AGRICULTURAL UNIVERSITY OF ATHENS
DEPARTMENT OF CROP SCIENCE
LABORATORY OF VEGETABLE PRODUCTION

Doctoral Thesis

Implementation of innovative farming systems for optimizing yield, and quality of stamnagathi (*Cichorium spinosum* L.,)

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«Εφαρμογή καινοτόμων γεωργικών συστημάτων
για τη βελτιστοποίηση της απόδοσης, ποιότητας και μετασυλλεκτικής ζωής του σταμναγκαθιού»

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Implementation of innovative farming systems for optimizing yield, quality and post-harvest behavior of stamnagathi (Cichorium spinosum L.,)

Department of Crop Science
Laboratory of Vegetable Production

Abstract

This thesis revolves around: the wild edible green *Cichorium spinosum* L., (Stamnagathi in Greek), the cultivation practices, and systems that could be utilized for its commercialization. A total of five experiments are presented in this thesis. The first two experiments were carried out under natural lighting conditions, in greenhouses, and the other three were carried out with solely artificial lighting, in climate chambers and vertical farms.

In the first experiment stamnagathi was cultivated in a floating-raft hydroponics system. In this experiment, the simultaneous effects of different potassium, calcium and magnesium ratios and different electrical conductivity (EC) levels on the growth and mineral composition of hydroponically grown C. spinosum were investigated. Four nutrient solutions (NS) were compared, two NS with low EC (L, 2.4 dS/m) and two with high EC (H, 3.6 dS/m) with K:Ca:Mg ratios of either 50:40:10 or 40:50:10. The results showed no interactions between the two factors. No significant effects were observed on the fresh and dry weight, leaf number and leaf area. High EC levels increased the K content and decreased the Mn and Zn content in the leaf tissues. The 40:50:10 ratio led to increased Ca content in plant tissues. The Nitrate-N was only affected by the EC level and was increased under H conditions, whereas the total-N was not affected.

The second study investigated the effects of nitrogen fertilization, irrigation, and biostimulant application on the growth and nutrient composition of stamnagathi. The experimental design included two nitrogen rates (100% and 30% of plant requirements, NR100 and NR30), two irrigation levels (100% and 50% of water availability, WA100 and WA50), and foliar application of a nitrogen-rich biostimulant (BS, NoBS). Results indicate that nitrogen deficiency had a more pronounced impact on stamnagathi compared to water stress. Biostimulant application partially mitigated the effects of nitrogen deficiency

but was ineffective against drought stress. Nitrogen limitation reduced leaf nitrate, calcium, and zinc content, while increasing iron, manganese, and copper concentrations. Water stress altered magnesium and zinc levels, raising the former and lowering the latter. The interaction between nitrogen and water stress notably affected calcium content, which was higher under adequate nitrogen combined with water stress. These findings suggest that stamnagathi is more vulnerable to nitrogen deficiency than water stress in soilless cultivation, and while biostimulants provide some relief, they are insufficient to fully counteract nitrogen stress.

In the third experiment, stamnagathi was cultivated in a climate-controlled chamber for five months. Peat-filled pots were used for the cultivation, light intensity was set at 100 µmol m^2 s^{-1}, and the effects of two different photoperiod treatments, 10 and 15 hours, were studied. The environmental conditions remained stable, with a temperature of 20 °C and CO_2 level at 400 ppm. At the end of the experiment, no flowering was observed, indicating that photoperiod alone was not sufficient to induce flowering. The aim of this experiment was to deepen the understanding of stamnagathi's response to different photoperiods in terms of flowering and growth, providing valuable insights for cultivating this wild edible vegetable in vertical farming systems.

The fourth experiment stamnagathi was cultivated in a vertical farm under three different types of white light. The three spectra used had different spectral compositions (blue:green:red:far-red), N:14:32:43:10, F:16:36:40:8, and S:21:34:36:7 respectively. The photoperiod was set to 12 hours and the plant density was 50 plants m^{-2}. Results showed no significant impact on agronomical parameters and leaf anatomy. The stomatal length and width decreased as the red:blue ratio of the light sources decreased, being greater in the N treatment (red:blue ratio of 3.1) compared to the F and S (red:blue ratios of 2.5 and 1.7 respectively). Based on these results the preferable "white light" product was the one with the highest efficiency and lowest market price at the time of the experiment.

The fifth experiment focused on the effects of different nutrient solutions on the agronomical and nutritional attributes of stamnagathi. Plants supplied with a control nutrient solution, "N10-Fe15" and were compared to plants cultivated under limited

nitrogen, “N4-Fe15” and elevated iron, “N10-Fe48”, EC was 1.5 ds m^{-1}, and pH was 5.6-6.5. The experiment simulated commercial practices by increasing the photoperiod to 15 hours and plant density to 100 plants m^{-2}. The results did not demonstrate significant effects of the nutrient solution differences on the agronomical characteristics except from a decrease in total Kjeldahl nitrogen under limited nitrogen conditions. Notably, leaf tissue phosphorus content increased under elevated iron conditions. The nitrate content remained within safe for consumption thresholds for all treatments.

The thesis contributes to the understanding of optimal cultivation methods for stamnagathi in commercial agriculture. The crop adapts well to different cultivation systems, but nutrient solution, irrigation, and environmental controls are essential for optimization. In perlite-based systems, nitrogen appeared beneficial, though vertical farming’s-controlled environment maintained high yields even under nitrogen starvation. In contrast, increased nutrient input in hydroponics did not significantly benefit stamnagathi, indicating its potential for low-input cultivation. This research lays the groundwork for further commercial integration of stamnagathi.

Scientific area: Horticulture

Keywords: stamnagathi, soilless culture, vertical farming, nitrogen, biostimulants, drought, plant nutrition, photoperiod, light, quality

Εφαρμογή καινοτόμων γεωργικών συστημάτων για την βελτιστοποίηση της απόδοσης, ποιότητας και μετασυλλεκτικής ζωής του σταμναγκαθιού

Τμήμα Επιστήμης Φυτική Παραγωγής
Εργαστήριο Κηπευτικών Καλλιεργειών

Περίληψη

Η παρούσα διατριβή μελετάει την καλλιέργεια του σταμναγκαθιού (*Cichorium spinosum* L.) ενός άγριου εδώδιμου φυτού (χόρτο). Η μελέτη αφορά στην εισαγωγή του σε διαφορετικά και καινοτόμα καλλιεργητικά συστήματα, με στόχο την αξιοποίηση και εμπορευματοποίησή του. Συνολικά, παρουσιάζονται πέντε πειράματα στη διατριβή αυτή. Δύο από τα πειράματα πραγματοποιήθηκαν σε θερμοκήπιο, ενώ τα άλλα τρία σε κάθετη καλλιέργεια.

Στο πρώτο πείραμα, το σταμναγκάθι καλλιεργήθηκε υδροπονικά σε σύστημα επίπλευσης. Σε αυτό το πείραμα διερευνήθηκαν οι ταυτόχρονες επιδράσεις διαφορετικών αναλογιών καλίου, ασβεστίου και μαγνησίου, καθώς και διαφορετικών επιπέδων ηλεκτρικής αγωγιμότητας (EC) ως προς την ανάπτυξη και τη θρεπτική κατάσταση του σταμναγκαθιού. Συγκρίθηκαν τέσσερα θρεπτικά διαλύματα, δύο με χαμηλή EC (L, 2,4 dS/m) και δύο με υψηλή EC (H, 3,6 dS/m) με αναλογίες K:Ca:Mg είτε 50:40:10 είτε 40:50:10. Δεν παρατηρήθηκαν αλληλεπιδράσεις μεταξύ των δύο παραγόντων. ούτε σημαντικές επιδράσεις των μεταχειρίσεων στο νωπό και ξηρό βάρος, τον αριθμό φύλλων και τη φυλλική επιφάνεια των φυτών. Τα υψηλά επίπεδα EC αύξησαν την περιεκτικότητα σε K και μείωσαν την περιεκτικότητα σε Mn και Zn στους φυτικούς ιστούς ενώ η αναλογία 40:50:10 οδήγησε σε αύξηση της περιεκτικότητας του Ca στους φυτικούς ιστούς. Το νιτρικό άζωτο (Nitrate-N) επηρεάστηκε μόνο από το επίπεδο της EC και αυξήθηκε υπό συνθήκες υψηλής EC, ενώ το συνολικό άζωτο δεν επηρεάστηκε.

Στο δεύτερο πείραμα, διερευνήθηκαν οι επιδράσεις της αζωτούχας λίπανσης, της άρδευσης και της εφαρμογής βιοδιεγερτών στην ανάπτυξη και τη θρεπτική κατάσταση του σταμναγκαθιού. Εφαρμόστηκαν δύο ποσοστά αζώτου (100% και 30% των απαιτήσεων του φυτού, NR100 και NR30), δύο επίπεδα άρδευσης (100% και 50% της διαθεσιμότητας νερού, WA100 και WA50) και διαφυλλική εφαρμογή ενός μη-

μικροβιακού βιοδιεγέρτη(BS, NoBS). Τα αποτελέσματα έδειξαν ότι η έλλειψη αζώτου είχε μεγαλύτερη επίδραση στο σταμναγκάθι σε σύγκριση με το υδατικό στρες. Η εφαρμογή του βιοδιεγέρτη μετρίασε μερικώς τις επιδράσεις της έλλειψης αζώτου αλλά δεν ήταν αποτελεσματική έναντι της υδατικής καταπόνησης. Ο περιορισμός του αζώτου μείωσε την περιεκτικότητα των φύλλων σε νιτρικά, ασβέστιο και ψευδάργυρο, ενώ αύξησε τις συγκεντρώσεις σιδήρου, μαγγανίου και χαλκού. Η υδατική καταπόνηση επηρέασε τα επίπεδα μαγνησίου και ψευδαργύρου, αυξάνοντας το πρώτο και μειώνοντας το δεύτερο. Η αλληλεπίδραση μεταξύ του αζώτου και της υδατικής καταπόνησης επηρέασε σημαντικά την περιεκτικότητα σε ασβέστιο, η οποία ήταν υψηλότερη υπό συνθήκες επάρκειας αζώτου σε συνδυασμό με υδατική καταπόνηση. Αυτά τα ευρήματα υποδηλώνουν ότι το σταμναγκάθι είναι πιο ευάλωτο στην έλλειψη αζώτου παρά στην υδατική καταπόνηση. Αντίστοιχα, η χρήση του συγκεκριμένου βιοδιεγέρτη παρέχει κάποια προστασία απέναντι στην έλλειψη αζώτου αλλά δεν είναι επαρκή για την πλήρη αντιμετώπιση της προκληθείσας καταπόνησης λόγω μειωμένης παροχής αρδευτικού νερού.

Στο τρίτο πείραμα, το σταμναγκάθι καλλιεργήθηκε σε κλιματικά ελεγχόμενο θάλαμο για πέντε μήνες. Για την καλλιέργεια χρησιμοποιήθηκαν γλάστρες με τύρφη, η ένταση φωτισμού ήταν 100 μmol m^2 s^{-1} και μελετήθηκε η επίδραση δύο διαφορετικών επεμβάσεων φωτοπεριόδου, 10 και 15 ωρών. Οι κλιματικές συνθήκες παρέμειναν σταθερές, θερμοκρασία 20 °C, και επίπεδο CO_2 400 ppm. Στη λήξη του πειράματος δεν παρατηρήθηκε ανθοφορία, υποδεικνύοντας ότι η φωτοπερίοδος από μόνη της δεν ήταν αρκετή για να προκαλέσει την άνθιση. Σκοπός αυτού του πειράματος ήταν η εμβάθυνση στην κατανόηση της απόκρισης του σταμναγκαθιού σε διαφορετικές φωτοπεριόδους ως προς την ανθοφορία και την ανάπτυξής του, παρέχοντας πολύτιμες πληροφορίες για την καλλιέργεια αυτού του άγριου εδώδιμου λαχανικού σε συστήματα κατακόρυφης γεωργίας (vertical farming).

Στο τέταρτο πείραμα, το σταμναγκάθι καλλιεργήθηκε σε κατακόρυφη καλλιέργεια υπό τρία διαφορετικά είδη λευκού φωτός. Τα τρία φάσματα που χρησιμοποιήθηκαν είχαν διαφορετικές συνθέσεις (μπλε:πράσινο:κόκκινο:σκοτεινό ερυθρό), N:14:32:43:10,

F:16:36:40:8 και S:21:34:36:7 αντίστοιχα. Η φωτοπερίοδος ορίστηκε στις 12 ώρες και η πυκνότητα των φυτών στα 50 φυτά m^{-2}. Τα αποτελέσματα δεν έδειξαν σημαντική επίδραση στα αγρονομικά χαρακτηριστικά ή στην ανατομία των φύλλων. Το μήκος και το πλάτος των στομάτων μειώθηκε καθώς μειωνόταν η αναλογία κόκκινου:μπλε στο φάσμα του φωτός, με τις μεγαλύτερες τιμές να παρατηρούνται σε φυτά που καλλιεργήθηκαν κάτω από το φάσμα N με αναλογία κόκκινου:μπλε 3.1, σε σύγκριση με αυτού που μεγάλωσαν κάτω από το φάσμα F και S με αναλογίες κόκκινου:μπλε 2.5 και 1.7 αντίστοιχα. Με βάση αυτά τα αποτελέσματα, ο προτεινόμενος λαμπτήρας λευκού φωτός ήταν αυτός με τη μεγαλύτερη απόδοση και τη χαμηλότερη τιμή αγοράς κατά την πειραματική περίοδο.

Το πέμπτο πείραμα εστίασε στις επιδράσεις διαφορετικών θρεπτικών διαλυμάτων στις αγρονομικές και θρεπτικές ιδιότητες του σταμναγκαθιού. Τα φυτά τροφοδοτήθηκαν με ένα θρεπτικό διάλυμα μάρτυρα, “N10-Fe15” και συγκρίθηκαν με φυτά που καλλιεργήθηκαν υπό συνθήκες μειωμένου άζωτου, “N4-Fe15” και αυξημένης συγκέντρωσης σιδήρου, “N10-Fe48” στο θρεπτικό διάλυμα Κατά τη διάρκεια του πειράματος η EC ήταν 1,5 ds m^{-1} και το pH ήταν 5,6-6,5. Το πείραμα προσομοίωσε συνθήκες εντατικής καλλιέργειας αυξάνοντας τη φωτοπερίοδο στις 15 ώρες και την πυκνότητα των φυτών στα 100 φυτά m^{-2}. Δεν παρατηρήθηκαν σημαντικές επιδράσεις εξαιτίας της σύνθεσης των θρεπτικών διαλυμάτων ως προς τα αγρονομικά χαρακτηριστικά. Η θρεπτική κατάσταση των φυτών επηρεάστηκε σε ορισμένο βαθμό. Παρατηρήθηκε μείωση της συγκέντρωσης του συνολικού αζώτου υπό συνθήκες μειωμένου αζώτου. Αξιοσημείωτα, η περιεκτικότητα σε φώσφορο στους φυτικούς ιστούς αυξήθηκε υπό συνθήκες αυξημένης συγκέντρωσης σιδήρου στο θρεπτικού διάλυμα. Η περιεκτικότητα των φύλλων σε νιτρικά παρέμεινε εντός των ασφαλών ορίων για κατανάλωση για όλες τις επεμβάσεις.

Η διατριβή αυτή συμβάλλει στον προσδιορισμό των βέλτιστων μεθόδων καλλιέργειας του σταμναγκαθιού με σκοπό στην εισαγωγή του στη παραγωγική γεωργία. Ως φυτό προσαρμόζεται με επιτυχία σε διαφορετικά συστήματα, αλλά παράγοντες όπως η θρέψη, η άρδευση, και ο περιβαλλοντικός έλεγχος είναι απαραίτητα για την

βελτιστοποίηση της παραγωγής. Η αυξημένη παροχή αζώτου φάνηκε ωφέλιμη σε σύστημα καλλιέργειας σε περλίτη. Στο σύστημα επίπλευση η αυξημένη χρήση λιπασμάτων δεν οδήγησε σε αύξηση της παραγωγής. Αντίστοιχα σε συνθήκες κατακόρυφης καλλιέργειας η έλλειψη αζώτου δεν επηρέασε την παραγωγή. Αυτά τα χαρακτηριστικά σκιαγραφούν τη δυναμικότητα του σταμναγκαθιού ως φυτό με χαμηλές εισροές πόρων. Η έρευνα αυτή ενισχύει τα θεμέλια της έρευνας σχετικά με την ενσωμάτωση του σταμναγκαθιού στην εμπορική γεωργία.

Επιστημονικός τομέας: κηπευτικές καλλιέργειες

Λέξεις κλειδιά: σταμναγκάθι, καλλιέργειες εκτός εδάφους, κατακόρυφη γεωργία, άζωτο, βιοδιεγέρτες, ξηρασία, θρέψη φυτών, φωτοπερίοδος, φάσμα φωτός

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1. Introduction

1.1 Agricultural systems and sustainable agriculture

The agricultural sector has long faced challenges in meeting the needs of populations. Today, these challenges have escalated and taken on a global scale. According to FAO (Food and Agriculture Organization), it is dire for the agricultural industry to intensify food production to meet the demands of the rising global population by 2050 [5]. In addition, demand for stable year-round production, the dwindling farmers' population, and urbanization, has placed immense pressure on the remaining farmers and arable land [6]. At the same time, the impacts of climate change, including unpredictable weather patterns and shifting growing seasons [7], as well as soil degradation and desertification, have significantly heightened the difficulties the agricultural sector must overcome. Given that natural resources are finite, the agricultural sectors challenges are amplified by the need to produce more food with fewer resources [8,9] as efforts focus on meeting the demands in a sustainable way [10].

Efficient management of energy and resources is vital for achieving more sustainable agricultural practices [11]. Improving mineral efficiency in crops, as approximately 40% of the world's arable land suffers from mineral deficiencies, has been of great importance [12]. Such deficiencies, often necessitate the use of inorganic fertilizers to maintain high crop yields. Nevertheless, dietary surveys suggest that around 10% of the well-nourished adult population in developed countries has a suboptimal intake of essential macronutrients like potassium (K), calcium (Ca), and magnesium (Mg), putting them at risk of deficiency [13]. Meanwhile, the significant increase in the fertilizers' cost and growing concerns regarding the environmental impact of the recently excessive fertilizer inputs further enhances the importance of both nutrient-efficient cultivars and agricultural systems [14,15]. Innovative solutions and farming practices, better management of natural resources, such as water and nutrients, utilization of biodiversity and resilient crops will all be tools for surpassing the current challenges.

Even though, in the past 30 years, many experiments have been conducted in order to identify nutrient-efficient species and cultivars, or even landraces and ecotypes [16–20], there has been little success in breeding and introducing those cultivars into mainstream agriculture. Soilless culture, in either greenhouses or indoor growing modules, is becoming a more popular alternative to open-field production both due to energy use efficiency and also due to the increasing risk of open-field systems induced by climate change [21–23]. Year-round production, regardless of the outdoor conditions or soil quality, can nowadays be achieved through protected, controlled environment agricultural systems, such as hydroponic greenhouses [24,25] or even vertical farms [26,27], covering the rising demand for high-value fresh leafy vegetables [28].

1.2 Stamnagathi, a wild leafy green

In today's world, the increasing focus on consumer health has sparked a significant interest in "optimal nutrition" and "functional foods" [29]. The need to diversify a monotonous diet and consume foods with nutraceutical compounds played a critical role in this increase [30–32]. This shift in dietary preferences has created opportunities for exploring crops with potential health benefits. Throughout history, many plants have been highly regarded for their health enhancing properties, largely attributed to their abundance of mineral elements and bioactive compounds [33,34]. Although only a few plant-based foods have been extensively researched, and even less meet the rigorous standards of scientific health claims, the evidence supporting the health benefits of various plant foods, including wild leafy greens, is growing [35–38].

The Mediterranean basin is highly vulnerable to climate change, further intensifying the challenges faced by its agricultural sector [39]. Moreover, agriculture in the Mediterranean is predominantly small-scale, and local products are essential to the region’s diet. Moreover, the Mediterranean basin is known for its abundance of wild, edible underutilized horticultural species [40]. These plants grow spontaneously in nature and were traditionally included in the locals’ diets [41]. Several wild edible vegetables offer potential advantages, such as greater resilience to environmental stresses like

drought, extreme temperatures, and pests, along with the ability to maintain high yields if given the right inputs [42,43]. Hence, the cultivation of wild edible vegetables could contribute to food security, while stabilizing agricultural incomes, especially for local and small-scale growers. Nowadays, even though urbanization has alienated people from the lifestyle associated with the gathering of these crops, consumer demand for these species has increased [44].

Cichorium spinosum L., is a wild leafy edible plant belonging to the Asteraceae family. It is also known as spiny chicory or "stamnagathi" in Greek, and is native to the Mediterranean coastal regions, including rocky shores and mountainous areas [45]. The plant's leaves form a rosette, and they are typically hand-harvested at different growth stages for consumption. Leaves are either boiled or, less commonly, eaten raw in salads [46–48]. Moreover, stamnagathi commands high prices on the market thanks to its unique taste and richness in health-promoting compounds [49].

Despite its nutritional potential, the body of research on this wild plant is limited, with some studies focusing on open-field production and others examining its cultivation in soilless systems [50–53]. In Greece, stamnagathi is mainly grown in Crete, where different ecotypes have been introduced to commercial cultivation, both in soil and soilless culture systems [54–57]. However, upscaling efforts of the cultivation of stamnagathi, with a potential for it to become a highly profitable crop, are limited. In addition, the information regarding its response to various environmental conditions is largely fragmented [58]. Even though stamnagathi has an estimated demand of 2000 tons per year, there are few to none commercial growers that are currently supplying the mainstream markets. This also led to lack of standardization of the quality of the products. There have been few projects for large scale open-field, and perlite-based cultivation the past decades but those companies are no longer actively present in the market. The demand is met by gathering stamnagathi from the wild, or the borders of growers' fields, which raises a lot of questions regarding quality and safety. "Kouroupakis, personal communication 2019".

Moreover, the natural origin of wild foraged plants does not always mean “safe” nor “healthy”. It is crucial to recognize potential risks such as toxic compounds and high heavy metal concentrations [59–61]. By documenting the chemical profiles of these plants, the toxic compounds can be characterized and therefore plants with toxic attributes can be avoided. On the other hand, being grown spontaneously in the wild where none of the environmental parameters are controlled, nor monitored, the concentrations of certain elements and the health promoting compounds might also vary. Leafy vegetables are known to be the top nitrate-containing plant-based foods [62]. Towards that end, some wild leafy vegetables have often high nitrate content even when gathered from virgin lands [63]. Due to several concerns regarding the implications of nitrate intake in human health, such as gastric cancer, [64–67], nitrate accumulation in vegetables has been a topic of interest since the 70s [68,69].

1.3 Nitrogen

Excessive use of fertilizers, particularly nitrogen-based ones, is a major contributor to environmental pollution through nitrate leaching. As the global population grows, the demand for nitrogen fertilizers has surged, while concerns over their environmental impact have significantly increased in recent decades [70]. In plants, nitrogen is absorbed as nitrate or ammonium [71,72]. In the initial stage of nitrate assimilation, nitrate reductase [73] converts nitrate into nitrite, followed by the conversion of nitrite to ammonium by nitrite reductase [74,75]. Ammonium is then utilized in amino acids through reactions mediated by glutamine synthetase and glutamate synthase [76,77]. The cultivation of horticultural crops is especially prone to nitrate losses due to their high fertilizer requirements and shallow root zones. Therefore, improving nitrogen utilization efficiency is a difficult task affected by several factors, such as the hydraulic characteristics of the growing medium, the nitrogen and other nutrient levels in the irrigation water, and the timing of irrigation. Moreover, the needs for water and nutrients in plants are frequently determined by genetics and are specific to each species. Furthermore, root morphology, and the interaction between canopy and the surrounding environment also

affect the transpiration rate and therefore nutrient uptake. Precision fertigation, by delivering nutrients through irrigation, can improve the absorption of plant nutrients, such as nitrogen, while reducing nitrate runoff [78]

Several parameters, such as light and photoperiod, fertilization strategies, and genetics affect nitrate assimilation and therefore its concentration in plants [79–81]. Nitrogen plays a crucial role in various physiological and metabolic processes, serving as a fundamental component of proteins, enzymes, and nucleic acids [82,83]. The availability of nitrogen influences morpho-physiological plant traits, thereby impacting marketability and visual quality [84].

Certain thresholds have been established by the European Commission regarding nitrate levels in different leafy vegetables (regulation No 1258/2011) [85]. This precautionary measure derived from indications in epidemiological studies demonstrating a probable connection between nitrate intake from food sources and the appearance of a range of cancers [86–88]. Depending on the crop type and seasonality, the nitrate threshold can be as high as 5000 mg per kg of fresh vegetable weight. Preventing high nitrate levels in vegetables has been the focus of several studies when it comes to agricultural systems [89,90].

Towards this end, precise nitrogen supply can mitigate high nitrate levels in leaf tissues at the time of harvest, while also maintaining high yields. Soilless culture methods are ideal for exerting high control over nutrient supply to the plants. Previous studies have demonstrated that some stamnagathi ecotypes can be cultivated under total-N concentration of 4 mM without reduction of its biomass, compared to total-N concentration of 16 mM, demonstrating its genotypic resilience to low-N supply levels [91]. In addition, fertilization strategies, such as altering the total nitrogen concentration of the supplied nutrient solution [92], or replacing a percentage of the supplied nitrogen as nitrate with ammonium [91], have been found to be good agricultural practices that could help set the foundations for vegetables with low nitrate content.

1.4 Drought stress

Drought poses a formidable challenge for modern agriculture, particularly in the context of climate change. As the availability of water for agriculture is increasingly constrained worldwide, temperatures are rising, precipitation patterns shift, often resulting in short and acute rainfalls, water scarcity increasingly threatens global food accessibility. These issues necessitate the adoption of effective irrigation management strategies, cultivation systems, and more resilient crops [93,94]. Hence, effective management of irrigation water becomes paramount in ensuring a consistent food supply by conserving water resources [95]. Another key ingredient to better use of water is identifying the water stress thresholds of crops and utilizing reduced irrigation practices. This practice has emerged as a necessary strategy in water-scarce regions as a means to water usage optimization [96]. Soilless culture methods and deficit irrigation emerge as a viable approach. In soilless systems, precise control over irrigation allows for the adaptation of water supply to plant needs, minimizing water wastage and maximizing resource utilization [97]. The impact of water deficiency itself, on plants is multifaceted, influenced by factors such as intensity, duration, and the plant's developmental stage. In order to avoid the disruption of plant water relations, which can in turn decrease water use efficiency, impair photosynthesis, and compromise water and nutrient uptake, understanding each plants needs is crucial in soilless culture and deficit irrigation practices [98–100].

1.5 Biostimulants in Agriculture

Biostimulants are considered a promising novel technology that can be applied via foliar spray or fertigation, that could be utilized to reinforce nutrient uptake, crop yield and quality, as well as modulating plant metabolic processes [101–107]. As an upcoming technology, the global biostimulants market is experiencing rapid growth. The projections indicated could reach a value of $2.24 billion by 2018 [108] whereas it is expected to increase to $5.1 billion by 2027 [109]. Protein hydrolysates, a subset of biostimulants, comprise free amino acids or oligo- and polypeptides formed through chemical processes

like enzymatic hydrolysis. When administered via foliar spray, they are absorbed through the cuticle, epidermal cells, and stomata, ultimately reaching the foliar mesophyll [110]. The resultant plant growth is frequently attributed to hormone-like activities induced by the application of protein hydrolysates [111–114]. Protein hydrolysates have been reported to be capable of reducing yield loss under sub-optimal nitrogen conditions [115]. Moreover, protein hydrolysates have also demonstrated promising results under drought conditions for crops with high water demands, such as grapevines, especially in Mediterranean vineyards [116]. Taking all the above into consideration a study was designed with the aim to explore how a wild edible leafy vegetable could be utilized as a cultivated crop under limited nitrogen, or limited irrigation conditions and whether novel biostimulant application could counteract some of the negative effects of the induced stresses. To this day, no experiments combining biostimulants and the cultivation of *Cichorium spinosum* L., have been conducted.

1.6 Vertical farming / Artificial lighting

"Vertical farming" is an umbrella term referring to the protected cultivation of plants in soilless culture systems with artificial lighting. These vertical farms can either have multiple horizontally stacked layers or vertical plant panels. From a commercial perspective, this technology allows for cultivation of a diversity of crops, [117–120]. Studies from the early 2000s revealed that red and blue LEDs could produce plant growth comparable to natural sunlight, owing to their effective photon absorption [121]. By 2010, advancements in phosphor technology enabled the development of white LEDs, which provide a broad spectrum light similar to that of natural sunlight [122,123]. This control over the light qualities, such as spectrum, photoperiod, and intensity opened up numerous opportunities towards regulating physiological properties in controlled environment agriculture [124,125]. Nonetheless, this development has created ample room for experimentation and has sparked a complex quest to find the optimal light characteristics for each plant, cultivar, and cultivation stage [126]. Furthermore, the extensive spectrum combinations explored in existing literature, including comparisons of

R:B ratios ranging from 1 to 8 using monochromatic blue and red LEDs, combinations with green and white LEDs, or even fluorescent light [127–129], render it impossible to define one “optimum” spectrum. Nevertheless, LED efficiency remains a factor of high importance when it comes to choosing the right lighting for commercial production [130–132]. Cultivation within vertical farms commonly involves the application of extended photoperiods and relatively low photosynthetic photon flux density (PPFD) ranging from 15 to 18 hours and 180 to 300 umol m^{-2} s^{-1}, respectively. This practice seeks to reach the daily light integral (DLI) goals of certain crops by utilizing the energy-efficient attributes of lower PPFD levels, while maintaining high photosynthetic capacity over prolonged durations, thereby achieving optimum growth rates [133,134].

