting hormones like auxins.

L-tryptophan is an essential amino acid and a metabolic precursor of indole-3-acetic acid (IAA). Beyond its role in auxin biosynthesis, L-Tryptophan has been shown to contribute to redox regulation by modulating ROS levels and preserving cellular integrity under saline conditions, as reported in crops such as spinach, red pepper, and potato (Gull et al., 2023; Jamil et al., 2018; San-Francisco et al., 2005; Turfan et al., 2024). In onion, foliar application of L-Tryptophan under saline conditions at doses comparable to those used in the present study resulted in increased fresh and dry biomass while maintaining leaf number (Hussein et al., 2014). A similar response was observed in our experiment, where L-Tryptophan increased total fresh biomass by 16.5% compared to NaCl, without significantly affecting leaf number, total leaf area, and SLA. Moreover, L-Tryptophan's effect on maintaining the highest chlorophyll index suggests it specifically protects the photosynthetic apparatus from ion-induced senescence. L-Alanine (1 mM; Ala) proved particularly effective, increasing biomass accumulation, leaf number, lamina expansion, and RGR under saline conditions. L-Alanine is a non-essential proteinogenic amino acid involved in the regulation of photosynthetic activity and chlorophyll biosynthesis (Kumar et al., 2017; Parthasarathy et al., 2019). Interestingly, previous studies using foliar alanine at higher doses failed to stimulate tomato growth (15 mM) (Alfosea-Simón et al., 2021) or enhance lettuce salinity (5.6 mM) (Abdelkader et al., 2023) and L-Alanine treatments were associated with reduced biomass accumulation. Our success with a lower dose via root application suggests that supplying Ala directly to the rhizosphere at a concentration that supports photosynthetic efficiency and light interception (higher SLA) is a superior strategy for lettuce. This response supports the positive relationship between specific leaf area (SLA) and RGR, whereby higher SLA enhances photosynthetic efficiency and improves light interception (Vitale et

al., 2021), ultimately promoting plant growth under saline conditions. Overall, these results indicate that the effectiveness of L-Alanine is strongly dependent on both concentration and application method and suggest that root application at low doses may represent a more effective strategy than foliar treatments for improving lettuce tolerance to salinity stress. Even with some significant mitigation effects, unlike SA, L-Alanine and L-Tryptophan did not fully restore leaf area, indicating that while they protect cell viability, they are less effective at maintaining the cell expansion rates required to match non-saline growth.

The other exogenous compounds failed in supporting leaf lettuce TKI-122 growth under salinity and showed inhibitory effects and phytotoxicity.

In stark contrast to the other phytohormone tested (SA), Methyl Jasmonate (MeJA) induced severe growth inhibition and phytotoxicity, particularly at higher concentrations (MeJA-H). Methyl Jasmonate is a phytohormone that plays a central role in the activation of plant defence mechanisms under both biotic and abiotic stresses (Huang et al., 2017). Exposure to salt stress in combination with MeJA has been shown to activate the Jasmonic Acid (JA) signaling pathway, which can exert deleterious effects on plant growth and development (Ahmad et al., 2019; Valenzuela et al., 2016), a phenomenon known as the "growth-defense trade-off". Previous studies on exogenous foliar application of MeJA in radish reported that low concentrations were largely ineffective in mitigating salt-induced stress, whereas higher concentrations caused pronounced growth inhibition (Henschel et al., 2023). Thus, our trial confirmed that the combination of salinity and elevated root applied MeJA likely sustained Jasmonic Acid signaling pathways that prioritize defense over growth, ultimately limiting cellular expansion and development (Sakamoto and Suzuki, 2019). In the case of TKI-122, the root application of MeJA likely triggered an over-activation of senescence-related pathways, leading to the observed drastic reduction in leaf number and area.

Similarly, high concentrations of Sodium Nitroprusside (SNP) and Glutathione (GSH) failed to mitigate stress and, in some cases, exacerbated biomass loss.

