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SEEDLING PERFORMANCE AND PHENOLOGICAL DEVELOPMENT OF BASIL CULTIVARS IN SUBTROPICAL PROTECTED CULTIVATION

DESEMPENHO DE MUDAS E DESENVOLVIMENTO FENOLÓGICO DE CULTIVARES DE MANJERICÃO EM CULTIVO PROTEGIDO SUBTROPICAL

DESEMPEÑO DE PLÁNTULAS Y DESARROLLO FENOLÓGICO DE CULTIVARES DE ALBAHACA EN CULTIVO PROTEGIDO SUBTROPICAL

Cristina Batista de Lima
Docente do Setor de Produção Vegetal
Universidade Estadual do Norte do Paraná (UENP)
Campus Luiz Meneghel (CLM), Brazil
E-mail: crislima@uenp.edu.br

Jael Simões Santos Rando
Docente do Setor de Produção Vegetal
Universidade Estadual do Norte do Paraná (UENP)
Campus Luiz Meneghel (CLM), Brazil
E-mail: jael@uenp.edu.br

João Gabriel Naime de Godoy
Discente do Curso de Agronomia
Universidade Estadual do Norte do Paraná (UENP)
Campus Luiz Meneghel (CLM), Brazil
E-mail: naimedegodoyj@gmail.com

Luís Eduardo Inacio da Silva
Discente do Curso de Agronomia
Universidade Estadual do Norte do Paraná (UENP)
Campus Luiz Meneghel (CLM), Brazil
E-mail: luis.silva1@discente.uenp.edu.br

Abstract

Basil (*Ocimum basilicum* L.) supplies critical raw materials to the pharmaceutical and food industries, yet escalating climatic instability increasingly threatens open-field production. Protected cultivation offers a viable strategy to guarantee consistent, high-quality yields. To identify genotypes suited for subtropical greenhouse environments, this study characterized the initial growth and

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phenological development of five basil cultivars ('Grecco a Palla', 'Limoncino', 'Basilicão', 'Folha de Alface', and 'Vermelho Rubi'). Evaluations tracked seedling establishment alongside vegetative and reproductive progression. During the seedling phase, 'Basilicão' and 'Vermelho Rubi' demonstrated superior initial vigor and highly efficient dry biomass partitioning. Conversely, 'Grecco a Palla' developed a compact architecture and a dense root system, morphophysiological traits ideal for high-density planting models. Phenological progression remained uniform across all evaluated genotypes. Plants reached peak vegetative growth at 60 days after sowing, establishing an optimal harvest window for maximum biomass accumulation. The reproductive transition initiated at 65 days of cultivation, culminating in full bloom by day 90. Within the experimental setup, physical isolation via insect-exclusion nets severely restricted natural pollen flow, precluding a comprehensive assessment of seed maturation. Successful basil production in protected environments ultimately relies on matching specific genotypes to targeted commercial outcomes, as the cultivation system effectively stabilizes crop phenology. While insect-exclusion nets provide necessary phytosanitary control, blocking natural entomophilous pollination dictates a clear need to develop assisted pollination protocols to support viable seed production.

Keywords: *Ocimum basilicum* L.; Medicinal and aromatic plants; Phenotypic plasticity; Protected cultivation; Reproductive biology.

Resumo

O manjericão (*Ocimum basilicum* L.) é um recurso valioso para indústrias farmacêuticas e alimentícias; contudo, a crescente instabilidade climática ameaça cada vez mais a produção em campo aberto. O cultivo protegido oferece uma estratégia viável para garantir rendimentos consistentes e de alta qualidade. Para identificar genótipos adaptados a ambientes de estufa em condições subtropicais, este estudo caracterizou o crescimento inicial e o desenvolvimento fenológico de cinco cultivares de manjericão ('Grecco a Palla', 'Limoncino', 'Basilicão', 'Folha de Alface' e 'Vermelho Rubi'). As avaliações acompanharam o estabelecimento das mudas paralelamente à progressão vegetativa e reprodutiva. Durante a fase de muda, 'Basilicão' e 'Vermelho Rubi' demonstraram vigor inicial superior e partição de biomassa seca altamente eficiente. Em contrapartida, 'Grecco a Palla' desenvolveu uma arquitetura compacta e um sistema radicular denso, características morfofisiológicas ideais para modelos de plantio em alta densidade. A progressão fenológica manteve-se uniforme em todos os genótipos avaliados. As plantas atingiram o pico de crescimento vegetativo aos 60 dias após a semeadura, estabelecendo uma janela de colheita ideal para o máximo acúmulo de biomassa. A transição reprodutiva teve início aos 65 dias de cultivo, culminando no pleno florescimento no 90º dia. Dentro do arranjo experimental, o isolamento físico por meio de telas de exclusão de insetos restringiu severamente o fluxo natural de pólen, inviabilizando uma avaliação abrangente da maturação das sementes. O sucesso da produção de manjericão em ambientes protegidos depende, em última análise, do alinhamento de genótipos específicos aos objetivos comerciais almejados, uma vez que o sistema de cultivo estabiliza efetivamente a fenologia da cultura. Embora as telas de exclusão de insetos proporcionem o controle fitossanitário necessário, o bloqueio da polinização entomófila natural determina uma necessidade evidente de desenvolver protocolos de polinização assistida para viabilizar a produção de sementes.

