



## Research Paper

# Suitability of *Apium graveolens* L. var. *secalinum* Alef. to hydroponic cultivation for baby leaf production

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## ARTICLE INFO

## Keywords:

Leaf celery  
Underutilized leafy vegetables  
Plant density  
Nutrient solution  
Ready-to-eat vegetables  
Sensory analysis  
Shelf-life

## ABSTRACT

Consumers are increasingly seeking innovative, healthy foods rich in nutraceuticals, driving the search for new or underutilized leafy vegetables. Leaf celery (*Apium graveolens* L. var. *secalinum* Alef.), a promising candidate for new food sources, stands out from ribbed celery with its smaller size and enhanced aroma. It is gaining global interest due to its high concentration of bioactive compounds, but it is presently cultivated only on soil in restricted regions. Significant knowledge gaps still exist regarding optimal agronomic management for its hydroponic baby leaf production and post-harvest cold storage as a minimally processed product. The necessity of adopting hydroponic systems for ready-to-eat leafy salads requires specific studies on techniques and nutrient management for novel vegetables like leaf celery. This research, for the first time, investigates the feasibility of producing fresh-cut suitable leaf celery baby leaves using a hydroponic ebb-and-flow cultivation system. We studied the effects of two plant densities (615 and 947 plants m<sup>-2</sup>) and three nutrient solution concentrations (NS) (only water, half strength, and full strength) on leaf celery growth, yield, and postharvest quality over two growing seasons (S1: winter/spring and S2: spring/summer). The experiment included two mowings per season to test the plant's regrowth capability, with morphological, biochemical, and yield characteristics assessed after each. Leaves from the first mowing of each trial were tested via sensory analysis and evaluated for shelf-life following minimal processing and cold storage (21 days at 4 °C). This research provides essential, globally transferable data for sustainable Controlled Environment Agriculture (CEA) by quantifying the yield, nutritional stability, and post-harvest longevity of this novel crop across critical seasonal and resource management variables. Results showed higher total yields in S1 (5.25 kg m<sup>-2</sup>) compared to S2 (2.76 kg m<sup>-2</sup>) using the full-strength NS, with nutrient availability effects varying by season and density. The full-strength NS maximized total yield, while the half-strength NS achieved the highest NUE (35.6 g DW g<sup>-1</sup> N in S1). Importantly, the baby leaves exhibited good vitamin and mineral content with consistent stability across growing seasons and mowings. Their sensory profile showed only minor differences between seasons, generally maintaining a good overall evaluation. Crucially, the leaves maintained a shelf-life exceeding 14 days across all tested treatments. Overall, leaf celery proved well-suited for hydroponic cultivation, yielding baby leaves with excellent shelf-life and nutritional quality, offering a viable high-value option for the fresh-cut market.

## 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 (Giménez et al., 2020; Hyldelund 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., 2013). 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 (Hyldelund et al., 2020; Nassivera and Sillani, 2015; Ricci et al., 2018). To further expand

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<https://doi.org/10.1016/j.scienta.2026.114619>

Received 28 August 2025; Received in revised form 18 December 2025; Accepted 7 January 2026

Available online 16 January 2026

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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; Rožek 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 (Rožek, 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, anti-rheumatic, 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* (Rožek 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; Rožek, 2013; Rožek 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; it 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<sup>-3</sup> 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<sup>-2</sup>) 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<sup>-1</sup>) with the following mineral elements: 19 mM NO<sub>3</sub><sup>-</sup>, 1.25 mM NH<sub>4</sub><sup>+</sup>, 2 mM H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, 11 mM K<sup>+</sup>, 4.5 mM Ca<sup>2+</sup>, 1 mM Mg<sup>2+</sup>, 1.1 mM SO<sub>4</sub><sup>2-</sup>, 40 µM Fe<sup>3+</sup>, 30 µM BO<sub>3</sub><sup>3-</sup>, 5 µM Mn<sup>2+</sup>, 4 µM Zn<sup>2+</sup>, 0.75 µM Cu<sup>2+</sup>, and 0.50 µM Mo (Sonneveld et al., 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<sup>-2</sup>, with a day

length ranging between 10 and 13 h. In S2, the temperature ranged between  $20.2 \pm 0.4$  °C and  $24.7 \pm 0.5$  °C, with an average noon net solar radiation of  $712 \text{ W m}^{-2}$  and a day length from 13 to 15 h. The average hourly air temperature inside the greenhouse was  $15.3 \pm 1.0$  °C during the first growing cycle and  $22.8 \pm 0.8$  °C during the second. The temperature ranged between an average minimum of  $9.6 \pm 0.3$  °C and maximum of  $25.3 \pm 0.5$  °C in the first cycle, and between an average minimum of  $18.2 \pm 0.3$  °C and maximum  $29.9 \pm 0.5$  °C in the second (Fig. 1). The relative humidity was  $75.0 \pm 4.0$  % and  $82.3 \pm 2.6$  % on average during the first and second cycle, respectively. Inside the greenhouse, noon light intensity averaged  $44,080 \pm 2845$  lx (range: 3410–70,740 lx) in S1 and  $54,933 \pm 3789$  lx (range: 5805–74,021 lx) in S2, with fluctuations driven by cloudiness.

The ebb and flow system was configured to deliver a 15-minute irrigation cycle once every 24 h. 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  $\text{WUE} (\text{g DW L}^{-1} \text{H}_2\text{O}) = \text{plant dry weight (g)}/\text{H}_2\text{O (L)}$ , and nitrogen use efficiency as  $\text{NUE} (\text{g DW g}^{-1} \text{N}) = \text{plant dry weight (g)}/\text{supplied N (g)}$  (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 (Fig. 2).

