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Early Physiological and Transcriptomic Responses of Container-Grown Blueberry *(Vaccinium corymbosum* L. 'Sierra') to Partial Substrate Replacement With Biochar

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ABSTRACT

Biochar has been widely investigated for its potential to improve substrate properties and plant performance, offering a potential partial replacement for peat in soilless cultivation amid declining peat availability. However, information on its short-term physiological and molecular effects during the early establishment of container-grown blueberry plants remains limited. This study evaluated the short-term effects of biochar incorporation (0% (BC0), 5% (BC5), and 10% (BC10)), as a partial replacement of a peat-based substrate, on growth, photosynthetic traits, and gene expression in potted highbush blueberry (*Vaccinium corymbosum* L.), cv. "Sierra", at the establishment stage (25–40 cm in height).

A non-fertilized commercial biochar was incorporated into the substrate in April 2024, and plant responses were evaluated in September 2024. Plant height was not affected by BC5, while BC10 reduced it by 10%. Leaf chlorophyll content (SPAD) decreased following both biochar treatments, while maximum PSII efficiency (Fv/Fm) remained unaffected. Gas-exchange analyses indicated that BC10 plants exhibited altered light-response traits, including lower dark respiration and a reduced light-compensation point, although no significant changes were observed in V_{cmax} or J_{max}. Gene expression analysis showed consistent upregulation of growth-related genes (SAUR49, STP, TCH4, DRM1) in biochar-treated plants, whereas defence-related genes (AOS, CHIB, JAS5, NPR1) displayed heterogeneous responses. Overall, biochar application modulated physiological and transcriptional processes in young blueberry plants, highlighting early responses occurring during establishment rather than direct improvements in growth. Within the tested range, results suggest that partial substitution of the substrate with biochar (5–10% v/v) does not enhance short-term growth and may induce dose-dependent effects. These findings should be interpreted as preliminary and hypothesis-generating, supporting the need for further studies integrating substrate chemistry, plant physiology, and long-term performance across multiple growing seasons under northern European climate conditions.

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1 | Introduction

Highbush blueberry (*Vaccinium corymbosum* L.) is an economically important perennial shrub of the *Ericaceae* family, native to North America. This crop is highly valued and sought after by consumers not only for its appealing taste but also for its exceptional nutritional profile. Blueberries are recognized as one of the richest sources of polyphenols, particularly flavonoids such as anthocyanins, flavonols, and flavanols, which underlie their notable antioxidant properties and related health benefits (Pérez et al. 2022). The blueberry crop is widely cultivated worldwide, with production expanding significantly over the past two decades (Petridis et al. 2020). This growing demand has driven advancements in cultivation techniques to optimize yield and fruit quality, particularly in regions with less favourable climatic conditions, such as northern Europe. However, in these environments, blueberry yields can be highly variable, largely due to fluctuations in seasonal light intensity (Petridis et al. 2021). For these reasons, various strategies have been tested, including the introduction of new cultivation methods, such as soilless systems, and the use of soil amendments, such as biochar.

Soilless systems are increasingly adopted to support more efficient and flexible crop management (Milivojevic et al. 2025). In contrast, traditional soil-based blueberry cultivation requires substantial labour and inputs, and is often constrained by soilborne diseases, pests, and monoculture practices, which have limited the development of the blueberry industry (Yasin et al. 2020). For blueberry plants, containerized production using a substrate made from a blend of various materials, such as different combinations of peat, sphagnum moss, coconut coir, and bark, is a relatively recent approach that has gained popularity in recent years (Yang and Lin 2025). Because no single substrate can provide all the physical and chemical properties required for optimal plant growth, several studies have demonstrated that combining different components into mixed substrates offers more favourable conditions and results in improved crop performance (Yang and Lin 2025).

Among the emerging strategies, biochar has been proposed not only as a soil amendment, but also as a potential partial replacement of conventional substrates in soilless systems, such as peat moss, bark, vermiculite, perlite, and coir (Huang and Gu 2019).

Biochar is a carbon-rich material derived from the pyrolysis of organic residues, such as pruning waste, commonly used as a soil amendment (Sharma et al. 2022). The interest behind the use of biochar is largely due to its proven benefits in improving plant growth on a wide range of crops (Huang and Gu 2019). Specifically, biochar has been shown to enhance soil structure by increasing porosity and water-holding capacity, supply essential nutrients (such as nitrogen, phosphorus, potassium, and calcium), stimulate beneficial microbial activity, improve root development, and immobilize heavy metals and organic pollutants in contaminated soils, thereby creating a healthier environment for plant growth with the ultimate objective to improve plant production (Huang and Gu 2019). A meta-analysis by Jeffery et al. (2011) found that biochar application can enhance plant growth by approximately 50% and yield by 10%.

Biochar application on blueberry plants has so far been investigated primarily under field conditions (Sales et al. 2020; 2022; Zhang et al. 2020). Evidence on the use of biochar as a substrate component in soilless blueberry systems is still extremely limited, with only one recent study addressing this topic (Agosti et al. 2025). In contrast, the use of biochar in soilless systems has been widely explored in several agronomically important species, including tomato, pepper, and strawberry (Aali et al. 2024; Graber et al. 2010).

Given that blueberry plants require at least 7 years to reach full production (Julian et al. 2012), early substrate management strategies, such as biochar application, may be crucial for optimizing growth and long-term productivity.

The effects of biochar on plant photosynthesis have been investigated in several species, yet results remain highly variable and often contradictory. For example, Changxun et al. (2016) and Vannini et al. (2022) reported improvements respectively for *Poncirus trifoliata* L. and in both *Fagus sylvatica* L. and *Quercus cerris* L., whereas other studies reported neutral or negative effects (Nguyen et al. 2017; Speratti et al. 2018). While biochar effects on plant growth and yield have been extensively investigated, its influence at the molecular level has received far less attention (Polzella et al. 2019). The limited available evidence suggests that biochar generally induces growth-related genes, whereas responses of defence-related genes are more variable and context dependent, as observed for tomato, lettuce, wheat, and maize (Chen et al. 2019; He et al. 2024; Tartaglia et al. 2020; Viger et al. 2015). In blueberry, however, information on the physiological and molecular responses to biochar application is still largely lacking, particularly under controlled container conditions and during the early establishment phase. As a result, the initial plant-level responses to biochar incorporation in blueberry substrates remain poorly characterized, preventing the formulation of robust, mechanism-driven hypotheses.