Palavras-chave: *Ocimum basilicum* L.; Biologia reprodutiva; Cultivo protegido; Plantas medicinais e aromáticas; Plasticidade fenotípica.

Resumen

La albahaca (*Ocimum basilicum* L.) provee materias primas críticas para las industrias farmacéutica y alimentaria; sin embargo, la creciente inestabilidad climática amenaza cada vez más su producción a campo abierto. El cultivo protegido ofrece una estrategia viable para garantizar rendimientos consistentes y de alta calidad. Para identificar genotipos adaptados a ambientes de

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invernadero subtropical, este estudio caracterizó el crecimiento inicial y el desarrollo fenológico de cinco cultivares de albahaca ('Grecco a Palla', 'Limoncino', 'Basilicão', 'Folha de Alface' y 'Vermelho Rubi'). Las evaluaciones monitorearon el establecimiento de las plántulas junto con la progresión vegetativa y reproductiva. Durante la fase de plántula, 'Basilicão' y 'Vermelho Rubi' demostraron un vigor inicial superior y una partición de biomasa seca altamente eficiente. En contraste, 'Grecco a Palla' desarrolló una arquitectura compacta y un denso sistema radical, rasgos morfofisiológicos ideales para modelos de plantación de alta densidad. La progresión fenológica se mantuvo uniforme en todos los genotipos evaluados. Las plantas alcanzaron el pico de crecimiento vegetativo a los 60 días después de la siembra, estableciendo una ventana de cosecha óptima para la máxima acumulación de biomasa. La transición reproductiva inició a los 65 días de cultivo, culminando en plena floración el día 90. Dentro del diseño experimental, el aislamiento físico mediante mallas de exclusión de insectos restringió severamente el flujo natural de polen, impidiendo una evaluación exhaustiva de la maduración de las semillas. El éxito de la producción de albahaca en ambientes protegidos depende, en última instancia, de la adecuación de genotipos específicos a los objetivos comerciales previstos, ya que el sistema de cultivo estabiliza eficazmente la fenología del cultivo. Si bien las mallas de exclusión de insectos proporcionan el control fitosanitario necesario, el bloqueo de la polinización entomófila natural dicta una clara necesidad de desarrollar protocolos de polinización asistida para respaldar la producción viable de semillas.

Palabras clave: *Ocimum basilicum* L.; Biología reproductiva; Cultivo protegido; Plantas medicinales y aromáticas; Plasticidad fenotípica.

1. Introduction

A prominent member of the Lamiaceae family, basil (*Ocimum basilicum* L.) is an herbaceous aromatic native to the Middle East and India (GOSSA; ASFAW, 2025). Cultivation across Brazil serves both the fresh-cut market and the essential oil sector, supplying linalool and eugenol to the pharmaceutical, cosmetic, and food industries. The crop also sustains a critical socio-economic function, providing a reliable income stream for family-farming operations (FERNANDES, 2014; OLIVEIRA, 2022;).

Frequent cross-pollination and natural hybridization make *Ocimum* one of the most highly polymorphic genera within its family. This genetic diversity manifests as broad morphophysiological plasticity, particularly regarding canopy architecture and leaf pigmentation (MINAMI *et al.*, 2007; PATON, 1992). Consequently, tall-statured cultivars typically maximize vegetative biomass accumulation, while compact genotypes adapt more readily to high-density planting models or ornamental use (BLANK *et al.*, 2004; FERNANDES, 2014).

