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

Orfeas Voutsinos-Frantzis [1], Ioannis Karavidas [1], Georgios Liakopoulos [2], Costas Saitanis [3], Dimitrios Savvas [1] and Georgia Ntatsi [1]*

[1] Agricultural University of Athens, Department of Crop Science, Laboratory of Vegetable Crops, Iera Odos 75, 11855 Athens, Greece, vorfeas@aua.gr
[2] Agricultural University of Athens, Department of Crop Science, Laboratory of Plant Physiology, Iera Odos 75, 11855 Athens, Greece, gliak@aua.gr
[3] Agricultural University of Athens, Department of Crop Science, Laboratory of Ecology and Environmental Science, Iera Odos 75, Athens 11855, Greece, saitanis@aua.gr
* Correspondence: ntatsi@aua.gr

Abstract: Vertical farming is gaining attention for urban agriculture and sustainable food production, but mainstream crops may not be economically viable in this system, prompting a shift to high-value crops. This study explores the potential of *Cichorium spinosum* L. (spiny chicory), a wild edible green, for vertical farming. When cultivated on open field and greenhouses, spiny chicory tends to flower prior vernalization deeming the flowered plants unsalable, necessitating an investigation on its flowering responses. *C. spinosum* L. plants were cultivated and for 5 months in peat-filled pots, under low light (100 umols m^2 s^{-1}), and two photoperiods (10 and 15 hours) with stable temperature (20°C) and CO_2 level (400 ppm). No flowering occurred at the end of the first experiment, indicating that photoperiod alone did not induce flowering. Next, *C. spinosum* L. was hydroponically cultivated under a 15-hour photoperiod, light intensity of 300 umols m^{-2} s^{-1}, temperature between 25 and 30°C, CO_2 levels of 350 to 400 ppm, and plant density of 100 plants m^{-2}. At the end of the one-month cultivation the yield of the salable fresh weight was approximately 1.7-2 kg per m^2. Moreover, gas exchange measurements were conducted to analyze CO_2 uptake and evapotranspiration. This study aims to enhance understanding of spiny chicory's flowering response and growth performance, providing valuable insights for cultivating this wild edible vegetable in vertical farming systems.

Keywords: vertical farming; photoperiod; spiny chicory; wild edible greens; underutilized crops

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1. Introduction

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 [1–4]. Despite of the rapid expansion of the vertical farming sector, criticism often occurs due to the high energy use and increased carbon footprint compared to open field or greenhouse production [5–7], further emphasized by the fact that key businesses have faltered to sustain their growth and financial viability [8,9]. 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 [10,11]. This strategic integration aims to mitigate the considerable operational expenses and contribute significantly to the attainment of profitability.

Cichorium spinosum L. (spiny chicory) is a wild edible plant that can be found near the sea, on rocks or coastal sand as well as rocky mountains of the Mediterranean basin [12,13]. Being part of the Mediterranean diet, *C. spinosum* L. has been gaining attention thanks to its rich phytonutrient content and anti-carcinogenic properties [14–16]. As a result, the commercial cultivation of *C. spinosum* L. has also been gaining attention. 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 [17]. 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 [18]. 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 [19–21]. It has been also suggested that high temperature (20-25°C) could have a devernalization effect on chicory plants [21] but on the other hand, very high temperatures, (28-35°C) could hasten flowering independently of vernalization [22,23]. 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 [19]. 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 [19–25].

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.

2. Materials and Methods

2.1. Cultivation conditions

In the first experiment, sowing took place during May of 2020 inside polyester trays filled with TS 1 fine peat (Klasmann-Deilmann GmbH, Geest, Germany). Subsequently, the trays were placed in the glasshouse of the Laboratory of Vegetable Production's during the germination process. The moisture level of the substrate was checked daily and irrigation was administered manually. After 4 weeks, 30 seedlings per treatment were transplanted into individual 0.5 L pots containing peat and were relocated on 3 horizontal trays, inside each of the two climate chambers of the Laboratory of Ecology. Temperature, relative humidity, carbon dioxide concentration, and PPFD at the canopy level, were set to 20 °C, 65-60%, 400 ppm, and 70-80 umol m^{-2} s^{-1}, respectively. For the experiment, the photoperiod was established at 10 hours (short day, SD) within one chamber, and 15 hours (long day, LD) in the other. The nutrient solution used was tailored for the cultivation of spiny chicory as recommended by the decision support system "NUTRISENSE DSS and fertigation was administrated manually using syringes of different volume depending on the plant stage. As a treatment, the photoperiod was set to 10 hours (short day, SD) in one chamber and 15 hours (long day, LD) on the other. The plants were maintained inside the chambers for 7 months.