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 ( $h^\circ$ ) as  $C^* = (a^{*2} + b^{*2})^{1/2}$  and  $h^\circ = 180 + \arctan(b^*/a^*)$  (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. Tokyo, 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,  $\text{cm}^2 \text{g}^{-1} \text{DW}$ ) 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 muffle furnace at 550 °C for 8 h. Subsequently, acid digestion (2 %  $\text{HNO}_3$ ) 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 h. The ashes were later recovered through acid digestion using 10 mL of 1 M HCl on a hotplate at 100 °C for 15 min. 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  $\text{H}_2\text{SO}_4$ . 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

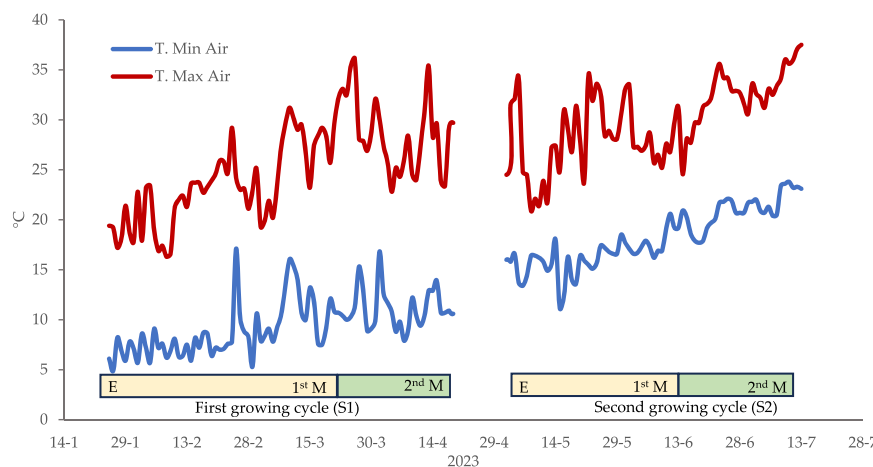


Fig. 1. Daily average maximum and minimum temperatures of the air recorded inside the greenhouse with a data logger during leaf celery cultivation with a hydroponic ebb and flow system. The two growing cycles (S1 – winter-spring, S2 – spring-summer) are reported in the horizontal bars (E = emergence;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

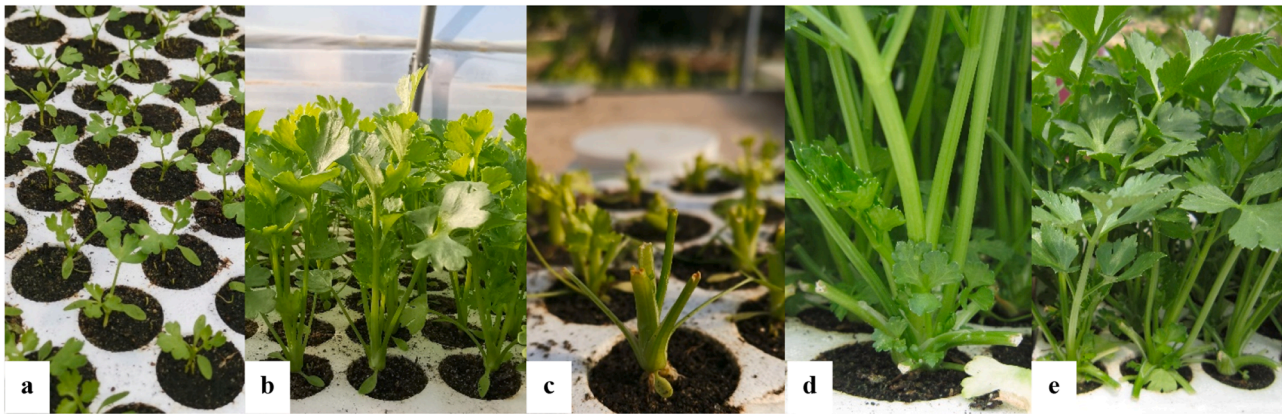


Fig. 2. Leaf celery plants grown on polystyrene trays in an ebb-and-flow hydroponic system at different growth stages: a) plants at the two-leaf stage; b) plants before the first mowing; c) plants soon after the first mowing; d) plant regrowth; e) plants ready for the second mowing.

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 (Fig. 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 min two times and dried by manual centrifugation for 1 min



Fig. 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.

with a handheld salad spinner. Twelve samples of approximately 50 g 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 ( $\text{g } 100 \text{ g}^{-1}$  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.

### 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 three-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 (Fig. 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.

#### 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 ( $0.22 \text{ g plant}^{-1}$  on average in the first cycle and  $0.05 \text{ g plant}^{-1}$  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 ( $1.70 \text{ g plant}^{-1}$  on average) during the first season, and when using both HS



**Fig. 4.** Differences in size and canopy structure of plants grown under varying plant densities (low density: 615 plants  $\text{m}^{-2}$ ; high density: 947 plants  $\text{m}^{-2}$ ) 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.