To address this knowledge gap, the present study was designed as a preliminary assessment of the short-term physiological and transcriptional responses of young blueberry plants to biochar, considering its potential use as a partial replacement of the commercial ericaceous substrate. Rather than testing specific mechanistic hypotheses, this study was designed as an exploratory, hypothesis-generating assessment of early plant-level physiological and transcriptional responses during establishment, providing a baseline for future studies integrating substrate chemistry, plant physiology, and long-term performance. To this end, potted plants of *Vaccinium corymbosum* L. cv. 'Sierra' was grown with two biochar incorporation rates (5% and 10% v/v) under low-light conditions typical of northern European environments. Specifically, the study aimed to (i) evaluate early photosynthetic adjustments associated with biochar amendment and (ii) explore whether growth- and defence-related genes previously identified as biochar-responsive in other species show similar modulation in blueberry during establishment. This approach provides a necessary baseline for future, hypothesis-driven studies linking substrate chemistry, plant physiology, and long-term performance.

2 | Materials and Methods

2.1 | Site Description and Experimental Design

The experiment took place during the 2024 growing season, from April to September, at the James Hutton Institute's experimental site in Dundee, Scotland (56°27′25′′N, 34°′11′′W).

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During the growing season, the mean monthly air temperature varied from 1.1°C/11.5°C to 7.1°C/19.4°C (mean min/max), with July and August being the warmest months in mean values. Regarding the total sunlight hours, 139.9 h in total were recorded during April, whereas the maximum was recorded in August (total of 193.4 h). Full meteorological data are reported in Table S1.

Eighteen newly established highbush blueberry plants (cv. Sierra; 6–12 months old; 25–40 cm height) were transplanted into 10-L pots. Each pot containing a single plant was considered the experimental unit. Pots were assigned to three treatments based on the volumetric proportion of biochar incorporated into the substrate: BC0 (0% v/v), BC5 (5% v/v), and BC10 (10% v/v), with six replicate pots per treatment ($n = 6$). Where multiple measurements were taken in the same plant (e.g., repeated leaf-level gas-exchange readings or height measurements on multiple stems), values were averaged to obtain a single mean per pot prior to statistical analyses, thereby avoiding pseudoreplication.

The biochar used in this study is a commercial product purchased from SoilFixer Ltd. (Brockham, UK) (https://www.soilfixer.co.uk/). The feedstock consists of virgin wood chips derived from a mixed hardwood and softwood source. The material is produced through an advanced two-stage thermochemical process: an initial gasification phase at approximately 900°C for several seconds to oxidize syngases, followed by a secondary pyrolytic phase at ~600°C under oxygen-limited conditions for several minutes. The feedstock moisture content is maintained at ~8%, with chip size ranging between 5 and 25 mm to ensure optimal conversion efficiency. The biochar used is highly carbonized, with a total carbon content of 86.2%, a low hydrogen-to-organic-carbon ratio (H/C = 0.07), and a molar O/C ratio of 0.02, indicating a highly aromatic and stable structure consistent with high-temperature gasification–pyrolysis biochars. Additional information about the biochar is reported in Table S2. Plants were grown in a commercial ericaceous growing medium (Levington Advance CNS Ericaceous, ICL, Ipswich, UK), specifically formulated for acid-loving plants and characterized by a naturally low pH (4.4–5.0), composed of peat fractions with particle size ranges of 0–10 mm and 6–12 mm. The biochar was incorporated into the substrate at the beginning of the experiment and remained in the soil for the entire growing season (April–September) before subsequent analyses. Pots were placed outdoors under natural environmental conditions. Irrigation relied primarily on natural rainfall and was supplemented when required by additional watering and fertigation (DOFF Ericaceous Fertiliser Feed) as needed according to manufacturer recommendations and applied uniformly across all treatments; fertilization was not experimentally manipulated. To minimize uncontrolled variability, pots were randomly positioned within the experimental area.

2.2 | Agronomical Parameters Collection

Plant height was measured from the base of the plant to the tips of the three tallest stems within the canopy. The final plant height was expressed in centimeters as the average of these three measurements per plant. The number of branches per plant was measured by counting all the primary branches emerging from the main stem or base of the plant. Projected canopy area and projected canopy perimeter were quantified by digital image analysis using ImageJ software, an open-source platform for biological-image analysis. At the end of the experiment, standardized front-view images of each plant were acquired against a uniform background under consistent lighting conditions. A reference object with a known length was included in each image and used to calibrate pixel dimensions to real units (cm).

Images were converted to 8-bit and thresholded to obtain binary masks of the plant canopy (plant pixels vs. background). Small gaps within the canopy were removed using hole-filling and morphological closing operations to merge all aboveground structures into a single object per plant. Projected canopy area (cm^2) and projected canopy perimeter (cm) were then extracted using the "Analyze Particles" function, excluding objects touching image borders and applying a minimum size filter to remove background noise.

2.3 | Photosynthetic Measurements

In each plant, measurements of relative chlorophyll content were taken at midday on equivalent, fully expanded, and well-lit leaves using the leaf-clip chlorophyll metre SPAD-502DL Plus (Konica Minolta Inc., Osaka, Japan). The photosynthetic efficiency (Fv/Fm) was recorded using the Handy PEA instrument (Handy PEA, Hansatech Ltd., Norfolk, UK). Leaves were analysed by exposing the samples for 1 s with a saturating 3000 μmol/m^2/s red light pulse after 15 min of dark adaptation. Measurements were carried out on fully developed leaves from the 4th and 5th nodes from the bottom of the canopy. Fifteen leaves per treatment were randomly selected from different plants within the experimental unit.