Sodium Nitroprusside, a Nitric Oxide (NO) donor, is known to modulate antioxidant enzyme activity, thereby reducing oxidative damage and promoting growth in several crop species (Sardar et al., 2023; Shang et al., 2022; Tahjib-Ul-Arif et al., 2022). In our experiments, the lowest SNP concentration (0.02 mM; SNP-L) partially alleviated the negative effects of salinity, resulting in plants with 11.4% higher total fresh biomass compared with NaCl, likely by preserving photosynthetic pigments and reducing ROS-induced damage (Khademi Astaneh et al., 2022). From a morphological perspective, these plants were characterized by increased leaf area per leaf and higher specific leaf area, suggesting improved leaf expansion under moderate NO availability. Conversely, the highest SNP concentration (0.5 mM; SNP-H) negatively affected all growth parameters, likely due to excessive NO release into the nutrient solution. These results indicate that the stress-mitigating capacity of NO is strongly

concentration-dependent (Gupta and Huang, 2014). At elevated concentrations, Nitric Oxide (NO) can become phytotoxic by promoting peroxynitrite formation (Chen et al., 2004), ultimately impairing cellular functions and inhibiting plant growth. Moreover, excessive nitrogen availability can trigger physiological disorders, including nitrate accumulation and root toxicity, further restricting biomass production (Aydinsakir et al., 2019; Bian et al., 2020; Wang and Li, 2004).

Similarly, Glutathione root application markedly reduced all growth parameters. Several studies have demonstrated that exogenous application of Glutathione, particularly via foliar spraying at concentrations ranging from 0.4 to 1 mM, can effectively alleviate salinity-induced stress by reducing ROS accumulation and enhancing antioxidant enzyme activity in various crop species (Akram et al., 2017; Al-Elwany et al., 2020; Rehman et al., 2021). However, as already mentioned, many exogenous substances may induce toxicity and growth inhibition when applied at excessive doses. For instance, root application of 100 μM (0.1 mM) GSH in Chinese cabbage has been reported to mitigate cadmium toxicity (Huang et al., 2019), highlighting the importance of dose optimization. In contrast, in our study, GSH root application markedly reduced both physiological and morphological parameters. These results suggest that, to achieve a beneficial effect, GSH concentrations applied via the root system should be substantially lower than those used in the present trial, emphasizing the strong dose and application-dependent nature of GSH-mediated stress responses.

5. Conclusions

This study provides a comprehensive evaluation of salt stress responses in lettuce, bridging the gap between genotypic screening and targeted physiological mitigation. Trial 1 demonstrates that lettuce responses to salinity in hydroponic systems are strongly genotype-dependent, encompassing morphological, physiological, and biochemical traits. Leaf lettuce genotypes TKI-126 and TKI-125 exhibited effective maintenance of total leaf area, relative growth rate (RGR), and biomass under salt stress, indicating superior stress resilience. Among wild genotypes, TKI-257 combined moderate biomass reduction with effective physiological adaptation, whereas TKI-252 and TKI-122 were the most sensitive, showing marked declines in leaf area, RGR, and dry biomass. Enhanced productivity under optimal conditions was closely linked to greater total leaf area and higher RGR, as observed in TKI-126 and TKI-468, underscoring the role of canopy development in light interception and carbon assimilation. Salinity-induced morphological adaptations, including reductions in leaf expansion and SLA modulation, alongside biochemical responses such as chlorophyll retention and flavonoid/anthocyanin accumulation, contributed to genotype-specific strategies for coping with osmotic and oxidative stress. Notably, TKI-125 displayed an unusual SLA increase under salinity, suggesting a predominantly osmotic adjustment mechanism. From an applied perspective, TKI-468 emerges as a promising candidate for ready-to-eat (RTE) production, combining high productivity, attractive leaf morphology, and moderate stress resilience. These findings provide a framework for

selecting and breeding lettuce genotypes with improved salinity tolerance, balancing growth potential under optimal conditions with adaptive stress responses in hydroponic cultivation systems. Overall, trial 1 highlighted a high degree of variability in salinity tolerance across the *Lactuca* germplasm. While the wild relative TKI-257 and the leaf lettuce TKI-126 demonstrated robust resilience, the leaf lettuce genotype TKI-122 was identified as highly sensitive, making it a critical model for testing rescue strategies.