Regardless of the current advances of the lighting technology, the rapid growth of the industry has not been without its criticisms. High energy consumption and carbon emissions of vertical farms, in contrast to open field and greenhouse production [135–137], coupled with the challenges faced by leading companies in maintaining their growth and financial stability [138,139] have both been subjects of debate within scientific and industrial circles. Due to this reason, novel crops characterized as "niche", that command elevated prices in the market, compared to mainstream crops, are progressively being incorporated into vertical farming systems [140]. These wild edible greens offer a range of phytonutrients, bioactive compounds and potential anti-carcinogenic properties, and could play a key role in solidifying the success of certain vertical farming enterprises [141–143]. Moreover, given the degree of control over light quality and quantity in vertical farms, maintaining low nitrate levels in leaf tissues through the alteration of the spectral composition [144], light intensity [145] and photoperiod [79] has been found to be an effective way for the production of safe-for-consumption leafy greens.

1.6.1 Long photoperiod might initiate flowering of stamnagathi in vertical farms

Extended photoperiods can potentially trigger flower initiation, subsequently inducing changes in the chemical composition of the leaves, flavor profile, and ultimately rendering the yield unmarketable [50]. As a result, it becomes imperative to ascertain the

feasibility of *C. spinosum* L. cultivation within vertical farms, particularly under prolonged photoperiod conditions. Presently, the absence of documented research regarding the growth cycle of spiny chicory in controlled environments from seed to harvest, deems the optimum conditions rather unclear. Knowledge from *C. intybus* L., (chicory) could perhaps be implemented in the cultivation of *C. spinosum* L since these two are genetically similar, yet their morphological characteristics delimit the two species [146]. Unfortunately, studies on distinct varieties within the *Cichorium intybus* L. group have shown that chicory plants can either be of absolute or facultative cold requirements with regard to flowering. In addition, the prevailing temperature, during various stages—ranging from seed production in maternal plants, seed storage, germination, and seedling cultivation—can hasten the processes of bolting and flowering [147–149]. It has been also suggested that high temperature (20-25°C) could have a devernalization effect on chicory plants [149] but on the other hand, very high temperatures, (28-35°C) could hasten flowering independently of vernalization [150,151]. It is unclear, whether flowering initiation is attributed to temperature, light intensity, or their interaction. Since low temperatures are easy to avoid in vertical farms, flowering due to vernalization does not appear to be of primary concern. Conversely, *C. intybus* has been known to have an absolute long day requirement, therefore photoperiod is the primary determinant for triggering bolting and flowering [147]. In addition, the developmental stage of the plant has been suggested to contribute to its sensitivity to the interplay between low temperatures and extended photoperiods, highlighting the complexity of these regulatory mechanisms [147–153]. In order to clarify weather un-vernalized seeds can be used for the commercial cultivation of spiny chicory in vertical farms, two experiments were carried out. The first experiment took place in climate chambers and explored whether long days could initiate flowering under low light intensity. The second experiment applied the findings from the first, and explored the commercial potential of spiny chicory when cultivated in a small-scale vertical farm while utilizing long photoperiod.

1.7 Scope of study

The aim of this study was to explore the cultivation of *Cichorium spinosum* L., in different agricultural systems and produce applicable protocols and agricultural practices for the commercial cultivation in each of the studied systems. The focus was set on understanding how *C. spinosum* L., responds to different types of stresses or limitations in each system. Firstly, sufficient and excessive fertilization was explored and defined on a floating raft hydroponic system. Based on those outcomes, the next experiments focus on further reducing and fine-tuning the fertilizer use. As nitrogen has a key role in modern agriculture, the effects of its limitation were studied in greenhouse and vertical farming cultivation. In the scope that drought is another main challenge of today's agriculture, its effects were also studied in a greenhouse experiment. Moreover, the possibility to alleviate the nitrogen limitation stress through biostimulant application was explored as a possible solution for greenhouse growers. The indoor cultivation of *C. spinosum* L., in a vertical farming setup was investigated for the first time. Given, challenges that have been verbally communicated by growers regarding the flowering of this species, its photoperiodic responses were investigated. Furthermore, the experiments focused on different lighting profiles, and nutrient solution recipes. The plant's response to nitrogen limitation was also investigated in vertical farming conditions.

Through this research, constant efforts were made to take the cultivation of *C. spinosum* L., further and increase its productivity in each agricultural system. Small-scale farmers could benefit from the cultivation of this wild edible vegetable thanks to the high prices that commands in the local markets and its resilience towards different types of stresses. Our results can further be utilized for feasibility studies of commercial cultivation of *C. spinosum* L.

2. Materials and Methods

2.1 Plant Material

As plant material, stamnagathi (*Cichorium spinosum* L.) achenes of the mountainous ecotype were used after breaking down the achenes in a commercial blender and separating the seed from the debris (Figure 1) using a Endecotts Fluid Bed Dryer model FBD2000 (Hope Valley, United Kingdom) and a sieve until the remaining mixture had mostly clean seeds and few debris (Figure 2a-e). Seeds where then sown in rockwool or peat depending on the type of the experiment.

Figure 1: Stamnagathi achenes broken down using a commercial blender and sieve to separate seeds from the debris.

a b c d e

Figure 2: Depiction of achene cleaning process; a) bag of stamnagathi achenes, b) individual achenes, c) separation of debris using a fluid bed dryer, d) small debris flying out of the cone of the fluid bed dryer, e) clean seed with few debris used for sowing

2.2 Greenhouse Experiments

2.2.1 Exploring the Simultaneous Effect of Total Ion Concentration and K:Ca:Mg Ratio of the Nutrient Solution on the Growth and Nutritional Value of Hydroponically Grown Cichorium spinosum L.

2.2.1.1 Seedling and glasshouse chamber preparation

The experiment took place in a glasshouse of the Laboratory of Vegetable Production located at the Agricultural University of Athens (latitude 37.98°, longitude 23.7°, and elevation 38 meters, Figure 3a). The chamber inside the glasshouse was equipped with 16 stainless steel tanks measuring 1.5 m in length, 0.6 m in width, and 0.4 m in depth, developed by Intelagro (Thessaloniki, Greece).

a b

Figure 3: a) Top view of the glasshouse and chambers location on the experimental field of the Laboratory of Vegetable Production, b) view of the inside of experimental chamber of the glasshouse, the stainless-steel tanks, and their black plastic replenishment tanks, and aerial hot pipes are visible.

On April 5, 2021, seeds of *Cichorium spinosum* L. (stamnagathi) were sown in rockwool plugs (AO Plug, Grodan, Roermond, Netherlands) and covered lightly with vermiculite (Figure 5a). Climate was maintained through a heating system that consisted of aerial hot water pipes, while cooling was achieved by opening the roof and side windows (Figure 3b). The entire structure was protected from insect invasion by covering all windows and door openings with insect netting. No supplementary lighting was applied.

Before transplanting took place, the stainless-steel tanks were cleaned and each replication was filled with 180 L of a specific starter nutrient solution tailored to the treatment (Figure 5b). The different nutrient solution was prepared by dilution equally amount of the A and B nutrient solutions into a tank and recirculating the solution with a pump (Figure 4a). When the EC values and pH values were adjusted accordingly, the solution was pumped to the tank according to the experimental design (Figure 4b).

a b

Figure 4: a) preparation of the nutrient solutions, and b) filling the tanks with the solution of each treatment.

To ensure the oxygenation and homogeneity of the nutrient solution, an air stone and an aquarium pump were installed in each tank Figure 5c). To ensure the stability of the 180-liter tank's nutrient solution volume, a secondary plastic tank with a capacity of 50 liters was connected. This auxiliary tank replenished the nutrient solution as needed, through a float valve mechanism.

a b c

Figure 5: a) Dr. Karavidas fertigating the rockwool slabs after sowing, b) Neofytou, Msc student, filling the tank with nutrient solution, c) aquarium pump and airstone combination for the oxygenation of the tank's solution.

Figure 6: Separating the seedling into 3 groups based on their size and leaf number before transplanting them to the tanks.

By May 10, 2021, the seedlings were separated into groups based on the number of the developed true leaves (Figure 6). Seedlings that had reached the stage of 4 true leaves, which is an appropriate size and developmental stage for transplanting stamnagathi to the floating rafts, a total of 704 seedlings were transplanted onto the 16 floating rafts designed for the experiment. The rockwool plugs were placed into 55 mm plastic net pots, which were subsequently inserted into holes on each raft (Figure 7). Each accommodated up to 48 plants, resulting in a plant density of 53 plants per square meter. The average day/night temperatures and humidity values (± standard deviations) inside the glasshouse were recorded at 33 ± 9 °C and 32 ± 10%, respectively, while the night temperatures were 19 ± 3 °C, with relative humidity at 61 ± 10%.

Figure 7: View of the chamber where the experiment took place, with the 16 tanks and floating rafts, right after transplanting took place.

2.2.1.2 Experimental Design

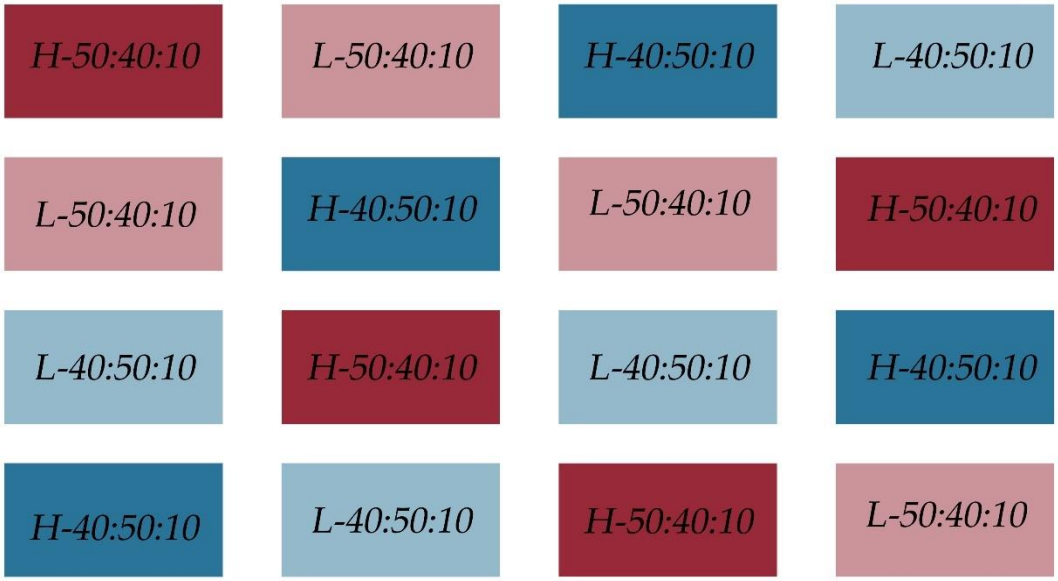


Figure 8: Positioning of the different hydroponic rafts for the current experimental design. exploring the simultaneous effect of total ion concentration and k:ca:mg ratio. H: high EC, L: low EC, 50:40:10 and 40:50:10 refer to the ratio of K:Ca:Mg of each solution.

The experiment was structured as a Randomized Complete Block Design. This investigation focused on the simultaneous effects of two factors, the total electric conductivity (EC), and the macrocation ratio of potassium, calcium, and magnesium (K:Ca:Mg). The cultivation was carried out under two EC levels, a high (3.6 dS m^{-1}, H-) and a low (2.4 dS m^{-1}, L-), and two macrocation ratios, one with high potassium concentration (K:Ca:Mg: 50:40:10) and one with high calcium concentration (K:Ca:Mg: 40:50:10). This design led to 4 unique treatment combinations (2 EC x 2 K:Ca:Mg). The four treatments were designated as L-50:40:10, L-40:50:10, H-50:40:10, and H-40:50:10, with each treatment replicated four times, resulting in 16 tanks and a total of 768 plants). The analytical composition of the nutrient solution for each treatment, along with the replenishment solution which was common for all treatments, is shown in Table 1.

The control level for the EC treatment was considered the one with the highest concentration, and as control for the K:Ca:Mg ratio, the 50:40:10 was selected, given that higher electrical conductivity levels have been found to be beneficial for the cultivation of spiny chicory, and that ratio was found to be similar to the molar ratio of these nutrients

in leaf tissue from previous experiments from our group [91]. It is well-established that adequate calcium uptake by plants requires maintaining a significantly higher calcium concentration in the root zone compared to its uptake concentration (i.e., the Ca/water ratio in mmol/L) [154]. Thus, our study compared this standard K ratio (50% K, 40% Ca, 10%Mg) with a ratio where calcium was increased relative to potassium and magnesium (40% K, 50% Ca, 10%Mg). This comparison was crucial for fine-tuning the nutrient solution for cultivating spiny chicory, as larger ratio differences could lead to significant alterations in plant anatomy, physiology, and biochemistry, particularly affecting photosynthetic carbon assimilation, which was not desired in this study [155].

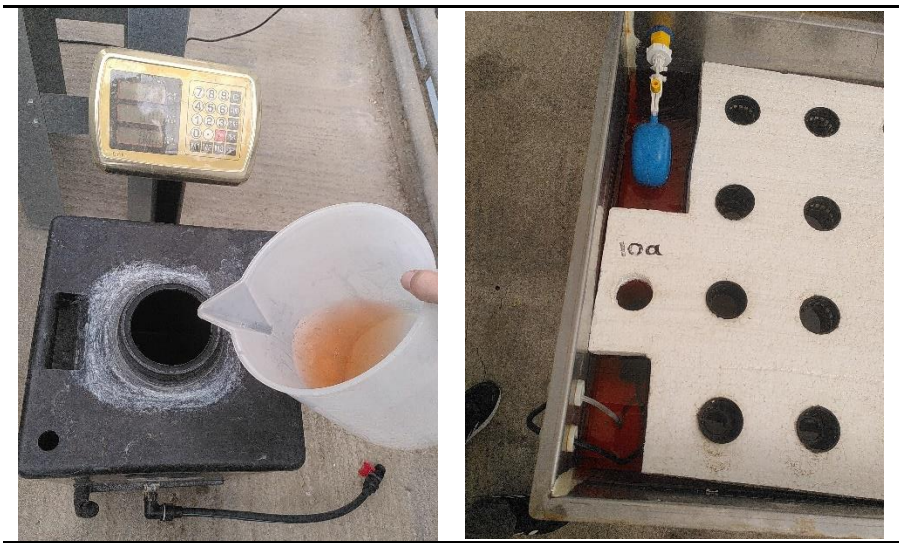
To determine the required fertilizer quantities for the precise composition of each nutrient solution, the online software "NUTRISENSE" was used (accessed on 1 April 2021, https://nutrisense.online/) [156]. After the preparation of the stock solutions A and B, the final nutrient solution was prepared by diluting equal amounts of A and B with tap water until the desired EC levels (2.4 dS/m or 3.6 dS/m) were achieved. For the acidity control, nitric acid was added to adjust the pH between 5.5 and 6.5. The dilution process was carried out in a 300 L barrel connected to a pump that could either recirculate the solution or distribute it through rubber tubing into the experimental tanks.

During the experiment, electrical conductivity (EC) and pH levels were monitored twice daily using Bluelab pH and EC pens (Tauranga, New Zealand), with readings manually recorded. To measure the consumption of the replenishment solution, each tank was weighed when empty, after the addition of 15 liters of solution, and periodically throughout the growing period (Figure 9a). Tanks were disconnected and re-weighed to track the decrease in solution volume, and were refilled to 15 liters when the solution levels were low. At the experiment's conclusion, the total amount of replenishment solution consumed was calculated by summing the differences in weight measurements.

Table 1: Chemical composition of the starter nutrient solutions and the replenishment solution

Element	Units	L-K50	L-Ca50	H-K50	H-Ca50	Replenishment
EC*	dS m^{-1}	2.4	2.4	3.6	3.6	2.2
K:Ca:Mg ratio		50:40:10	40:50:10	50:40:10	40:50:10	60:32:8
pH		5.6	5.6	5.6	5.6	6.1
K^+	mmol L^{-1}	6.93	5.14	10.69	7.9	7.54
Ca^{2+}	mmol L^{-1}	5.54	6.52	8.55	10.07	4.02
Mg^{2+}	mmol L^{-1}	1.39	1.30	2.14	2.01	1.01
SO_4^{2-}	mmol L^{-1}	3.08	3.08	5.18	5.18	1.98
NH_4^+	mmol L^{-1}	1.15	1.15	1.87	1.87	1.68
NO_3^-	mmol L^{-1}	14.17	14.17	21.97	21.97	11.4
$H_2PO_4^-$	mmol L^{-1}	1.4	1.4	1.4	1.4	1.12
Fe	µmol L^{-1}	20	20	20	20	14.88
Mn^{2+}	µmol L^{-1}	9	9	9	9	8.37
Zn^{2+}	µmol L^{-1}	5	5	5	5	3.72
Cu^{2+}	µmol L^{-1}	0.8	0.8	0.8	0.8	0.65
B	µmol L^{-1}	30	30	30	30	23.25
Mo	µmol L^{-1}	0.5	0.5	0.5	0.5	0.47
Cl^-	mmol L^{-1}	0.4	0.4	0.4	0.4	0.4
Na^+	mmol L^{-1}	0.6	0.6	0.6	0.6	0.6
HCO_3^-	mmol L^{-1}	0.4	0.4	0.4	0.4	0.4

*Electric Conductivity

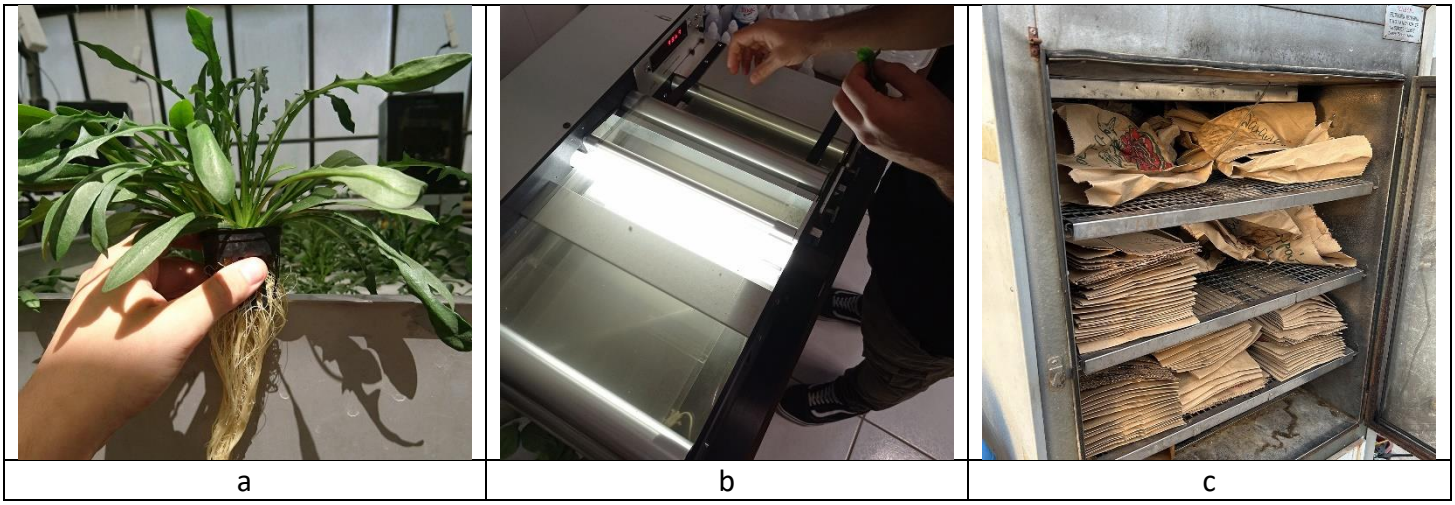


a b

Figure 9: a) Filling the plastic tanks with replenishment nutrient solution, b) mechanism for nutrient solution replenishment of the stainless-steel tank

2.2.1.3 Agronomical measurements

At the end of the experiment all plants from each of the 4 tanks, per treatment, were harvested, and their fresh weight was measured to determine yield. Additionally, five plants from each tank were used to measure leaf number, leaf area, and dry weight (Figure 10a-c). Leaf number was measured by separating leaves of each plant manually. Each leaf was meticulously placed on the transparent conveyor belt of a leaf area meter LI-3100C by LI-COR (Lincoln, NE, USA). After concluding the leaf area measurement, the leaves were of each plant, along with the stem, were weighed on a precision scale (model Mettler PE 3600, Mettler-Toledo, Columbus, OH, USA) and placed in a paper bag. To determine the dry weight the paper bags were placed in a ventilated oven (STF-N 400, FALC Instruments S.L.R, Treviglio, Italy) at 65°C until a stable dry weight was achieved. After 7 days, the dry weight stabilized for all plants and the measurement of dry weight were recorded.

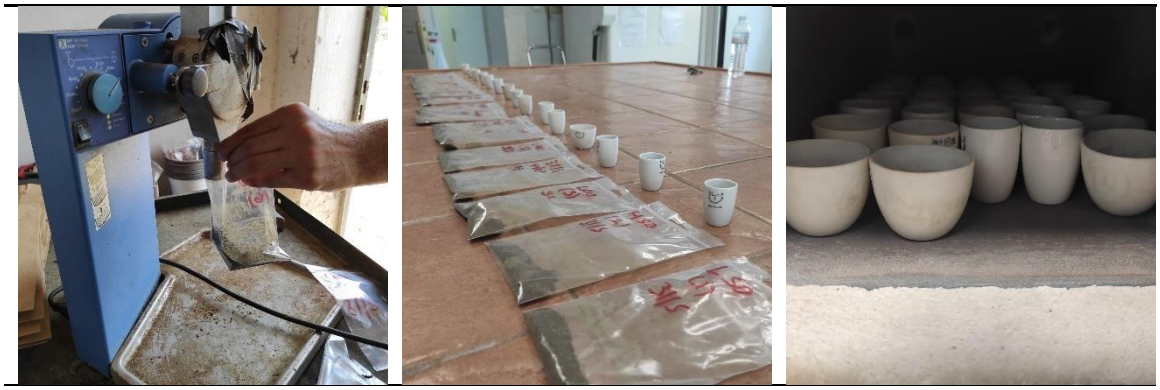


a b c

Figure 10: a) stamnagathi plant at harvest, b) separating leaves on the conveyor belt of LI-3100C to measure leaf area, c) paper bags containing fresh leaves placed in the ventilated drying oven for 7 days.

2.2.1.4 Chemical composition measurements

Dry samples were grounded using an MF 10 Microfine grinder (IKA Werke, Staufen, Germany) at the highest speed setting (6000–6500 rpm). The ground tissue samples were then sealed in plastic bags to prevent moisture degradation (Figure 11a). For nutrient content analysis, the dry ashing method was employed. A 0.5 g sample of grounded dry plant tissue was placed in porcelain cups and incinerated in a chamber furnace (LM-112, Linn High Therm, Hirschbach, Germany) at 550°C for 8 hours until it was reduced to ash (Figure 11b-c). The ash was dissolved by introducing 10 mL of 0.25 N HCl into each porcelain cup, and the resulting solution was subsequently filtered through 125 mm Macherey-Nagel filter paper into volumetric flasks. These flasks were then brought to a final volume of 100 mL with distilled water. The prepared tissue extracts were transferred to 100 mL plastic bottles and stored at 4°C until subjected to chemical analysis. The nutrient concentrations within the aqueous tissue extracts were determined using analytical methods specifically designed for each individual element.



a b c

Figure 11: a) grounding the dried plant tissue through the MF10 Microfine grinder and storing in sealable plastic bags, b) transferring 0.5 grams of each sample to porcelain cups, c) porcelain cups in the furnace during the dry ashing procedure

2.2.1.4.1 Colorimetrical Photometry: Determination of phosphorus, boron, and nitrates

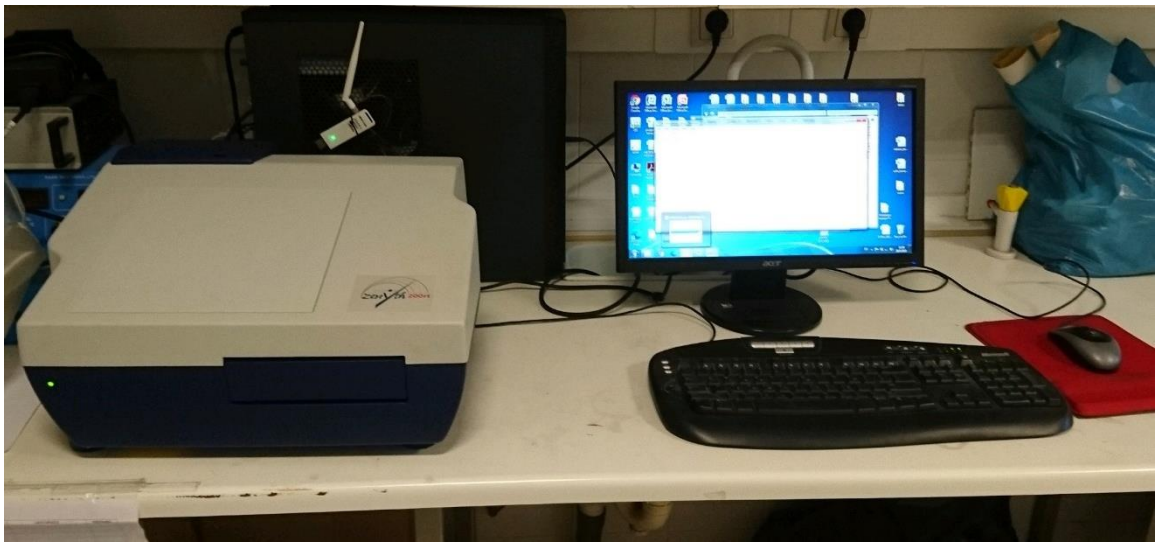


Figure 12: Anthos Zenyth 200 photometer (Biochrom Ltd., Cambridge, UK).

Phosphorus content was measured using the molybdenum blue reaction for orthophosphate determination [157], boron content was determined spectrophotometrically using an azomethine H derivative [158] and the nitrate (NO_3^-) content in dry leaf tissues, was determined calorimetrically, through the nitration of salicylic acid method [159], with photometry performed using an Anthos Zenyth 200 photometer (Biochrom Ltd., Cambridge, UK).

For phosphorus analysis, the following materials were used: Ammonium Molybdate 4-hydrate, Potassium antimonyl, Potassium di-Hydrogen Phosphate, Sulfuric acid, L(+)-Ascorbic acid, Sodium hydroxide and 4-Nitrophenol (Figure 13). Also, a 1000 mL flask, 100mL flask, 50mL flasks (as many as samples plus 7 extra for the calibration curve), 100 mL conical flask, 250mL conical flask, 1000mL conical flask, microplate for photometry, pipettes (Figure 14).