Gas exchange measurements were conducted on three fully expanded leaves located at half the height on the edge of the bush. All measurements were conducted on six independent replicate plants. Net carbon assimilation rate (A), stomatal conductance (gs), and intercellular CO_2 concentration (Ci) were measured using a LI-6800 Portable Photosynthesis System (LI-COR Biosciences Inc., Lincoln, NE, USA). Leaf temperature was maintained at 25°C and relative humidity at 60%. Except for A/Ci curves, where CO_2 was increased at regular intervals, CO_2 concentration was supplied at 400 μmol mol^{-1} with a gas flow rate of 500 mL min^{-1}. To generate light response curves, light irradiance was decreased using the following photosynthetic photon flux density (PPFD) sequence: 1,500, 1,200, 900, 600, 400, 200, 100, 75, 50, 25, and 0 μmol m^{-2} s^{-1}. The P_N/I curves were fitted using Equation 6 from de Lobo et al. (2013), using a non-rectangular hyperbola-based model of the Microsoft Excel spreadsheet and the Solver function for fitting the models. This approach was chosen over alternative models as it yielded the best fit for the collected data, resulting in the lowest sum of squared errors (SSE). To generate a dynamic response curve (A–Ci curves), leaves were maintained at 1200 μmol m^{-2} s^{-1} PPFD using the following stepwise gradients: 400, 200, 100, 50, 400, 500, 600, 800, 1000, 1200 μmol mol^{-1}.

2.4 | RNA Extraction and Quantitative RT-PCR

Ten fully developed leaves, located at the 4th or 5th node of blueberry shoots, were collected, and immediately snap frozen in liquid N_2, at the beginning of September, when

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photosynthetic capacity was still relatively high before transitioning from vegetative/reproductive growth to physiological acclimation for dormancy. Leaves were then frozen in liquid nitrogen, ground to a fine powder with a mortar and pestle, and stored at −80°C until analysis. Total RNA was extracted from blueberry leaves using the cetyl-trimethyl-ammonium bromide (CTAB) method described by Petridis et al. (2020). Residual genomic DNA was removed using RQ1 RNase-free DNase (Promega, Madison, WI, USA). RNA concentration and purity were checked using an ND1000 UV NanoDrop spectrophotometer (Thermo-Fisher Scientific, Waltham, Massachusetts, USA) and then stored at −80°C until cDNA synthesis. cDNA was synthesized from a 1 μg aliquot of total RNA using RNA to cDNA EcoDry™ Premix (Double Primed) (Takara Bio USA Inc. 2560 Orchard Parkway, San Jose, CA, USA). Subsequently, qPCR reactions were performed in a final mixture comprising 1× SsoAdvanced™ SYBR® Green Supermix (Bio-Rad Laboratories Ltd, Watford, UK), 1 μM of each gene-specific primer, and 40 ng of cDNA template. The amplification was monitored on an Applied Biosystems StepOnePlus™ Real-Time PCR System (Thermo-Fisher Scientific, Waltham, Massachusetts, USA). For each treatment, three biological and three technical replicates were used. Transcription levels were normalized using the GAPDH2 gene (glyceraldehyde 3-phosphate dehydrogenase 2), in accordance with Deng et al. (2020), whose levels remained stable in all analysed samples.

2.5 | Target Gene Selection and Primers Design for RT-PCR

Primer sequences were designed using orthologous sequences identified in blueberry (*Vaccinium* spp.) from *Arabidopsis thaliana*. The nucleotide coding sequences (CDS) for the genes of interest were identified in the *Arabidopsis thaliana* database (https://www.arabidopsis.org/). They were used as queries in the nucleotide-to-protein BLAST (blastx) tool (https://www.vaccinium.org/blast) of the Genome Database for Vaccinium (GDV) (https://www.vaccinium.org/) against the *V. caesariense* clone W85-20 P0 v2.0 genome peptides. Both the protein and nucleotide sequences of the best blueberry gene candidates were verified by cross-comparisons with those of Arabidopsis using the CLUSTALW Multiple Sequence Alignment tool (http://www.clustal.org/).

The primer sequences, listed in Table S3, were generated using the QuantPrime primer design tool (Arvidsson et al. 2008).

2.6 | Statistical Analysis

Photosynthetic parameters derived from gas-exchange measurements were first calculated using a modified version of the "Photosynthesis" R package (https://github.com/cdmuir/photosynthesis). The computational workflow incorporated the algorithms described by Lobo et al. (2013) (de Lobo et al. 2013), using their dynamic Excel spreadsheet as an additional reference tool for curve fitting and parameter estimation.

All calculated gas-exchange variables, the morphological parameters, and the gene expression data (analysed using ΔCt values) were subsequently subjected to one-way analysis of variance (ANOVA) using the General Linear Model (GLM) procedure in IBM SPSS Statistics 29.0.1.0 (SPSS Inc., Chicago, IL, USA). When significant differences were detected, mean separation was performed using Tukey's HSD test at $p \leq 0.05$.

To summarize the overall photosynthetic response of blueberry plants to different levels of biochar, a Hierarchical Clustering Heatmap was generated using MetaboAnalyst 6.0. Pearson's distance was used to evaluate similarity among treatments, and the heatmap included the following parameters: P_N (Net photosynthesis rate at saturation), Rd (dark respiration), Icomp (light compensation point), Igmax (maximum gross photosynthetic rate), Imax (Light saturation point), Vcmax (Maximum carboxylation rate of Rubisco), and Jmax (Maximum electron transport rate). Data were $\log_{10}$-transformed to reduce the influence of extreme values and allow consistent comparison across variables.