Open-field basil production in subtropical climates, such as the Norte Pioneiro region of Paraná, frequently suffers from frost, wind damage, severe thermal fluctuations, and intense phytosanitary pressure. These environmental

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constraints inherently destabilize supply chains. Protected cultivation mitigates these abiotic and biotic stressors through precise microclimate regulation, significantly reducing physiological stress risks (GUERRA *et al.*, 2020). Even so, commercial success in greenhouse systems depends heavily on genotype × environment (G × E) interactions, requiring cultivars specifically adapted to these controlled conditions (YOKOTA; SOUZA, 2022).

Effective crop management also demands a thorough understanding of basil floral biology. The species exhibits protandry and relies heavily on external pollinators for outcrossing (MAČUKANOVIĆ-JOCIĆ *et al.*, 2007). Greenhouse environments frequently employ insect-exclusion nets to reduce pest pressure and limit agrochemical applications. While phytosanitary beneficial, these physical barriers obstruct natural pollinator visitation (GELMINI, 2014). This mechanical isolation severely restricts reproductive potential and viable seed set. Under these conditions, rigorous phenological monitoring becomes essential for identifying optimal harvest windows before energy diverts from vegetative growth to stalled reproductive efforts.

To address these specific agronomic challenges, this study characterized seedling establishment and phenological progression across five basil cultivars grown under protected cultivation. By quantifying early-stage biomass allocation and mapping the phenological timeline, this research aims to identify genotypes with the highest morphophysiological potential for sustainable, high-yield greenhouse production in subtropical environments.

2. Materials and Methods

2.1 Location and Environmental Characterization

The experiment was conducted under protected cultivation at the Regional Research Center for Vegetable Crops, Medicinal Plants and Seeds (CROPS), situated at the Universidade Estadual do Norte do Paraná (UENP), Campus Luiz Meneghel (CLM), in Bandeirantes, Paraná, Brazil (23°06′36″ S, 50°21′37″ W; 420 m a.s.l.) (Figure 1). The regional climate classifies as humid subtropical (Cfa)

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according to the Köppen-Geiger system. During the experimental period, the internal greenhouse temperature ranged from 28 to 33 °C, while relative humidity remained between 85 and 90%. The study encompassed two complementary phases: initial seedling production and the subsequent monitoring of cultivar phenological dynamics.

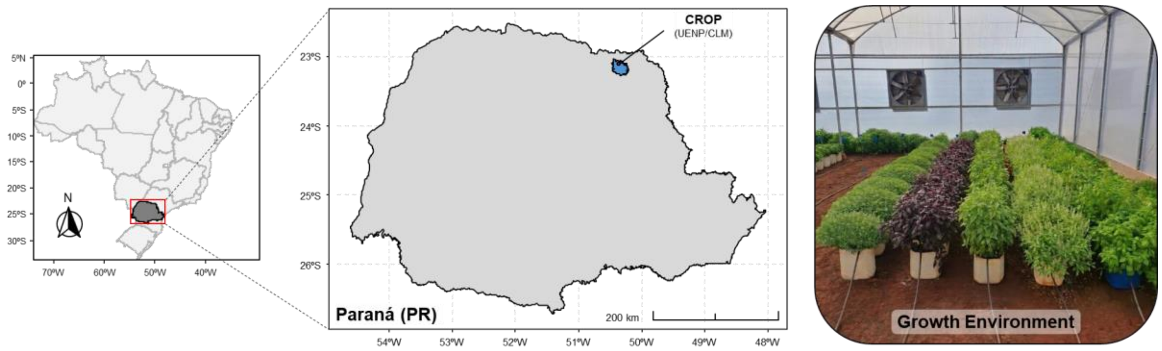

Figure 1 – Geographic location of the experimental site and an overview of basil plants under protected cultivation. CROPS/UENP/CLM, Bandeirantes, PR, Brazil, 2026.

2.2 Seedling Production and Evaluation

The experiment utilized commercial seeds from five basil cultivars: 'Grecco a Palla', 'Limoncino', 'Basilicão', 'Folha de Alface', and 'Vermelho Rubi'. Trials followed a completely randomized design consisting of five treatments (cultivars) with four replicates, assigning 36 seedlings per experimental unit.

Seeds were sown in 144-cell plastic plug trays filled with Tropstrato HT®, a commercial substrate composed of pine bark, peat, and vermiculite formulated specifically for vegetable seedling production. Trays remained on raised greenhouse benches throughout this initial growth phase. The irrigation protocol involved twice-daily applications (morning and afternoon), delivering sufficient volume to reach container capacity and ensure excess water drainage through the cell base.