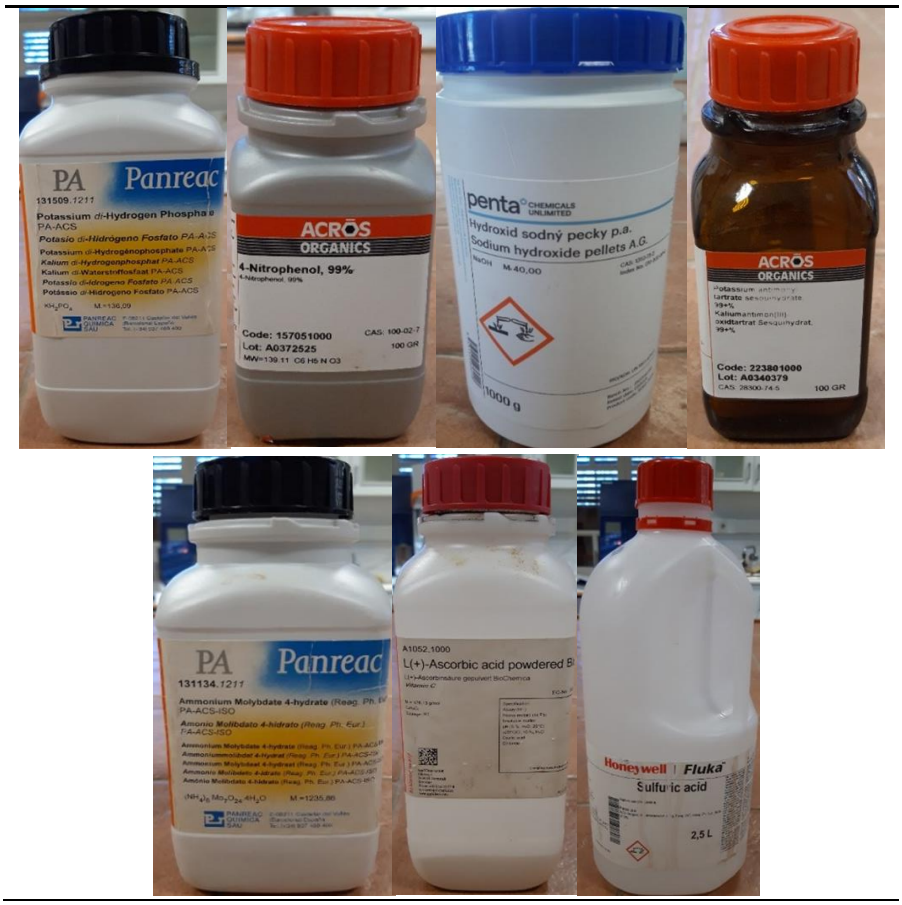


Figure 13: Reagents used for the molybdenum blue reaction for orthophosphate determination

Figure 14: Materials used for the molybdenum blue reaction for orthophosphate determination

1. For the calibration curve, 0.4395g of potassium dihydrogen phosphate (KH_2PO_4) were dissolved with distilled water in a 1L flask, to prepare the stock solution (100ppm). For further dilution, 10mL of this stock solution were diluted in a 100mL flask to acquire the 10ppm solution.
2. For the nitrophenol reagent, 0.5g of 4-Nitrophenol were dissolved with 60mL of distilled water in a 100 mL conical flask and stirred on a magnetic plate until the solution turned yellow.
3. For the sodium hydroxide (NaOH) reagent, 10g of NaOH were dissolved in a 250mL conical flask with distilled water.
4. The ascorbic acid reagent was prepared in regards to the amount of analyzed samples. For each sample, 10mL of Ascorbic Acid reagent are used. The reagent was prepared by adding 0.4g Ascorbic Acid per 100mL of the 7th reagent (for approximately 90 samples).
5. For the ammonium molybdate reagent 4.8g of ammonium molybdate 4-hydrate were dissolved in distilled water in a 100mL conical flask and stirred on a heated magnetic plate.
6. The potassium antimonyl tartrate reagent was prepared by dissolving 0.1097g of potassium antimonyl in a 100mL conical flask with distilled water.
7. The sulfuric acid reagent was finally prepared by adding 55 mL of sulfuric acid (H_2SO_4) to 500mL of distilled water inside of a 1 L flask. After letting the solution cool down, approximately after 30 minutes, the reagents 4, 5, and 6, (ascorbic acid, ammonium molybdate and potassium antimonyl) were added and the remaining volume was filled with distilled water until it reached 1 L.

For the calibration curve, specific ml were transferred from the 10ppm potassium dihydrogen phosphate solution to 50 mL flasks so the final solution would have concentrations of 0, 0.05, 0.1, 0.2, 0.4, 0.6, and 1 ppm, according to the dilution formula:

$$C_1V_1 = C_2V_2 \implies V_1 = C_2 * V_2 \,/\, C_1.$$

Equation 1: Dilution formula. C: Concentration, V: Volume

For the dilution of the samples, prior to the analysis, 50 mL flasks were also used. A dilution of 1:100 ratio was conducted so that the concentration of the samples would be in the range of the calibration curve. Hence, 0.5 mL of each sample was transferred into 50 mL flasks. In both the calibration solutions and the samples, the subsequent reagents were added in the following order: 2-3 drops of nitrophenol (2nd reagent), a small amount of distilled water, 2-3 drops of NaOH (3rd reagent) which turned the solution yellow, 10mL of sulfuric acid reagent (7th reagent) which turned the solution blue, and finally distilled water until the solution of every flask was filled up to the 50 mL mark. The samples and calibration curve standards were left at room temperature for 20 minutes until the color stabilized. Then, 300 µL per sample were transferred into the well of the micro-plate and loaded into the spectrophotometer. The analysis run at 880nm for 10 cycles, with 60-second intervals. By using the known concentrations of the standard solutions, the measured absorption was calculated to ppm. The measured concentration of the samples (expressed in ppm) was then calculated to mg g^{-1} according to the following equation.

$$C_{El} = C_M * D * \left(\frac{V_s}{g_s}\right) * \frac{1}{1000}$$

Equation 2: Formula for calculating the concentration of an element in the leaf tissues. C_{El}: Concentration of element (mg/g), C_M: measured concentration (ppm), D: dilution rate, V_s: Volume of aqueous leaf extract (ml), g_s: grams of dry leaf tissue in the aqueous leaf extract (g), and 1/1000 unit conversion factor to change µg/g to mg/g

Figure 15: Color gradient of standard solutions of the molybdenum blue reaction for orthophosphate determination

Figure 16: Color change from yellow to blue during the phosphorus analysis

For the analysis of leaf nitrate nitrogen content the following materials were used: sulfuric acid, salicylic acid, sodium hydroxide pellets, potassium nitrate puriss. The equipment used was: a 1000 mL conical flask, 400 mL beakers, 15 mL test tubes (twice as many as samples, plus 10 for standards), 125 mm Macherey-Nagel filter paper, 100mL flask, 25 mL flasks (as many as samples, plus 10 for standards), micro-plate, pipettes, and funnels.

The following reagents were prepared:

1. Calibration curve: 0.326g of potassium nitrate puriss were dissolved in a 100 mL flask with distilled water to produce a 500ppm stock solution.
2. NaOH (Sodium Hydroxide) solution: 80g of NaOH were dissolved with distilled water in a 1000 mL conical flask (sufficient for approximately 50 samples, or 2 plates).

3. Salicylic/Sulfuric acid solution: In a 400 mL beaker, 60 mL of sulfuric acid were added, 5g of salicylic acid, and finally filled up to 100 mL with more sulfuric acid again (about 40 mL more). No distilled water was added to this solution (sufficient for approximately 125 samples, or 35 plates).

The calibration curve was prepared according to the dilution formula (Equation 1), by diluting the standard solution of 500 ppm to prepare solutions of 25, 50, 75, 100, 150, 200, 300, 400, and 500 ppm. For the extraction of the samples, two technical replicates were prepared by transferring 0.1 grams of dried and ground leaf tissue into 15 mL centrifuge tubes. Each sample was then mixed with 10 mL of distilled water. This process was repeated for every sample to ensure consistent extraction results. Tubes were immersed in a 45°C water bath for 1 hour, stirring every 15 minutes. After filtering through 125 mm Macherey-Nagel filters, the sample solution was placed in sterilized 15 mL tubes. For both the sample solution and the standards, 0.2 mL were transferred to 25 mL centrifuge tubes, and 0.8 mL of the salicylic and sulfuric acid solution added. The mixture was stirred for 20 minutes using an orbital shaker Unitwist 300 (Biotech, Madrid, Spain). After adding 19 mL of 2N NaOH solution, the samples were stirred for an additional 20 minutes. All samples and standards were then loaded on the micro-plate and photometrically analyzed at 410 nm using the Biochrom® Anthos Zenyth 200 (Harvard Bioscience, Inc, MA, US). Results were then converted to mg per gram of dry weight through Equation 2. For the calculation of nitrate expressed as mg per Kg of fresh weight the values were multiplied with the dry weight/fresh weight ratio.

Figure 17: Colorimetrical determination of the nitrate-nitrogen through the nitration of salicylic acid method

Figure 18: Reagents for the colorimetrical determination of the nitrate-nitrogen through the nitration of salicylic acid

2.2.1.4.2 Atomic Absorption: Determination of calcium, magnesium, iron, manganese, zinc, and copper concentrations

Calcium, magnesium, iron, manganese, zinc, and copper concentrations were measured using an Atomic Absorption Spectrophotometer (Shimadzu AA-7000, Shimadzu, Kyoto, Japan), either with diluted or undiluted tissue extracts according to the dilution formula (Equation 1). The calibration curve was obtained by using standard solutions of the respective metallic salts (1000 ppm) provided by Shumadzu. For the operation of AA-700, the acetylene gas flow was set to 1.5 L min^{-1} and vacuum pressure to 3.5 bar.

Figure 19: Atomic Absorption Spectrophotometer, Shimadzu AA-7000.

2.2.1.4.3 Flame photometry: Determination of potassium and sodium

Flame photometry was used to measure potassium and sodium of the aqueous tissue extracts using a Sherwood Flame Photometer 410 (Sherwood, Cambridge, UK) [160]. The calibration curve for the flame photometer was prepared by diluting the stock solution (Figure 20)of potassium and sodium (1000 ppm) according to the dilution formula (Equation 1), so that the final concertation was 5 and 10 ppm. The samples were also diluted accordingly, in order for their potassium and sodium concentrations to be within the range of the calibration curve. The measured concentration (ppm) was then calculated to mg/g of dry weight through Equation 2.

Figure 20: Flame Photometer by Sherwood and standard solution for the preparation of the calibration curve

2.2.1.4.4 Determination of total Kjeldahl nitrogen

The total Kjeldahl nitrogen was determined through digestion, distillation and manual titration. This process was carried out using the Labtec DT 220 apparatus in conjunction with the Scrubber Labtec SR 210 and Tecator Kjeltec 8200 equipment ([161], FOSS Analytical A/S, Hillerød, Denmark). For the recovery success, 100 mg of glycine (18%) were used. Moreover, blank samples were also placed in every digestion tube. For the leaf tissues samples used, 250 mg of dried and powdered leaves were place into the digestion

tubes (Figure 21a). In each digestion tube 12 mL of sulfuric acid were added and 2 Kheltabs (Cu/3.5 (3.5 g K_2SO_4 + 0.4 g $CuSO_4 \times 5\ H_2O$). The digestion was carried out at 440 °C for 60 minutes (Figure 21b). During digestion, fumes were passed through the exhaust to a scrubber (Figure 21c). After the 60-minute period the digestion tubes were left to cool down to avoid fume inhalation.

Figure 21: a) scrubber unit, b) digestion block, c) digestion tubes, d) distilation

After digestion of the samples, distillation was carried out (Figure 21d). The glycine samples were used to verify the accuracy of the digestion process aiming for recovery above 99.5%. After distillation, each sample was manually titrated by measuring the volume (mL) of 0.05 N HCl solution required to change the solution's color from green to pink, thereby completing the determination of total Kjeldahl nitrogen. The results were expressed in μg g^{-1} of fresh weight through Equation 3, and then multiplied by 1000 to express in mg g^{-1}.

$$\frac{(ml_{sample} - ml_{blank}) * N * 14.007}{mg_{sample}}$$

Equation 3: Total nitrogen recovered from samples. ml_{sample}: mL of used to titrate the sample, ml_{blank}: mL used to titrate the blank, N: Normality of titrant (0,05), 14.007: molar mass of nitrogen, mg_{sample}: mg of sample used in the digestion process.

2.2.1.5 Statistics

Experimental data were analyzed using a two-way ANOVA to assess the main effects of individual factors, EC and K:Ca:Mg, as well as their interaction (EC × K:Ca:Mg). For multiple comparisons of means following the two-way ANOVA, Duncan's Multiple Range Test was applied at a 0.05 confidence level. Statistical analyses were performed using STATISTICA software version 9.0 for Windows (StatSoft Inc., Tulsa, OK, USA). Both parameters satisfied the assumption of normality, eliminating the need for data transformations.

2.2.2 Impact of nitrogen levels, irrigation, and biostimulant application on yield and chemical traits of stamnagathi grown in perlite bags

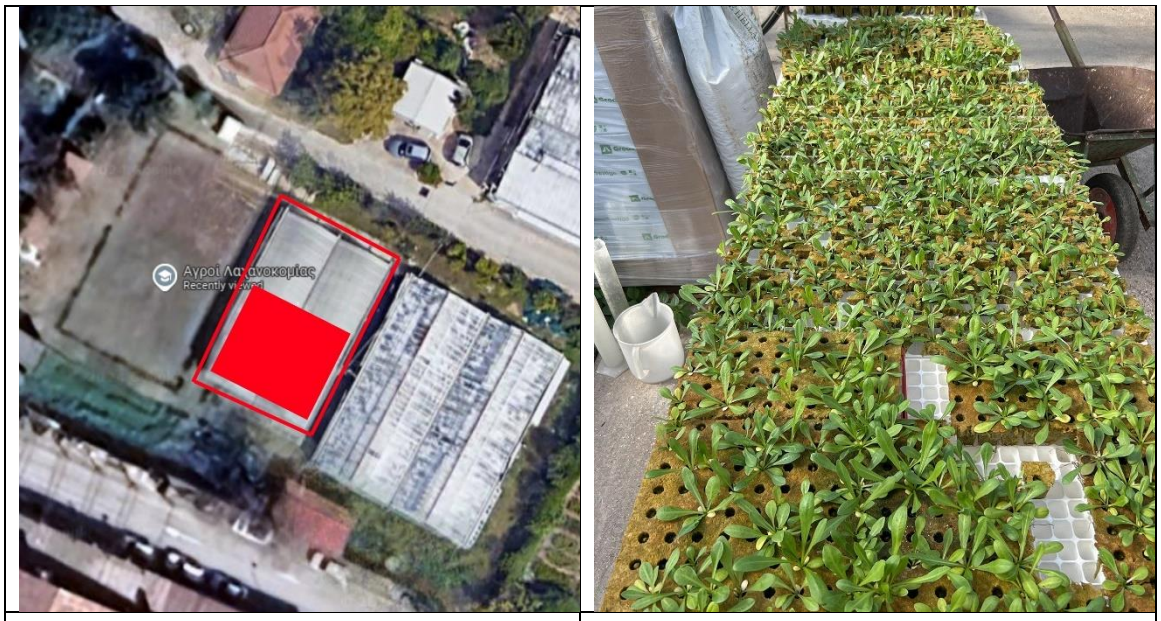
2.2.2.1 Seedling and greenhouse chamber preparation

A greenhouse experiment was set up on December 30th 2022 for a total of 38 days. The cultivation was carried out in an unheated, plastic, semitransparent greenhouse of the Laboratory of Vegetable Production of the Agricultural University of Athens (AUA), located at coordinates 37° 59′ 2″ N and 23° 42′ 19″ E (Figure 22a). As plant material, clean seeds of the mountainous ecotype of stamnagathi (*Cichorium spinosum* L.) were used (section 2.1). Sowing took place on AO 25/40 rockwool plugs. Each plug was 25*25*40 mm and connected to the others forming a sheet of 200 plugs. During the seedling preparation stage these rockwool sheets were placed on seedling trays and fertigated once at the beginning of the germination phase with the control nutrient solution. Moisture was maintained by top spraying every 2-3 days. The seedlings were separated and transplanted 35 days after sowing (Table 2). Seedling plugs were re planted

directly into perlite bags in a plant density of 20 plants m^{-2}. During the transplanting phase all seedlings used for the had 5 or 6 true leaves (Figure 22b). The greenhouse's chamber was cleaned, leveled and the cultivation gullies were carefully positioned so that the angle would results in the removal of the excess nutrient solution. Prior to transplanting, the perlite bags were fertigated with the nutrient solution designated for each treatment (Figure 23a-c).

Table 2: Timetable of the experiment

Cultural Practice	Date	Days After Sowing
Sowing	30/12/2022	0
Transplanting	3/2/2023	35
Harvest	13/3/2023	73



a b

Figure 22: a) plastic, semitransparent greenhouse of the Laboratory of Vegetable Production of the Agricultural University of Athens, and b) seedlings prepared on rockwool on the day of transplanting.

Figure 23: Preparation of the experimental setup; a) testing the inclination and outflow of the channels before placing the perlite bags, b) placement of 3 perlite bags per channel, c) setting up the irrigation and pre-fertigating the perlite bags before transplanting took place.

2.2.2.2 Experimental design

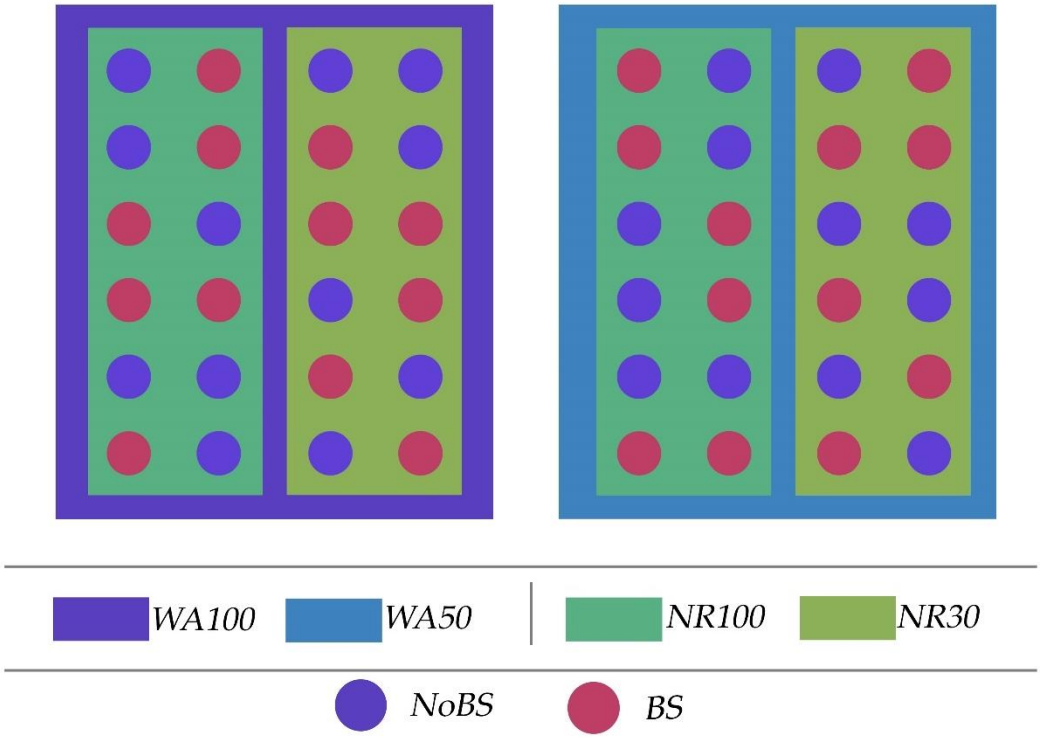


Figure 24: Experimental design exploring the simultaneous effects of three factors, nitrogen rates (NR), water availability (WA), and biostimulant application (B). NR100 and NR30: 100% and 30% of plants needs in nitrogen, WA100 and WA50: 100% and 50% of irrigation needs, NoBs and BS: No biostimulant application and biostimulant application.

The simultaneous effects of three factors, nitrogen rates (NR), water availability (WA), and biostimulant application (B) were investigated. The cultivation was carried out under two isosmotic solutions with different nitrogen rates (NR), the control nutrient solution (N100), with a total nitrogen at 13.64 mmol L^{-1}, and a limited nitrogen treatment with 30% (N30) that of the control, 4.55 mmol L^{-1} (Table 3). Water availability was applied through two levels, the control treatment which supplied 100% of the plant's needs (WA100), and the drought treatment which was applied by reducing the irrigation supply to 50% (WA50). In the control group, plants were irrigated so that the target drainage fraction substituted by a suitable value of 30%, whereas drought treatments had a drainage of 0%. The drought treatment was initiated on week after transplanting. In addition, biostimulant application (B) with a hydrolyzed plant protein was carried out via foliar spray (BS) whereas the control plants received only water (NoBS). The biostimulant applied in this study was Tyson® by Mugaver Fertilizers (Italy), a protein hydrolysate biostimulant rich in nitrogen (total nitrogen, 5.0%; organic nitrogen, 4.5%; organic carbon, 25 %; and free amino acids, 13.4%), which was selected for its demonstrated beneficial effects in previous trials [105–107,114,120]. The solution was prepared as recommended (3 mL Tyson per L of tap water) and 0.2 mL per plant were applied every 10 days (3 times throughout the experiment).

A split-split plot design was applied with three main factors. The main plots contained two levels of nitrogen rates (NR100 and NR30). Subplots were allocated two levels of water availability (WA100 and WA50), and sub-subplots were dedicated to the biostimulant treatment (NoBS and BS). This design led to 8 unique treatment combinations (2 NR x 2 WA x 2 B), each treatment was repeated in six perlite grow-bags, with 6 plants per replicate, resulting in 288 plants.

Table 3: Chemical characteristics of the supplied nutrient solutions.

Element	units	Control (NR100)	Limited Nitrogen (NR30)
EC	dS m^{-1}	2.5	2.5
pH		5.6	5.6
NO_3^-	mmol L^{-1}	12.00	4.00
NH_4^+	mmol L^{-1}	1.64	0.55
NH_4^+/Total-N		0.12	0.12
K^+	mmol L^{-1}	6.71	6.98
Ca^{2+}	mmol L^{-1}	3.70	3.85
Mg^{2+}	mmol L^{-1}	2.07	2.16
SO_4^{2-}	mmol L^{-1}	3.11	4.41
$H_2PO_4^-$	mmol L^{-1}	1.46	1.46
Fe	µmol L^{-1}	17.89	17.89
Mn^{2+}	µmol L^{-1}	9.36	9.36
Zn^{2+}	µmol L^{-1}	4.47	4.47
Cu^{2+}	µmol L^{-1}	0.73	0.73
B	µmol L^{-1}	27.56	27.56
Mo	µmol L^{-1}	0.52	0.52
Cl^-	mmol L^{-1}	0.40	6.00
Na^+	mmol L^{-1}	0.60	0.60
HCO_3^-	mmol L^{-1}	0.40	0.40
Ψs (MPa)		-0.20	-0.20

2.2.2.3 Measurements and Statistical analysis

In total, 18 plants per treatment were sampled from 6 grow-bags. Each plant was collected to assess its individual agronomic traits. Leaf number, leaf area, fresh weight, and dry weight of each plant was recorded as in section 2.2.1.3. After the drying process the 3 plants per grow-bag were combined and treated as a single sample, resulting in 6 replicates per treatment. The process followed for the determination of leaf nitrate nitrogen, calcium, potassium, magnesium, iron, manganese, zinc and sodium was the same as in section 2.2.1.4. Experimental data were analyzed using a multifactorial ANOVA to assess the effects of 3 individual factors, nitrogen rates (NR), water availability (WA) and biostimulant application (B), as well as their interactions per two and three factors respectively (NR x WA, NR x B, WA x B, NR x WA x B). For multiple comparisons of means

following the two-way ANOVA, Duncan's Multiple Range Test was applied at a 0.05 confidence level. Statistical analyses were performed using STATISTICA software version 9.0 for Windows (StatSoft Inc., Tulsa, OK, USA). Both parameters satisfied the assumption of normality, eliminating the need for data transformations.

2.3 Exploring the cultivation of *Cichorium spinosum* L. in vertical farms

2.3.1 Can long photoperiods be utilized to integrate *Cichorium spinosum* L. into vertical farms?

In the first experiment carried out with complete lack of natural sunlight, the effect of elongated photoperiod on growth and flowering response of *Cichorium spinosum* L., was investigated. After breaking the achenes (as in section 2.1) clean seed of the mountaineous ecotype of stamnagathi were sown in fine peat trays (type TS1, Klasmann-Deilmann GmbH, Geest, Germany). Sowing took place during May of 2021 and the seedling preparation stage was carried out inside a glasshouse of the Laboratory of Vegetable Production. The moisture level of the substrate was checked daily and irrigation was administered manually. After 4 weeks, seedlings had reached the stage of 4 true leaves and 96 seedlings were transferred to into individual 0.5 L pots containing peat. Then the pots were separated into two groups and relocated on 3 horizontal trays inside two climate chambers of the Laboratory of Ecology of the Agricultural University of Athens (Figure 25a). In total, 48 plants were placed inside each chamber and each tray accommodated 24 plants (Figure 25b). Each chamber had a different photoperiod treatment, a short one, of 10 hours (short day, SD) and a long one of 15 hours (long day, LD). Both chambers had the same temperature, relative humidity, carbon dioxide concentration, and light intensity levels of 20 °C, 65-60%, 400 ppm, and 100 µmol m^{-2} s^{-1}, respectively. Fertigation was applied manually throughout the experiment with the tailored nutrient solution for stamnagathi according to Nutrisense's database of 2021. The nutrient solution was administrated manually using volumetric syringes of 20 ml (Figure 25c). The plants were maintained in the chamber for 5 months. At harvest, the agronomical characteristics were measured as previously described (2.2.1.3.).

a b c

Figure 25: Photoperiod experiments. a) two climate-controlled chambers of the Laboratory of Ecology, b) one tray of 27 pots representing one replicate, c) manual irrigation with a volumetric syringe

2.3.1.1 Leaf gas exchange measurements and Chlorophyll fluorescence

Leaf gas exchange analysis was carried out three days prior to harvest using the LCpro T analyzer (ADC BioScientific, UK). Gas exchange measurements were performed on a total of four plants in three consecutive days to acquire sufficient date. In this measurement, the net photosynthetic rate (A) and transpiration rate (E) were measured. A represents the rate at which carbon dioxide is assimilated through photosynthesis. It is often referred to as the net photosynthetic rate because it derives from the carbon dioxide that is assimilated through photosynthesis minus the carbon dioxide that is lost through respiration. Hence, the units of A are micromoles of carbon dioxide per square meter per second (µmol CO_2 m^{-2} s^{-1}). On the other hand, the E is the rate of water vapor being lost from the surface of the leaf through the stomata, as a result of transpiration. E is influenced by factors such as stomatal conductance, temperature, humidity, and light. E is measured as millimoles of water vapor per square meter per second (mmol H_2O m^{-2} s^{-1}). For the analysis, only fully expanded leaves that could fully cover the gas chamber surface were used. The measurements were carried out between 09:00 and 14:00 hours. The illumination source was directed toward the adaxial side of the leaf, comprising 35% blue

5% green and 60% red light. The initial light intensity (Q_{leaf}) was set at 0 µmol m^{-2} s^{-1}, followed by subsequent values of 43, 87, 174, 261, 435, 696, 870 and 1044 µmol m^{-2} s^{-1}. During the analysis, the PPFD level increased every 4 minutes and one measurement per minute was recorded.

The in vivo chlorophyll fluorescence was conducted on the day of harvest. The key parameters of electron transport rate (ETR), non-photochemical quenching (qN), photochemical quenching (qP), and the quantum yield of photosystem II (ΦPSII) were measured. ETR measured the rate at which electrons are transported in the photosynthetic chain through PSII. Even though the actual rate is unitless it can also be reported in µmols electrons m^{-2} s^{-1}. Moreover, qN represents the dissipation of excess light energy as heat that cannot be ascribed to photochemical mechanisms. On the other hand, qP indicated the proportion of PSII reaction centers that are capable of photochemistry and it reflects how efficiently light is absorbed and used for electron transport. Both qN and qP are unitless. Finally, ΦPSII measures the efficiency of the PSII to convert the absorbed photons into chemical energy under the given conditions. ΦPSII is also unitless. Simillarly to leaf gas exchange, fully expanded leaves were used. For this measurement, PAM-2100, a portable chlorophyll fluorometer by Heinz Walz GmbH (Effeltrich, Germany) was used as described by Liakopoulos et al. [162]. Prior to each measurement cycle, each leaf was acclimated for 20 minutes. For the dark acclimation leaf clips were used. The light response curve was measured at different light intensities. The initial light intensity (Q_{leaf}) was 0 µmol m^{-2} s^{-1}, followed by subsequent 40, 74, 120, 192, 302, 412, 631 and 938 µmol m^{-2} s^{-1}.

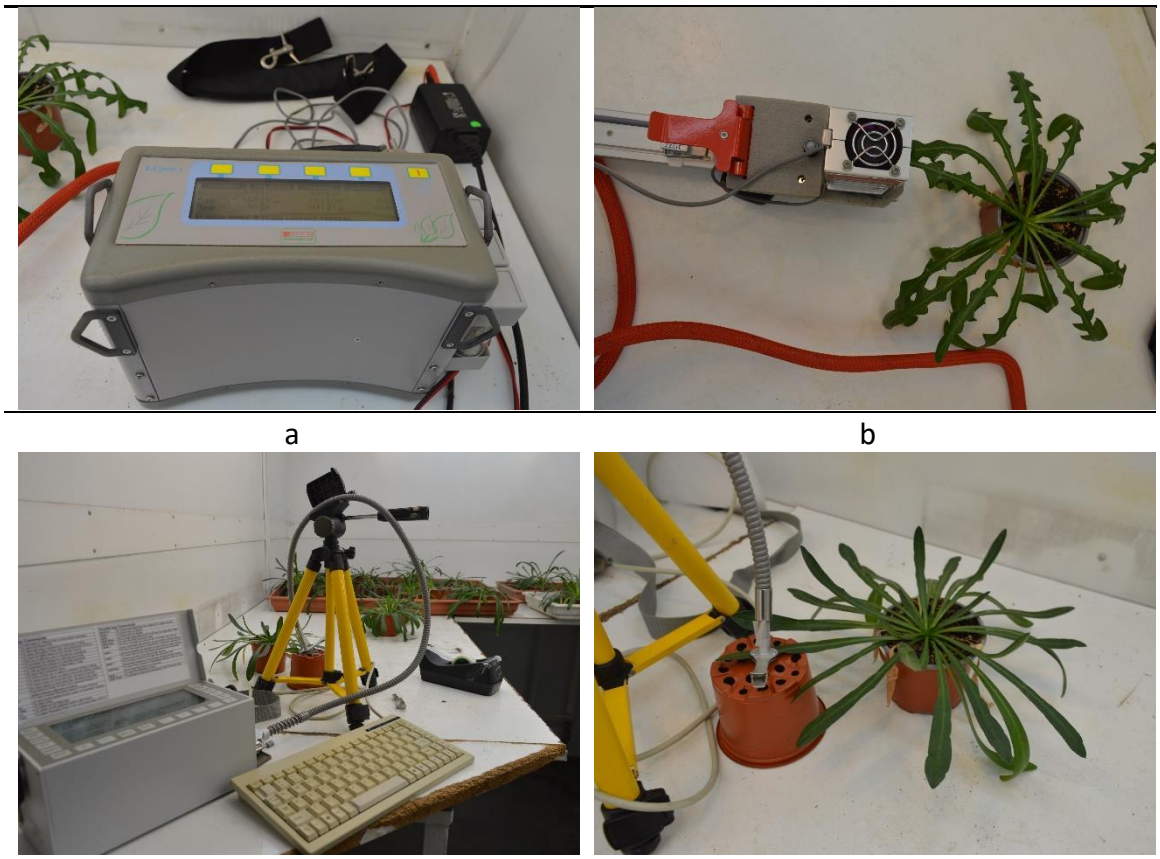


Figure 26: a) Gas exchange LCpro T analyzer and b) measurement on Cichorium spinosum. c) PAM-2100 chlorophyl fluorescence analyzer and d) measurement on Cichorium spinosum.