3 | Results and Discussion

3.1 | Morpho-Physiological Plant Response to Biochar Application

In this study, growth assessment was limited to non-destructive vegetative traits potentially related to yield establishment, rather than direct measurements of biomass accumulation or fruit production. No significant differences were observed between control plants and those grown with 5% biochar-enriched substrate for either plant height or number of branches (Table 1). Increasing the biochar concentration to 10%, however, significantly reduced plant height, while the number of branches remained unaffected (Table 1). These results suggest that, under the tested conditions, biochar did not enhance blueberry plant growth. Similar findings have been reported by Zhang et al. (2020), who observed no negative effects on plant height in blueberry plants grown in open field conditions with 1.5% and 3% biochar. Likewise, other studies have shown that biochar application does not always promote plant growth (Graber et al. 2010; Meller Harel et al. 2012), likely depending on factors such as biochar concentration or the developmental stage of the plants. The plants at the final stage of the experiment are shown in Figure 1.

Image-based analysis showed no significant differences among treatments in projected canopy area or projected canopy perimeter (Table 1). The reduction in plant height observed at 10% biochar was therefore not accompanied by detectable changes in overall canopy projected size or outline.

Although blueberries are known to be highly sensitive to root-zone pH, the growth response observed at the highest biochar rate cannot be unambiguously attributed to pH-related stress. Because substrate chemistry was not measured in this study, potential changes in substrate pH cannot be quantified, and any pH-related interpretation must therefore remain speculative. Previous studies have shown that woody biochar, despite its alkaline nature, does not necessarily induce substantial increases in soil or substrate pH when applied at moderate rates. For example, Sales et al. (2022) observed pH shifts ≤ 0.3 units at biochar incorporation levels up to 20% (v/v) in field-grown highbush blueberry. However, such findings cannot be directly extrapolated to the present experiment, as the biochar used in the present study differed markedly in ash content (more than sixfold higher than that reported by Sales et al.) and was not characterized for CCE or liming value, parameters that

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TABLE 1 | The effect of biochar treatment on the morphological parameters and foliar indices of blueberry plants.

Treatment	Height (cm)	N° of branches	Canopy projected area (cm^2)	Canopy perimeter (cm)	SPAD	Fv/Fm
BC0	81.4 ± 5.8^a	9.0 ± 0.4^a	1073.3 ± 161.6^a	435.3 ± 52.8^a	55.3 ± 2.9^a	0.74 ± 0.02^a
BC5	86.5 ± 5.8^a	7.4 ± 0.9^a	1146 ± 107.6^a	518.4 ± 54.4^a	50.4 ± 2.7^b	0.75 ± 0.02^a
BC10	64.0 ± 3.7^b	8.4 ± 0.8^a	1108.6 ± 219.3^a	459.6 ± 66.4^a	49.7 ± 2.2^b	0.75 ± 0.02^a

Note: One-way ANOVA, Tukey's test, $p \leq 0.05$. Data are presented as mean ± standard error (SE). Different letters indicate statistically different values.
BC0: substrate with 100% coir, BC5: substrate with 5% biochar and 95% coir, BC10: substrate with 10% biochar and 90% coir. SPAD: Soil Plant Analysis Development; Fv/Fm: maximum quantum efficiency of PSII.



FIGURE 1 | Image representing the blueberry plants (*Vaccinium corymbousm* L.) cv Sierra used in the experiment, with a total of 18 plants across 3 different treatments (six plants per treatment). The treatments performed were Control-BC0 (0% v/v), BC5 (5% v/v), and BC10 (10% v/v). The scale bar represents 20 cm.

govern acid-neutralizing capacity and cannot be inferred from ash content or pH alone.

For these reasons, the reduction in plant height observed at 10% biochar should be interpreted with caution and viewed within a broader dose-dependent response framework rather than attributed to a specific pH-driven mechanism. Within this context, the results more appropriately support a substrate-substitution perspective, evaluating whether partial replacement (5–10% v/v) of a commercial ericaceous substrate with biochar influences early plant establishment.

Both biochar treatments resulted in a significant reduction in SPAD values (approximately -9%) compared with the Control (Table 1), indicating lower leaf chlorophyll content. In fact, SPAD values are known to correlate linearly with leaf greenness and are

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therefore used to estimate leaf tissue chlorophyll content (Lafond 2023). In this study, biochar-treated plants exhibited lower SPAD values than the control (Table 1), but the reduced chlorophyll content was not associated with decreased plant growth, particularly in BC5 plants, which maintained similar height compared with BC0 plants. In environments characterized by low light intensity, plants may compensate by increasing chlorophyll content to enhance light absorption, energy transfer, and conversion efficiency (Sun et al. 2023). The results of this study suggest that the addition of biochar to the substrate may reduce the need for plants to activate adaptive mechanisms typically associated with low-light environments (Long et al. 2024), similar to the conditions under which this experiment was conducted. In contrast with what was observed in this study, He et al. (2020) reported that biochar application increases chlorophyll content, largely due to improvements in soil nutrient availability and water status. These contrasting outcomes highlight the strong context-dependence of biochar effects, which are influenced by factors such as application rate, biochar properties, and soil–crop compatibility. Excessive biochar dosages, for example, may induce nutrient imbalances or immobilization of key elements such as nitrogen and phosphorus, both essential for chlorophyll biosynthesis (He et al. 2020; Joseph et al. 2021). All these considerations suggest that while biochar can enhance chlorophyll content under optimal conditions, inappropriate application strategies or other physiological mechanisms may counteract or even (hypothetically) reverse the benefit, highlighting the need for site-specific evaluations and careful dosage management.