( $1.00 \text{ g plant}^{-1}$  on average) and FS NS ( $1.86 \text{ g plant}^{-1}$  on average) in the second growing cycle. The highest total biomass accumulation ( $3.46 \text{ g plant}^{-1}$ ) was observed with the combination of FS NS, a density of 615 plants  $\text{m}^{-2}$ , 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  $0.03 \text{ g plant}^{-1}$  on average with 0 NS to  $1.56 \text{ g plant}^{-1}$  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 ( $1.49 \text{ g plant}^{-1}$  on average). However, FS NS significantly increased this parameter, reaching  $2.31 \text{ g plant}^{-1}$  with 947 plant  $\text{m}^{-2}$  and even higher with 615 plant  $\text{m}^{-2}$  ( $3.12 \text{ g plant}^{-1}$ ) (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  $164.2 \text{ mg plant}^{-1}$  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  $\text{m}^{-2}$ ) (total dry weight:  $323.3 \text{ mg plant}^{-1}$  with FS NS and  $214.7 \text{ mg plant}^{-1}$  with HS NS) compared to the higher density (947 plant  $\text{m}^{-2}$ ) (total dry weight:  $232.3 \text{ mg plant}^{-1}$  with FS NS and  $169.0 \text{ mg plant}^{-1}$  with HS NS) (Table 1). The dry biomass accumulated in the stem was higher during S1 and

**Table 1**

Effects of growing season (S1 – winter-spring, S2 – spring-summer), plant density (615, 947 plants m<sup>-2</sup>) 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			1.64 <sup>a</sup>	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 <sup>-2</sup>			1.43	0.20a	1.23	142.3	16.7a	125.6	12.0a
947 plants m <sup>-2</sup>			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
		HS	1.87c	0.23	1.64c	214.7b	22.3	192.3b	11.7
		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
		HS	1.52c	0.17	1.35c	169.0c	16.3	152.7c	11.3
		FS	2.57b	0.26	2.31b	232.3b	20.3	212.0b	9.2
	615	0	0.05e	0.02	0.03e	5.3f	1.3	4.0e	12.2
		HS	1.08d	0.20	0.89d	100.0d	15.0	85.0d	9.6
		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	
	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 <sup>x</sup>									
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

<sup>a</sup> 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.

<sup>x</sup> Significance: ns = not significant; \* significant at  $p < 0.05$ ; \*\* significant at  $p < 0.01$ ; \*\*\* significant at  $p < 0.001$ .

when adopting the lowest plant density and linearly increased up to an average of 24.0 mg plant<sup>-1</sup> (FS NS) with increasing NS concentration.

The leaf dry matter percentage was highest in the plants grown at 615 plant m<sup>-2</sup> (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).

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 3.9 (0 NS) to 6.2 (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<sup>-2</sup> (64.9 cm<sup>2</sup> plant<sup>-1</sup> and 10.5 cm<sup>2</sup> leaf<sup>-1</sup> for total and average leaf area, respectively) compared to 947 plant m<sup>-2</sup> (53.8 cm<sup>2</sup> plant<sup>-1</sup> and 9.0 cm<sup>2</sup> leaf<sup>-1</sup> for total and average leaf area, respectively) (Table 2). Moreover, a decreasing trend in average leaf area was

**Table 2**

Effects of growing season (S1 – winter-spring, S2 – spring-summer), plant density (615, 947 plants m<sup>-2</sup>) 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		SLA (cm <sup>2</sup> g DW <sup>-1</sup> )	L*	Chroma	Hue°
				(cm <sup>2</sup> plant <sup>-1</sup> )	(cm <sup>2</sup> leaf <sup>-1</sup> )				
Growing season (S)									
S1 – winter-spring	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 <sup>-2</sup>	5.4a	10.6	7.1	35.6	6.0	312.6	50.2	37.6	118.8
947 plants m <sup>-2</sup>	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 <sup>x</sup>									
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

<sup>z</sup> 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.

<sup>x</sup> Significance: ns = not significant; \* significant at  $p < 0.05$ ; \*\* significant at  $p < 0.01$ ; \*\*\* significant at  $p < 0.001$ .

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<sup>2</sup> g DW<sup>-1</sup>). 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).

### 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<sup>-2</sup> (+46.6 %) than during S2 (+33.0 %) or when using 947 plant m<sup>-2</sup> (+39.4 %). Despite these different response rates, no significant differences were observed between the 615-FS and 947-FS treatments (3.31 g plant<sup>-1</sup> on average).

Stem FW was higher in the first cycle compared to the second (1.09 and 0.45 g plant<sup>-1</sup> 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).

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

**Table 3**

Effects of growing season (S1 – winter-spring, S2 – spring-summer), plant density (615, 947 plants m<sup>-2</sup>) 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 (Second 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			<sup>z</sup> 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 <sup>-2</sup>			4.32	0.94	3.38	406.3	78.5	327.8	9.8
947 plants m <sup>-2</sup>			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 <sup>x</sup>									
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

<sup>z</sup> 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.

<sup>x</sup> Significance: ns = not significant; \* significant at  $p < 0.05$ ; \*\* significant at  $p < 0.01$ ; \*\*\* significant at  $p < 0.001$ .

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<sup>2</sup> plant<sup>-1</sup>) or by 34.0 % when using 615 plants m<sup>-2</sup> (73.7 cm<sup>2</sup> plant<sup>-1</sup>). However, the NS effect was less pronounced and non-significant in S2 (45.3 cm<sup>2</sup> plant<sup>-1</sup> on average for HS NS and FS NS) and with 947 plants m<sup>-2</sup> (46.3 cm<sup>2</sup> plant<sup>-1</sup> 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<sup>2</sup> leaf<sup>-1</sup> on average) and with the lower plant density (14.7 cm<sup>2</sup> plant<sup>-1</sup> 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<sup>2</sup> g DW<sup>-1</sup>), by 12.3 % at the highest density compared to the

lowest density (206.1 cm<sup>2</sup> g DW<sup>-1</sup>), and by 11.4 % with the supply of HS NS compared to FS NS (207.0 cm<sup>2</sup> g DW<sup>-1</sup>).