Seedling assessments occurred 25 days after sowing (DAS). Six plants per replicate were randomly sampled, yielding 120 total sampling units. Evaluated morphometric parameters included leaf number (NL), maximum leaf length (LL),

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primary root length (RL), and shoot height (SH). For biomass partitioning, fresh and dry weights were recorded for both shoot (SFM, SDM) and root tissues (RFM, RDM). Dry mass values were determined after samples reached constant weight in a forced-air oven set to 50 °C. The shoot dry mass fraction (SDMF) was then calculated as the percentage ratio of dry to fresh biomass.

Prior to analysis of variance (ANOVA), the data underwent testing for normality of residuals (Shapiro-Wilk test) and homogeneity of variances (Bartlett test). Following assumption validation, significant differences detected via the F-test ($p \leq 0.05$) were separated using the Scott-Knott test at a 5% probability threshold. All statistical analyses were executed in the SISVAR® software environment (FERREIRA, 2019).

2.3 Phenological Characterization

2.3.1 Setup and Observation Units

Seedlings were transplanted into 20-L plastic containers arranged in rows within the greenhouse (Figure 1). To establish the cultivation layout, rows were separated by 1.0 m, with a 0.3 m spacing maintained between individual containers. The experimental setup for this phase included 12 independent observation units (containers) per cultivar, with four plants allocated to each container. This configuration yielded 48 monitored individuals per genotype, totaling 240 plants across the entire trial. Given the purely descriptive nature of phenological tracking, statistical hypothesis testing was not applied to this phase.

Containers received a substrate formulated in a 2:1:1 (v/v/v) ratio of filter cake (a sugarcane industry byproduct), commercial medium sand, and a clayey subsoil (69.8% clay, 21.9% sand, and 8.3% silt). Chemical analysis of the final substrate mixture yielded the following profile: pH ($CaCl_2$) 6.5; organic matter 25,3 $g.kg^{-1}$; P (Mehlich) 153,2 $mg.dm^{-3}$; K 1,6 $cmol_c.dm^{-3}$; Ca 9,4 $cmol_c$ dm^{-3}; Mg 4,2 $cmol_c.dm^{-3}$; potential acidity (H+Al) 2,8 $cmol_c.dm^{-3}$; sum of bases (SB) 15,2 $cmol_c.dm^{-3}$; cation exchange capacity (CEC) 18,0 $cmol_c.dm^{-3}$; and base saturation (V%) 84,7%.

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2.3.2 Cultural Practices

Nutritional management relied on nitrogen supplied via urea, following the recommendations of Ferreira *et al.* (2016). Initial fertilization during the seedling stage consisted of a substrate drench using a solution of 10 g urea per 10 L water. Following transplanting, at the onset of leaf expansion, 15 g of solid urea was side dressed per container; this application was immediately followed by irrigation to minimize ammonia volatilization. Due to higher nutrient demands, cultivars 'Folha de Alface' and 'Basilicão' required supplemental nitrogen and potassium (10 g each of urea and potassium chloride per container) at 45 DAS. This intervention corrected visual symptoms of incipient chlorosis and restored vegetative vigor to match the other evaluated genotypes.

A drip irrigation system delivered water twice daily (morning and afternoon). Application volumes were calibrated to replace 100% of crop evapotranspiration (ETc), an established optimum for maximizing basil yield (PRAVUSCHI *et al.*, 2010). This strategy successfully maintained substrate moisture near container capacity, ensuring adequate water availability while preventing nutrient leaching from the rhizosphere.

Pest management was strictly curative and targeted sporadic aphid infestations. Interventions utilized a 1.5% (v/v) neutral detergent solution applied via foliar spray. Applications were made to the point of runoff, ensuring thorough coverage of the abaxial leaf surfaces where the insects primarily aggregate (GELMINI, 2014).

2.3.3 Phenological Monitoring

Phenological development across the basil cultivars was characterized by using an adapted BBCH scale (HACK *et al.*, 1992). These descriptive and exploratory evaluations aimed to determine the overall viability of completing the crop cycle within a subtropical greenhouse environment. The experimental cultivation period lasted approximately 100 days, utilizing weekly site inspections

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to track developmental progression. A phenological stage transition was officially recorded once 50% or more of the plants in each observation unit met the specific morphological criteria outlined in Table 1. By establishing this population threshold, the methodology provided a chronological timeline of crop development, ensuring the recorded data accurately reflected the general phenological behavior under the established greenhouse microclimate.