To further assess the physiological response of blueberry plants to biochar treatments, the maximum quantum efficiency of photosystem II (F_V/F_M) in dark-adapted leaves was also evaluated, in addition to SPAD measurements. The maximum quantum efficiency of photosystem II (PSII) in dark-adapted leaves. This parameter provides an indicator of the functional and physiological status of the photosynthetic apparatus (Strasser et al. 2004). No significant differences in F_V/F_M were observed among treatments (Table 1), suggesting that the PSII functionality was maintained despite the reduction in SPAD values observed in biochar-treated plants. This result is consistent with the findings of Vannini et al. (2022), who reported that F_V/F_M remained stable in European beech and Turkey oak plants grown on a substrate containing up to 20% biochar, despite an increase in chlorophyll content. This is a promising result since, particularly at the lower concentration tested, BC5, the reduced chlorophyll content did not affect either photosynthetic capacity or plant growth. This is consistent with previous findings from a study involving a rice mutant with reduced chlorophyll content, in which no decline in photosynthetic efficiency was observed; in fact, the absorbed light in the mutant was utilized very efficiently by the photosynthetic apparatus, resulting in an overall improvement in photosynthetic efficiency (Wang et al. 2022).

Gas-exchange measurements revealed treatment-dependent trends in photosynthetic performance (Table 2).

The net photosynthesis rate (P_N) serves as a key indicator for assessing how plants respond to their environment and how efficiently their leaf photosynthetic mechanisms function (Chen et al. 2014). Although net photosynthesis at saturating light (P_N) did not differ significantly among treatments, blueberry plants grown with 10% biochar (BC10) showed a tendency toward higher P_N values compared with BC0 and BC5 ($p = 0.054$). This result suggests that a 10% biochar amendment may enhance photosynthetic efficiency in blueberry, aligning with the findings that have been reported for other species, including peanut (Wang et al. 2021; Zulfiqar et al. 2021) and switchgrass. P_N values were used to build curves illustrating the relationship between net CO_2 assimilation (P_N) in a plant leaf and the increasing photosynthetic photon flux density (I), ranging from complete darkness to a high light intensity, in this experiment, 1500 μmol (photon) m^{-2} s^{-1} (P_N/I curve, Figure S1), as described by de Lobo et al. (2013). The analysis of the light-response curves showed that, between 200 and 1500 μmol m^{-2} s^{-1}, BC10 plants exhibited higher values than both the control and BC5 plants. Moreover, biochar incorporation significantly reduced dark respiration (R_d) at the highest application rate ($p = 0.039$) and decreased the light compensation point I_{comp} at both biochar levels ($p = 0.003$), while increasing light saturation point (I_{max}) in BC10 plants ($p = 0.017$, Table 2).

Reduced R_d indicates lower respiratory carbon losses, suggesting improved energy-use efficiency in BC10 plants. Similar reductions were reported in quinoa and maize grown on soils biochar-amended soils (Speratti et al. 2018; Kammann et al. 2011).

The Light Compensation Point (I_{comp}) is a key physiological parameter that represents the light intensity at which CO_2 uptake through photosynthesis matches CO_2 release from respiration and photorespiration, resulting in a net photosynthesis (P_N) of zero (de Lobo et al. 2013). It has previously been demonstrated that different light compensation points can be observed within the same species under varying light exposure conditions (Zhao et al. 2012). The decrease in I_{comp} further indicates that biochar-treated plants were able to achieve a positive carbon balance at lower light intensities, a trait typically associated with shade-tolerant species (Joesting et al. 2009) and particularly relevant for blueberry cultivation under northern European light conditions. As for other parameters evaluated, the plant physiological response to biochar is highly

TABLE 2 | Effect of biochar in substrate on photosynthetic light response and dynamic response in blueberry plants.

Treatment	P_N μmol m^{-2} s^{-1}	R_d μmol m^{-2} s^{-1}	I_{comp} μmol m^{-2} s^{-1}	I_{gmax} μmol m^{-2} s^{-1}	I_{max} μmol m^{-2} s^{-1}	V_{cmax} μmol m^{-2} s^{-1}	J_{max} μmol m^{-2} s^{-1}
BC0	4.02 ± 0.40^{a}	1.08 ± 0.18^{a}	20.31 ± 1.45^{a}	5.52 ± 0.23^{a}	270.40 ± 21.02^{b}	154.70 ± 9.47^{a}	69.93 ± 5.30^{a}
BC5	3.76 ± 0.29^{a}	0.81 ± 0.14^{ab}	15.65 ± 1.25^{b}	5.09 ± 0.09^{a}	298.40 ± 47.33^{b}	140.50 ± 10.13^{a}	70.70 ± 11.96^{a}
BC10	5.53 ± 0.70^{a}	0.46 ± 0.08^{b}	11.11 ± 1.72^{b}	6.38 ± 0.65^{a}	352.20 ± 35.40^{a}	149.10 ± 9.85^{a}	86.19 ± 10.69^{a}

Note: One-way ANOVA, Tukey's test, $p \leq 0.05$. Data are presented as mean ± standard deviation (SD). Different letters indicate statistically different values.
BC0: substrate with 100% coir, BC5: substrate with 5% biochar and 95% coir, BC10: substrate with 10% biochar and 90% coir. P_N: Net Photosynthesis Rate at saturation; R_d: Dark respiration; I_{comp}: Light Compensation Point; I_{gmax}: Maximum Gross Photosynthetic Rate; I_{max}: Light Saturation Point, V_{cmax}: Maximum Carboxylation Rate of Rubisco; J_{max}: Maximum Electron Transport Rate.