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<sup>-2</sup>. 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<sup>-2</sup>, 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<sup>-2</sup> (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<sup>-2</sup> 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<sup>-2</sup>) 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		SLA (cm <sup>2</sup> g DW <sup>-1</sup> )	L*	Chroma	Hue <sup>o</sup>
				(cm <sup>2</sup> plant <sup>-1</sup> )	(cm <sup>2</sup> leaf <sup>-1</sup> )				
Growing season (S)									
S1 – winter-spring	<sup>z</sup> 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 <sup>-2</sup>	5.1a	17.7a	10.6a	64.3	12.6	206.1b	46.0	27.6	126.1
947 plants m <sup>-2</sup>	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
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
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
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
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
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
FS	4.4	16.1	10.0	49.3bc	11.2b	214.2	46.0b	27.3c	125.1a
Significance <sup>x</sup>									
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

<sup>z</sup> 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.

<sup>x</sup> 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<sup>-2</sup> 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<sup>-2</sup> on average), which was 68.6 % higher than the yield from the same NS in S2 (1.22 kg m<sup>-2</sup> on average) (Fig. 5). This latter value did not significantly differ from the yield obtained with the HS NS in S1 (1.14 kg m<sup>-2</sup> 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<sup>-2</sup>, 0.78 and 1.44 kg m<sup>-2</sup>, 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<sup>-2</sup>) (Fig. 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 (Fig. 5).

The total baby leaf yield mirrored the trends observed in the first and second mowings (Fig. 5). A slight but significant increase in yield was found with increasing plant density (from 2.14 kg m<sup>-2</sup> at 615 plants m<sup>-2</sup> to 2.29 kg m<sup>-2</sup> at 947 plants m<sup>-2</sup>, on average). Increased nutrient supply also had a positive effect, though yield responses varied across growing seasons (Fig. 5). The highest yield (5.25 kg m<sup>-2</sup>) was recorded in the first growing cycle when the full-strength nutrient solution (NS) was applied (Fig. 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.

### 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<sup>-1</sup> H<sub>2</sub>O for the first mowing and 5.9 g DW L<sup>-1</sup> H<sub>2</sub>O 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<sup>-1</sup> H<sub>2</sub>O for the first mowing and 5.3 g DW L<sup>-1</sup> H<sub>2</sub>O 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<sup>-1</sup> H<sub>2</sub>O). Considering both mowings, the WUE in S2 increased significantly with increasing NS concentration, rising from 0.1 g DW L<sup>-1</sup>

**Table 5**

Effects of growing season (S1 – winter-spring, S2 – spring-summer) plant density (615, 947 plants m<sup>-2</sup>) 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 <sup>-2</sup> )		
	1 <sup>st</sup> mowing	2 <sup>nd</sup> mowing	Total
Growing season (S)			
S1 – winter-spring	<sup>z</sup> 1.11	2.70	2.90
S2 – spring-summer	0.63	1.35	1.53
Plant density (PD)			
615 plants m <sup>-2</sup>	0.76	2.08	2.14b
947 plants m <sup>-2</sup>	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			
S1			
0	0.12d		0.12e
HS	1.14b	2.20b	3.34b
FS	2.05a	3.19a	5.25a
S2			
0	0.03d		0.03e
HS	0.64c	1.16d	1.80d
FS	1.22b	1.55c	2.76c
PD x NS			
615			
0	0.06e		0.06
HS	0.78d	1.70	2.47
FS	1.44b	2.46	3.90
947			
0	0.09e		0.09
HS	1.01c	1.66	2.67
FS	1.84a	2.28	4.11
Significance <sup>x</sup>			
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

<sup>z</sup> 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.

<sup>x</sup> Significance: ns = not significant; \* significant at  $p < 0.05$ ; \*\* significant at  $p < 0.01$ ; \*\*\* significant at  $p < 0.001$ .

H<sub>2</sub>O (0 NS) to 2.7 g DW L<sup>-1</sup> H<sub>2</sub>O (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<sup>-1</sup> N (Fig. 6).

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<sup>-1</sup> N) was achieved using HS NS combined with the 947 plants m<sup>-2</sup> density. In S2, the lowest NUE was recorded with the 0 NS treatment (1.8 g DW g<sup>-1</sup> 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 (Fig. 7). Overall, for S2, the NUE averaged 12.2 g DW g<sup>-1</sup> N using 615 plants m<sup>-2</sup> and was significantly higher using 947 plants m<sup>-2</sup> (15.9 g DW g<sup>-1</sup> N). The second mowing recorded a greater NUE in S1 with HS NS (51.7 g DW g<sup>-1</sup> N) compared to FS NS (42.8 g DW g<sup>-1</sup> N), while there was no significant effect of NS concentration in S2 (29.9 g DW g<sup>-1</sup> N, on average) (Fig. 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<sup>-1</sup> N, on average) and showed similar values with HS NS or FS NS (21.1 g DW g<sup>-1</sup> N, on average) in S2. In S1, supplying 0 NS resulted in a NUE of 11.7 g

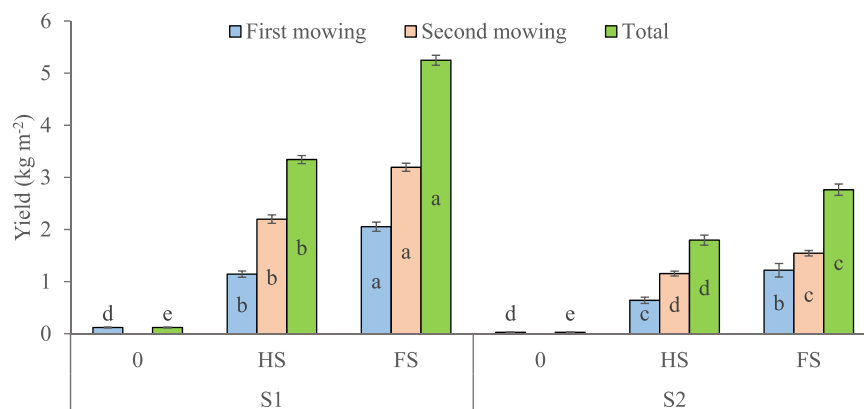
**Table 6**

Effects of growing season (S1 – winter-spring, S2 – spring-summer), plant density (615, 947 plants m<sup>-2</sup>) 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.