The results of Trial 2 underscore the potential of root-zone biostimulation to counteract salinity-induced growth inhibition in sensitive genotypes. Root-applied compounds can differentially modulate lettuce responses to salinity stress, with effectiveness strongly dependent on both the type of compounds and its applied concentration. Overall, treatments that promoted root system development were more effective in sustaining shoot growth under saline conditions, confirming the central role of root functionality in mitigating salt-induced growth limitations in hydroponically grown lettuce. Among the diverse exogenous compounds tested Salicylic Acid (SA) showed a strong capacity to counteract salinity-induced growth inhibition in a dose-dependent manner. The intermediate SA concentration fully restored fresh and dry biomass, relative growth rate, and nitrogen balance, although this recovery was accompanied by modifications in leaf morphology, suggesting that stress compensation may occur through altered growth strategies rather than full structural recovery. While L-Alanine, L-Tryptophan, and low-dose Ascorbic Acid (0.1 mM) significantly improved salt tolerance, they were less effective than SA in restoring structural leaf expansion.

Conversely, this study reveals the risks associated with dose and application methods. The severe phytotoxicity observed with Methyl Jasmonate and high concentrations of Sodium Nitroprusside and Glutathione indicates that root-applied mitigants must be carefully calibrated to avoid exacerbating stress.

Collectively, these findings demonstrate that root-applied exogenous compounds can effectively mitigate salinity stress in lettuce, but only within a narrow and compound-specific dosage range. Root-applied Salicylic Acid at low concentrations represents a promising, low-cost strategy for maintaining the productivity of high-value, salt-sensitive lettuce cultivars in hydroponic systems. Future research should focus on the molecular pathways behind SA-mediated compensatory growth and investigate the long-term effects of these compounds on the nutritional quality of ready-to-eat vegetables.

6. References

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VI. FINAL CONCLUSIONS

The experimental activities undertaken in this thesis demonstrate that the sustainable production of fresh-cut vegetables in the face of increasing soil salinization and climate instability relies on a strategic integration of underutilized germplasm, optimized hydroponic protocols, and targeted physiological mitigation. By characterizing the productive and physiological potential of *Tetragonia tetragonioides*, leaf celery (*Apium graveolens* var. *secalinum*), and diverse *Lactuca* genotypes, this work confirms that neglected plant resources are not merely alternatives to traditional crops, but high-performance candidates for modern, resilient, and resource-efficient agriculture.

A primary finding of this research is that these species exhibit a high degree of adaptability to soilless cultivation, although their performance is heavily governed by the interaction between genetics and environmental management. For Tetragonia, yield and nutritional quality were maximized through high plant density and full-strength nutrient solutions, though the species remained sensitive to seasonal climatic shifts. Similarly, leaf celery was identified as a robust multi-cut vegetable, with a remarkable regrowth capacity that saw biomass increase by up to 44% in the second mowing under cooler winter-spring conditions. These results suggest that optimizing the "timing" of cultivation is as critical as the technical setup, with leaf celery and Tetragonia both proving capable of meeting the rigorous 14-to-21-day shelf-life standards required by the ready-to-eat industry.

The core of this thesis explored the dual nature of salinity as both a stressor and a strategic tool. While severe salt stress (100 mM NaCl) eventually limits growth across all species, moderate salinity (50 mM NaCl) acted as a beneficial eustressor, particularly in Tetragonia. At this concentration, the plants achieved a physiological "priming" that not only optimized water and nitrogen use efficiency but also enhanced the sensory and commercial quality of the final product. These saline-grown plants exhibited a more compact morphology, more intense coloration, and superior post-harvest resilience. This demonstrates that moderate salt stress can be leveraged as an agronomic tool to deliver a more resilient product to the cold chain while simultaneously conserving freshwater resources.

However, the research also highlights that natural resilience has its limits, particularly when ionic toxicity accumulates over multiple harvests. In leaf celery, the transition from the first to the second mowing revealed a significant decline in salt tolerance as cumulative stress surpassed the plant’s natural recovery mechanisms. To address this, the thesis successfully validated the use of exogenous protective compounds. The application of 10 mM citrulline emerged as a potent strategy to bridge the economic gap, effectively restoring the yield of salt-stressed plants to the levels of non-saline controls. In a similar vein, the screening of *Lactuca* genotypes allowed for the identification of salt-sensitive models where root-zone biostimulation with low-dose salicylic acid could restore biomass and nitrogen balance. These findings underline the necessity of a compound-specific approach, as

improper dosages of certain mitigants (such as Methyl Jasmonate) were found to exacerbate rather than alleviate stress.

In conclusion, this work represents a scientific contribution to the field of controlled-environment agriculture, offering a sustainable production system that transforms environmental challenges into opportunities for enhancing the quality, resilience, and diversity of our food supply.