2.3.2 Exploring the effects different white light spectra and nutrient solutions

2.3.2.1 Seedling preparation

As plant material, stamnagathi (*Cichorium spinosum* L.) seeds of the mountainous ecotype were used after breaking down achenes and separating the seed from the debris (as in section 2.1). Sowing took place on AO 25/40 rockwool plugs (Grodan, Roermond, The Netherlands). Each plug was 25*25*40 mm and connected to the others forming a sheet of 200 plugs. Four rockwool sheets per layer were used during the seedling preparation stage, and were arranged in the ebb and flow trays of a Vegeled trolley (Collasse SA, Seraing, Belgium). The seedlings destined for the spectrum experiment were placed from seed to seedling under the treatment's light spectrum (section 2.3.2.2). The seedlings destined for the nutrient solution experiment were cultivated under the control

spectrum (section 2.3.2.3). Key environmental factors including were measured with the aid of a Sigrow Pro sensor (Sigrow B.V., Wageningen Campus, Wageningen, The Netherlands). The average temperature, relative humidity and CO_2 values were 25°C, 60%, and 400 ppm during the light period and 18°C, 50%, and 360 ppm during the dark period. For the seedling preparation stage the photoperiod was 12 hours and a light intensity of 180 µmol m^{-2} s^{-1}. The rockwool sheets were fertigated once at the beginning of the germination phase and then sprayed every 2-3 days to maintain moisture. Almost a week after sowing, as the seeds started to sprout and the cotyledons were visible, fertigation automatically applied through the ebb and flow system of the Vegeled trolley with a control solution (N10-Fe15). Meanwhile, in plugs that had more than one sprouted seed, the extra seedlings were removed with tweezers. For the next 10 days, fertigation was applied for 2 minutes every 2 days. After that, and for another 10 days, fertigation frequency was increased to 2 minutes per hour. Separation and transplanting of the seedlings were conducted 27 days after sowing, when the plants had reached the stage of 4 true leaves.

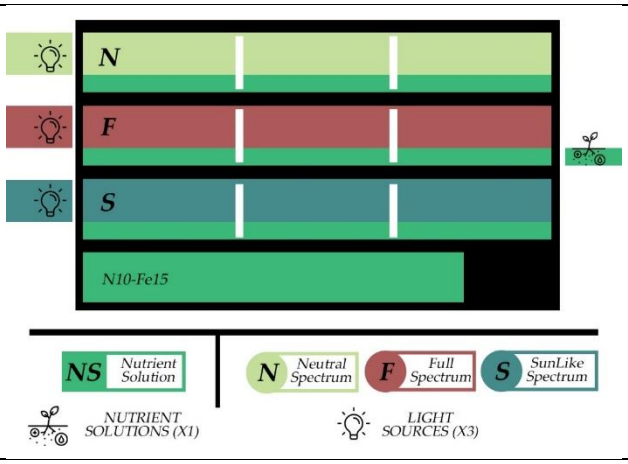
Table 4: Chemical composition of the nutrient solution applied from seed to harvest

Nutrient solution parameter	Units	N10-Fe15
EC*	ds m^{-1}	1.49
pH		5.6
K^+	mmol L^{-1}	4.23
Ca^{2+}	mmol L^{-1}	2.32
Mg^{2+}	mmol L^{-1}	1.01
NH_4^+	mmol L^{-1}	1.64
NO_3^-	mmol L^{-1}	9.28
$H_2PO_4^-$	mmol L^{-1}	1.06
SO_4^{2-}	mmol L^{-1}	0.70
Fe	µmol L^{-1}	15.36
Mn^{++}	µmol L^{-1}	8.64
Zn^{++}	µmol L^{-1}	3.84
Cu^{++}	µmol L^{-1}	0.67
B	µmol L^{-1}	24

Mo	µmol L^{-1}	0.48
Cl^-	mmol L^{-1}	1.92
Na^+	mmol L^{-1}	0.6
HCO_3^-	mmol L^{-1}	0.4

*Electric conductivity

2.3.2.2 Experimental Design, measurements and statistical analysis: Different spectra experiment



a

b

Figure 27: a) Illustrative depiction of the lighting experiment. In this experiment each layer had a different spectrum of "white light", a "neutral" (N), a "full" (F) and a "SunLike" (S) but all layers were supplied from the same tank, with the same nutrient solution (NS). Each treatment had 3 replicates per layer. No randomization was implemented in this experiment. b) seedling preparation on the three levels of the Vegeled Trolley.

As seedlings were prepared under the light spectrum that they would continue to grow after separation and transplanting, the treatment's effects had started from sowing (Figure 27). Transplanting tool place 27 days after sowing and the cultivation lasted for 36 days after transplanting (Table 5). The lighting systems were Vegeled™ Eos series and had the commercial names "Neutral" (N), "Full" (F) and "SunLike™" (S). The light composition (Blue:Green:Red:Far-red) of the three spectral treatments was 14:32:43:10, 16:36:40:8, and 21:34:36:7 for the N, F and S treatments respectively (Table 6). The efficacy of the LEDs was 2.4 µmol J^{-1} for N and F, and 1.8 µmol J^{-1} for S. Each layer had only one spectrum and there was no randomization in the lighting conditions. The seedlings were transplanted into plastic net pots at a density of 50 plants m^{-2}. During the cultivation phase the climatic conditions were measured on each layer by an Air-Pro Sigrow sensor.

During the light period, the average temperature was 27°C, dropping to 25°C during the dark period, with relative humidity at 68% and 55%, and CO_2 levels at 450 ppm and 360 ppm, respectively. The photoperiod was 12 hours long and the light intensity 300 µmol m^{-2} s^{-1}. A minor temperature difference of about 1°C, between the bottom and the top layer. The irrigation method used was, as in the seedling stage, "ebb and flow" and the nutrient solution was supplied to all layers simultaneously from one tank, drained back to the tank and recirculated. The chemical composition of the nutrient solution was consistent with that used in the seedling stage (N10-Fe15). The irrigation frequency was 5 minutes every half hour. The first irrigation was at 09:00-09:05 and the last at 21:00-21:05, at the end of the photoperiod (Table 7). During each irrigation cycle the water level reached the bottom half of the rockwool plugs. The experiment continued on the 135 x 56 cm Vegeled™ trolley with three layers and one common tank. Each layer was divided into three sections measuring 45 x 56 cm, with each section considered as one replicate.

Table 5: Timeline of each experiment and experimental phase.

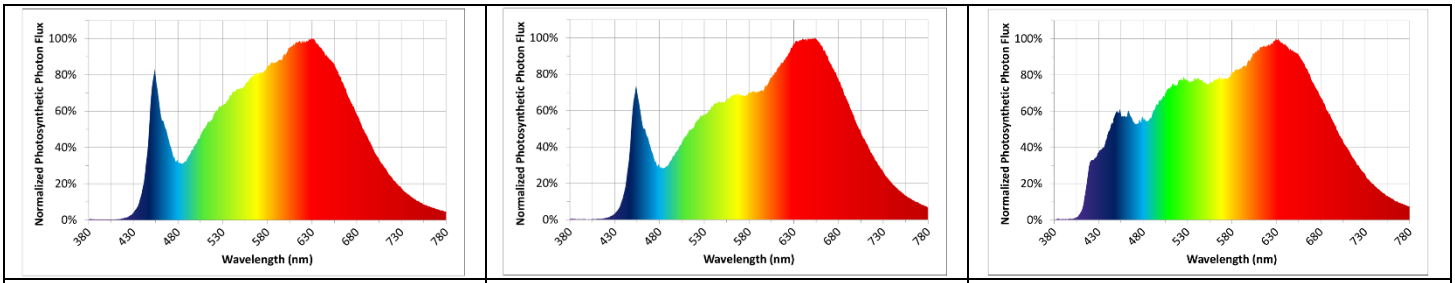
Practice	Experiment 1
Sowing	23/12/2022
Transplanting	19/1/2023 (27 DAS)
Harvest	24/2/2023 (36 DAT)
Total days	63 (DAS)

DAS stand for "days after sowing" and DAT for "days after transplanting".

Table 6: Spectral composition of the three different "white lights"

Color	Neutral	Full Spectrum	Sunlike
Blue %	14	16	21
Green %	32	36	34
Red %	43	40	36
Far Red %	10	8	7
R:B	3.1	2.5	1.7
G:B	2.3	2.2	1.6
R:FR	4.3	5	5.1
Efficiency µmol J^{-1}	2.4	2.4	1.8

R: red, B: blue, G: green, FR: Far red



a	b	c

Figure 28: Normalized Photosynthetic Photon Flux for spectra (a) Neutral, (b) Full spectrum, and (c) Sunlike provided by Colasse.

Table 7: Synopsis of average environmental conditions during the light and dark periods (day and night) of the different spectra experiment

Parameter	Seedling stage	Spectra Experiment
Temperature °C (Day/Night)	25/18	27/25
Humidity % (Day/Night)	60/50	68/55
CO_2 ppm (Day/Night)	400/360	450/360
Light Intensity µmol m^{-2} s^{-1}	180	300
Irrigation method	Top spray, then Ebb and flow	Ebb and flow
Photoperiod (hours)	12	12
Irrigation frequency	Depends on stage	5 minutes 0.5 $hour^{-1}$
Plant density (plants m^{-2})	1100	50
Spectra	Depends on experiment	N, F, S
Nutrient solutions	N10-Fe15	N10-Fe15

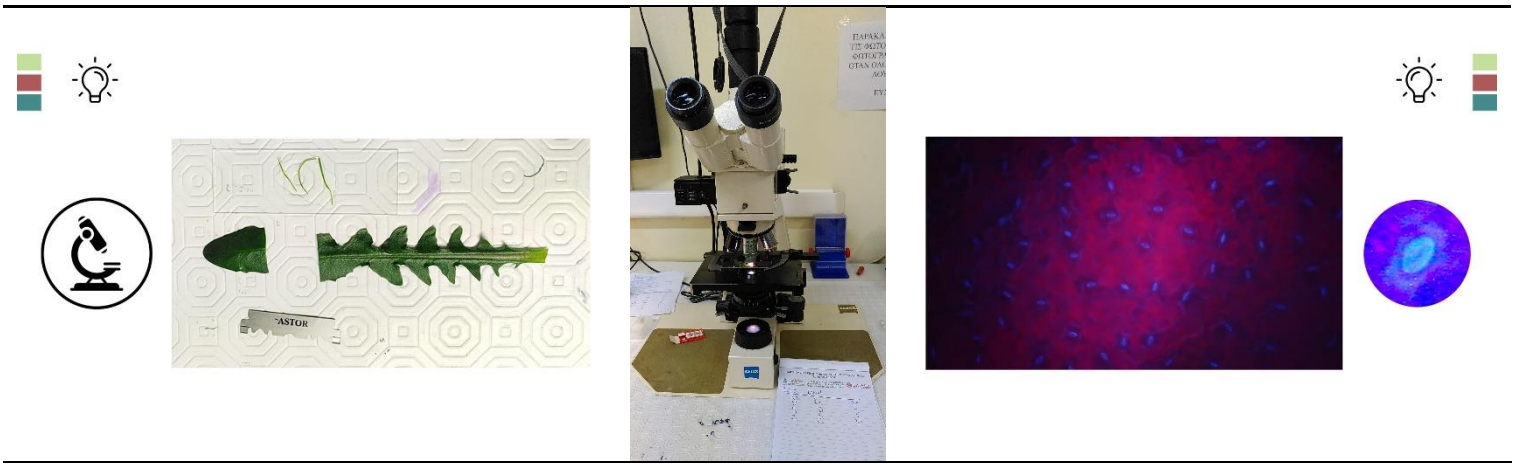
The spectra refer to commercial names "Neutral" (N), "Full" (F) and "SunLike™" (S). The spectral composition (Blue:Green:Red:Far-red) of the three light treatments was 14:32:43:10, 16:36:40:8, and 21:34:36:7 respectively. The nutrient solution names refer to the concentrations of total nitrogen and iron in the nutrient solutions, N10-Fe15 (10 mmol L^{-1} and 15 µmol L^{-1})

During the cultivation period of the lighting experiment, and the nutrient solution experiment (see next section, 2.3.2.3), the nutrient solution volume, EC and pH were measured diurnal. For the measurement of the volume a ruler was used whereas Bluelab pens (Bluelab, Tauranga, New Zealand) were used to check the electrical conductivity and

pH. Adjustments were made when values deviated from the target range (EC 1.5-2 ds/m and pH 5.5-5.6), and additional nutrient solution was added to the tank when the water level dropped significantly, potentially compromising the pump's ability to uniformLy supply the solution.

Harvest took place 36 days after transplant. At harvest, the leaf quantitative characteristics (number, area, and fresh weight) were measured as described in section 2.2.1.3. During harvest 8 plants per layer, per replicate were collected (resulting in 24 plants per treatment). Drying took place in a ventilated oven as previously described in section 2.2.1.3.

In addition to the previously described measurements, leaf characteristics such as leaf thickness, spongy and palisade parenchyma thickness, stomata density and stomata size were performed in 2 plants per replicate. The second youngest and fully expanded leaf was chosen for this measurement. For measuring leaf thickness, fresh transverse sections from the middle of the lamina were examined under a Zeiss Axiolab microscope (Carl Zeiss, Jena, Germany), with the thickness of the palisade and spongy parenchyma measured using a calibrated eyepiece (Figure 29a-c). Stomatal density, length, and width were measured on the abaxial surface of 6 fresh leaf samples under incident UV light (maximum energy of 365 nm). To enhance the accuracy of stomatal pore measurements, 6 images per sample were captured (3 at 10x and 3 at 40x magnification) using a SSCD 38P/45 digital camera (SONY Corporation, Japan) and stored in digital format. The images were then edited in Image Pro-Plus to determine the number (at the 10x magnification) and the length of the stomata (40x magnification).



a b c

Figure 29: a) leaf slices of the second youngest and fully expanded leaf for the microscopic observation of leaf total thickness, and palisade and spongy parenchyma thickness. b) Zeiss Axiolab microscope used for the determination of leaf thickness and stomatal density, c) measurement of stomatal density stomatal length and width of the abaxial surface of fresh leaf specimens observed with incident UV light

Experimental data were analyzed using a ONE-WAY ANOVA to assess the effects of a single factor, the light spectrum. Duncan's Multiple Range Test was applied at a 0.05 confidence level. Statistical analyses were performed using STATISTICA software version 9.0 for Windows (StatSoft Inc., Tulsa, OK, USA). The parameters satisfied the assumption of normality, eliminating the need for data transformations

2.3.2.3 Experimental Design, measurements and statistical analysis: Different nutrient solutions

Figure 30: The three experimental modules (right) that were used for the nutrient solution experiment.

For the nutrient solution experiment, the seedlings were prepared only under the "Neutral" spectrum with the following Blue:Green:Red:Far-red compostion 14:32:43:10 and a light intensity of 180 μmol m^{-2} s^{-1} as in section 2.3.2.1. This experiment was carried out from June to July of 2023 (Table 8) in an acclimated room with modular vertical farms at the Laboratory of Vegetable Production, after the results of the experiment of section **Σφάλμα! Το αρχείο προέλευσης της αναφοράς δεν βρέθηκε.** was completed.

Table 8: Timeline of each experimental phase.

Practice	Nutrient solution experiment
Sowing	02/06/2023
Transplanting	29/06/2023 (27 DAS)
Harvest	22/07/2023 (24 DAT)
Total days	51 (DAS)

DAS stand for "days after sowing" and DAT for "days after transplanting".

This experiment explored solely the nutrition aspect of the cultivation and was carried out on three 71.5 x 56.5 cm Vegeled™ trolleys. Each trolley had three layers and each layer was connected with one nutrient solution tank independently from the rest (Figure 30, Figure 31). Moreover, in the scope of increasing the productivity per square meter, the seedlings were transplanted into plastic net pots at a density of 100 plants m^{-2}. The nutrient solution was supplied to each layer by "ebb and flow". All nutrient recipes used in this experiment were prepared using the online software "NUTRISENSE DSS" (www.nutrisense.online, [156]) to have the same EC level (1.5 dS m^{-1}). The three treatments were named after their total nitrogen and iron content and were as follows: the control treatment, N10-Fe15, with a total nitrogen content of 10 mmol L^{-1} and an Fe content of 15 μmol L^{-1}, the limited total nitrogen treatment (N4-Fe15) which had a N content of 4 mmol L^{-1} and Fe 15μmol L^{-1}, and the elevated iron treatment (N10-Fe48), which had a total nitrogen content of 10 mmol L^{-1} and a Fe content of 48 μmol L^{-1} (Table 9). In all treatments, Fe was supplied as Fe-EDDHA (6% Fe). The treatments were randomLy positioned in the three trolleys following a randomized design. The irrigation

frequency was increased to 20 minutes every hour. The first irrigation was at 09:00-09:30 and the last at 00:00-00:30, at the end of the photoperiod. The photoperiod was set to 15 hours, after results of section 2.3.1 demonstrated that stamnagathi could be cultivated under long photoperiods without flowering before reaching the commercial stage [3]. After the results regarding the effect of light spectrum of the three lighting products (section 2.3.2) on stamnagathis' growth, the lighting fixtures used in the nutrient solution experiment were Vegeled™ Eos series solely of the "Neutral" spectrum with the Blue:Green:Red:Far-red composition 14:32:43:10 and 2.4 µmol J^{-1} efficacy. The light intensity was 300 µmol m^{-2} s^{-1}. The environmental parameters averaged 28°C and 25°C for temperature, 70% and 60% for relative humidity, and 450 ppm and 360 ppm for carbon dioxide concentration during the light and dark periods, respectively (Table 10).

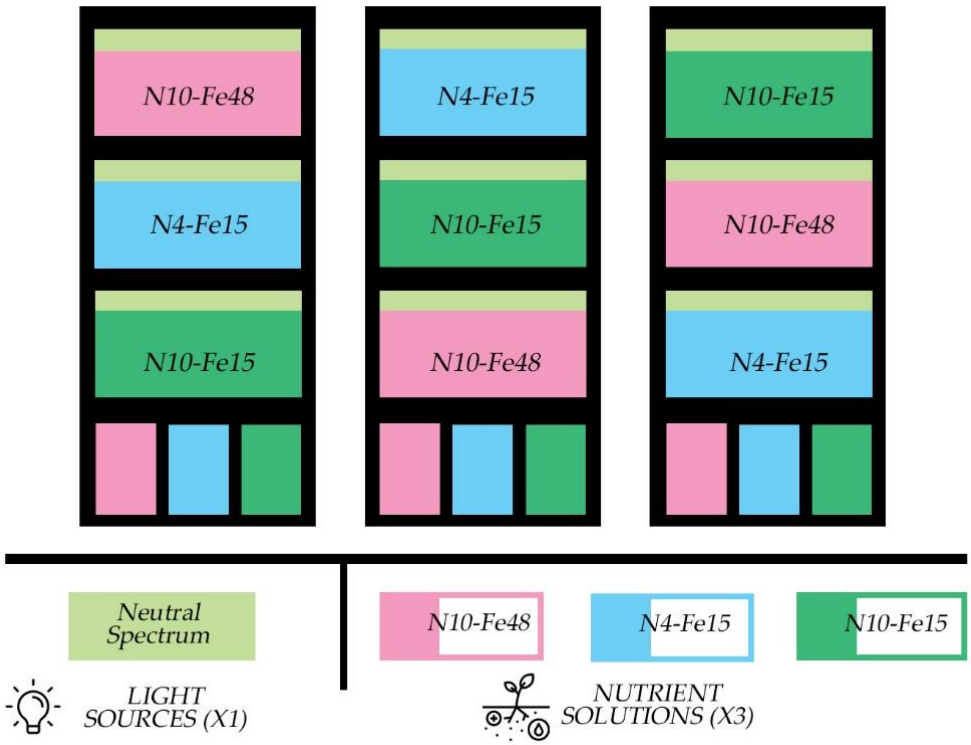


Figure 31: Illustrative depiction of the nutrient solution experiment. In this experiment the N spectrum was used for all layers and all cultivation units. Three NS treatments were carried out, based on their total-N and Fe content. The control (10 mmol L⁻1 and 15 µmol L⁻1, N10-Fe15), the limited total-N (4.55 mmol L⁻1 and 15 µmol L⁻1, N4-Fe15) and the elevated Fe (10 mmol L⁻1 and 48 µmol L⁻1, N10-Fe48). Each treatment had 3 replicates placed randomLy in the three cultivation units.

Table 9: Chemical composition of the nutrient solution of each treatment.

Nutrient solution parameter	Units	N10-Fe15	N4-Fe15	N10-Fe48
EC*	ds m^{-1}	1.49	1.48	1.49
pH		5.6	5.6	5.6
K^+	mmol L^{-1}	4.23	4.81	4.23
Ca^{2+}	mmol L^{-1}	2.32	2.48	2.32
Mg^{2+}	mmol L^{-1}	1.01	1.09	1.01
NH_4^+	mmol L^{-1}	1.64	0.55	1.64
NO_3^-	mmol L^{-1}	9.28	4	9.28
$H_2PO_4^-$	mmol L^{-1}	1.06	1.06	1.06
SO_4^{2-}	mmol L^{-1}	0.70	2.85	0.70
Fe	µmol L^{-1}	15.36	15.36	48
Mn^{++}	µmol L^{-1}	8.64	8.64	8.64
Zn^{++}	µmol L^{-1}	3.84	3.84	3.84
Cu^{++}	µmol L^{-1}	0.67	0.67	0.67
B	µmol L^{-1}	24	24	24
Mo	µmol L^{-1}	0.48	0.48	0.48
Si	mmol L^{-1}	0	0	0
Cl^-	mmol L^{-1}	1.92	1.92	1.92
Na^+	mmol L^{-1}	0.6	0.6	0.6
HCO_3^-	mmol L^{-1}	0.4	0.4	0.4

*Electric conductivity

During the cultivation period, the nutrient solution volume, EC and pH were measured and re-adjusted as described in section 2.3.2.2.

Plants were harvested 24 days after transplant. Measurements during harvest (leaf number, area, fresh and dry weight) were carried out as in section 2.2.1.3. During harvest 12 plants per layer, per replicate (resulting in 36 plants per treatment) were collected (resulting in 24 plants per treatment). Drying took place in a ventilated oven as previously described in section 2.2.1.3.

The chemical composition of the leaf tissues at the harvest stage was determined. For the determination of the leaf nitrate nitrogen, total Kjeldahl nitrogen, leaf phosphorus, calcium, magnesium, iron, potassium, and sodium content, the grated leaf tissues for each replicate were processed as described in section 2.2.1.4.

Recorded data underwent One-Way ANOVA analysis using the Statistica 12 software package for Windows (StatSoft Inc., Tulsa, OK, USA). Experimental data were analyzed using a ONE-WAY ANOVA to assess the effects of the different nutrient solition. Duncan's multiple range test was administered at a significance level of $p \leq 0.05$ for all measured variables. The parameters satisfied the assumption of normality, eliminating the need for data transformations

Table 10: Synopsis of average environmental conditions during the light and dark periods (day and night) of the nutrient solution experiment

Parameter	Seedling stage	Nutrient Solution Experiment
Temperature °C (Day/Night)	25/18	28/25
Humidity % (Day/Night)	60/50	70/60
CO_2 ppm (Day/Night)	400/360	450/360
Light Intensity µmol m^{-2} s^{-1}	180	300
Irrigation method	Top spray, then Ebb and flow	Ebb and flow
Photoperiod (hours)	12	15
Irrigation frequency	Depends on stage	30 minutes $hour^{-1}$
Plant density (plants m^{-2})	1100	10
Spectra	Depends on experiment	N
Nutrient solutions	N10-Fe15	N10-Fe15, N4-Fe15 and N10-Fe48

The spectra refer to commercial names “Neutral” (N), “Full” (F) and “SunLike™” (S). The spectral composition (Blue:Green:Red:Far-red) of the three light treatments was 14:32:43:10, 16:36:40:8, and 21:34:36:7 respectively. The nutrient solution names refer to the concentrations of total nitrogen and iron in the nutrient solutions, N10-Fe15 (10 mmol L^{-1} and 15 µmol L^{-1}), N4-Fe15 (4 mmol $L^{-}1$, and 15 µmol L^{-1}), and N10-Fe48 (10 mmol L^{-1} and 48 µmol L^{-1})

3. Results

3.1 Results: Exploring the Simultaneous Effect of Total Ion Concentration and K:Ca:Mg Ratio of the Nutrient Solution on the Growth and Nutritional Value of Hydroponically Grown Cichorium spinosum L.

The EC of the nutrient solution in the four different treatments increased gradually for each EC level (Figure 32). Nutrient solutions of different initial electrical conductivity levels, L and H, remained statistically similar to their K:Ca:Mg counterpart. The EC increase from day zero to day 21 was less than 0.5 dS m^{-1}. Moreover, he pH of each nutrient solution gradually became more acidic (Figure 33). All four treatments started from the same pH values and without significant differentiations, resulted in reduced pH values of almost 1 pH unit.

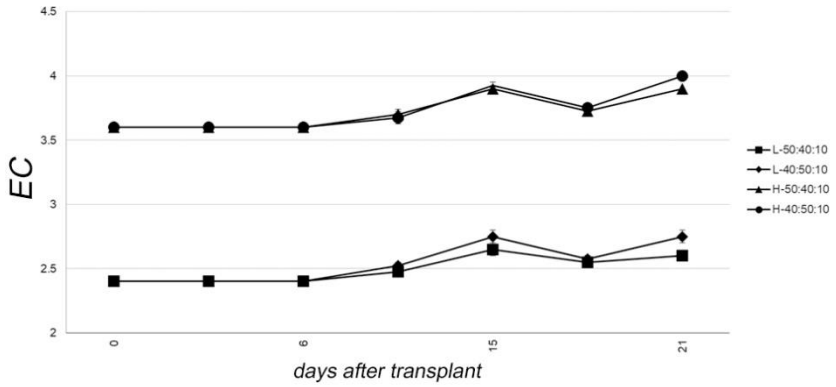


Figure 32: Mean weakly EC levels during the cultivation period. Vertical bars denote ± standard errors of means (n = 4).

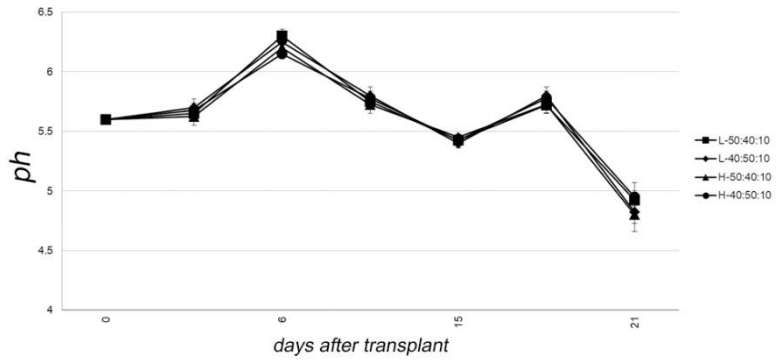


Figure 33: Mean weakly pH levels during the cultivation period. Vertical bars denote ± standard errors of means (n = 4).

To assess the effects on the yield of stamnagathi, we measured fresh and dry weight, as well as leaf number and leaf area at the harvest stage. The analysis revealed no significant impact from the independent variables of electrical conductivity (EC) levels or the ratio of potassium, calcium, and magnesium (K:Ca:Mg), nor from their interaction (EC × K:Ca:Mg), as shown in Table 11.

Table 11: Impact of different EC levels (low and high, as L and H respectively) and K:Ca:Mg ratios (40:50:10 and 50:40:10) on the on the fresh weight (FW), dry weight (DW), leaf number (LN) and leaf area (LA) of stamnagathi cultivated in a hydroponic floating raft system

Main Effects					
Factor	Treatment	FW (g plant^{-1})	DW (g plant^{-}1)	LN No plant^{-}1	LA (cm2 plant^{-}1)
EC level	L	9.179 ± 0.662	0.815 ± 0.136	20.98 ± 1.517	137.75 ± 9.253
	H	7.863 ± 0.711	0.823 ± 0.154	17.56 ± 0.836	123.43 ± 9.849
K:Ca:Mg ratio	40:50:10	7.763 ± 0.614	0.766 ±0.154	18.19 ± 1.100	123.67 ± 8.133
	50:40:10	9.279 ±0.744	0.873 ± 0.136	20.34 ± 1.394	137.50 ± 10.807
Statistical Significance					
EC level		ns	ns	ns	ns
K:Ca:Mg ratio		ns	ns	ns	ns
EC × K:Ca:Mg ratio		ns	ns	ns	ns

The values are means (n = 4). No significant differences were observed according to Duncan's multiple comparison test ($p < 0.05$): ns indicates "non significance" at $p < 0.05$.