Flowering initiation was not observed in the first experiment, which led to the implementation of a 15-hour photoperiod in the second experiment which was conducted from June to July of 2023 in an acclimated room with modular vertical farms at the Laboratory of Vegetable Production. In this experiment, sowing took place on rockwool sheets (AO Plug, Grodan, Roermond, the Netherlands) which were placed inside the horizontal layers of Vegeled trolleys (Colllasse SA, Seraing, Belgium). Before sowing, achenes were broken by using a house blender and separated from the debris using a Fluid Bed Dryer (Endecotts Limited, London, UL). A month from sowing, seedlings were transplanted to plastic net pots and distributed to the 3 layers of the modular farm at a plant density of 100 plants per m^2. The temperature, relative humidity, carbon dioxide concentration, photoperiod, and PPFD at the canopy level, were set to 25-30 °C, 60-70%, 400 ppm, 15 hours, and 300 umol m^{-2} s^{-1}. The nutrient solution recipe was designed using "NUTRISENSE DSS". EC and pH were checked daily and maintained to 1.5-2 (mS/cm), 5.5 to 6.5 respectively.

2.2. Measurements

In both experiments leaf gas exchange analysis was carried out one week prior to harvest using the LCpro T analyzer (ADC BioScientific, UK). The plants were then harvested and their leaf number (LN), leaf area (LA), fresh and dry weight (FW, DW) were measured using the LI-3100C (LI-COR, Inc. Lincoln, NE, USA) and a Mettler PE-3600 scale (Mettler Toledo LLC, Columbus, Ohio, USA).

2.3. Statistical Analysis

All experimental data underwent One-Way ANOVA analysis employing the Statistica 12 software package for Windows (StatSoft Inc., Tulsa, OK, USA) for each experiment separately. Duncan's multiple range test was administered at a significance level of $p \leq 0.05$ for all measured variables.

3. Results and Discussion

Experiment 1: Does extended photoperiod alone induces flowering in Chicorium spinosum L,?

In the first experiment, leaf area (LA), leaf fresh weight (FW) and leaf dry weight (DW) statistically differed between plants cultivated under long and short days, whereas leaf number (LN) and DW/FW did not show any significant differences. As seen in Table 1, plants that grew under LD conditions had increased LA, FW, and DW compared to plants grew under SD. This was expected, since increased daily light integrals are linked to increased yields [26]. Moreover, as seen in Table 2, leaf gas exchange was significantly affected only between the 460-920 umol m^{-2} s^{-1} range. Hence, CO_2 assimilation and transpiration were not affected within the light intensity range in which the plants were cultivated, regardless of the photoperiod treatment.

The low light intensity (70-80 umol m^{-2} s^{-1}), being slightly greater than the light intensity of the photosynthetic compensation point (around 50 umol m^{-2} s^{-1}) appeared to be the primary limiting factor in terms of growth. Moreover, it is suggested that the plants never surpassed the juvenile stage during the 7 months of the experiment. 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 [27]. This supports findings from research on *Cichorium intybus* L., that suggests that the developmental stage exerts a significant influence on the sensitivity of chicory to extended photoperiods [19–25].

Table 1. 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	LA	FW	DW	DW/FW
LD	15 ± 1.14	123.57 ± 13.82 a	6.04 ± 0.72 a	0.422 ± 0.06 a	6.91% ± 0.37%
SD	14.7 ± 0.74	76.19 ± 5.73 b	3.3 ± 0.25 b	0.216 ± 0.01 b	6.84% ± 0.50%
Statistical Significance	ns	*	*	*	ns

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

Table 2. Leaf gas exchange (assimilation (A), and transpiration (E)) of spiny chicory plants cultivated under long and short photoperiods in a climate chamber.