Table 1 - Adapted phenological scale characterizing basil development under protected cultivation. CROPS/UENP/CLM, Bandeirantes, PR, Brazil, 2026.

Phase	Stage	Process	Identification Criteria
Vegetative	V1	Germination and emergence	Cotyledon emergences through the substrate surface until full cotyledon expansion
	V2	Seedling establishment	Full expansion of the first true leaf pair
	V3	Leaf expansion	Onset of accelerated post-transplant vegetative growth, marked by a visible increase in total leaf area
	V4	Stem elongation and branching	Maximum main stem development and lateral shoot emergence
	V5	Vegetative peak	Maximum canopy expansion and peak leaf density establishment
Reproductive	R1	Floral bud emergence	Appearance of the first floral bud at the apical meristem
	R2	Inflorescence development	Elongation of inflorescences and initial flower opening
	R3	Full bloom	Full anthesis, defined by peak floral opening across all main and lateral inflorescences

3. Results and Discussion

3.1 Phenotypic Variability and Biomass Allocation in Seedlings

Except for root dry mass (RDM), all evaluated growth parameters differed significantly across cultivars, confirming substantial genetic variability within the tested group (Table 2). This phenotypic diversity reflects underlying genetic

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control over fundamental physiological processes, specifically resource-use efficiency and biomass partitioning, which collectively drive genotype-specific adaptive responses to the cultivation environment (PURUSHOTHAMAN *et al.*, 2018).

Seedling phenotypes clearly displayed these morphological divergences (Figure 2). Notably, 'Vermelho Rubi' exhibited the highest mean values for both shoot height (SH) and leaf length (LL) (Table 2). Such attributes are characteristic of purple-leaf basil genotypes, which typically feature an upright growth habit and vigorous leaf area expansion (BLANK *et al.*, 2004).

Table 2 - Biometric characterization of basil seedlings under protected cultivation. CROPS/UENP/CLM, Bandeirantes, PR, Brazil, 2026.

Cultivar	NL	LL	SH	RL	SFM	RFM	SDM	RDM
			cm			g		
Grecco a Palla	9,6 ±2,2^{a}	1,8 ±0,3^{c}	3,0 ±0,5^{c}	11,3 ±1,6^{a}	0,2749 ±0,0636^{a}	0,1932 ±0,1149^{b}	0,0279 ±0,0069^{b}	0,0180 ±0,0047^{ns}
Limoncino	5,5 ±1,1^{b}	2,3 ±0,3^{b}	4,1 ±0,9^{b}	10,1 ±1,7^{b}	0,2085 ±0,0611^{b}	0,2411 ±0,1378^{b}	0,0321 ±0,0120^{b}	0,0205 ±0,0065
Basilicão	5,5 ±1,2^{b}	2,2 ±0,4^{b}	3,9 ±0,4^{b}	9,9 ±1,3^{b}	0,2938 ±0,0697^{a}	0,3741 ±0,1795^{a}	0,0451 ±0,0155^{a}	0,0216 ±0,0055
Folha de Alface	4,6 ±0,9^{b}	2,2 ±0,3^{b}	4,1 ±0,6^{b}	8,9 ±1,6^{b}	0,2951 ±0,0706^{a}	0,1551 ±0,0754^{b}	0,0300 ±0,0070^{b}	0,0136 ±0,0045
Vermelho Rubi	5,4 ±1,1^{b}	3,5 ±0,4^{a}	8,4 ±0,8^{a}	9,8 ±1,3^{b}	0,3258 ±0,0767^{a}	0,2440 ±0,1067^{b}	0,0463 ±0,0205^{a}	0,0166 ±0,0072
CV (%)	14,9	7,0	8,6	6,7	15,5	31,0	24,0	38,3

Means within a column sharing the same letter are not significantly different (Scott-Knott test, $p \leq 0.05$). NL: number of leaves; LL: maximum leaf length; SH: shoot height; RL: primary root length; SFM: shoot fresh mass; RFM: root fresh mass; SDM: shoot dry mass; RDM: root dry mass; CV: coefficient of variation; ns: non-significant. Values represent the mean ± standard deviation ($n = 4$).