On the other hand, the high EC level (H) had a positive effect of 10.73% increase of the potassium content of the leaf tissues compared to the low EC level (L). Moreover, the 40:50:10 also demonstrated a positive effect of 39.03% on leaf calcium concentration compared to the 50:40:10 treatment. Nevertheless, interactions between EC and K:Ca:Mg were not observed according to the statistical analysis (Table 12).

Table 12: Impact of different EC levels (low and high, as L and H respectively) and K:Ca:Mg ratios (40:50:10 and 50:40:10) on the concentration of macronutrient (K, Ca, Mg and P) in leaf tissues of hydroponically grown stamnagathi.

Factor	Treatment	Main Effects K (mg g^{-1})	Ca (mg g^{-1})	Mg (mg g^{-1})	P (mg g^{-1})
EC level	L	51.25 ± 1.50 b	3.68 ± 0.37	2.27 ± 0.11	7.66 ± 0.78
	H	56.75 ± 1.92 a	3.72 ± .38	2.07 ± 0.09	8.62 ± 0.41
K:Ca:Mg ratio	40:50:10	54.00 ± 1.77	4.31 ± 0.37 a	2.17 ± 0.11	8.24 ± 0.60
	50:40:10	54.00 ± 2.23	3.10 ±0.22 b	2.17 ± 0.12	8.04 ± 0.69
Statistical Significance					
EC level		*	ns	ns	ns
K:Ca:Mg ratio		ns	*	ns	ns
EC × K:Ca:Mg ratio		ns	ns	ns	ns

The values presented are means (n = 4). For each individual factor, or combination of factors where interactions were significant, means within the same column followed by different letters denote significant differences as determined by Duncan's multiple comparison test ($p < 0.05$). Asterisks (*) indicate significance at $p < 0.05$, while "ns" denotes results that are not statistically significant.

Analysis from the micronutrients, iron, manganese, boron, zinc, and copper, and sodium demonstrate a significant increase on the Mn and Zn leaf composition of plants cultivated under low EC conditions (Table 13 and Table 14).. The manganese concentration was increased under L conditions by 16.77% compared to that of plants cultivated under H EC conditions. Zinc concentration of plants that underwent the H treatment was higher by 13.41% compared to plants cultivated under the L treatment. No effects of the micronutrient concentration of the leaf tissues was observed due to the different K:Ca:Mg ratios. Moreover, no interactions were observed between EC levels and K:Ca:Mg ratios.

Table 13: Impact of different EC levels (low and high, as L and H respectively) and K:Ca:Mg ratios (40:50:10 and 50:40:10) on the concentration of micronutrients (Fe, Mn, B) in leaf tissues of hydroponically grown spiny chicory.

Factor	Treatment	Main Effects Fe (µg g⁻1)	Mn (µg g⁻1)	B (µg g⁻1)
EC level	L	114.69 ±5.24	131.7 ± 3.55 a	63.21 ± 5.03
	H	110.75 ± 4.23	112.79 ± 3.47 b	62.87 ± 5.71
K:Ca:Mg ratio	40:50:10	116.11 ± 5.64	122.68 ± 5.21	64.53 ± 6.20
	50:40:10	109.33 ± 3.39	121.82 ± 4.79	61.55 ± 4.34
Statistical Significance				
EC level		ns	*	ns
K:Ca:Mg ratio		ns	ns	ns

EC × K:Ca:Mg ratio	ns	ns	ns

The values presented are means (n = 4). For each individual factor, or combination of factors where interactions were significant, means within the same column followed by different letters denote significant differences as determined by Duncan's multiple comparison test ($p < 0.05$). Asterisks (*) indicate significance at $p < 0.05$, while "ns" denotes results that are not statistically significant.

Table 14: Impact of different EC levels (low and high, as L and H respectively) and K:Ca:Mg ratios (40:50:10 and 50:40:10) on the concentration of micronutrients (Zn, Cu, Na) in leaf tissues of hydroponically grown spiny chicory.

Factor	Treatment	Main Effects Zn ($\mu g\ g^{-1}$)	Cu ($\mu g\ g^{-1}$)	Na ($mg\ g^{-1}$)
EC level	L	112.59 ± 2.61 a	22.5 ± 3.99	0.68 ± 0.04
	H	99.28 ± 4.40 b	16.88 ± 1.14	0.66 ± 0.04
K:Ca:Mg ratio	40:50:10	105.84 ± 3.51	21.12 ± 4.31	0.69 ± 0.05
	50:40:10	106.03 ± 5.16	18.26 ± 0.43	0.66 ± 0.03
		Statistical Significance		
EC level		*	ns	ns
K:Ca:Mg ratio		ns	ns	ns
EC × K:Ca:Mg ratio		ns	ns	ns

The values presented are means (n = 4). For each individual factor, or combination of factors where interactions were significant, means within the same column followed by different letters denote significant differences as determined by Duncan's multiple comparison test ($p < 0.05$). Asterisks (*) indicate significance at $p < 0.05$, while "ns" denotes results that are not statistically significant.

The nitrate-N ($mg\ kg^{-1}$ of fresh weight) was solely affected by the EC levels (Table 15). Higher EC led to higher leaf nitrate content by 29.40% compared to that of plants cultivated under L EC conditions. Additionally, the different K:Ca:Mg ratios did not results in any differences on the nitrate-nitrogen leaf content. Finally, the total nitrogen ($mg\ g^{-1}$ of dry weight) was not affected by neither EC levels, K:Ca:Mg or their interaction.

Table 15: Impact of different EC levels (low and high, as L and H respectively) and K:Ca:Mg ratios (40:50:10 and 50:40:10) on the concentration of nitrates in fresh tissues and total nitrogen in dry tissues of hydroponically grown spiny chicory.

Factor	Treatment	Main Effects NO_3-N ($mg\ kg^{-1}$ fw)	Total N ($mg\ g^{-1}$ dw)
EC level	L	721.21 ± 57.52 b	48.99 ± 1.12
	H	933.28 ± 33.95 a	47.98 ± 0.84
K:Ca:Mg ratio	40:50:10	822.53 ± 49.63	49.05 ± 1.13
	50:40:10	833.53 ± 82.95	47.92 ± 0.84
		Statistical Significance	

EC level	*	ns
K:Ca:Mg ratio	ns	ns
EC × K:Ca:Mg ratio	ns	ns

The values presented are means (n = 4). For each individual factor, or combination of factors where interactions were significant, means within the same column followed by different letters denote significant differences as determined by Duncan's multiple comparison test ($p < 0.05$). Asterisks (*) indicate significance at $p < 0.05$, while "ns" denotes results that are not statistically significant.

3.2 Results: Impact of nitrogen levels, irrigation, and biostimulant application on yield and chemical traits of stamnagathi grown in perlite bags

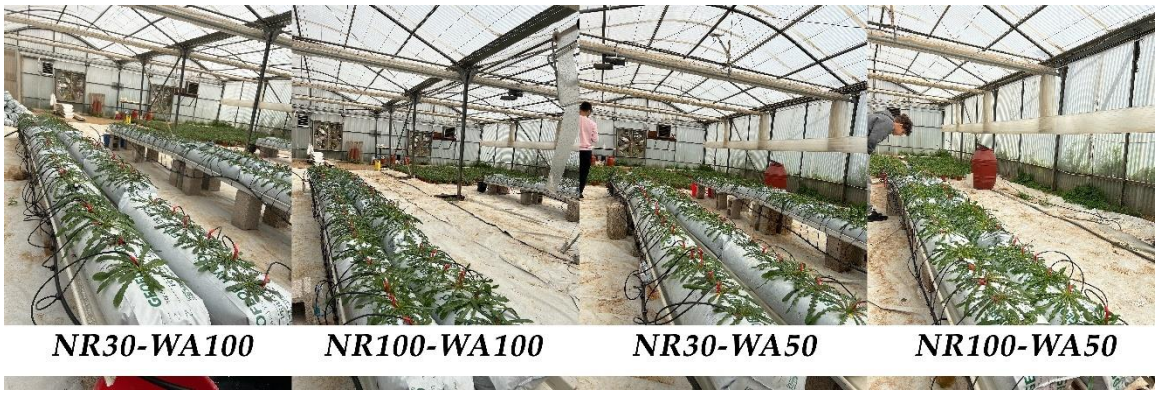


Figure 34: Cultivation of C.spinosum L., under 2 different nitrogen rates and irrigation regimes in on the day of harvest.

Nitrogen rates (NR) significantly influenced the agronomical characteristics of hydroponically cultivated stamnagathi. Low nitrogen application (NR30) led to significantly inferior quantitative agronomical characteristics compared to the control group (NR100). Specifically, plants subjected to nitrogen stress exhibited reduced leaf number, leaf area, fresh weight, and dry weight relative to those grown under NR100 conditions. However, an interesting observation was that dry matter content was higher in the NR30 group compared to NR100. Similarly, water availability (WA) had a significant impact on plant growth parameters, mirroring the effects of reduced nitrogen rates. Plants experiencing drought stress (WA50) demonstrated reduced leaf number, leaf area, fresh weight, and dry weight compared to those that received sufficient irrigation (WA100). Biostimulant application (B) also affected plant growth in a similar manner to that of nitrogen rates. Plants sprayed with the nitrogen-rich biostimulant (BS) exhibited increased

leaf number, leaf area, fresh weight, and dry weight, though dry matter content was not significantly affected by the biostimulant application (Table 16).

Table 16: Main effects of nitrogen rate (NR), water availability (WA) and biostimulant application (B) on the leaf number (LN), leaf area (LA), leaf fresh weight (LFW), leaf dry weight (LDW) and dry matter content (DMC) of hydroponically grown stamnagathi.

Factor	Sources of variation	LN (No plant^{-1})	LA (cm^2 plant^{-1})	LFW (g plant^{-1})	LDW (g plant^{-1})	DMC (%)
NR	NR100	16.78 ± 0.39 a	252.88 ± 4.96 a	17.87 ± 0.39 a	1.4 ± 0.03 a	7.85 ± 0.09 b
	NR30	14.51 ± 0.28 b	189.84 ± 5.65 b	14.16 ± 0.31 b	1.19 ± 0.02 b	8.45 ± 0.12 a
WA	WA100	16.35 ± 0.47 a	229.61 ± 9.37 a	16.47 ± 0.65 a	1.33 ± 0.04 a	8.23 ± 0.17
	WA50	14.93 ± 0.28 b	213.11 ± 7.01 b	15.56 ± 0.32 b	1.26 ± 0.03 b	8.07 ± 0.05
B	NoBS	15.08 ± 0.46 b	212.84 ± 9.59 b	15.46 ± 0.59 b	1.26 ± 0.04 b	8.25 ± 0.15
	BS	16.21 ± 0.32 a	229.88 ± 6.68 a	16.57 ± 0.4 a	1.33 ± 0.03 a	8.05 ± 0.08
		Statistical Significance				
	NR	***	***	***	***	***
	WA	**	*	*	*	ns
	B	**	**	**	*	ns
	NR*WA	**	*	***	ns	***
	NR*B	**	*	*	ns	*
	WA*B	ns	ns	ns	ns	*
	NR*WA*B	ns	ns	ns	ns	*

Values are means (n=24). In each column, mean within the same factor interaction followed by different letters indicate significant differences according to Duncan' s multiple range test for $p \leq 0.05$: *, **, ***, and "ns" indicate significance at $p < 0.05$, 0.005, 0.001, and non-significant, respectively.

The simultaneous effect of nitrogen rates and water availability demonstrated significant differences in terms of the leaf fresh weight of the cultivated stamnagathi plants (Figure 35a). When the plants were cultivated under sufficient nitrogen conditions, the drought stress (NR100-WA50) significantly reduced leaf fresh weight. When plants were cultivated under limited nitrogen conditions the fresh weight was further reduced but was not further diminished by the drought stress (NR30-WA100, and NR30-WA50). On the other hand, the biostimulant application appeared to have a significantly positive effect on the leaf fresh weight of plants cultivated under limited nitrogen conditions (NR30-BS) even though that effect was not strong enough to equalize the leaf fresh weight

to values as high as those of plants cultivated with sufficient nitrogen (NR100-BS, NR100-NoBS) as illustrated in (Figure 35b).

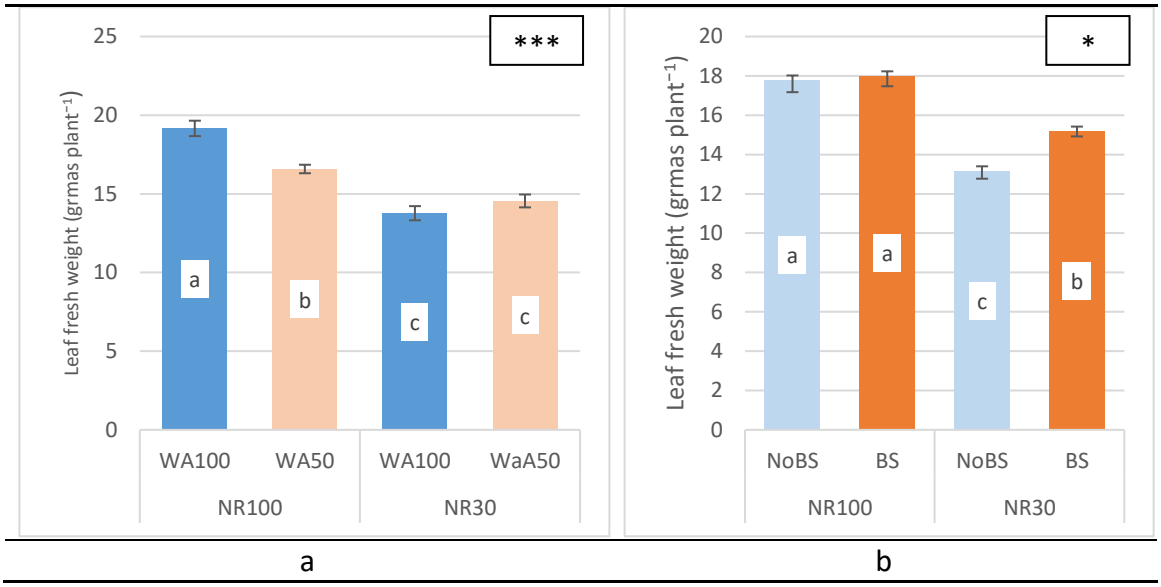


*Figure 35: a) Simultaneous effects of nitrogen rate and drought levels, on the leaf fresh weight of hydroponically cultivated stamnagathi plants. b) Simultaneous effects of nitrogen rate and biostimulant application, on the leaf fresh weight of hydroponically cultivated stamnagathi Bars are mean values ± standard error (n=12). Means with different letters indicate significant differences according to Duncan's multiple range test: *, and ***, indicate significance at $p < 0.05$, and 0.001, respectively.*

The interaction between nitrogen rate and water availability (NR*WA) revealed significant variations for leaf number and leaf area (Table 17). Plants under the combination of high nitrogen and full irrigation (NR100-WA100) exhibited the highest leaf number and leaf area compared to its water-starved counterpart. Plants that underwent the low nitrogen treatment and were fully irrigated (NR30-WA50) scored as low values as the ones of reduced irrigation (NR30-WA50) for fresh weight and leaf area. The leaf number of the NR30-WA100 treatment was statistically similar to both that of NR30-WA50 and NR100-WA50. The simultaneous effect of nitrogen rate and biostimulant application (NR*B) demonstrated some significant differences. The highest leaf area, and leaf number values were observed in the treatment where the supplied nitrogen was at a sufficient level (NR100) regardless of the biostimulant application. On the other hand, biostimulant application demonstrated a significant increase of the leaf number and leaf area of plants that were cultivated under limited nitrogen conditions (NR30-BS) compared to plant that were not sprayed with the biostimulant (NR30-NoBS). Nevertheless, the biostimulant

application partially mitigated the nitrogen stress since the observed values were lower compared to the plants cultivated under the NR100 treatments. The leaf dry weight on the other hand appeared unaffected between these interactions. Additionally, the interaction between drought stress and biostimulant application (WA*B) did not demonstrate significant effects on the growth parameters.

*Table 17: Simultaneous effect of nitrogen rates and water availability (NR*WA), nitrogen rates and biostimulants application (NR*B), and water availability and biostimulant application (WA*B) on leaf number (LN), leaf area (LA) and leaf dry weight (LDW) of hydroponically cultivated stamnagathi plants.*

Factor Interaction	Sources of variation		LN (No plant^{-1})	LA (cm^2 plant^{-1})	LDW (g plant^{-1})
NR*WA	NR100	WA100	18.06 ± 0.48 a	267.99 ± 6.27 a	1.46 ± 0.04
		WA50	15.5 ± 0.31 b	237.77 ± 4.68 b	1.34 ± 0.02
	NR30	WA100	14.65 ± 0.40 bc	191.23 ± 7.74 c	1.21 ± 0.03
		WA50	14.36 ± 0.40 c	188.45 ± 8.55 c	1.18 ± 0.04
NR*B	NR100	NoBS	16.71 ± 0.58 a	252.55 ± 6.14 a	1.38 ± 0.04
		BS	16.85 ± 0.53 a	253.21 ± 8.07 a	1.41 ± 0.04
	NR30	NoBS	13.44 ± 0.21 c	173.13 ± 7.75 c	1.14 ± 0.03
		BS	15.57 ± 0.26 b	206.55 ± 4.74 b	1.25 ± 0.02
WA*B	WA100	NoBS	15.82 ± 0.77	221.53 ± 14.58	1.32 ± 0.05
		BS	16.89 ± 0.52	237.69 ± 11.95	1.35 ± 0.05
	WA50	NoBS	14.33 ± 0.41	204.15 ± 12.58	1.2 ± 0.04
		BS	15.53 ± 0.28	222.07 ± 5.72	1.31 ± 0.03
Statistical Significance					
NR*WA			**	*	ns
NR*B			**	*	ns
WA*B			ns	ns	ns

Values are means (*n*=12). In each column, mean within the same factor interaction followed by different letters indicate significant differences according to Duncan' s multiple range test: *, **, and "ns" indicate significance at $p < 0.05$, 0.005, and non-significant, respectively.

Dry matter content was affected significantly in all three interactions of the factors. As illustrated in Figure 36a, the simultaneous effect of nitrogen rates and water availability demonstrated that plants under the combination of high nitrogen and full irrigation (NR100-WA100) exhibited the lowest dry matter content whereas the combination of nitrogen stress and sufficient irrigation (NR30-WA100) showed the highest dry matter content.

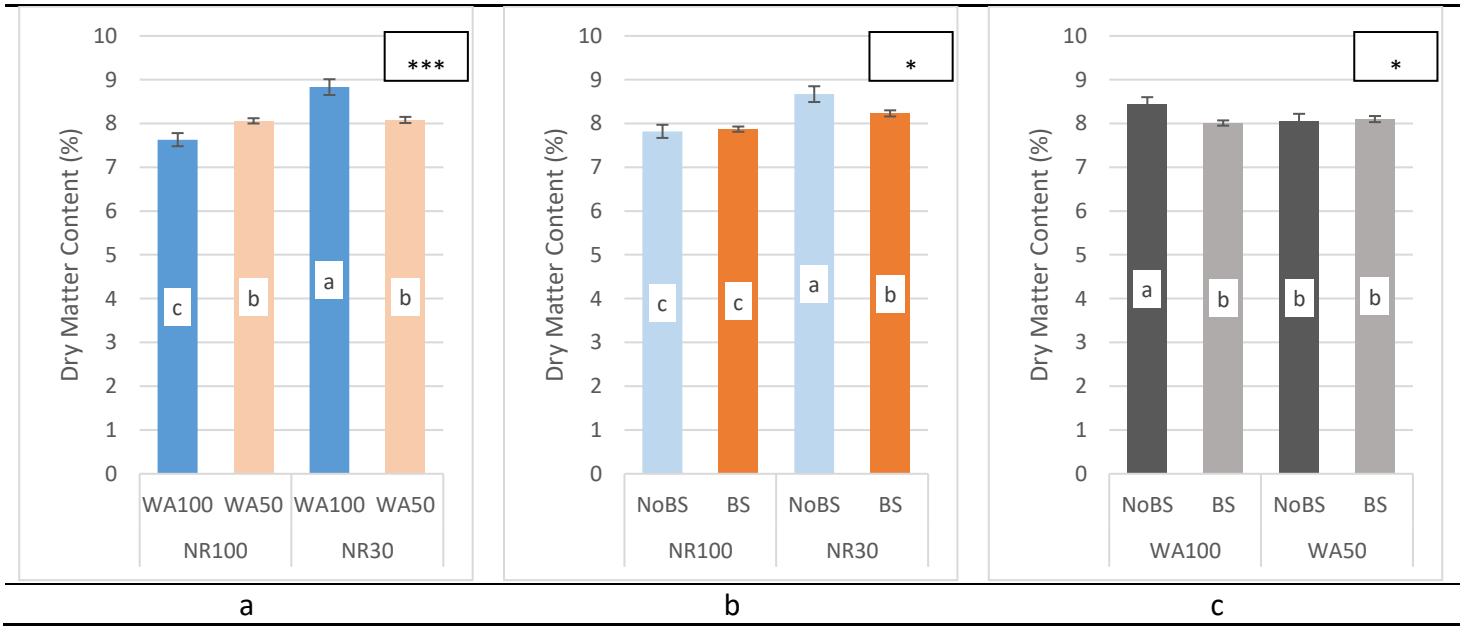


*Figure 36: Effects on dry matter content of the simultaneous effect of a) nitrogen rates and water availability (NR*WA), b) nitrogen rate and biostimulant application (NR*B), c) water availability and biostimulant application (WA*B) of hydroponically cultivated stamnagathi Bars are mean values ± standard error (n=12). Means with different letters indicate significant differences according to Duncan's multiple range test: *, and ***indicate significance at p < 0.05, and 0.001, respectively.*

The interaction between nitrogen rate and biostimulant application (NR*B) significantly affected the dry matter content (Figure 36b). Under sufficient nitrogen, dry matter content values remained low for both sprayed (NR100-BS) and not sprayed plants (NR100-NoBS) and did not significantly differ from each other. On the other hand, under low nitrogen rates, non-sprayed plants (NR30-NoBS) demonstrated the highest values, whereas biostimulant application (NR30-NoBS) lowered the dry matter content to an intermediate level. The simultaneous effect of water availability and biostimulant application (WA*B, Figure 36c) demonstrated that under sufficient irrigation conditions the biostimulant (WA100-BS) reduced the dry matter content to levels statistically similar

to those of plants cultivated under drought conditions regardless of the biostimulant application (WA30-BS, WA30-NoBS), while the highest dry matter content was observed on plants that were sufficiently irrigated and no biostimulant application took place (WA100-BS).

The simultaneous effect of all three factors nitrogen rate, water availability, and biostimulant application (NR*WA*B) affected significantly the dry matter content (Figure 37). The highest dry matter content was observed in the NR30-WA100-NoBS treatment. NR30-WA100-BS was significantly lower compared to the forementioned treatment but higher compared to the statistically similar NR100-WA100-NoBS and NR100-WA100-BS. The dry matter content of the rest of the treatments lingered between NR30-WA100-BS and NR100-WA100-NoBS/BS. On the other hand, the differences on leaf number, leaf area, leaf fresh weight and leaf dry weight could not be attributed to the interaction of the factors according to Duncan's multiple range test (Table 18)

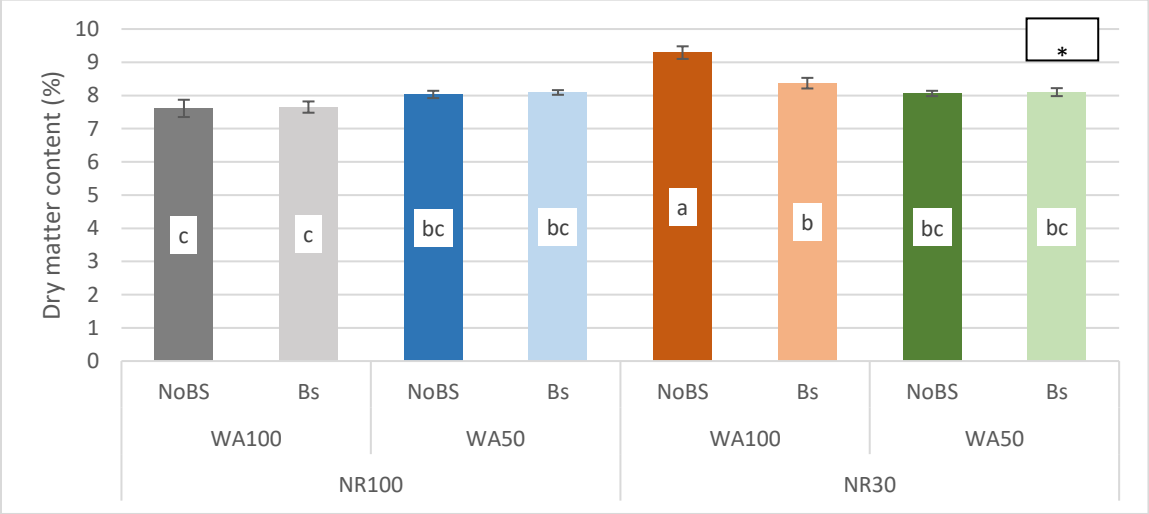


*Figure 37: Effect on dry matter content of the simultaneous effect of nitrogen rate, water availability, and biostimulant application (NR*WA*B). Bars are mean values ± standard error (n=6). Means with different letters indicate significant differences according to Duncan's multiple range test: * indicates significance at $p < 0.05$.*

*Table 18: Simultaneous effect of nitrogen rates, water availability, and biostimulant application (NR*WA*B), on leaf number (LN), leaf area (LA) and leaf fresh weight (LFW), and leaf dry weight (LDW) of hydroponically cultivated stamnagathi plants.*

NR	WA	B	LN (No plant^{-1})	LA (cm^2 plant^{-1})	LFW (g plant^{-1})	LDW (g plant^{-1})
NR100	WA100	NoBS	18.08 ± 0.71	264.08 ± 8.22	19.22 ± 0.74	1.46 ± 0.05
		BS	18.03 ± 0.72	271.91 ± 9.98	19.1 ± 0.73	1.46 ± 0.07
	WA50	NoBS	15.33 ± 0.49	241.02 ± 6.71	16.31 ± 0.43	1.31 ± 0.04
		BS	15.67 ± 0.42	234.51 ± 6.87	16.85 ± 0.34	1.36 ± 0.02
NR30	WA100	NoBS	13.56 ± 0.28	178.99 ± 12.00	12.7 ± 0.61	1.18 ± 0.05
		BS	15.75 ± 0.36	203.46 ± 7.74	14.84 ± 0.20	1.24 ± 0.02
	WA50	NoBS	13.33 ± 0.34	167.27 ± 10.33	13.61 ± 0.42	1.1 ± 0.04
		BS	15.39 ± 0.41	209.63 ± 5.93	15.49 ± 0.44	1.25 ± 0.04
			Statistical Significance			
	NR*WA*B		ns	ns	ns	ns

Values are means (n=6). In each column, means followed by different letters indicate significant differences according to Duncan's multiple range test for $p \leq 0.05$.

The nitrogen rates (NR) of the supplied nutrient solution significantly affected leaf nitrate, calcium and iron content (Figure 38a-c) as well as manganese, zinc and copper content (Figure 38d-e). Plants that were cultivated under limited nitrogen conditions (NR30) had significantly reduced nitrate and calcium content compared to the control (NR100). On the contrary, iron content increased in nitrogen deprived plants. In addition, manganese and copper content of nitrogen stressed plants demonstrated a statistically significant increase compared to plants cultivated under sufficient nitrogen conditions. On the contrary, leaf zinc content was lower in nitrogen stressed plants.

The drought levels, also affected the leaf nitrate content, magnesium and zinc content (Figure 39a-c). Plants that were cultivated under water stress (WA30) conditions demonstrated reduced nitrate and zinc levels, but magnesium levels in the leaves were increased compared to sufficiently irrigated plants (WA100).