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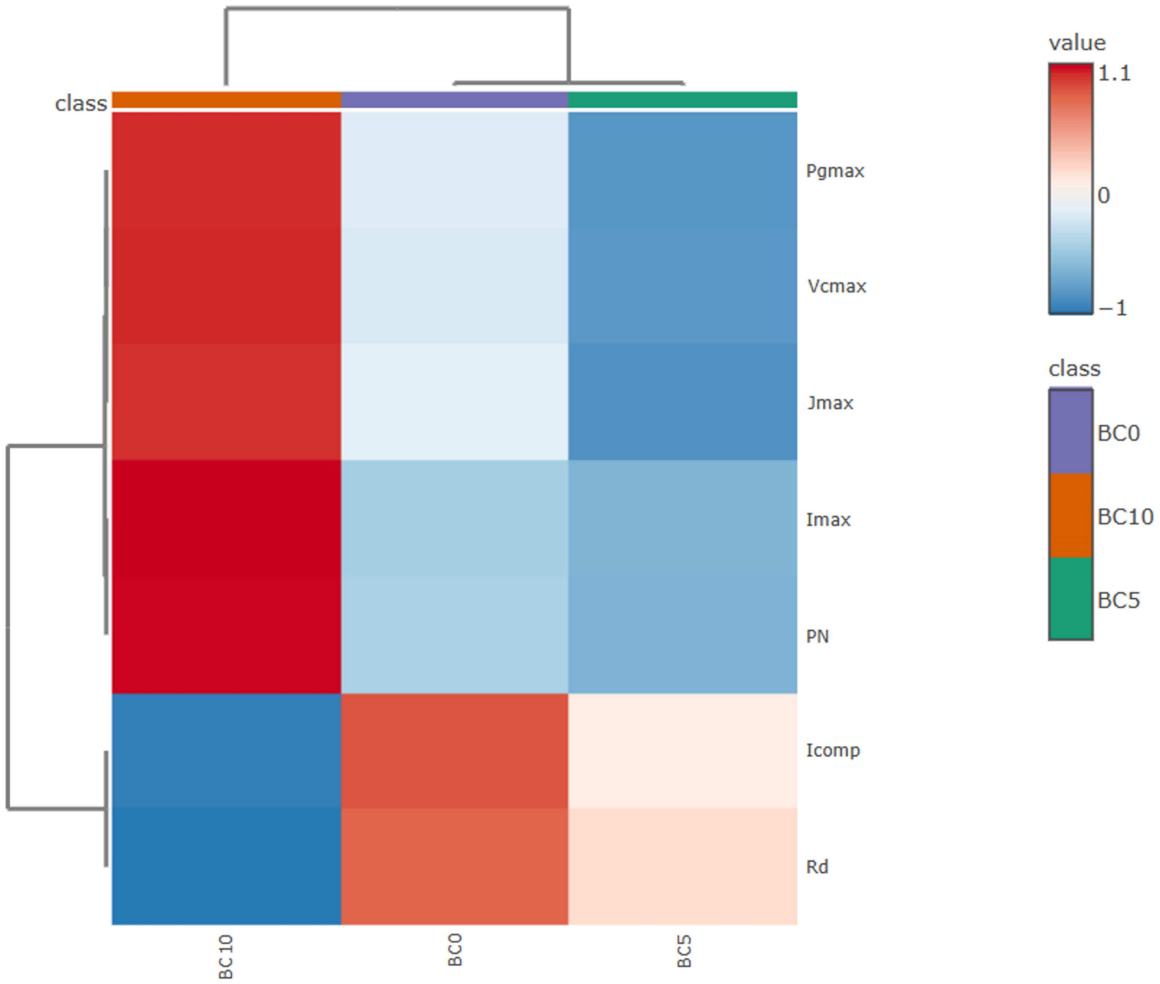


FIGURE 2 | Heatmap of Spearman correlation coefficients between biochar treatments (BC0, BC5 and BC10) and photosynthetic parameters measured in blueberry plants. Columns represent biochar treatments (0%, 5%, and 10% v/v), and rows represent photosynthetic traits derived from light-response and gas-exchange analyses (LI-COR 6800). Red and blue colours indicate positive and negative correlations, respectively, with intensity reflecting correlation strength. Hierarchical clustering based on Spearman distance was applied to photosynthetic variables to highlight similarity patterns.

variable and dependent on the species, type, and concentration of biochar considered. In fact, Wen et al. (2024) did not find any differences in I_{comp} when biochar is applied to switchgrass (*Panicum virgatum* L.).

I_{max} typically refers to the light intensity at which the maximum net photosynthetic rate (P_N) is achieved (de Lobo et al. 2013). Plants grown on BC10 substrate manifested a significant increase in the I_{max} parameter, with values significantly higher than those of control and BC5 plants. This result may indicate that the light saturation point, which leads to the arrest of photosynthetic activity, is reached in BC10 plants at light intensities higher than those required for control and BC5 plants (Table 2). As noted for I_{comp}, the plant response for I_{max} is variable and depends on several factors; for switchgrass, contrary to what was observed in this study, biochar treatment did not affect I_{max} (Wen et al. 2024).

For other parameters, such as maximum gross photosynthesis (P_{gmax}), the highest rate at which a plant can convert light energy into chemical energy through photosynthesis under optimal light conditions, and those of the dynamic response, measured under rapidly changing in CO_2 concentrations (Stinziano et al. 2017), maximum carboxylation rate (V_{cmax}) and the maximum electron transfer rate (J_{max}) (Walker et al. 2014), no statistically significant differences were found between the control and the biochar-treated plants (respectively $p = 0.104$, $p = 0.332$, and $p = 0.370$). This indicates that biochar did not alter the intrinsic enzymatic capacity of the photosynthetic machinery, such as Rubisco activity or electron transport efficiency, consistent with findings reported for switchgrass (Wen et al. 2024).

To summarize the overall effect of biochar on blueberry plants, a Hierarchical Clustering Heatmap was generated using key photosynthetic parameters obtained from Licor-6800 measurements, specifically parameters related to both light and dynamic response curves. This visual approach provides an overview of the treatment effects, highlighting patterns that may not be immediately evident when analysing individual variables (Campana et al. 2025; Dasgan et al. 2024). Furthermore, Figure S2 presents the correlation coefficients and corresponding significance levels between the various photosynthesis-related parameters analysed in the present study.