'Grecco a Palla' seedlings exhibited the lowest SH and LL, offset by a higher leaf initiation rate (NL) per plant (Table 2). This allocation pattern typifies genotypes with a compact growth habit, directing resources toward canopy densification (LORENZI; MATOS, 2021). Such architectural traits potentially optimize light interception in high-density cropping systems, a hypothesis warranting validation in future yield trials. This cultivar also produced the longest

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primary roots (RL). This specific morphological conformation, prioritizing tissue elongation over bulk biomass accumulation, aligns with established substrate exploration strategies for maximizing water and nutrient uptake (PAIVA *et al.*, 2011; RAJANIEMI, 2022; YANG *et al.*, 2023).

In terms of biomass partitioning, 'Basilicão' and 'Vermelho Rubi' accumulated the highest shoot dry mass (SDM) (Table 2). Although 'Grecco a Palla' developed an extensive root system, this morphophysiological investment did not translate into proportional shoot mass, further emphasizing its compact nature. The robust performance of 'Basilicão' under these greenhouse conditions corroborates findings by Yokota and Souza (2022), who documented similar high-biomass potential and early developmental vigor for this genotype. In contrast, 'Folha de Alface' ranked among the highest for shoot fresh mass (SFM) but yielded the lowest SDM. This divergence reveals a lower relative accumulation of

structural carbon compared to total tissue volume, indicating the production of highly hydrated seedlings with reduced structural density during early establishment.

Figure 2 – Phenotypic divergence and root development of basil seedlings among cultivars under protected cultivation. CROPS/UENP/CLM, Bandeirantes, PR, Brazil, 2026.

Although 'Grecco a Palla' produced the longest primary roots, 'Basilicão' accumulated significantly more RFM, revealing distinctly different genetic

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strategies for early substrate exploration (Table 2). The high coefficients of variation recorded for both fresh and dry root biomass likely stem from the inherent sensitivity of root tissue to micro-variations in substrate density and moisture distribution (PAIVA *et al.*, 2011). Notably, the lack of significant statistical differences in RDM across cultivars strongly suggests a physical confinement effect. The restricted volume of the plug tray cells establishes an absolute ceiling for early root biomass expansion, ultimately masking potential genetic divergence at this developmental stage.

Shoot dry mass fraction (SDMF) analysis exposed striking contrasts in initial tissue composition (Figure 3). Mean values ranged from 10.2% ('Folha de Alface' and 'Grecco a Palla') up to 15.3% ('Basilicão'), confirming the superior structural development of 'Vermelho Rubi', 'Basilicão', and 'Limoncino'. As a direct indicator of seedling vigor and source-sink balance, SDMF strongly correlates with abiotic stress tolerance and field establishment rates (WELTER *et al.*, 2024). Tissues presenting a lower dry mass fraction possess less structural lignin and fewer stored energy reserves; consequently, these highly hydrated seedlings remain significantly more susceptible to mechanical damage during transplanting and post-handling dehydration (GOMES; PAIVA, 2011).