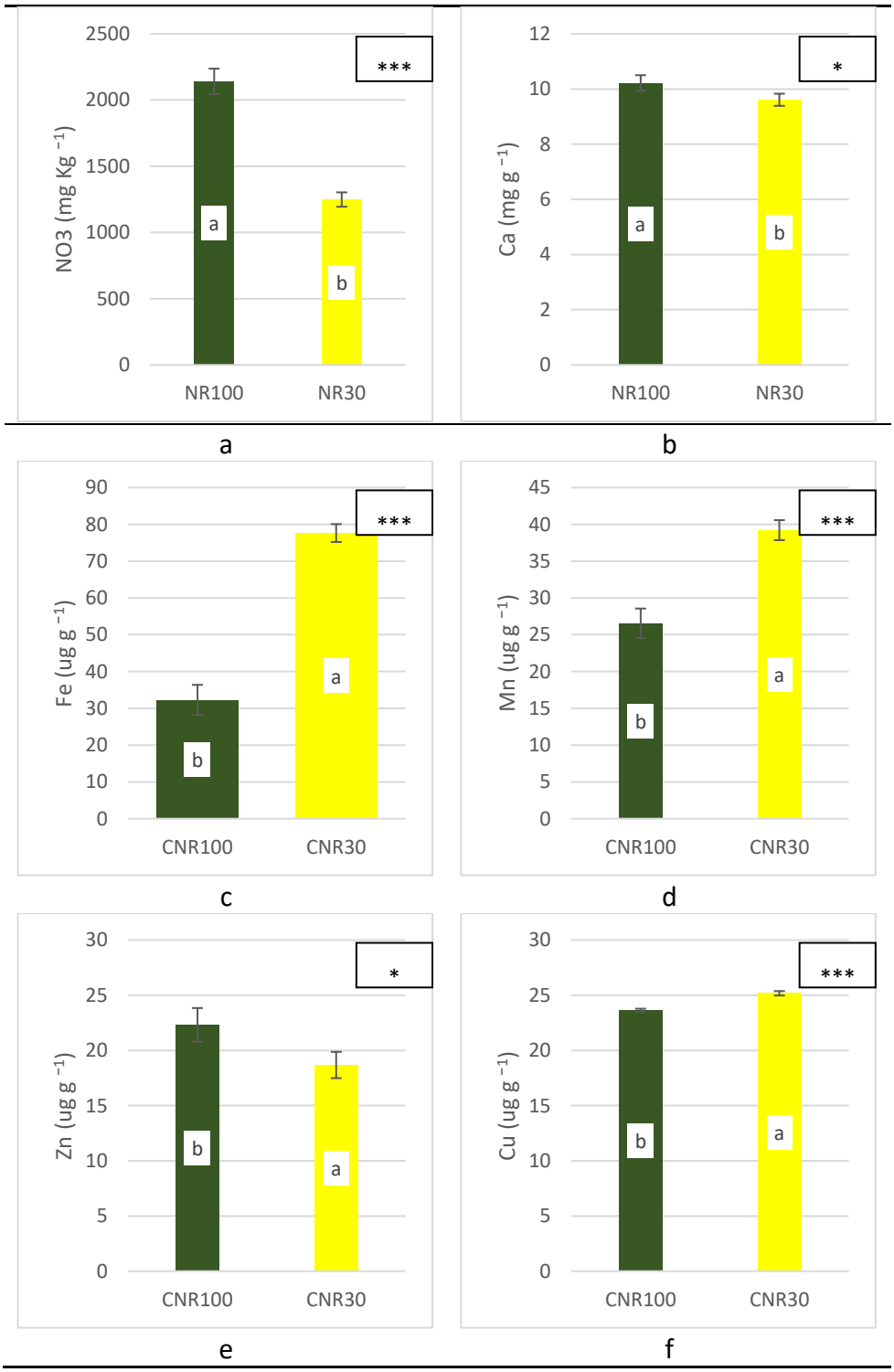


*Figure 38: Main effects of nitrogen rate on leaf a) nitrate, b) calcium, and c) iron leaf d) manganese, e) zinc, and f) copper content of hydroponically cultivated stamnagathi Bars are mean values ± standard error (n=24). Means with different letters indicate significant differences according to Duncan's multiple range test: *, and ***indicate significance at p < 0.05, and 0.001, respectively.*

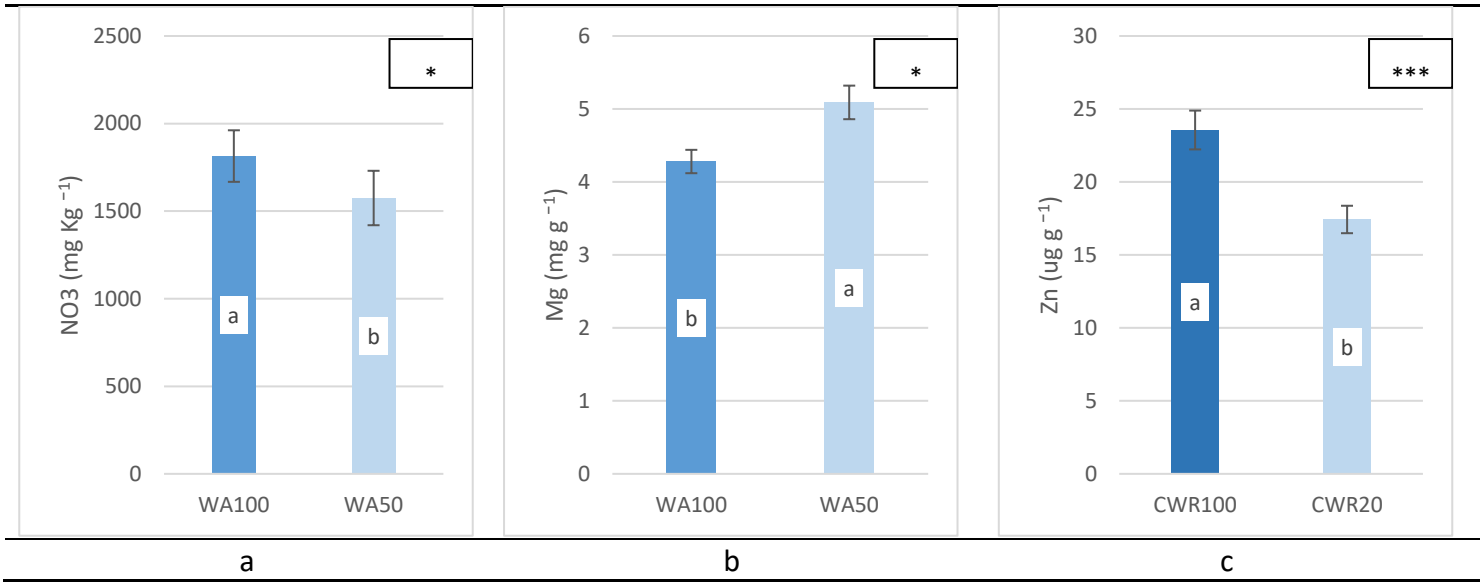


*Figure 39: Main effects of drought level on leaf a) nitrate, b) magnesium and c) zinc content of hydroponically cultivated stamnagathi Bars are mean values ± standard error (n=12). Means with different letters indicate significant differences according to Duncan's multiple range test: *, and ***indicate significance at p < 0.05, and 0.001, respectively.*

The simultaneous effect of nitrogen rates and water availability (NR*WA) had significant effects on the leaf calcium content (Figure 40). Under sufficient nitrogen (NR100), drought stress (WA50) lead to an increase in the calcium content of the leaves, whereas under limited nitrogen (NR30) drought did not significantly affect the calcium content. Hence, the highest calcium content was observed in plants that were sufficiently fertilized but were inadequately irrigated (NR100, WA50) compared to the rest of factor combinations.

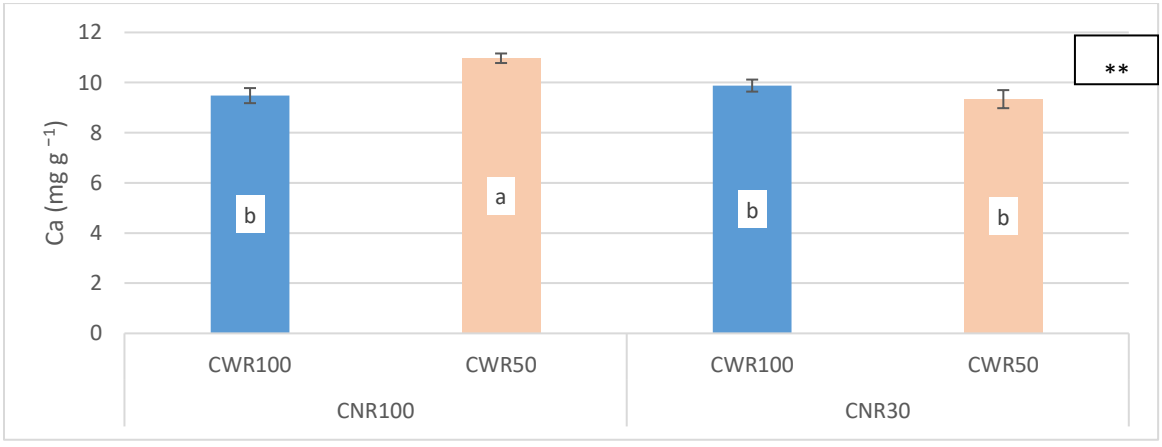


Figure 40: Simultaneous effects of nitrogen rates and drought levels on leaf calcium concentration of hydroponically cultivated stamnagathi. Bars are mean values ± standard error (n=12). Means with different

*letters indicate significant differences according to Duncan's multiple range test: **indicates significance at p < 0.005.*

The differences in the leaf nutrient content due to the simultaneous effect of all three factors nitrogen rate, water availability, and biostimulant application (NR*WA*B) could not be attributed to the interaction of the factors according to Duncans test (Table 20 and Table 21).

*Table 19: Simultaneous effect of nitrogen rates, water availability, and biostimulant application (NR*WA*B), on leaf nitrate, potassium, magnesium and calcium content of hydroponically cultivated stamnagathi*

NR	WA	B	NO_3 (mg Kg^{-1})	K (mg g^{-1})	Mg (mg g^{-1})	Ca (mg g^{-1})
NR100	WA100	NoBS	2186.71 ± 181.75	70 ± 1.15	4.2 ± 0.1	10 ± 0.24
		BS	2301.36 ± 247.95	58 ± 4.16	3.96 ± 0.38	8.95 ± 0.35
	WA50	NoBS	1990.53 ± 158.94	69.33 ± 4.67	5.04 ± 0.26	11.06 ± 0.18
		BS	2084.01 ± 240.42	67.33 ± 3.53	4.83 ± 0.61	10.88 ± 0.39
NR30	WA100	NoBS	1464.52 ± 33.14	66.67 ± 1.33	4.41 ± 0.48	9.39 ± 0.03
		BS	1305.19 ± 43.17	61.33 ± 0.67	4.56 ± 0.23	10.37 ± 0.23
	WA50	NoBS	1132.46 ± 79.41	63.33 ± 5.21	5.01 ± 0.22	9.38 ± 0.5
		BS	1092.81 ± 108.86	64 ± 1.15	5.48 ± 0.73	9.3 ± 0.64
			Statistical Significance			
NR*WA*B			ns	ns	ns	ns

Values are means (*n*=6). No significant differences were found according to Duncan's multiple range test for $p \leq 0.05$.

*Table 20: Simultaneous effect of nitrogen rates, water availability, and biostimulant application (NR*WA*B), on leaf iron, sodium, manganese, zinc and copper content of hydroponically cultivated stamnagathi*

NR	WA	B	Fe (ug g^{-1})	Na (mg g^{-1})	Mn (ug g^{-1})	Zn (ug g^{-1})	Cu (ug g^{-1})
NR100	WA100	NoBS	31.89 ± 8.18	2.64 ± 0.22	26.83 ± 6.42	23.61 ± 2.64	23.95 ± 0.31
		BS	31.97 ± 3.36	2.56 ± 0.36	26.73 ± 1.47	27.74 ± 3.14	23.77 ± 0.25
	WA50	NoBS	40.61 ± 10.69	2.93 ± 0.37	28.46 ± 4.27	20.05 ± 0.54	23.05 ± 0.08
		BS	24.65 ± 10.65	3.12 ± 0.6	24.16 ± 4.7	17.86 ± 2.49	23.73 ± 0.27
NR30	WA100	NoBS	72.58 ± 4.02	2.48 ± 0.52	39.31 ± 2.31	21.64 ± 2.13	25.16 ± 0.48
		BS	84.66 ± 0.36	2.08 ± 0.08	44.95 ± 2.11	21.25 ± 2.04	25.46 ± 0.44

WA50	NoBS	76.77 ± 3.7	2.4 ± 0.24	37.77 ± 0.53	18.14 ± 0.34	25.11 ± 0.05
	BS	76.52 ± 7.94	2.45 ± 0.14	34.83 ± 1.92	13.67 ± 1.48	24.98 ± 0.65
		Statistical Significance				
NR*WA*B		ns	ns	ns	ns	ns

Values are means (n=6). No significant differences were found according to Duncan's multiple range test for $p \leq 0.05$.

3.3 Results: Can long photoperiods be utilized to integrate *Cichorium spinosum* L. into vertical farms?

In this experiment the agronomical characteristics were greatly affected by the elongation of the photoperiod. Leaf number was the only agronomical parameter that was not affected. The other parameters, leaf area, fresh weight and dry weight were significantly increased by 62.19%, 83.04% and 95.37% (Table 21). This was expected, since increased daily light integrals are linked to increased yields [163].

Table 21: Effect of photoperiod on the agronomical characteristics, namely leaf number (LN), leaf area (LA), leaf fresh weight and dry weight (FW, DW) and their ration (DW/FW), of plants cultivated on peat inside a climate chamber.

Treatment	**LN (No plant^{-1})**	**LA (cm^2 plant^{-1})**	**LFW (g plant^{-1})**	**LDW (g plant^{-1})**
LD	15 ± 1.14	123.57 ± 13.82 a	6.04 ± 0.72 a	0.422 ± 0.06 a
SD	14.7 ± 0.74	76.19 ± 5.73 b	3.3 ± 0.25 b	0.216 ± 0.01 b
Statistical Significance	ns	*	*	*

Means followed by different letters within a column are significantly different as determined by Duncan's test ($P \leq 0.05$; n = 10)

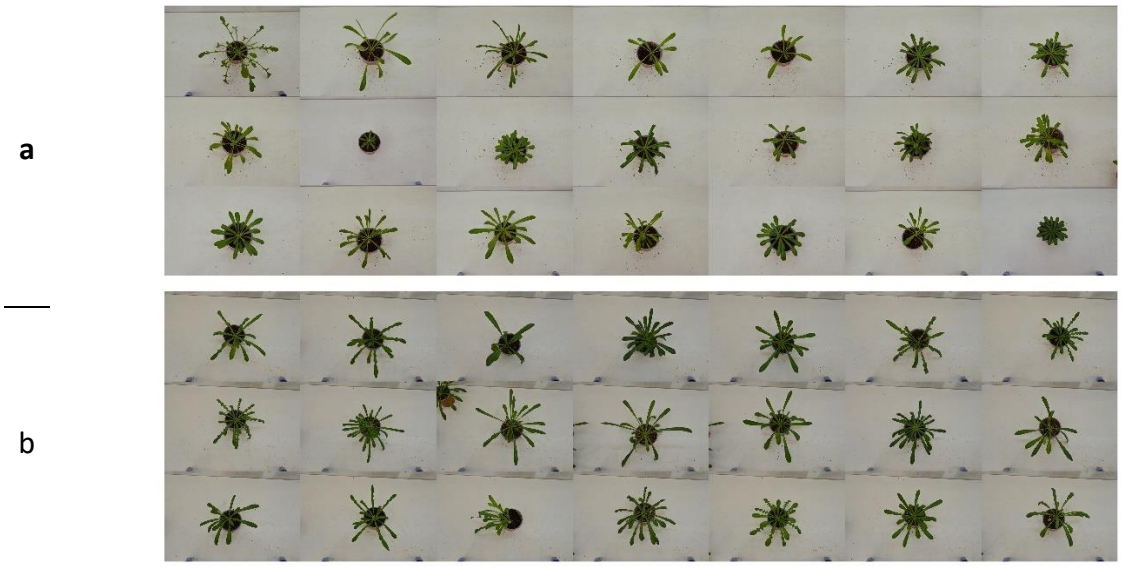


Figure 41: Pictures of individual Cichorium spinosum L., plants at harvest cultivated under a) long photoperiod and b) short photoperiod.

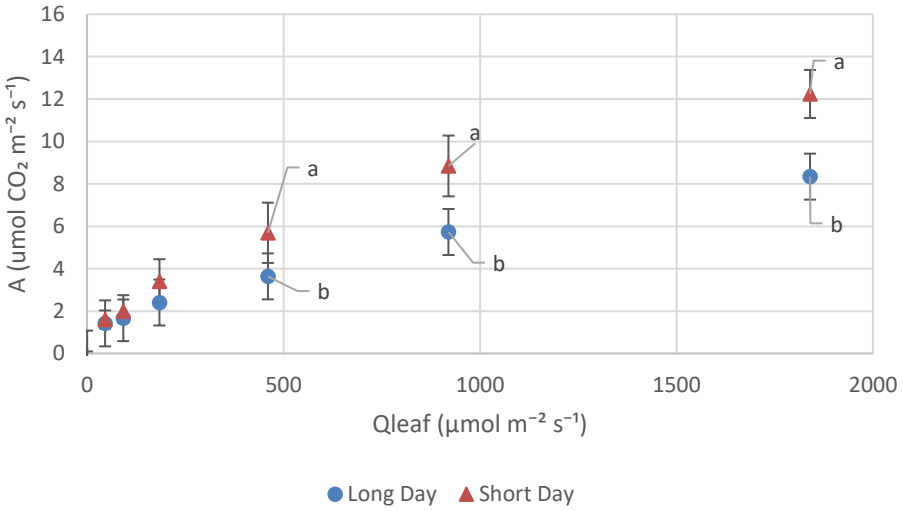


Figure 42: Photosynthetic curve (A, µmol CO_2 m^{-2} s^{-1}) of plant cultivated under long and short photoperiods. Qleaf: light intensity (µmol m^{-2} s^{-1}). Statistically significant differences according to Duncan's multiple range test for p≤0.001 were observed for the recorded values 460, 920 and 1840 µmol m^{-2} s^{-1}.

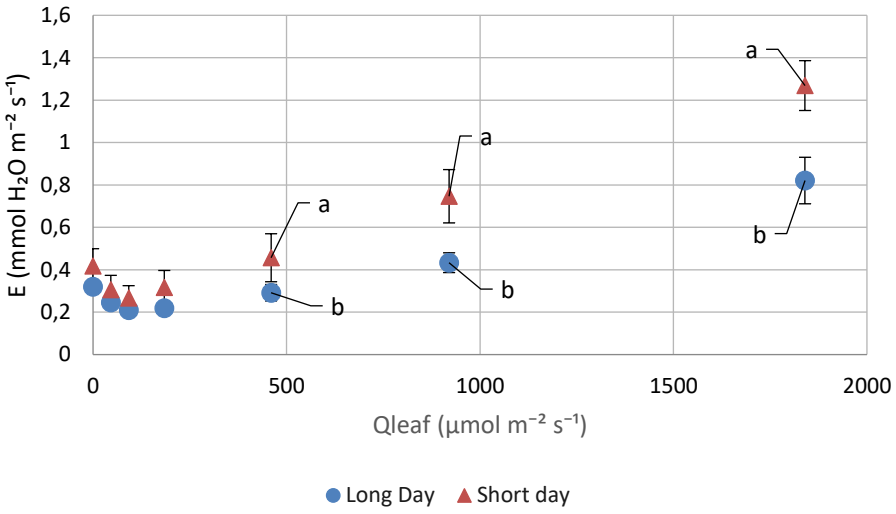


Figure 43: Evapotranspiration rate (E, mmol H_2O m^{-2} s^{-1}) of plant cultivated under long and short photoperiods. Qleaf: light intensity (µmol m^{-2} s^{-1}). Statistically significant differences according to Duncan's multiple range test for p≤0.005 were observed for the recorded values 460, 920 and 1840 µmol m^{-2} s^{-1}.

Leaf gas exchange was significantly affected when the lighting intensity increased to the 460 µmol m^{-2} s^{-1}. The photosynthetic compensation point was observed around the light intensity of 50 µmol m^{-2} s^{-1}. Photosynthetic capacity of plants cultivated under a 10-hour photoperiod (Short day) was 36.12%, 35.21%, and 31.01% less compared to that of plant cultivated under 15 hours photoperiod (Long day) for measurements recorded at 460, 920, and 1840 µmol m^{-2} s^{-1}. Evapotranspiration rate of Short day plants was also observed to be 36.13%, 41.96%, and 34.26% less compared to that of long day plants for measurements recorded at 460, 920, and 1840 µmol m^{-2} s^{-1}, respectively.

Chlorophyll fluorescence measurements of the parameters ΦPSII, ETR, and qN did not demonstrate any significant differences between the measured parameters per light intensity of incident light. For values of light intensity of *74, plant that grew under an extended photoperiod demonstrated an increase in qP by 6% compared to short day plants. On the other hand, for light intensities 631 and 938 µmol m^{-2} s^{-1},* plants of long day plants demonstrated reduced values by 5% and 9% compared to short day plants.

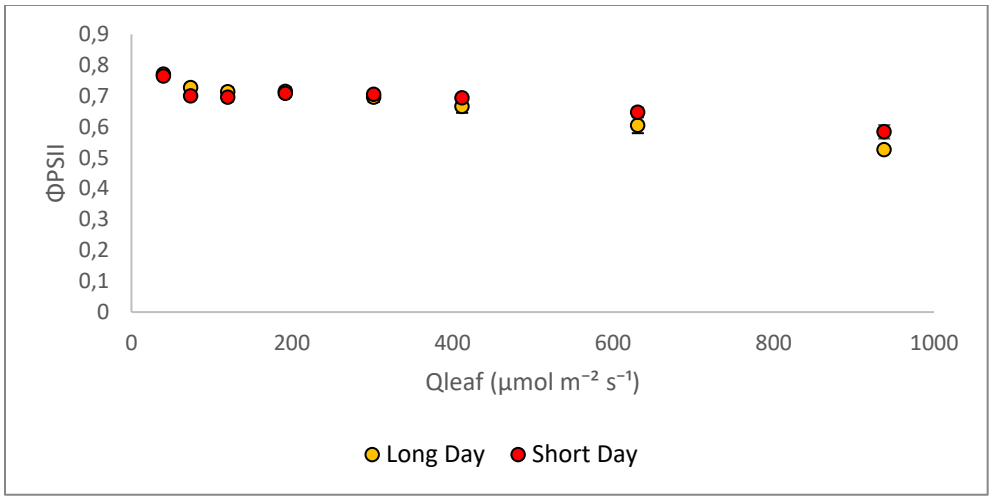


Figure 44: Quantum yield of photosystem II of plants cultivated under long and short photoperiods. Long Day (15 hours) and Short Day (10 hours). For each parameter, values are means of 5 plants per replicate of each light treatment (n=3). There were no statistically significant differences according to Duncan's multiple range test for p≤0.05.

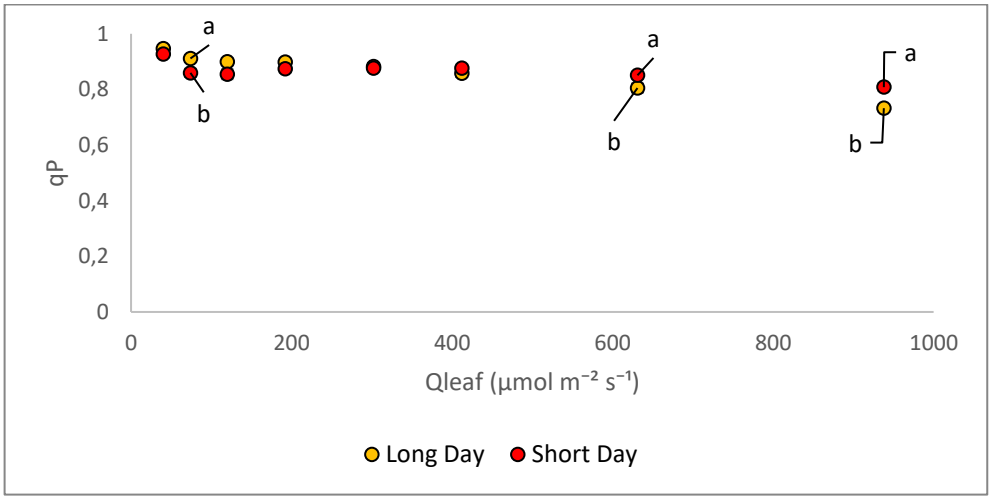


Figure 45:Photochemical quenching of plants cultivated under long and short photoperiods. Long Day (15 hours) and Short Day (10 hours). For each parameter, values are means of 5 plants per replicate of each light treatment (n=3). Statistically significant differences according to Duncan's multiple range test for p≤0.005 were observed for the recorded values 74, 631 and 938 µmol m⁻² s⁻¹.

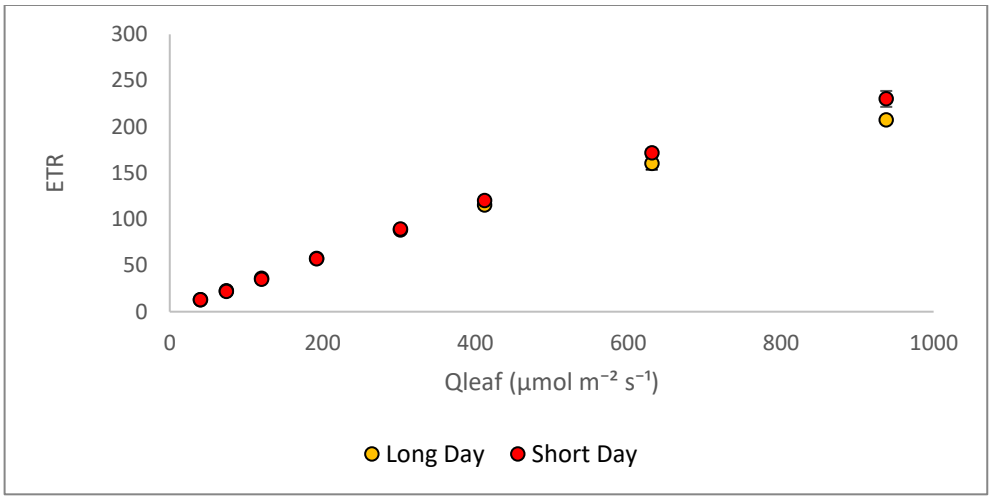


Figure 46: Electron transport rate of plants cultivated under long and short photoperiods. Long Day (15 hours) and Short Day (10 hours). For each parameter, values are means of 5 plants per replicate of each light treatment (n=3). There were no statistically significant differences according to Duncan's multiple range test for p≤0.05.

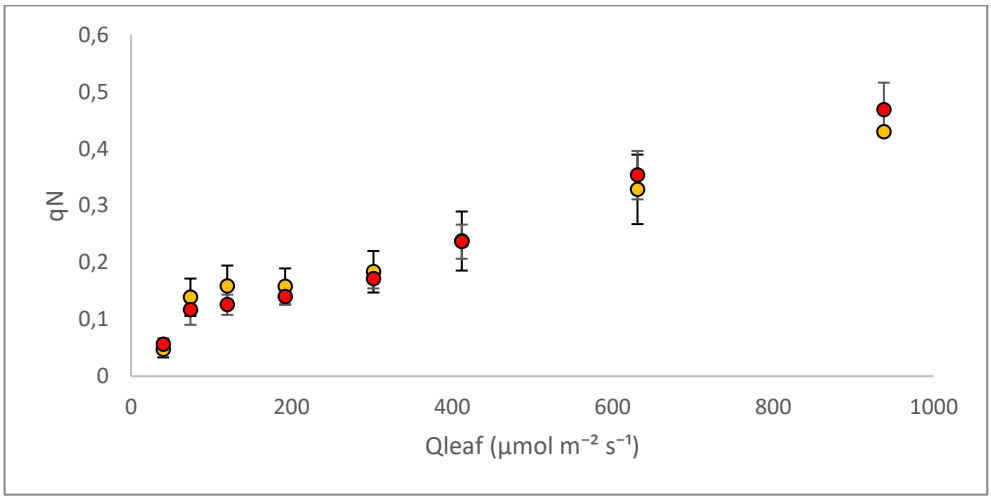


Figure 47:Non photochemical quenching of plants cultivated under long and short photoperiods. Long Day (15 hours) and Short Day (10 hours). For each parameter, values are means of 5 plants per replicate of each light treatment (n=3). There were no statistically significant differences according to Duncan's multiple range test for p≤0.05.

3.4 Results: Exploring the effects different white light spectra

At the end of the lighting experiment, plants from all treatments had reached a stage of an average 18, 19, and 17 leaves plant $^{-1}$, covering a leaf area of 151, 173, and 166 cm^2 plant^{-1} a fresh weight an average of 9.8, 10.1 and 10.2 g plant $^{-1}$ and having a dry weight of 1.0, 1.2 and 1.3 g plant^{-1} for S, N and F spectra respectively. Nevertheless, these differences were not statistically significant (Table 22).

Figure 48: Figure 2: Growth stages of stamnagathi plants grown at a 50 plants m–1 density, from the transplanting day to harvest from the Neutral, Full and SunLike™ spectra respectively. Pictures are indicative. No visual differences between the "white" light spectra treatments were observed.

Figure 49: Side and top view of stamnagathi plant at the harvest stage cultivated under the SunLike™ spectrum. A ruler was used for scaling. No significant differences were observed between plants of different lighting treatments. Figure is indicative of stamnagathi's morphology.

Table 22: Effect of three different kinds of "white light", a "SunLike" (S), a "neutral" (N), a "full" (F) and with B:G:R:FR ratios 21:34:36:7, 14:32:43:10 and 16:36:40:8, respectively, on leaf number (LN), leaf area (LA), leaf fresh weight (LFW), and leaf dry weight (LDW) of stamnagathi.