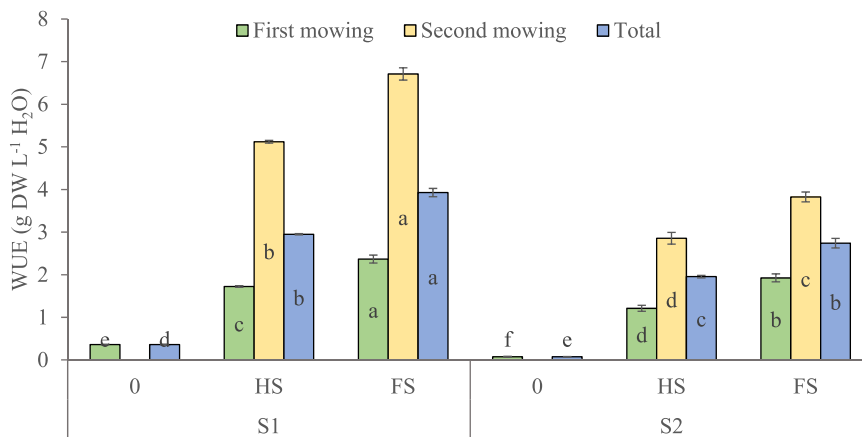
Source of Variance	WUE (g DW L <sup>-1</sup> H <sub>2</sub> O)		
	1 <sup>st</sup> mowing	2 <sup>nd</sup> mowing	Total
Growing season (S)			
S1 – winter-spring	<sup>z</sup> 1.5a	5.9a	2.4
S2 – spring-summer	1.1b	3.3b	1.6
Plant density (PD)			
615 plants m <sup>-2</sup>	1.2b	4.7	2.0
947 plants m <sup>-2</sup>	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 <sup>x</sup>			
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

<sup>z</sup> 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.

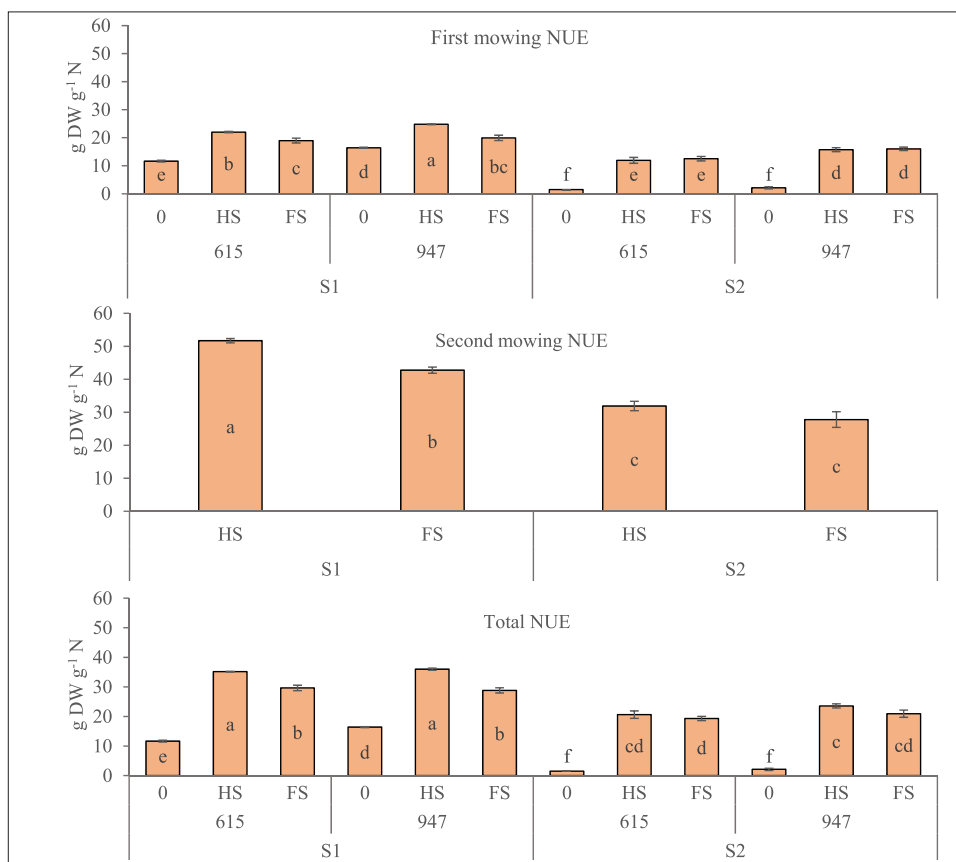
<sup>x</sup> Significance: ns = not significant; \*\*\* significant at  $p < 0.001$ .



**Fig. 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).



**Fig. 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).



**Fig. 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).

DW g<sup>-1</sup> N using 615 plants m<sup>-2</sup>, which increased up to 16.4 using 947 plants m<sup>-2</sup>. In this growing season (S1), NUE increased with HS NS up to the highest values (35.6 g DW g<sup>-1</sup> N, on average) then significantly declined with FS NS (29.2 g DW g<sup>-1</sup> N, on average) with no significant difference ascribed to plant density (Fig. 7).

### 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<sup>-2</sup> and supplied with HS NS compared to the other treatments (2.82 g 100 g<sup>-1</sup> 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<sup>-1</sup> FW on average) was recorded in S1 in plants grown at 947 plants m<sup>-2</sup> supplemented with both HS and FS NS and in S2 in the plants grown at 615 plants m<sup>-2</sup> and supplemented with FS NS. The lowest protein content (2.01 g 100 g<sup>-1</sup> FW on average) was recorded in S1 using 615 plants m<sup>-2</sup> and HS NS. Increasing NS concentration, the protein content significantly increased in both growing seasons in the plants grown at 615 plants m<sup>-2</sup>, 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<sup>-2</sup>.