The heatmap (Figure 2) reveals trends in photosynthetic responses across treatments (BC0, BC5, BC10). Although no

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significant differences were observed in P_N and P_{gmax}, the trend toward higher CO_2 assimilation in BC10-treated plants suggests a potential positive adjustment of the photosynthetic apparatus. Dark respiration (R_d) decreased with increasing biochar concentration, indicating a reduction in respiratory carbon losses and suggesting that BC10 plants may use energy more efficiently. Similarly, the progressive reduction in light compensation point (I_{comp}) with higher biochar levels suggests that treated plants can achieve a positive carbon balance under lower light conditions, whereas the increase in light saturation point (I_{max}) in BC10 plants indicates improved tolerance to higher light intensities. V_{cmax} and J_{max} did not show significant variation, suggesting that biochar did not alter the biochemical or photochemical capacities of the photosynthetic machinery (e.g., Rubisco activity, electron transport efficiency). Additional information on the relationships among photosynthetic parameters is provided in the correlation heatmap (Figure S2). Overall, these results indicate that replacing part of the substrate with biochar, particularly at 10%, may optimise energy use and light response in blueberry plants, improving photosynthetic performance through adjustments in physiological rather than enzyme-level processes.

3.2 | Modulation of Gene Expression in Response to Biochar Application

This study represents the first investigation of gene expression responses to biochar in blueberries. Previous research has shown that biochar can induce substantial transcriptomic reprogramming in other plant species: for example, He et al. (2024) reported 2568 differentially expressed genes in maize, with 1697 up-regulated and 871 down-regulated in biochar-treated plants compared with the control. Similarly, Viger et al. (2015) identified 507 up-regulated and 571 down-regulated genes in *Arabidopsis* and lettuce following biochar application. Building on this evidence, for the present study, a targeted set of growth- and defence-related genes was selected from data published by Viger et al. (2015) to assess whether comparable transcriptional shifts also occur in blueberry during early plant establishment.

3.3 | Growth-Related Genes Response

All four growth-related genes analysed (SAUR49, STP1, TCH4, and DRM1) were significantly upregulated in biochar-treated plants compared with the control (Figure 3). This consistent induction suggests that blueberry responds to biochar in a manner broadly similar to that described for *Arabidopsis* and lettuce by Viger et al. (2015) and for wheat by Racioppi et al. (2020). As shown in Figure 3a, SAUR49, a member of the SMALL AUXIN-UP RNA family (Viger et al. 2015), showed a clear increase in expression in both BC5 and BC10 ($p = 0.017$). Its induction is consistent with its role in auxin-responsive growth regulation and supports the hypothesis that biochar may activate pathways associated with cell expansion and early developmental processes. Figure 3b shows the effect of biochar application on STP1, a monosaccharide transporter involved in sugar uptake and redistribution (Viger et al. 2015). STP1 was significantly upregulated in biochar-treated plants ($p = 0.019$). Sugar transporters are often responsive to changes in carbon availability and root–shoot communication; thus, their induction may reflect an adjustment of carbon allocation in response to modified substrate conditions. TCH4, which encodes a xyloglucan endotransglucosylase/hydrolase involved in cell wall remodelling (Viger et al. 2015), showed the strongest significant induction ($p = 0.012$), particularly in plants treated with the highest biochar concentration (Figure 3c). Upregulation of cell wall–modifying enzymes has been previously associated with biochar-mediated changes in root architecture and tissue growth in other species. Finally, DRM1, a gene associated with auxin signalling and dormancy (Viger et al. 2015), was significantly upregulated ($p = 0.040$) under both levels of biochar (Figure 3d). Its induction may point to altered hormonal balance or developmental cues triggered by changes in substrate properties.

In summary, the upregulation of growth-related genes in biochar-treated blueberry plants suggests an early stimulation of growth-associated pathways. However, these molecular changes were not accompanied by corresponding morphological effects, as plant height remained comparable to the control in BC5 and even decreased in BC10. Because the plants were still in the establishment phase, it is likely that transcriptional adjustments precede visible phenotypic outcomes. As noted by Harrison et al. (2012), gene-level responses can occur rapidly and sensitively in reaction to environmental cues, whereas morphological or physiological traits often require longer periods to manifest.

3.4 | Defence-Related Genes

Defence-related genes were analysed under unstressed conditions, in the absence of any biotic challenge, following the same experimental rationale adopted by Viger et al. (2015), who demonstrated that biochar can modulate basal defence gene expression independently of pathogen presence.

In contrast to the consistent pattern observed for growth-related genes, defence-related genes (AOS, CHIB, JAZ5, and NPR1) displayed a heterogeneous response to biochar (Figure 4).

AOS, a key enzyme in jasmonic acid (JA) biosynthesis (Viger et al. 2015), was significantly down-regulated ($p = 0.001$) in BC-treated plants (Figure 4a), consistent with previous observations in *Arabidopsis* grown on biochar-amended soils (Viger et al. 2015). Under non-stress conditions, reduced JA signalling may reflect the absence of defence activation rather than an impairment of plant resistance. Similarly, CHIB, a basic chitinase involved in ethylene- and jasmonic acid-mediated signalling (Viger et al. 2015), showed a decrease in expression, although this reduction was not statistically significant ($p = 0.057$), as shown in Figure 4b. Differences in the magnitude of CHIB modulation compared with the pattern reported by Viger et al. (2015) may reflect species-specific responses or differences in the characteristics of the biochar used, as suggested by Olmo and Villar (2018). This contrasts with the up-regulation of the ethylene-related gene, such as *Pti5*, observed in tomato by Mehari et al. (2015); however, in that case, plants were exposed to *Botrytis cinerea*, a biotic stress absent both in Viger's experiment and in the present study. Figure 4c shows the