Parameter	Treatment	0	46	92	184	460	920
E	LD	0.32 ± 0.03	0.25 ± 0.02	0.21 ± 0.02	0.22 ± 0.02	0.29 ± 0.04 b	0.43 ± 0.05 b
	SD	0.42 ± 0.08	0.31 ± 0.07	0.27 ± 0.06	0.32 ± 0.08	0.46 ± 0.11 a	0.75 ± 0.13 a
A	LD	-0.01 ± 0.04	1.42 ± 0.15	1.67 ± 0.21	2.4 ± 0.33	3.64 ± 0.56 b	5.73 ± 1.12 b
	SD	-0.13 ± 0.23	1.6 ± 0.43	1.99 ± 0.55	3.4 ± 1.04	5.69 ± 1.42 a	8.84 ± 1.43 a
Statistical Significance	E	ns	ns	ns	ns	*	*
	A	ns	ns	ns	ns	*	*

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

Experiment 2: Yield and photosynthetic capacitly of Chicorium spinosum L. cultivated commercially in a vertical farm

In the second experiment, the plants grew rapidly. A yield of 17.65 grams per plant was reached within 1 month of cultivation in the vertical farming system, as seen in Table 3. Through this cultivation design the yield is estimated to be around 1.7 Kg per m^2 per harvest. The increased light intensity was crucial for photosynthesis and plant development as it is also supported by the results from the leaf gas exchange analysis showed in Table 4. Moreover, flowering appeared to less than 4% of the plants, deeming the 15-hour photoperiod and PPFD of 300 umol m^{-2} s^{-1}, a viable cultivation design for spiny chicory.

Table 3. 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.

Treatments	#leaves	Leaf Area (cm2)	FW (g)	DW	DW/FW
DLI->15-300	20 ± 1	346.56 ± 29.69	17.65 ± 1.34	1.29 ± 0.1	7% ± 0.24%

Values are mean of n=30 followed by the standard error.

Table 4. Leaf gas exchange (assimilation (A), and transpiration (E)) of spiny chicory plants cultivated under long and short photoperiods in a climate chamber.

Parameter	0	46	92	184	460	920
E	1.07 ± 0.07	1 ± 0.09	0.92 ± 0.09	0.9 ± 0.09	1.07 ± 0.08	1.49 ± 0.07
A	-1.03 ± 0.14	0.94 ± 0.16	3.04 ± 0.2	5.59 ± 0.47	11.25 ± 0.76	15.8 ± 0.8

Values are mean of n=30 followed by the standard error.

Our results support that the cultivation of spiny chicory can be feasible in vertical farms and that the cultivation time can be drastically decreased compared to other agricultural systems. Petropoulos et al., [17,28] report preparing seedling for 90 days, while by breaking the achenes as reported above, the process was reduce to 30 days. In addition, the cultivation phase lasted for another 30 days, leading to a total of 60 days from seed to harvest, whereas Petropoulos et al., report 133 days after sowing (DAS). In other experiments conducted from our group, Ntatsi et al., had previously reported yields of less than 6 grams per plant, after 56 days from transplanting in a floating raft hydroponic system [29]. Furthermore, Voutsinos et al., in another research conducted from our group on hydroponically cultivated spiny chicory, the yields were close to 9 grams per plant after less than a month of cultivation [30]. These comparisons portray how vertical farming can decrease the time needed for crops to reach certain yields.

4. Conclusions

In conclusion, our study suggests that flowering initiation of non-vernalized and non-stressed *Cichorium spinosum* L. plants is primarily controlled by the developmental stage. Under very low PPFDs, the plant development is stagnant and plants fails to flower even after 7 months of cultivation under a 15-hout photoperiod. When spiny chicory plants are cultivated in vertical farms, the 15-hour photoperiod can be utilized since plants managed to reach high yields, in just 2 months from seed to harvest, while maintaining a flowering percentage of less than 4% of the population. Nevertheless, even though the cultivation of spiny chicory in vertical farms can greatly reduce the time needed to reach high yields, the profitability of such a system remains to be analyzed.

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