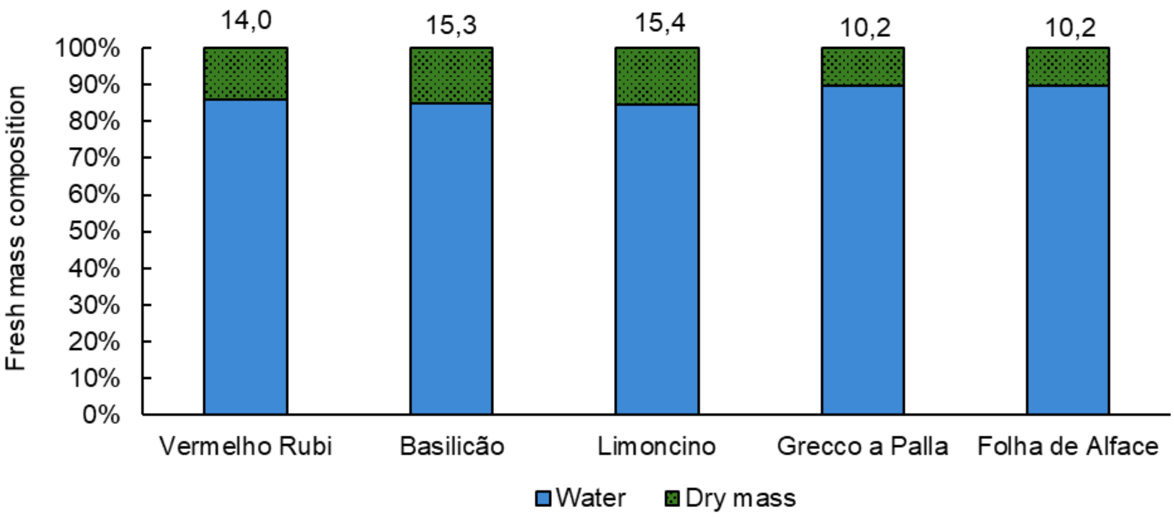

Figure 3 - Shoot dry mass fraction of basil seedlings at transplanting, expressed as the ratio of dry to fresh biomass. CROPS/UENP/CLM, Bandeirantes, PR, Brazil, 2026.

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The relationships among shoot components reveal contrasting resource allocation strategies among the genotypes. A clear functional compensation mechanism operates in 'Grecco a Palla': despite exhibiting the lowest stature and leaf length, its leaf initiation rate exceeds that of 'Basilicão' by approximately 78% (Table 2). This behavior indicates a strategy of maximizing light interception area via canopy density rather than individual organ expansion. The magnitude of the genotype effect on biomass accumulation is clearly evident in 'Vermelho Rubi' and 'Basilicão'. Both cultivars produced significantly higher SDM than the others, demonstrating greater efficiency in converting resources into structural tissue.

The decoupling of fresh volume and dry density, observed most prominently in 'Folha de Alface', serves as a critical indicator for assessing seedling vigor. Dry mass accumulation reflects seedling metabolic efficiency and the capacity to build energy reserves, factors intrinsically linked to successful post-transplant establishment (GROSSNICKLE; MacDONALD, 2018). However, the relationship between biomass accumulation and morphological quality remains complex. A lower dry mass fraction does not inherently dictate reduced structural integrity; biomechanical properties depend heavily on tissue-specific density and genotypic strategies for cell expansion (MICKKY; ALDESUQUY, 2017; SELLIN *et al.*, 2015; YANG *et al.*, 2026). Genotypes prioritizing rapid light interception often maintain high tissue hydration and turgor pressure to support canopy architecture, temporarily sacrificing accelerated lignified tissue densification.

Although the high initial water content in 'Folha de Alface' and 'Grecco a Palla' indicates lower structural density, this physiological state did not compromise transplanting success under the experimental conditions. The full establishment and 100% seedling survival rate across all genotypes demonstrate that, given adequate greenhouse management, variations in initial dry mass partitioning do not act as limiting factors. Consequently, while the dry mass fraction functions as a reliable indicator of acclimation potential and energy reserves, its ultimate impact on field establishment is heavily modulated by the

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microclimate of the post-transplant growth environment.

3.2 Phenological Evolution and Reproductive Dynamics under Protected Cultivation

The evaluated basil cultivars exhibited strict temporal synchrony throughout the transition between vegetative and reproductive stages. All five genotypes reached corresponding phenological milestones concurrently within the same weekly evaluation windows. This uniformity demonstrates that protected cultivation effectively neutralizes interspecific phenological delays under the climatic conditions of the Norte Pioneiro region. Consequently, this synchronized progression justifies using a unified chronological timeline (days after sowing, DAS) and establishing 'Basilicão' as the representative model to describe the crop cycle. This approach avoids analytical redundancy while accurately reflecting the consolidated developmental pattern across all tested genotypes.

Protected greenhouse conditions clearly favored vigorous phenological dynamics. Visual tracking of morphological progression (Figure 4) revealed rapid leaf emergence and expansion beginning at stage V3, followed by maximum lateral branching during stage V4. This canopy architecture consolidated at the vegetative peak (stage V5) at 60 DAS. Under the adopted management protocol, this milestone marked the period of absolute maximum biomass accumulation and highest canopy density. Agronomically, stage V5 serves as the optimal indicator for relative biomass harvest immediately preceding floral bud emergence (BLANK *et al.*, 2004). While this specific timeline reflects containerized greenhouse production,

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it provides a highly reliable foundational baseline for future commercial-scale yield trials.

Figure 4 - Morphological progression and phenophase characterization of cultivar 'Basilicão' throughout the developmental cycle. CROPS/UENP/CLM, Bandeirantes, PR, Brazil, 2026.