The fiber content was not significantly affected by the experimental factors in the first mowing (1.41 g 100 g<sup>-1</sup> 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<sup>-1</sup> FW in S1 combined with 615 plants m<sup>-2</sup> to a minimum of 1.35 g 100 g<sup>-1</sup> FW in S2 combined with 947 plants m<sup>-2</sup>. The HS NS treatment showed contrasting effects: it determined the lowest fiber content (1.34 g 100 g<sup>-1</sup> FW) when applied in S2 or at the higher plant density (947 plants m<sup>-2</sup>). Conversely, it resulted in the highest fiber content (1.50 g 100 g<sup>-1</sup> FW) when applied in S1 or at the lower plant density (615 plants m<sup>-2</sup>).

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<sup>-1</sup> FW with HS NS in S1 to 2.19 g 100 g<sup>-1</sup> 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<sup>-1</sup> on average) were recorded in S1 with 615 plants m<sup>-2</sup> and FS NS and in S2 with 947 plants m<sup>-2</sup> and FS NS. Conversely, the lowest values (2.13 g 100 g<sup>-1</sup> on average) were observed in S1 with 947 plants m<sup>-2</sup> and FS NS and in S2 with 615 plants m<sup>-2</sup>, 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<sup>-1</sup>.

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<sup>-1</sup> FW in the first mowing and 223.7 µg 100 g<sup>-1</sup> FW in the second mowing. The highest values in the first mowing (250.1 µg 100 g<sup>-1</sup> 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<sup>-1</sup> FW on average. The second mowing confirmed a lower content (-3.6 %) of vitamin A in S2 (219.6 µg 100 g<sup>-1</sup> FW on average) compared to S1 (227.8 µg 100 g<sup>-1</sup> 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<sup>-1</sup> 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<sup>-1</sup> 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<sup>-2</sup>. The vitamin C averaged 32.3 mg 100 g<sup>-1</sup> FW on average in the first mowing. In S1, vitamin C was higher and not affected by the NS (33.6 mg 100 g<sup>-1</sup> FW on average). In S2, it showed a significant drop using FS NS (29.5 mg 100 g<sup>-1</sup> 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<sup>-1</sup> FW on average) and had a significant increase when supplying HS NS to the plants grown at 615 plants m<sup>-2</sup>.

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<sup>-1</sup> FW on average) and decreased in S2 with the same NS (272.2 mg 100 g<sup>-1</sup> FW on average). The Ca content was highest in S1 combined with 947 plants m<sup>-2</sup> (34.8 mg 100 g<sup>-1</sup> FW on average), while it averaged 29.8 mg

**Table 7**

Effects of growing season (S1 – winter-spring, S2 – spring-summer), plant density (615, 947 plants m<sup>-2</sup>) and nutrient solution (NS) concentration (0 %, only water - 0; 50 %, half strength - HS; 100 %, full strength - FS) on the vitamin content (mg 100 g<sup>-1</sup> 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		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 <sup>-2</sup>		238.8	0.21	0.06	33.1	223.8	0.20a	0.06	30.3a
947 plants m <sup>-2</sup>		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 <sup>x</sup>									
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

<sup>z</sup> 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.

<sup>x</sup> Significance: ns = not significant; \* significant at  $p < 0.05$ ; \*\* significant at  $p < 0.01$ ; \*\*\* significant at  $p < 0.001$ .

**Table 8**

Effects of growing season (S1 – winter-spring, S2 – spring-summer), plant density (615, 947 plants m<sup>-2</sup>) 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 <sup>-1</sup> FW)	Na (mg 100g <sup>-1</sup> FW)	Ca (mg 100g <sup>-1</sup> FW)	Mg (mg 100g <sup>-1</sup> FW)	P (mg 100g <sup>-1</sup> FW)	Fe (mg 100g <sup>-1</sup> FW)	Cu (mg 100g <sup>-1</sup> FW)	Zn (mg 100g <sup>-1</sup> FW)
Growing season (S)								
S1 – winter-spring	280.3 <sup>z</sup>	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 <sup>-2</sup>	278.6	138.8	29.8	14.9	48.8	0.35	0.11	1.30
947 plants m <sup>-2</sup>	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
S1 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
S2 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
S1 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
S2 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
S1 615 FS	281.0	137.0	29.3	15.0	48.7	0.36	0.11	1.29ab
S1 947 HS	280.3	143.7	32.7	15.0	48.7	0.36	0.11	1.29ab
S1 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
S2 615 FS	274.0	140.0	30.3	15.0	49.0	0.34	0.11	1.31a
S2 947 HS	277.3	141.3	29.7	14.7	46.3	0.35	0.11	1.30a
S2 947 FS	270.3	138.0	30.0	15.7	44.0	0.29	0.11	1.25b
Significance <sup>x</sup>								
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	**

<sup>z</sup> 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.

<sup>x</sup> Significance: ns = not significant; \* significant at  $p < 0.05$ ; \*\* significant at  $p < 0.01$ ; \*\*\* significant at  $p < 0.001$ .