Treatment	LN (No plant^{-1})	LA (cm^2 plant^{-1})	LFW (g plant^{-1})	LDW (g plant^{-1})
S	17.5 ± 1.06	151 ± 23.7	9.8 ± 1.78	1 ± 0.16
N	19.1 ± 2.03	173 ± 31.8	10.1 ± 1.64	1.23 ± 0.24
F	17.1 ± 0.63	166 ± 16.6	10.2 ± 1.00	1.28 ± 0.16
Statistical significance	ns	ns	ns	ns

For each parameter, values are means of 12 plants per replicate of each light treatment (*n*=3). There were no statistically significant differences according to Duncan's multiple range test for $p \leq 0.05$. No statistical significance difference is depicted with the "ns" in the last row of the table.

From the morphological characteristics' measurements (Table 23), the average measured values were 472, 460, and 460 nm for the leaf thickness, 255, 253, and 234 µm for the palisade parenchyma thickness, 236, 230, and 225 µm for the spongy parenchyma thickness, for S, N, F spectra respectively. According to the statistical analysis, the observed values could not be attributed to the spectral differences. The stomatal density

for the S, N, and F spectra was 146, 159 and 150 stomata mm^{-1}, respectively and did not differ significantly between plants cultivated in the three treatments (Table 24).

Table 23: Effect of three different kinds of "white light", a "SunLike" (S), a "neutral" (N), a "full" (F) and with B:G:R:FR ratios 21:34:36:7, 14:32:43:10 and 16:36:40:8, respectively, on leaf thickness (LT), palisade parenchyma thickness (PT), and spongy parenchyma thickness (ST) of stamnagathi

Treatment	LT (mm)	PT (mm)	ST (mm)
S	0.47 ± 0.02	0.25 ± 0.01	0.23 ± 0.01
N	0.46 ± 0.01	0.25 ± 0.01	0.23 ± 0.01
F	0.46 ± 0.01	0.23 ± 0.01	0.22 ± 0.00
Statistical significance	ns	ns	ns

For each parameter, values are means of 6 plants per treatment cultivated under each light source (*n*=3). There were no statistically significant differences according to Duncan's multiple range test for $p \leq 0.05$. No statistical significance difference is depicted with the "ns" in the last row of the table.

The values of the stomatal length and stomatal width were the only ones that were affected by the spectral qualities. Moreover, the stomatal length and stomatal width of the N treatment (20.0 and 9.13 μm) were greater compared to the F (25.9 and 12.9 μm), which in turn were greater compared to the S (21.9, and 10.1 μm).

Table 24: Effect of three different kinds of "white light", a "SunLike" (S), a "neutral" (N), a "full" (F) and with B:G:R:FR ratios 21:34:36:7, 14:32:43:10 and 16:36:40:8, respectively, on stomata density (SD), stomatal length (SL), and stomatal width (SW) of stamnagathi.

Treatment	SD (smm^{-2})	SL (μm)	SW (μm)
S	146 ± 7.34	20.0 ± 0.37 c	9.13 ± 0.21 c
N	159 ± 6.57	25.9 ± 0.47 a	12.9 ± 0.24 a
F	150 ± 4.30	21.9 ± 0.44 b	10.1 ± 0.22 b
Statistical significance	ns	*	*

For each parameter, values are means of 6 plants per treatment cultivated under each light source (*n*=3). Values of the same column accompanied by different letters indicate statistically significant differences according to Duncan's multiple range test: * indicates significance at $p < 0.05$, while "ns" non-statistically significant difference.

3.5 Results: Exploring the effects of different nutrient solutions

Stamnagathi's agronomical characteristics were unaffected by the different nutrient solution treatments (Table 25). The average observe leaf number was 20 leaves plant^{-1} for all treatments, the area was 347, 361, and 380 cm^2 plant^{-1}, average leaf fresh weight was 17.7, 18.9, and 20.6 g plant $^{-1}$ and dry weight was 1.3, 1.5, and 1.5 g plant^{-1}, for N10-Fe15, N4-Fe15, N10-Fe48 respectively. According to the statistical analysis these differences could not be ascribed to the different composition of the nutrient solution.

Table 25: Effect of the different nutrient solutions (NS) on leaf number (LN), leaf area (LA), leaf fresh weight (LFW), and leaf dry weight (LDW) of stamnagathi. The NS treatments were designed based on their N and Fe content. The control (10 mmol/L, and 15 µmol/L, N10-Fe15), the limited N (4 mmol/L and 15µmol/L, N4-Fe15) and the elevated iron (10 mmol/L and 48 µmol/L, N10-Fe48).

Treatment	LN (No plant^{-1})	LA (cm^2 plant^{-1})	LFW (g plant^{-1})	LDW (g plant^{-1})
N10-Fe15	20 ± 0.1	346.56 ± 29.69	17.65 ± 1.34	1.29 ± 0.1
N4-Fe15	20 ± 0.88	360.82 ± 29.37	18.87 ± 1.66	1.47 ± 0.12
N10-Fe48	20 ± 0.81	379.7 ± 20.36	20.55 ± 1.12	1.54 ± 0.08
Statistical significance	ns	ns	ns	ns

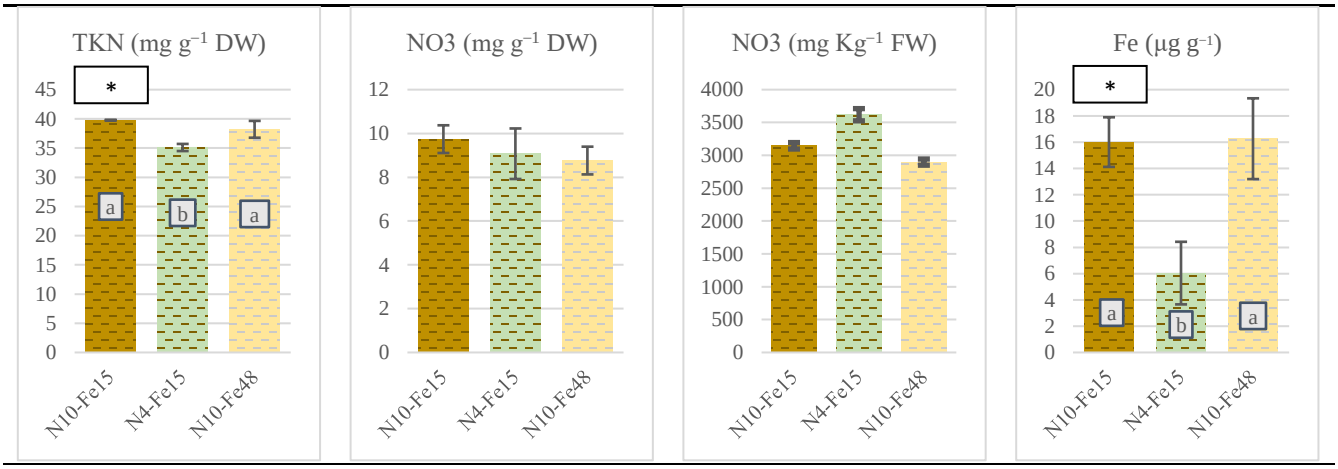
For each parameter, values are means of 12 harvested plants per replicate (*n*=3)., cultivated with the different nutrient solution recipes. There were no statistically significant differences according to Duncan's multiple range test for $p \leq 0.05$. No statistical significance difference is depicted with the "ns" in the last row of the table.

Figure 50: Side and top view of stamnagathi plant at the harvest stage cultivated under the control nutrient solution. For the top view photo, the plant was replaced into a bigger net pot. A ruler was used for scaling. No visual differences were observed between plants of different nutrient solution treatments. Figure is indicative of stamnagthi's morphology.

Figure 51: Growth stages of stamnagathi plants, grown at a 100 plants m¯1 density, from the transplanting day to harvest. Pictures are indicative of the growth rate. No visual differences were observed between the different nutrient solution treatments.

The chemical analysis of the total Kjeldahl N (Figure 52a) demonstrated that compared to the 39.76 mg g^{-1} dry weight observed in the control, N10-Fe15, the values of N4-Fe15 were significantly decreased to 35.09 mg g^{-1} dry weight, whereas the total Kjeldahl N of N10-Fe48, 38.21 mg g^{-1} dry weight, did not differ significantly from either of the previous treatments. The NO_3 concentration in the leaf tissues (Figure 52b-c) was not affected by neither the limited total-N nor the elevated Fe treatments.

The leaf iron content was significantly affected by the nutrient solution treatments (Figure 52d). In comparison to the control's observed value, 16.01 µg g^{-1} dry weight, the content of N4-Fe15 was reduced to 6.04 µg g^{-1} dry weight, whereas the content of the N10-Fe48, 16.26 µg g^{-1} dry weight, demonstrated a slight increase but not statistically significant to differentiate from the control. The phosphorus content was also significantly affected by the nutrient solution treatments. The leaf P content of N10-Fe15 and N4-Fe 15 (5.69 and 5.39 mg g^{-1} dry weight) respectively, and was significantly surpassed by the content of N10-Fe48 which reached 7.94 mg g^{-1} dry weight (Figure 52e). In addition, the K and Ca content (Figure 52f-g) of the leaf tissues were 29 and 0.85, 31.33 and 1.06, 30 and 1.00 mg g^{-1} dry weight for N10-Fe15, N4-Fe15, N10-Fe48 respectively and were not significantly affected by the different nutrient solutions. On the contrary Mg levels (Figure 52h) demonstrated an increase compared to the control (1.52 mg g^{-1} dry weight), in the leaf tissues of both the N4-Fe15 (2.09 mg g^{-1} dry weight) and N10-Fe48 (1.95 mg g^{-1} dry weight).



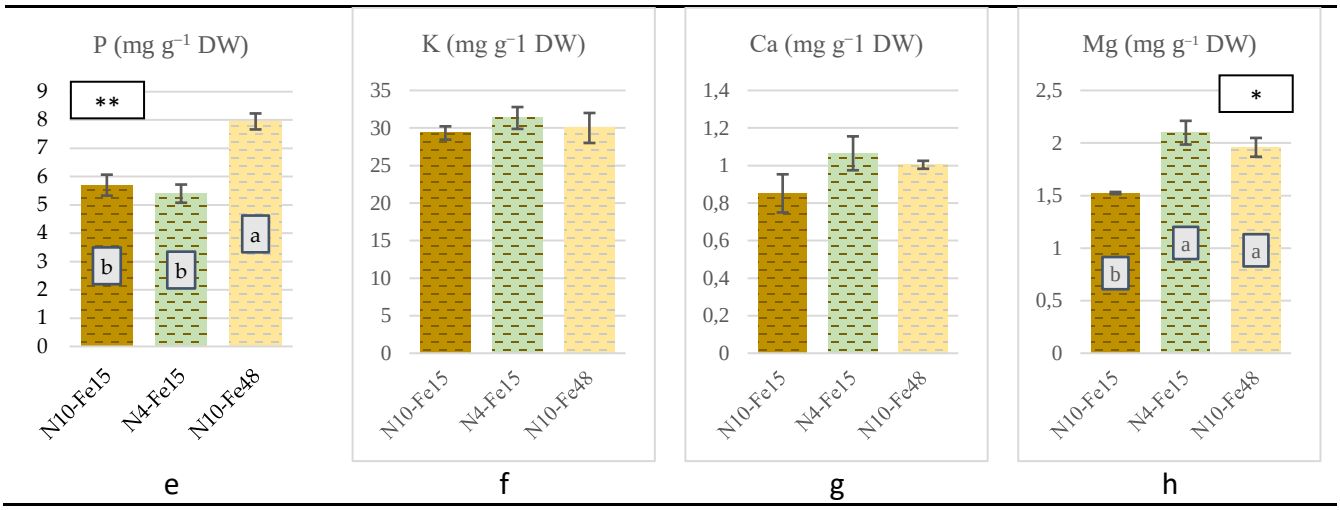


*Figure 52: Chemical composition of average leaves of stamnagathi plants cultivated with three different nutrient solutions, N10-Fe15, N4-Fe15, and N10-Fe48. (a) Total Kjeldahl Nitrogen (TKN), (b) Nitrate content (NO_3) in dry (b) and fresh (c) leaves of stamnagathi expressed, (d) Iron (Fe) content, (e) Phosphorus (P), (f) Potassium (K), (g) Calcium (Ca), and (h) Magnesium (Mg). Bars show means ± SE (n=3). Different letters indicate statistically significant differences according to Duncan's multiple range test: *, and **indicate significance at $p < 0.05$, and 0.005, respectively.*

4. Discussion

4.1 Discussion: Exploring the Simultaneous Effect of Total Ion Concentration and K:Ca:Mg Ratio of the Nutrient Solution on the Growth and Nutritional Value of Hydroponically Grown *Cichorium spinosum* L.

In recent years, few studies have been conducted on stamnagathi. Despite this, there has been a focus on its nutritional value, chemical composition and bioactive compounds content under salinity stress induced by NaCl [55] or other salts such as KCl, Na_2SO_4 and $CaCl_2$ [52]. Other studies have focused on the effects of different ecotypes, salinity and nitrogen supply on the biomass production of hydroponic spiny chicory [49] and on successive harvestings [51]. In every study, *C. spinosum* is identified as a highly adaptable wild edible vegetable, always rich in nutrients, especially when it comes to its potassium, calcium, iron, magnesium and zinc content [48]. In this study, the simultaneous effect of different EC levels (high, H and low, L) and K:Ca:Mg ratios (50:40:10 and 40:50:10) on the growth and nutrient concentration in dry leaf tissues of *C. spinosum* cultivated on a floating raft hydroponic system was evaluated. The two K:Ca:Mg ratios were chosen based on a previous study where the 50:40:10 ratio of those elements was also observed in the leaf tissues [91]. The uptake of sufficient amounts of Ca by plants requires the maintenance of a substantially higher Ca concentration in the root zone than the uptake concentration of Ca [154]. In order to avoid the starvation effects of either of the elements that would further induce changes in the anatomy, physiology and biochemistry of the plants, the 40:50:10 ratio was chosen. By studying the effect of this ratio on the growth of *C. spinosum,* we aimed to fine-tune the hydroponic solution supplied in hydroponics [155,164]. The findings of the present work also showcase *C. spinosum* as a highly adaptable edible green.

It has been suggested that increased K levels in the nutrient solution can increase the leaf area and number of leaves due to the effect that K ions have on cell division and in the control of cell expansion and turgor [165]. Moreover, it has been suggested that for lettuce and mustard, increased calcium in the nutrient solution could as also increase leaf

number, with or without the simultaneous increase in fresh weight [166]. Nevertheless, this was not observed in the current work, as our results indicate that none of the agronomical characteristics (fresh weight, dry weight, leaf number and leaf area) were affected by either the examined factors or their interaction (Table 11). These results indicate that stamnagathi has a wide range of adequacy for K and Ca, and the K and Ca levels applied through the NS in both tested ratios were within this range.

Throughout the experiment, the changes in EC and pH levels in the nutrient solution of each tank were monitored (Figure 32 and Figure 33). The EC levels increased slightly by the end of the growing period. This is ascribed to the accumulation of certain nutrients that were present in the starter solution and later supplied by the replenishment solution at a pace that was greater than the plants could absorb. The EC levels during a particular cropping period might also increase when the nutrient uptake rates remain stable, if the rates of water uptake increase due to changes in climatic conditions, since these two processes are physiologically independent [167]. This also underlines the importance of more balanced management of the nutrient solution, which should take into consideration the greenhouse microclimate.

Apart from the agronomical parameters, the nutrient concentrations in the leaf tissues were determined. The main finding is that there was no interaction between the two factors, but when looking only at the main effects, the EC factor affected the K concentration in the leaf tissues proportionally while the K:Ca:Mg ratio factor showed increased Ca concentration in the high Ca treatment (40:50:10). As seen from Tables 3–5, the different compositions of the nutrient solutions had a slight impact on the nutrient content of the plant tissues. The concentration of K in the leaf tissues increased from 51.25 mg g^{-1} in the L treatments to 56.75 mg g^{-1} in the H treatments, but the effect of the K:Ca:Mg ratio was insignificant (Table 12). On the contrary, the concentration of Ca in the leaf tissues increased under a lower K to Ca ratio from 3.10 mg g^{-1} under 50:40:10 conditions to 4.31 mg g^{-1} under 40:50:10 conditions and was not affected by the EC level. Towards the end, statistical analysis did not show any interaction between the EC and the K:Ca:Mg ratio for the macronutrient concentrations in the leaf tissues.

The effect of the composition of the nutrient solutions was even slighter on the plants for the micronutrient concentration (Table 13 and Table 14). The EC factor had a significant effect and only on the concentration of Mn (). Manganese concentrations were greater under low EC conditions. Mn concentration was 131.7 μg g^{-1} in the L treatment and 112.79 μg g^{-1} in the H treatment. It is clear that the K:Ca:Mg ratio did not affect any micronutrient. Furthermore, no interaction appeared between the two factors for the micronutrient concentration of the leaf tissues.

Finally, no interaction between the EC and the K:Ca:Mg ratio occurred for leaf nitrate-N concentration (Table 15). Nitrate appeared higher in the H treatment, 933.28 mg kg^{-1} of fresh weight, whereas it was 721.21 mg kg^{-1} of fresh weight in the L treatment. Furthermore, it was not affected by the K:Ca:Mg ratio. Nitrate concentration is very important to be kept under a certain threshold due to concerns that high amounts of NO_3^- in leafy vegetables could potentially result in life-threatening diseases such as methemoglobinemia or gastric cancer [168,169]. Since the high EC treatments had no significant impact on the yield, leaf number or leaf area, we consider the L treatments safer for consumption, even though both H and L maintained leaf nitrate levels dangerously close to the threshold for similar vegetables to *C. spinosum*, such as *C. intybus* [170]. The overall low nitrate level can also be ascribed to the design of the nutrient recipe and the use of a hydroponic floating raft system that can manipulate the root environment in a manner that contributes to the avoidance of increased nitrate levels in plant tissues [171,172]. Interestingly, the total nitrogen concentration of *C. spinosum* L. was not significantly affected by any of the studied factors or their interactions. The insignificant differences in the total nitrogen imply that the higher EC, which was imposed by increasing the concentration of N (and P, K, Ca, and Mg), did not further benefit the plants but only increased the nitrate concentration in the leaf tissues. Increased accumulation of nitrates in leafy vegetables occurs when the rate of absorbed nitrate is higher than the rate of its assimilation. Nitrate, being the main N source in hydroponic solutions and the main form that nitrogen is absorbed by plants, enters the plant cells and is reduced to nitrite via the nitrate reductase in the cytosol, and then nitrite, which is toxic to plant cells, is rapidly

reduced to ammonium in the plastids via the nitrite reductase. Ammonium then is assimilated into organic nitrogen via the actions of glutamate synthase, the glutamine synthetase cycle and the glutamate dehydrogenase pathway [173]. In the current experiment, the plants were grown under the same natural light conditions in all treatments. Hence, the increased amounts of unassimilated nitrogen in the leaves of plants treated with the higher EC is ascribed to excess nitrate in the nutrient solution and not to differences in lighting regimes or any other environmental factor [174–176].

As a wild plant, *C. spinosum* L. has high physiological efficiency in terms of produced biomass compared to domesticated commercial vegetables such as lettuce. Therefore, the unaffected macrocation concentration in the leaf tissues can be ascribed to its ability to easily acclimate to different conditions [49]. For instance, in an experiment carried out on two butterhead lettuce cultivars by Corrado et al. [28], the effect of three NSs differing in the K:Ca:Mg ratios were examined as follows, 68:16:16, 16:68:16 and 16:16:68, and the authors found that the nutrient concentration in the leaf tissue was significantly affected by the treatments. The difference between that study and our results is ascribed to both the plant material used, lettuce being a much more domesticated plant than spiny chicory, and the more extreme differences in the K:Ca:Mg ratios.

The fresh weight and dry weight of the current research appear lower compared to the outcome of Chatzigiani et al. [49], who explored the impact of different NaCl levels on the cultivation of montane and coastal ecotypes of spiny chicory grown in perlite bags. In addition, the leaf K, Mg and P concentrations in the current study appear close to those reported for the montane ecotype by Chatziginani et al. [49], while that for Ca was substantially lower in the current study. In another study by Petropoulos et al. [55], the leaf K and Mg concentrations were similar to those found in the current experiment, whereas that of Ca was higher compared to our study and closer to that reported by Chatzigiani et al. [49]. The differences in leaf Ca may be ascribed to the different growing systems and the differences in the nutrient solutions used in each experiment. Moreover, research carried out by Zamaniyan et al. [177] on *Cichorium intybus* L. demonstrated an increase in fresh weight when the K:Ca ratio reached 6:3, whereas further increase had a

negative effect on fresh weight. Huett's [178] results from examining two lettuce cultivars under different nutrient solutions, with K:Ca ratios ranging from 1:3.5 to 3.5:1, suggested that the level of the effect is tied to the cultivar. In agreement with Huett's findings, a positive effect of elevated K levels was reported by Barickman et al. [179], who observed an increase in fresh weight and dry weight of lettuce plants. These findings demonstrate the heterogeneity in the available literature in terms of the effect of macrocation ratios on plant growth. As it has been previously noted by El-Nakhel et al. [180], this may be due to the difference in the proportions of the accompanying ions, as well as their final concentration in the nutrient solution.

Overall, the study highlights the adaptability of *C. spinosum* in hydroponic systems, with the tested nutrient solutions having only minor effects on plant growth and nutrient composition. Despite its lower fresh and dry weight compared to previous studies, the nutrient concentrations in *C. spinosum* remained comparable, reinforcing its potential as a resilient and nutrient-rich edible plant. These findings emphasize the need for further fine-tuning nutrient solutions in hydroponic systems to optimize both yield and nutritional quality in wild edible plants like *C. spinosum*.

4.2 Discussion: Impact of nitrogen levels, irrigation, and biostimulant application on yield and chemical traits of stamnagathi grown in perlite bags

In order to maintain turgor at low water potential, plants invest in thicker and stiffer cell walls which is often expressed in higher dry matter content. This has higher carbon costs per unit leaf area and results to a slower growth but it has been found to increase the plants resilience against environmental factors but also decrease the possibility of being consumed by a herbivore in the wild [181]. Therefore, dry matter content can be used as an indicator of the degree of stress. Stamnagathi plants cultivated under nitrogen-deficient, fully irrigated and devoid of biostimulants (NR30-WA100-NoBS) had the highest dry matter content, which could define them as the treatment that underwent the most stress, regardless of being sufficiently irrigated. Additionally, drought stress applied in

perlite grow bags leads to the buildup of nutrients and salts, including the buildup of nitrogen, which could also have affected the cultivation in a positive way, given that stamnagathi is known to be salt and drought tolerant. Based in the current research, the growth of stamnagathi was primarily affected by the nitrogen rates. Foliar application on plant cultivated under nitrogen-deficit and fully irrigated conditions (NR30-WA100-BS) had a significant effect towards reducing the stress that was ascribed to nitrogen limitations, to a certain degree but not low enough to match that of NR100-WA100-NoBS or NR100-WA100-BS. The lowest dry matter content, was found in plants that were cultivated under sufficient nitrogen and water conditions, regardless of biostimulant application, (NR100-WA100-NoBS, and NR100-WA100-BS).

Even though in our experiment, the most profound effect on leaf dry matter continent should be attributed mainly to the supplied nitrogen, the effect of the nitrogen-rich biostimulant was shown to also have an effect. Notably, Petronia Carillo et al., [182] observed that the foliar application with a protein hydrolysate biostimulant strongly decreased leaf dry matter content independently of the supplied nitrogen rates, suggesting a partial alleviation of the stress. The increase of dry matter content was also observed in other experiments related mainly to nitrogen regimes rather than biostimulant application. For example, Christophe El-Nakhel et al., [115] reported that dry matter content was in fact increased under nitrogen limitation conditions but was not significantly affected by the biostimulant application. In another experiment, Maria Immacolata Schiattone et al., [183] reported that dry matter content was increased by water deficit, and by nitrogen limitation but also report that the seaweed derived biostimulant used in their experiment did not have a significant effect on the dry matter content whereas the azoxystrobin biostimulant did manage to cause a reduction. An observation by Beppe Benedetto Consentino et al., [114] the low water availability was believed to cause a low plant nitrogen uptake, similarly to our experiment, which consequently affected the dry matter content by reducing it, favoring the effect of nitrogen availability over water availability. In their report, even though the biostimulant

was capable of enhancing some of the agronomical characteristics, it did not appear to significantly affect the dry matter content.

Looking at the main effects, it was clearly demonstrated that reducing the available nitrogen of the fertigation solution led to the reduction of all agronomical values, with the exception of the dry matter content. The importance of nitrogen for photosynthesis, and therefore growth, has been well documented for staple crops and mainstream vegetables [184,185]. There is a strong asymptomatic relationship between biomass and nitrogen supply which is expressed through a linear relation until a certain plateau which is reached when the crops grows at the genetically determined potential rate and further nitrogen supplementation would only result in wastage or even negative consequences on the cultivation, through chemical imbalances or salinity increase [72,83,186]. Even though the yield reduction of stamnagathi was observed due to nitrogen limitation, the overall product was up to commercial standards in terms of size and yield. In a similar manner, the water limitations led to similar effects. Drought has also been known to hinder growth rates, shifting plant metabolism towards certain defense mechanisms that consume valuable resources [187,188]. Finally, biostimulant application demonstrated a significant role in stress alleviation by increasing all agronomical values and reducing the dry matter content. In the context that the main limiting factor was the nitrogen supply and that the biostimulant used in this experiment was a nitrogen rich protein hydrolysate, the stress alleviation through foliar application is a rather promising result. Other researchers have also observed beneficial effects of nitrogen rich biostimulants when applied to nitrogen stress crops [189].

Even though stamnagathi is a wild edible plant, and a relative resilient species to various types of stresses [53,55,58], its yield was been found to be significantly affected by nitrogen, and water limitations in this experiment that took place in a semitransparent greenhouse cultivation in a soilless culture system consisted of perlite bags. Additionally, the main effects demonstrated that nitrogen limitations led to also the reduction of nitrate, calcium, and zinc in the leaf tissues, whereas iron, manganese and copper levels were significantly increased. On the other hand, water limitation led to the decrease of

nitrates and zinc, but an increase of magnesium. Under drought conditions, magnesium, which is vital for energy conservation and protein synthesis, uptake and availability decreases, hindering normal growth [190]. The increase in magnesium in this case could perhaps play a role in the explanation of the resilience of stamnagathi towards drought stress. In both cases nitrate levels were measured as a quality indicator in regard to the EU regulations [85,191] and not as an indicator of the total nitrogen in the leaf tissues. Differences in nitrate among the treatments are related to the metabolism rate and the results are clearly justified by the amount of nitrate supplied through the irrigation integrals and the time period needed to sufficiently conduct nitrate assimilation [171,192,193].

The results derived from the two-way ANOVA demonstrated that the interactions between nitrogen and water availability as well as biostimulant application and nitrogen limitations were important. Stamnagathi is usually grown in areas where water scarcity is the main limiting factor [56]. Hence, its resilience towards water scarcity was expected. Moreover, its small size compared to the size of the perlite bags and hence their water storage, could also suggest that in order to apply drought stress conditions for stamnagathi the irrigation amount and frequency or the size of the perlite bags could be further reduced. On the one hand, it would be important to conduct measurements of the substrate moisture before initiating irrigation based rather than a pre-defined irrigation regime, as it has been suggested by Nemali et al., [194]. Nevertheless, its premise of the open-loop cultivation systems that an adequate drainage of nutrient solution is required in order to keep the salt accumulation under control. In other words, irrigating a perlite-based cultivation is not only based on substrate moisture but also EC levels, since salts tend to accumulate and plant might experience drought stress, in other words, osmotic stress symptoms due to the increased salinity if the substrate is not sufficiently irrigated. This has resulted in a leaching fraction that should range between 20 and 40% of the supplied volume [195,196]. In our experiment the leaching content of the drought stress was 0%.

It also appears that when cultivated commercially, in well fertilized lands and soilless culture systems, stamnagathi, and other wild edible greens benefits from the available nitrogen by enhancing its yield [197]. Based on our results, we consider that nitrogen availability outweighed the water stress since under nitrogen limitation the simultaneous limitation of water availability did not demonstrate any significant reduction on the agronomical characteristics, even though the dry matter content was affected. In fact, the dry matter content of nitrogen and irrigation stressed plants (NR30-WA50) was reduced compared to plants that were sufficiently irrigated (NR30-WA100). In our experiment, stamnagathi dry matter content in this occasion can be misleading, since the rest of the agronomical characteristics of NR30-WA50 and NR30-WA100 did not demonstrate significant differences between the two, but were clearly reduced compared to those of plants cultivated under sufficient nitrogen conditions. Towards that end, water limitations only played an important role in reducing the values of the agronomical characteristics when nitrogen was sufficiently available. In addition, this could be derived from the difference between the nitrogen amount between NR100-WA100 and NR100-WA50, which was around 0.6 mmol plant^{-1} per irrigation, whereas the difference between NR30-WA100 and NR30-WA50 was only 0.2 mmol plant^{-1} per irrigation.