The reproductive transition occurred synchronously across all genotypes, marked by the appearance of the first floral bud (stage R1) at 65 DAS. Following floral initiation, inflorescence elongation progressed steadily until the plants reached full bloom (stage R3) at 90 DAS. This uninterrupted phenological evolution confirms that the protected environment supplied the necessary stability to complete the entire reproductive cycle, successfully buffering the plants against the severe climatic fluctuations typical of open-field cultivation.

Characterizing these specific phenophases provides a highly strategic tool for optimizing crop management and maximizing yield. The growth curve displayed an exponential phase between stages V3 and V5, concentrating the highest input of shoot biomass. Consequently, the vegetative peak (V5) represents the ideal harvest window for the fresh culinary market, capturing maximum tissue volume before reproductive morphology alters the canopy structure. Upon floral induction, vegetative leaf expansion rates declined sharply. This shift perfectly illustrates classic source-sink dynamics (TAIZ *et al.*, 2024), where developing reproductive structures become the dominant metabolic sinks, actively diverting photoassimilates away from vegetative tissues. While this resource reallocation strictly limits fresh biomass yield, the reproductive transition often benefits industrial applications. Flowering in *O. basilicum* frequently triggers an increase in total essential oil yield and induces highly desirable alterations in the phytochemical profile (KHOLIYA *et al.*, 2022; MOUNIRA *et al.*, 2022).

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3.3 Experimental Limitations and Future Perspectives

While this phenological characterization provides a robust baseline for basil development under protected cultivation, specific limitations within the experimental design warrant discussion. Morphological documentation primarily utilized 'Basilicão' as the representative model. Although weekly site inspections confirmed consistent qualitative synchrony across all genotypes, the lack of daily, individualized quantitative tracking precludes formal statistical hypothesis testing of inter-cultivar phenological progression.

The observed phenotypic performance also remains tightly linked to the specific cultivation system, particularly the volumetric constraints of the 20-L containers. Furthermore, environmental monitoring tracked only air temperature and relative humidity; the absence of photosynthetically active radiation (PAR) data restricts deeper ecophysiological inferences. Consequently, these results reflect direct morphophysiological responses to this specific greenhouse microclimate rather than general crop parameters.

The corrective nitrogen and potassium supplementation applied to 'Folha de Alface' and 'Basilicão' at 45 DAS highlights a clear differential nutrient demand among cultivars. This intervention underscores the necessity of genotype-specific nutritional protocols for high-demand plants in containerized systems. While essential for maintaining physiological integrity, this mid-cycle adjustment introduces a management variable into the final performance comparisons. These findings should therefore be interpreted as indicators of genotypic potential under controlled conditions, avoiding direct extrapolation to open-field or intensive commercial cropping systems.

The use of insect-exclusion nets provided essential phytosanitary control but created a physical barrier that entirely restricted natural entomophilous pollination. This mechanical isolation severely reduced viable seed set, precluding a comprehensive characterization of the seed maturation and whole-plant senescence phenophases. This limitation does not imply reproductive inviability within greenhouse systems; rather, it dictates that successful seed production

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requires complementary pollination strategies. Future research targeting the complete reproductive cycle of protected basil must incorporate assisted manual pollination protocols or the controlled introduction of compatible pollinating insects into the greenhouse environment.

4. Conclusions

Cultivars 'Basilicão' and 'Vermelho Rubi' demonstrated superior initial vigor and highly efficient dry mass partitioning during seedling establishment. These phenotypic traits indicate strong potential for large-scale commercial biomass production, warranting validation in future yield trials. Specifically, 'Basilicão' accumulated the highest root fresh mass, highlighting an exceptional capacity for early substrate exploration and successful post-transplant establishment.

Conversely, 'Grecco a Palla' exhibited a highly compact architecture defined by dense root development and rapid leaf initiation, despite its reduced stature. This morphological profile identifies it as an ideal candidate for high-density cropping systems, container production, or vertical farming models where spatial optimization is critical, though empirical yield validation under these specific conditions remains necessary.

The uninterrupted phenological progression across all five genotypes confirms the high viability of subtropical protected cultivation for basil. Phenological tracking established the vegetative peak (stage V5) as the definitive harvest indicator, capturing maximum structural biomass immediately prior to the metabolic resource diversion triggered by floral induction. While insect-exclusion nets provided phytosanitary protection, this physical barrier effectively limited natural entomophilous pollination. Consequently, achieving viable seed production and full crop cycle completion within these greenhouse systems will require targeted reproductive management, strongly indicating the need to develop and implement assisted pollination protocols.

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