100 g<sup>-1</sup> FW in all the other treatments. The Mg content was positively affected only by the increase of NS concentration (15.4 mg 100 g<sup>-1</sup> FW on average). The baby leaves recorded a Fe content of 0.36 mg 100 g<sup>-1</sup> 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<sup>-1</sup> FW on average. The Zn content averaged 1.30 mg 100 g<sup>-1</sup> FW in all treatments except when supplying FS NS with 947 plants m<sup>-2</sup> in S2, which recorded 1.25 mg 100 g<sup>-1</sup> 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<sup>-1</sup> 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<sup>-2</sup> (0.32 mg 100 g<sup>-1</sup> FW on average) and significantly increased with 947 plants m<sup>-2</sup> up to 0.37 mg 100 g<sup>-1</sup> 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<sup>-1</sup> FW of K, 139.7 mg 100 g<sup>-1</sup> FW of Na 31.3 mg 100 g<sup>-1</sup> FW of Ca, 14.8 mg 100 g<sup>-1</sup> FW of Mg, 47.2 mg 100 g<sup>-1</sup> FW of P, 0.11 mg 100 g<sup>-1</sup> FW of Cu, and 1.30 mg 100 g<sup>-1</sup> FW of Zn (data not shown).

### 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) (Fig. 8). The different combinations of plant density and nutrient solution concentrations had a small effect on almost all the sensory parameter scores (Fig. 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 (6.4 score on average) compared to the HS NS, which reduced color scores at both plant densities, particularly in combination with 947 plants m<sup>-2</sup> (4.1 on average). NS concentration did not affect the odor score of baby leaves grown at 615 plants m<sup>-2</sup> (3.8 on average). However, at 947 plants m<sup>-2</sup> 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<sup>-2</sup> 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<sup>-2</sup>, scoring intermediate scores (4.6 on average). The average overall rating score was 6.3.

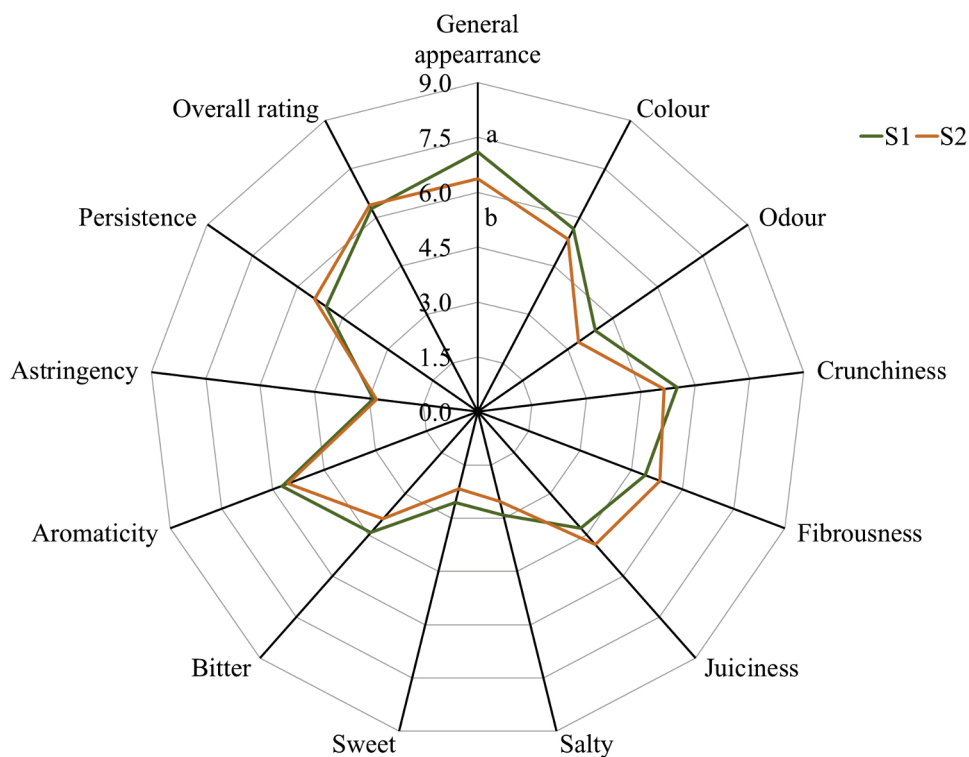


Fig. 8. Effects of growing season (S1 – winter-spring, S2 – spring-summer) on the baby leaf sensory profile of *Apium graveolens* L. var. *secalinum* Alef. plants grown using an ebb-and-flow hydroponic system (values within the same parameter with different letters are significantly different at  $p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