For domesticated cultivars that have lost their resilience to various stresses it is often observed that higher nitrogen rates could lead to better growth attributes, such as the number of leaves, leaf area, fresh biomass, and dry biomass [198]. Moreover, water deficit is known to hinder photosynthesis, nutrient absorption, and plant growth processes [199]. Nevertheless, Chen Ru et al., [200] have observed that plants cultivated under insufficient nitrogen supply are likely to cope with one or several abiotic stresses better than plants that are abundantly supplied with nitrogen, though the exact reasons and mechanism behind this notion is not clearly documented. Li Li and Yaosheng Wang [201], in proteomics focus study on combined stress on barley observed that compared to either drought or nitrogen stress, their combined effect led to the development of extensive signaling pathways related either to energy, carbohydrate or amino acid metabolism. Jiaxin Hu et al., [202] studied the short-term and long-term water and nitrogen use

efficiency of temperate grasslands under nitrogen and drought stress conditions. Their results demonstrated that in the long-term, drought presented a trade-off between nitrogen and water use efficiency, whereas nitrogen supply enabled a positive correlation between the two. Perhaps this positive correlation played a part in our experiment as well when it comes to the comparison of sufficiently fertilized plants.

As far as biostimulant application is concerned, it was found to partially alleviate the nitrogen stress when the biostimulant was applied on nitrogen deprived plants, by enhancing leaf number, leaf area and leaf fresh weight. The effect of the biostimulant was insignificant towards providing any level of resilience towards water stressed plants. Hence, a nitrogen-rich biostimulant such as the one used in this experiment could be an eco-friendly solution to counter the negative effects of nitrogen limitations, even though it was not capable of alleviating completely the negative effects. Our results are in accordance with El-Nakhel et al., [115] whose research stated that biostimulants are to be used complementary to fertilizers and not as replacements.

From the chemical analysis, only calcium demonstrated significant effect that could be attributed to the interactions of nitrogen and water availability. Towards that end, calcium was found to be higher in plants that were sufficiently fertilized but were irrigation starved. This could perhaps be attributed to the use of calcium in osmorythmisis since it is essential for plant recovery from dehydration, through the activation plasma membrane ATPase, which pumps nutrients back after stress-induced membrane damage [203].

The research highlights nitrogen's dominant influence on stamnagathi's growth and resilience, overshadowing water stress effects. While biostimulants partially reduced nitrogen stress, precise nitrogen management remains crucial for optimal cultivation outcomes. These findings provide a foundation for improving stamnagathi farming practices in soilless systems.

4.3 Discussion: Exploring the cultivation of *Cichorium spinosum* L. in vertical farms

4.3.1 Can long photoperiods be utilized to integrate *Cichorium spinosum* L., into vertical farms?

In the context that growers have observed what seems like a photoperiod induced flowering of *Cichorium spinosum* L., this experiment focused on clarifying whether flowering initiation is solely induced but long days. Our experiment succeeded in identifying long days (15-hour photoperiod) as a possible length for the cultivation of *C.spinosum* L., in vertical farms, since flowering was not induced.

In our experiment the plants were cultivated for an extended period of 5 months. The agronomical characteristics of this experiment (Table 21) demonstrated relative low growth compared to other experiments. Nevertheless, the significant differences observed were attributed to the increased photoperiod and therefore greater daily light integral (DLI). This was expected, since increased daily light integrals are linked to increased yields [163].

The CO_2 assimilation and transpiration (Figure 42and Figure 43) of plants cultivated either under long or short photoperiod were significantly affected, but for incident light intensities that were greater than *460 µmol m^{-2} s^{-1}.* The plants were cultivated under low light intensity, of an average 100 µmol m^{-2} s^{-1}. The observed compensation point was near the light intensity of 50 µmol m^{-2} s^{-1}. Hence, we believe that the low light intensity was the primary limiting factor in terms of growth. This raises the question whether the plants surpassed the juvenile stage during those 5 months. Research on *Cichorium intybus* L., that suggests that the developmental stage exerts a significant influence on the sensitivity of chicory to extended photoperiods [147–153]. Moreover, when cultivated in an open field, spiny chicory plants flower during May or June, depending on the ecotype, which could be a combination of developmental stage, vernalization, photoperiod and temperature [54].

In addition, the net photosynthetic rate, demonstrated that when stamnagathi plants are cultivated under short days, they are more capable of capturing carbon dioxide

as light intensity increases, whereas plant cultivated under long days become less efficient in utilizing light for carbon dioxide assimilation. Moreover, as the incident light increased the transpiration rate also increased in a similar manner to the photosynthetic rate. The ratio of photosynthetic rate to transpiration rate (Data not shown) demonstrated that the water use efficiency (WUE=A/E) was the same for both long day and short day plants for measurement recorded for the same incident light intensity.

Chlorophyll fluorescence analysis demonstrated some significant differences regarding the photochemical quenching (qP). Given that qP represent the ratio of active PSSI reaction centers that can participate in photochemical processes, this parameter indicated an equilibrium between light absorption and energy utilization for photosynthesis and therefore carbon assimilation. For incident light of a 74 μmol m^{-2} s^{-1} intensity, the observed increase of the qP in plants that underwent the long day treatment, compared to short day plants, suggests a higher ability to utilize the incident photons, and perhaps improved photosynthetic efficiency (Figure 45). Long-day plants in low light may have a higher qP due to adapting to longer exposure to light, enabling them to keep more open PSII centers and utilize the extended light more effectively. Plants that are adapted to prolonged light exposure can efficiently utilize light energy for extended periods, especially in environments with low light conditions. In addition, when the incident light of the measurement increased to high light intensities of 631 and 938 μmol m^{-2} s^{-1}, long-day plants showed a decrease in qP of 5% and 9% respectively. This decrease in qP indicates that long-day plants likely experience photoinhibition under high light intensities. Nevertheless, the rest of the measured parameters (ΦPSII, ETR, and qN, Figure 44, Figure 46, Figure 47) did not appear to be significantly affected by the photoperiod for each of the light intensity levels.

These results could be useful indicators when choosing lighting for the vertical farming cultivation of stamnagathi. Whether it makes sense for a grower to choose higher light intensities or longer photoperiods for the cultivation of crops in vertical farms can be affected but several parameters. Towards that end, the location of the vertical farm and connectivity to the power grid, renewable energy such as solar and wind, as well as the

energy demand at a given time, could affect the lighting schedule of a vertical farm as an effort to reduce lighting expenses [204–207]. Nevertheless, a crop in a vertical farm, at its simplest form, appears to benefit when longer photoperiods with lower light intensity are applied [208].

Further experiments on different photoperiod and light levels, therefore DLIs, could shed some light in the interaction of plant, photoperiod, and light intensity. Claudia et al., had experimented on implemented longer photoperiods with the same DLI and observed that the daily photochemical integral (DPI) increased as longer photoperiods were applied [209]. Another research by Fang Wang et al., investigated the simultaneous effects of different photoperiods (short, long, and continuous) and light intensities (133, 200, and 400 µmol m^{-2} s^{-1}) also demonstrated that a more balanced approach (16-hour photoperiod and 200 µmol m^{-2} s^{-1}) is beneficial for the cultivation of coriander in vertical farms [210]. On the other hand, Qianwen et al., observed that for celery cultivars, and light intensity of 200 µmol m^{-2} s^{-1}, a photoperiod of 12 hours resulted in better fresh weight values as well as qP compared to 8 or 16 hours of light [211]. For the cultivation of *Cichorium spinosum* L., further experimentation on light quality, quantity, and uniformity needs to be conducted in order to clarify the most energy and yield efficient light combinations.

4.3.2 Do spectral variations of different "white lights" justify product selection?

Light wavelengths of 450-495 nm (blue) and 620-750 nm (red) are often considered to be more efficient than wavelengths of 495-570 nm (green) in terms of quantum yield of CO_2 assimilation [212]. A review on the importance of green light by Smith et al., [213] proposed that plants utilize these wavelengths not only to fine-tune stomatal aperture but also to refine overall canopy performance. Smith's results suggest that various functions of green light significantly enhance plant productivity and resource utilization, advocating for the incorporation of green wavelengths in LED-based agriculture, and therefore vertical farming. Several "white light" solutions currently exist on the market, often differentiating slightly in their spectral qualities. The efficiency of light capture and

photosynthesis in a leaf is influenced by its structural components, their arrangement, and their intrinsic properties [214]. The absorption of photons varies with wavelength; green light, despite its lower absorption efficiency compared to red and blue light, can reach deeper into the leaves. This deeper penetration allows for more uniform chlorophyll excitation, potentially leading to higher yields, especially in high plant densities. [215]. In a research by Zheng et al., [216] examining the impact of light wavelengths including red, blue, red and blue, and white light on three ornamental pot plants, observed that blue light significantly affects the total leaf thickness, though this observation might be species depended and the effects on the palisade and spongy parenchyma ratios may vary. In the same study, blue light increased stomatal density without affecting the stomatal aperture area. Stomatal size has been linked to the ratio of red and blue photons. It appears that the stomatal size decreases with the decrease of the R:B ratio as it has been observed in lettuce [217], cucumber seedlings [218], and soybean [219]. This observation is in agreement with our results, where the stomatal size was decrease as the R:B ratio of the N, F and S treatments was reduced from 3.1, to 2.5 and 1.7 respectively. In this study, leaf thickness, as well as the thickness of the palisade and spongy parenchyma, along with stomatal density, were observed to be unchanged by the light treatments. The observed leaf similarities, and differences resulted in non-significant differences on the overall agronomical characteristics. In our experiment, it was found that 36 days after transplant (DAT) and a total of 63 days after sowing (DAS), the FW of stamnagathi was around 10 grams per plant, regardless of the light treatment. From an earlier greenhouse experiment conducted by our group, researchers reported yields of less than 6 grams per plant after 56 days from transplanting (DFT) in a floating raft hydroponic system [52]. On the other hand, a more recent greenhouse experiment of our group had reported an increase in the yield leading to 9 grams per plant after 21 DAT, and a total of 56 DAS [1]. In vertical farms it is important to utilize crops with small cultivation cycles or optimize the cultivation conditions to reduce the cultivation cycle [220]. The results of this experiment suggest that further optimization can be implemented for the reduction of the cultivation cycle of stamnagathi in vertical farms. Moreover, our results suggest that under “white light” the

slight spectral differences did not demonstrate any significant agronomical effects that could justify the use of one product over the other. Hence the decision between the three, was based upon the efficiency and market price at the time (data not shown). Further investigation on the spectral quality combined with other environmental parameters, such as the effects of monochromatic LEDs, light intensity, and photoperiod, could be carried out in the future for the better understanding of the physiology of *C. spinosum* L.

4.3.3 Does limited nitrogen or elevated iron in the nutrient solution affect the agronomical characteristics or nutrient composition of stamnagathi cultivated in a vertical farm?

Our results demonstrated that reducing the total nitrogen levels from 10.92 mmol L^{-1} (NO_3^-: 9.28 mmol L^{-1} and NH_4^+: 1.64 mmol L^{-1}) of the control (N10-Fe15) to 4.55 mmol L^{-1} (NO_3^-: 4.00 mmol L^{-1} and NH_4^+: 0.55 mmol L^{-1}) of the limited total-N treatment (N4-Fe15) did not have any significant effects on the agronomical characteristics of stamnagathi. In agreement to our results, in substrate based soilless culture of stamnagathi, Chatzigianni et al., [49] had observed that reducing the total-N from 16 mmol L^{-1} to 4 mmol L^{-1} in the nutrient solution of did not negatively affect the growth of stamnagathi plants. The leaf number and specific leaf area were not affected by the limited nitrogen, as it has been observed for lettuce [84]. The leaf fresh weight was similar for all treatments, suggesting that photosynthesis was maintained, either through prioritizing leaf expansion under limited nitrogen conditions [221] or stamnagathi, being a wild edible plant did not experience total nitrogen levels of 4.55 mmol L^{-1} as a significant limiting factor.

In a similar manner, the elevated Fe treatment (N10-Fe48) did not exhibit any discernible positive or negative effects. Filho et al., [222] while cultivating *Cichorium intybus* in an NFT soilless culture system, using different concentration of Fe (16 mmol L^{-1}, 48 mmol L^{-1}, 148 mmol L^{-1}, and 448 mmol L^{-1}) observed that the growth characteristics were negatively affected as the Fe concentrations increased but the optimal Fe concentration in the nutrient solution was identified in the range 48-148 mmol L^{-1}. Further experimentation on Fe levels could define the optimal composition of the nutrient

solution for stamnagathi cultivation, and enhance the database of the NUTRISENSE software. In addition, it has been observed by Hosseini et al., [223] that in vertical farming conditions the total fertilizer use, resulting in different EC levels, can be reduced in comparison to other soilless culture systems. This was also observed in the current research, where the EC level was decide to be less than that of previous experiments conducted by our group [1,91]. The current results, provide some initial details regarding the integration of stamnagathi in commercial vertical farming systems, suggesting that the nitrogen fertilizers could be reduced without affecting the overall growth, and elevating the iron levels does not result in yield losses. Future experiments could further focus on the EC levels and total fertilizer use per nutrient solutions recipe in order to define the parameters that could further enhance the agronomical characteristics, and primarily fresh weight per plant.

Findings on the total leaf nitrogen content in the presented study were in agreement with the experimental hypothesis. The plants from the limited nitrogen treatment (N4-Fe15) had significantly reduced total nitrogen values to the control nitrogen treatments (N10-Fe15 and N10-Fe48). The observed values and leaf nitrogen content were consistent with the findings of Chatzigiani et al.[91] where 4.00 mmol L^{-1} of total-N in the supplied nutrient solution in perlite bags led to a decrease in the total Kjeldahl nitrogen concentration in stamnagathi leaves, compared to 16.00 mmol L^{-1}. In contrast to our findings regarding the nitrate levels, where the different total nitrogen level of the nutrient solution did not affect the leaf nitrate content, Chatzigianni et al., [91] found that 4.00 mmol L^{-1} of total-N in the supplied nutrient solution in perlite bags decreased the nitrate concentration in stamnagathi leaves compared to those supplied with 16.00 mmol L^{-1} of total-N. In our case, this could perhaps be ascribed to the cultivation system. In vertical farms, the stability of the light intensity and photoperiod can maximize nitrate assimilation capacity of plants [224]. It is highly probable that the resulting unaffected nitrate content was primarily determined by the light conditions of the vertical farming lighting system rather than the nutrient solution characteristics [192]. Moreover, leaf nitrate content of stamnagathi plants in our experiment was below the suggested

threshold of 4000 mg of nitrate per kg of fresh weight, and therefore were safe for consumption. Even though the leaf nitrate content calculated in the leaf fresh weight did not demonstrate statistically significant differences, values ranged from 2897, 3145 and 3618 mg of nitrate per kg of fresh weight for the N10-Fe15, N4Fe15 and N10-Fe48 respectively.

Additionally, the elevated Fe in the supplied nutrient solution (48 μmol L^{-1}) did not affect the total Kjeldahl nitrogen levels of the N10-Fe48 treatment, suggesting that this observed increase was solely ascribed to total-N. An increase of the leaf Fe content was observed in the N10-Fe15 and N10-Fe48 treatments compared to the N4-Fe15. This observation suggests the importance of total-N rather than the Fe concentration in the nutrient solution. In nitrate assimilation, Fe assumes a pivotal role by acting as a cofactor for enzymes within the reductive assimilatory pathway. These enzymes include nitrate reductase (NR), nitrite reductase (NiR), and glutamate synthase (GOGAT), all of which require Fe in the form of either an Fe-heme group or an Fe-S cluster [225–229]. In this context, it is believed that the elevated Fe observed in the treatments supplied with high total-N (10 mmol L^{-1}) could be ascribed to the plant's need to assimilate the excess nitrate. This increase in assimilated nitrate is also evident in the total Kjeldahl nitrogen levels, which were higher in the treatments supplied with high levels of total-N and might be further reinforced by the insignificant differences in nitrate levels among the leaf tissues of the three treatments. It has been suggested that elevated Fe could increase the leaf N, P, K, Ca, and Mg contents of lettuce [230,231]. Nevertheless, in this research, only the P content of the leaves was increased solely due to elevated iron conditions. Towards that end, P content of stamnagathi plants cultivated under N10-Fe48 was significantly higher compared to N10-Fe15 and N4-Fe15, suggesting that higher Fe levels in the supplied nutrient were accompanied by higher leaf P content. Even though, K, Ca demonstrated insignificant differences between plants of the different treatments, the Mg content appeared lower in plants of the N10-Fe15 treatment.

These results demonstrate that limited nitrogen on the chemical composition of stamnagathi primarily affects total nitrogen and iron concentration. Future investigation

on the interaction of nutrient solution composition and light intensity could lead to cultivation protocols for stamnagathi plants with lower nitrate content. Increasing the iron content of the nutrient solution primarily affected the phosphorus content of the leaf tissues. Increasing the leaf content of essential micronutrient, such as iron, has been proposed as a means to combat hidden hunger. Given the growing interest for vegetables with fortified mineral and bioactive content, stamnagathi could be utilized as a wild edible plant with low nitrogen requirements and resilience towards iron concentration in the nutrient solution. Further investigation on elevated iron concentrations in the nutrient solution (up to 2.0mM Fe) could validate stamnagathi as a potential biofortified product.

5. Conclusions

5.1 Conclusions on the Simultaneous Effect of Total Ion Concentration and K:Ca:Mg Ratio of the Nutrient Solution on the Growth and Nutritional Value of Hydroponically Grown

In this experiment, we tested four different nutrient solution recipes on the growth of *Cichorium spinosum* L., particularly two of high total ionic content (H-40:50:10 and H-50:40:10) and two of low total ionic content (L-40:50:10 and L-50:40:10), one of each had a K:Ca:Mg ratio 40:50:10 and the other 50:40:10. It was speculated that as a highly adaptable wild edible green, spiny chicory's growth would not be affected by the different nutrient solutions. Apart from a few significant differences in the nutrient content of the plant tissues of the cultivated plants, such as increased K content under high EC levels and increased Ca content under the 40:50:10 K:Ca:Mg ratio, the overall fresh and dry weight, leaf number and leaf area were not affected by either factor or their interactions. The fresh weight ranged from 7.8 to 9.3 g per plant and the dry weight from 0.77 to 0.87 g per plant. The leaf number ranged from 17 to 21 leaves per plant, and the leaf area from 123 to 137 cm^2. Nitrate content appeared lower in the L treatments (L-40:50:10 and -50:40:10) even though both EC levels produced a plant with safe for consumption nitrate levels. The different ratios of K:Ca:Mg might have had a different effect on the crop if the differences were more extreme since 50:40:10 and 40:50:10 were relatively subtle ratios and were chosen for this indicative experiment. When compared to other studies, the total yield of the plants was relatively low. Therefore, we believe that there are still parameters that need to be considered to optimize the growth process of *C. spinosum* L. and help integrate it into highly productive commercial systems.

5.2 Conclusions on the Impact of nitrogen levels, irrigation, and biostimulant application on yield and chemical traits of stamnagathi grown in perlite bags

This study evaluated the individual and combined effects of nitrogen levels, drought stress, and biostimulant application on stamnagathi. The findings highlighted that even

for a wild edible plant like stamnagathi, nitrogen availability is a key determinant for a high yield cultivation. While water stress impacted the plants, nitrogen limitations had a more profound effect, leading to reduced growth and altered nutrient composition. The application of a nitrogen-rich biostimulant partially mitigated the negative effects of nitrogen deficiency but was less effective under water stress conditions. These findings suggest that for optimal stamnagathi cultivation, especially in soilless systems, careful management of nitrogen levels is essential. Future studies should explore more nuanced approaches to irrigation and biostimulant use to enhance plant resilience and productivity.

5.3 Conclusions on the cultivation of *Cichorium spinosum* L. in vertical farms

Given that extended photoperiods in vertical farms pay a significant role in reducing the energy cost by delivering the needed daily light integral with lower light intensities and ling photoperiods, it was crucial to determine whether or not *C. spinosum* L., is a strictly photoperiod plant. The experiment that took place in the climate chamber, demonstrated that *C. spinosum* L., could be cultivated under long photoperiods without inducing flowering. Moreover, it was also observed how plants that were cultivated under the 10-hour photoperiod were more capable of carbon capture under higher intensities whereas plant that were cultivated under the 15-hour photoperiod were less efficient in carbon capture as light intensity increased.

Different lighting products have been presented in the vertical faring industry resulting in chaos and confusion for growers and intrapreneurs. White light consists of photons throughout the visible spectrum and can be applied to range of different plants. In the first experiment, three different “white light” products were tested on the cultivation of stamnagathi in a vertical farm. The spectral differences did not appear to affect the agronomical characteristics of stamnagathi. The average plant weighted 10 grams, and at a plant density of 50 plants m^{-2} the yield (Kg m^{-2}) was estimated to be around 0.5 Kg m^{-2} regardless of the light source. The leaf morphology remained mostly unaffected as leaf and mesophyll thickness, and stomatal density did not differ

significantly between the three light sources. The dimensions of the stomatal pores, were the only parameters of the lighting experiment that demonstrated significant differences. The stomatal length and width were found to be positively affected by the red:blue ratio. As the red:blue ratio increased from 1.7, in the SunLike™, to 2.5, in the Full, and 3.1, in the Neutral treatments. Based on these results, the decisive factor towards the selection of the most suitable "white light" product was efficiency market price at the time of the current study.

The challenge of lowering fertilizer usage without impacting yields has been a key area of study over the past few decades. Moreover, the possibility of biofortifying plants with micronutrients, such as iron, has been suggested as a method to combat hidden hunger. Towards that end, the second experiment explored the effects of total nitrogen reduction (N4-Fe15) and increased iron concentration (N10-Fe48) in the supplied nutrient solution compared to a control solution (N10-Fe15). Additionally, increasing the plant density and photoperiod based on previous research, the second experiment aimed to further increase the overall yield of stamnagathi production in vertical farms beyond research conditions. The agronomical traits of stamnagathi were unaffected by differences in nutrient solution composition, demonstrating its resilience to nitrogen limitation and its tolerance to elevated iron levels. The average plant weighted 18 grams, at a plant density of 100 plants m^{-2}, resulting in a total yield of 1.8 Kg m^{-2} regardless of the supplied nutrient solution. Moreover, the chemical analysis of the leaf plant tissues indicated a decrease in total Kjeldahl nitrogen under limited nitrogen conditions (N4-Fe15), while elevated iron (N10-Fe48) did not show a significant impact. Notably, leaf tissue phosphorus was positively associated with elevated iron conditions (N10-Fe48) in the nutrient solution. The nitrate content remained within safe for consumption thresholds for all treatments. Our results showcase stamnagathi as a prime candidate for further experimentation in vertical farms, towards fertilizer use reduction and iron biofortification.

6. Originality of the study

The originality of the current dissertation lies on the combination of the studied plant, the studied agricultural systems and the agronomical practices. In more detail, *Cichorium spinosum* L., has been insufficiently studied in the past, and little efforts have been conducted for its research driven commercialization. Even though some initial studies have been conducted from our group, the fine tuning of the nutrient solution was far from determined. Nutrient-related EC levels, as well as potassium and calcium ratios have not been studied before for the cultivation of *Cichorium spinosum* L.

Even though the cultivation in open-cycle perlite bags has been previously studied from our group, that research had focused of non-nutrient-related salts, such as NaCl. Moreover, the effects of reducing the total nitrogen, and irrigation deficit have not been previously research on *Cichorium spinosum* L. In addition, the effects of foliar application of a nitrogen-rich biostimulant have never been studied on *Cichorium spinosum* L.

Furthermore, Cichorium spinosum L., has never before been cultivated in a vertical farm. The early experiment on photoperiod elongation have proven wrong the observation that early flowering was to be ascribed to "the elongation of the photoperiod". Moreover, nutrient solution recipes for *Cichorium spinosum* L., in vertical farms where not yet developed. This research has provided significant information regarding the different characteristics and thresholds of the supplied nutrient solution depending on the cultivation system. Finally, the effect of different white light spectra was identified in the cultivation of *Cichorium spinosum* L., in the scope of providing a suggestion to growers entering the vertical farming industry.

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8. CV

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Languages:

- Greek (native)
- English (C2 level)

Education:

- 2019-present: PhD student, Laboratory of Horticultural Crops, Agricultural University of Athens

- 2013-2019: Integrated M.Sc. Degree at the Agricultural University of Athens, Department of Crop Science, Applied Physiology and Microbial Biotechnology specialization (Grade of degree: 7.87). Thesis title "Theory and Application: Exploring the possibility of using solar powered light-emitting diodes (LEDs) for the year-round hydroponic cultivation of indoor lettuce (Grade of thesis: 10).

Published Research:

1. Orfeas Voutsinos, Maria Mastoraki, Georgia Ntatsi, Georgios Liakopoulos, Dimitrios Savvas "Comparative Assessment of Hydroponic Lettuce Production Either under Artificial Lighting, or in a Mediterranean Greenhouse during Wintertime" Agriculture 2021, 11, 503. DOI: https://doi.org/10.3390/agriculture11060503

2. Voutsinos-Frantzis, O.; Ntatsi, G.; Karavidas, I.; Neofytou, I.; Deriziotis, K.; Ropokis, A.; Consentino, B.B.; Sabatino, L.; Savvas, D. Exploring the Simultaneous Effect of Total Ion Concentration and K:Ca:Mg Ratio of the Nutrient Solution on the Growth and Nutritional Value of Hydroponically Grown Cichorium spinosum L. Agronomy 2022, 12, 2214. https://doi.org/10.3390/agronomy12092214

3. Voutsinos-Frantzis, O.; Karavidas, I.; Petropoulos, D.; Zioviris, G.; Fortis, D.; Ntanasi, T.; Ropokis, A.; Karkanis, A.; Sabatino, L.; Savvas, D.; et al. Effects of NaCl and CaCl2 as Eustress Factors on Growth, Yield, and Mineral Composition of Hydroponically Grown Valerianella locusta. Plants 2023, 12, 1454. https://doi.org/10.3390/plants12071454

4. Voutsinos-Frantzis, O.; Karavidas, I.; Liakopoulos, G.; Saitanis, C.; Savvas, D.; Ntatsi, G. Can long photoperiods be utilized to integrate Cichorium spinosum L. into vertical farms ? In Proceedings of the Proceeding of the 3rd International Electronic Conference on Agronomy; MDPI: Basel, Switzerland, 2023, 27, 8. https://doi.org/10.3390/IECAG2023-15172

5. Voutsinos-Frantzis, O.; Savvas, D.; Antoniadou, N.; Karavidas, I.; Ntanasi, T.; Sabatino, L.; Ntatsi, G. Innovative Cultivation Practices for Reducing Nitrate Content in Baby Leaf Lettuce Grown in a Vertical Farm. Horticulturae 2024, 10, 375. https://doi.org/10.3390/horticulturae10040375

6. Voutsinos-Frantzis, O.; Savvas, D.; Liakopoulos, G.; Karavidas, I.; Ntanasi, T.; Sabatino, L.; Marcelis, L.F.M.; Ntatsi, G. Optimizing vertical farm cultivation of Cichorium spinosum L.: White Light's influence and nutrition management. Heliyon 2024, 10, e37146. DOI: https://doi.org/10.1016/j.heliyon.2024.e37146

Work Experience

1. 2020-2023: Contract Plant Scientist / Researcher, Laboratory of Vegetable Production, Agricultural University of Athens.

    - RADIANT: Realising Dynamic Value Chains for Underutilised Crops, https://www.radiantproject.eu/, (European Union's Horizon 2020) under Georgia Ntatsi's supervision.

    - VF2FARM: Development and Implementation of an Automated, Energy Autonomous, Indoor Vertical Hydroponic Cultivation System: Evaluation in Urban Agriculture and Greenhouse Cultivation Applications, https://vf2farm.aua.gr/ (Operational Program Competitiveness, Entrepreneurship, and Innovation, under the call RESEARCH—CREATE—INNOVATE.

2. 2019-present: Co-Founder and Chief Scientific officer of startup FarmVent, https://farmvent.com/, (Hybrid work, Wageningen, Netherlands).
3. 2019-2021: Full time Agronomist at Modern Agricultural Enterprises, https://modernfarms.gr/, (Athens, Greece).

4. 2018: Four-month intern / Plant Scientist at Urban Crop Solutions, https://urbancropsolutions.com/, (Waregem, Belgium).

Conferences:

1. 31st Conference of the Hellenic Society for Horticultural Science (2023).
2. VertiFarm 2019, Wageningen University and Research (2019).
3. GreenSys 2019, Angers (2019).
4. LED Symposium, Bregenz (2018).
5. LED Light Symposium, Wageningen University and Research (2017).
6. LED Light Course, Wageningen University and Research (2017).

Entrepreneurship and Competitions related to Vertical Farming:

1. 2020: Featured in Forbes 30 under 30 with the startup FarmVent (https://www.forbesgreece.gr/forbes-30-under-30-2022/3596022/mpaxedes-se-estiatoria-xenodoxeion-kai-souper-market-apo-tin-farmvent).

2. 2019: Participated (V.Farms) in the Farming the Future Agrifood Acceleration Program, held by Orange Grove and IdeaHackers, Netherlands Embassy in Athens, https://farmingthefuture.eu/.

3. 2019-2020: 2nd Urban Greenhouse Challenge, Wageningen University & Research, Team leader of “Coexist,” partners prize.

4. 2017: Student LED Lighting Games by the Association for Vertical Farming, winner.