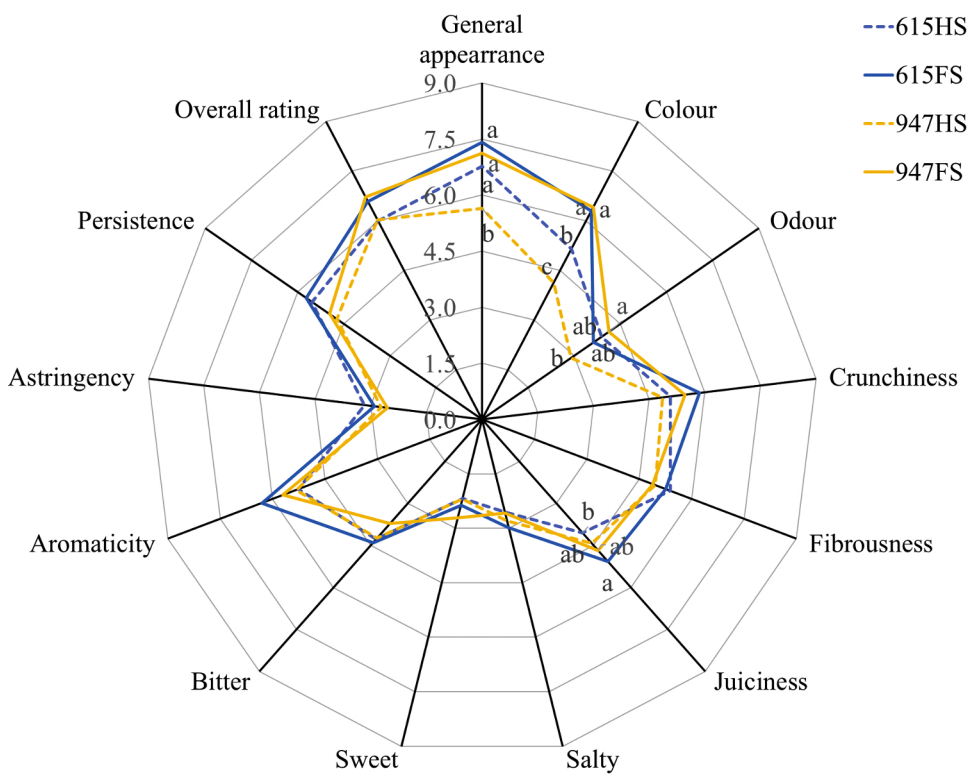


Fig. 9. Effects of plant density (615 and 947 plants  $m^{-2}$ ) and nutrient solution (NS) concentration (50 % half strength - HS; 100 % full strength - FS) on the baby leaf sensory profile of *Apium graveolens* L. var. *secalinum* Alef. plants grown using an ebb-and-flow hydroponic system (values within 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<sup>-2</sup> (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.

## 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

**Table 9**

Effect of growing season (S1 – winter-spring, S2 – spring-summer), plant density (615 and 947 plants m<sup>-2</sup>), 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°
<b>Growing season (S)</b>					
S1 – winter-spring	<sup>z</sup> 3.12	3.93	51.4	33.2	120.7
S2 – spring-summer	3.20	4.22	50.0	36.9	121.4
<b>Days at 4 °C (D)</b>					
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
<b>Plant density (PD)</b>					
615	3.52	4.23	50.7	35.1	121.2
947	2.80	4.03	50.5	35.0	121.2
<b>Nutrient Solution (NS)</b>					
HS	3.48	3.92	52.8	37.5	120.1
FS	2.84	4.23	48.6	32.6	122.1
<b>S x D</b>					
S1	0	5.00a	49.6c	35.1c	121.5
	7	1.78d	4.15c	50.6bc	32.5d
	14	3.30c	4.03c	51.0b	27.5e
	21	4.28b	2.54e	54.5a	37.8ab
S2	0	5.00a	50.7bc	37.0b	122.2
	7	1.30d	4.71b	49.3c	36.5b
	14	2.82c	4.22c	49.6c	36.0bc
	21	5.46a	2.96d	50.5bc	38.0a
<b>S x NS</b>					
S1	HS	3.44	3.74	53.7	35.4b
	FS	2.81	4.12	49.2	31.0d
S2	HS	3.51	4.10	52.0	39.6a
	FS	2.88	4.34	48.0	34.2c
<b>D x PD</b>					
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
	947	1.82	4.00c	50.4	33.0
14	615	3.68	4.26bc	51.6	27.6
	947	2.93	3.80d	50.3	27.4
21	615	4.67	2.58e	53.8	37.3
	947	3.90	2.50e	55.2	38.3
<b>D x NS</b>					
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
	FS	1.40	4.69b	47.5	31.5
14	HS	3.54	3.93d	52.2	34.1
	FS	2.59	4.31bc	48.3	29.4
21	HS	5.20	2.59f	55.0	40.5
	FS	4.54	2.91e	50.0	35.3
<b>PD x NS</b>					
615	HS	4.07a	4.01	52.9	37.5
	FS	2.96b	4.36	48.7	32.5
947	HS	2.88b	3.84	52.8	37.5
	FS	2.72b	4.10	48.5	32.7
<b>Significance<sup>x</sup></b>					
Growing season	ns	***	***	***	*
Day (D)	***	***	***	***	***
Plant density (PD)	***	***	ns	ns	ns

(continued on next page)

Table 9 (continued)

Source of Variance	Weight loss (%)	Overall Quality	L*	Chroma	Hue°
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

<sup>z</sup> 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.

<sup>x</sup> Significance: ns = not significant; \* significant at  $p < 0.05$ ; \*\* significant at  $p < 0.01$ ; and \*\*\* significant at  $p < 0.001$ .

moisture (Salehi et al., 2019). While the S1 mean temperature of  $17.5 \pm 0.4$  °C aligned with these optimal conditions, the S2 mean temperature of  $24.1 \pm 0.4$  °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<sub>2</sub> 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 lx) compared to S1 (10 to 13 h of light; 44,080 lx) 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<sup>-2</sup> compared to 615 plants m<sup>-2</sup>, 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) (Cavaliere et al., 2022). The increase in plant density from 615 to 947 plants m<sup>-2</sup> 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; Rožek, 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<sup>-2</sup>, 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 Rožek (Rožek, 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., 2021a). Similar trends are also observed in vertical farming systems regardless of photoperiod (Ciriello et al., 2023a), 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<sub>2</sub> 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., 2023a; Formisano et al., 2021b). 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<sub>2</sub> 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<sup>-2</sup> 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<sup>-2</sup> 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 <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<sup>-2</sup>. 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<sub>2</sub> 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., 2023b;

Formisano et al., 2021b). 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  $\beta$ -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., 2012a). 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., 2012b; Toivonen and Brummell, 2008a). 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, 2008b). 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 suggest 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. Conclusion

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.

## CRedit authorship contribution statement

**Alessandro Esposito:** Writing – review & editing, Writing – original draft, Validation, Investigation, Formal analysis, Data curation, Conceptualization. **Filippo Vetrano:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Alessandra Moncada:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Eristanna Palazzolo:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Caterina Lucia:** Writing – original draft, Investigation, Formal analysis, Data curation. **Alessandro Miceli:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

## Declaration of competing interest

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

## Data availability

Data will be made available on request.

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