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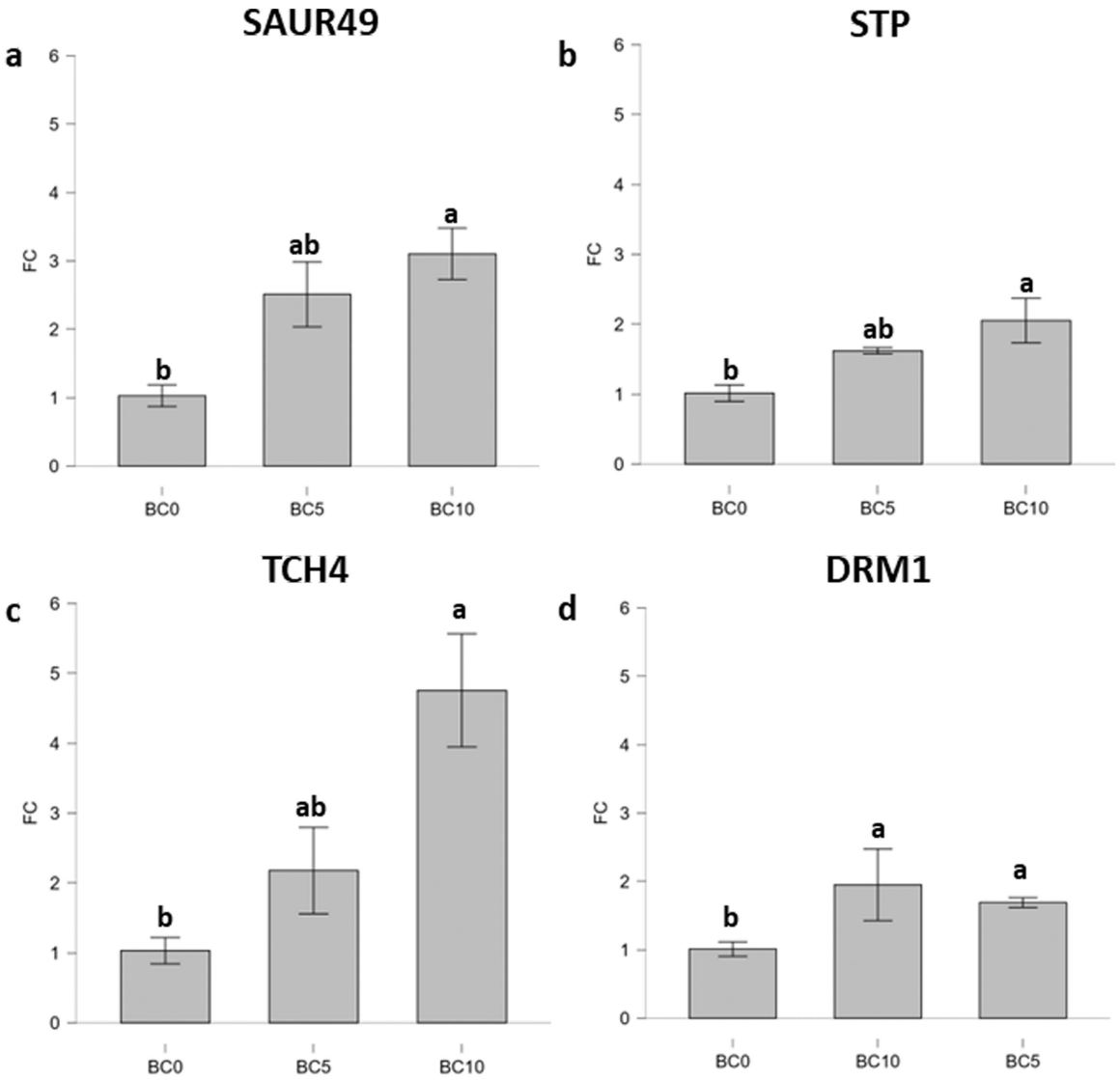


FIGURE 3 | Fold change (FC) in transcript abundance of growth-related genes in blueberry plants grown with different biochar rates: (a) *SAUR49*, (b) *STP*, (c) *TCH4*, and (d) *DRM1*. Gene expression was quantified using the ΔΔCt method, normalized to the reference gene *GAPDH2*, and expressed relative to the control treatment (BC0). Bars represent mean values ± standard error (SE). Treatments were BC0 (0% biochar), BC5 (5% biochar), and BC10 (10% biochar, v/v). Different letters indicate statistically significant differences among treatments (one-way ANOVA followed by Tukey's test, $p < 0.05$).

response of the JAZ5 gene to biochar application. The jasmonate-zim domain protein JAS5 (Viger et al. 2015) exhibited a non-linear, dose-dependent response to biochar application: with a significant up-regulation at the 5% rate (BC5; $p = 0.032$) and no difference from the control at the higher rate (BC10; Figure 4c).

NPR1, a central regulator of salicylic acid (SA)-mediated systemic acquired resistance (Viger et al. 2015), remained unchanged across treatments ($p = 0.641$, Figure 4d), indicating that SA-dependent defence pathways were not activated under the experimental conditions.

Overall, the observed variability highlights the context-dependent nature of biochar effects on plant defence regulation. In the absence of a biotic challenge, no consistent induction of defence-related genes was observed, in agreement with previous findings by Viger et al (2015), who reported a general up-regulation of growth-related genes and a down-regulation of defence-related genes under non-stress conditions. In contrast, several studies have shown that biochar can enhance defence responses and disease resistance when plants are exposed to biotic stress (Kasak et al. 2018; Poveda et al. 2021), as reported as reported for strawberry infected with *Botrytis cinerea (*Meller Harel et al. 2012*)*, wheat infested by *Sitobion avenae (*Chen et al. 2014*)*, and more recently by Bhatt et al. (2024) who reviewed multiple host–pathogen systems across different crops, showing that biochar-induced defence responses are primarily evident under biotic stress conditions. Taken together, these observations suggest that biochar-induced modulation of defence pathways is strongly influenced by pathogen presence. Accordingly, the transcriptional changes observed in the present study should be interpreted as context-dependent adjustments of basal signalling pathways rather than as indicators of altered plant resistance, whose functional consequences cannot be inferred in the absence of a biotic challenge.

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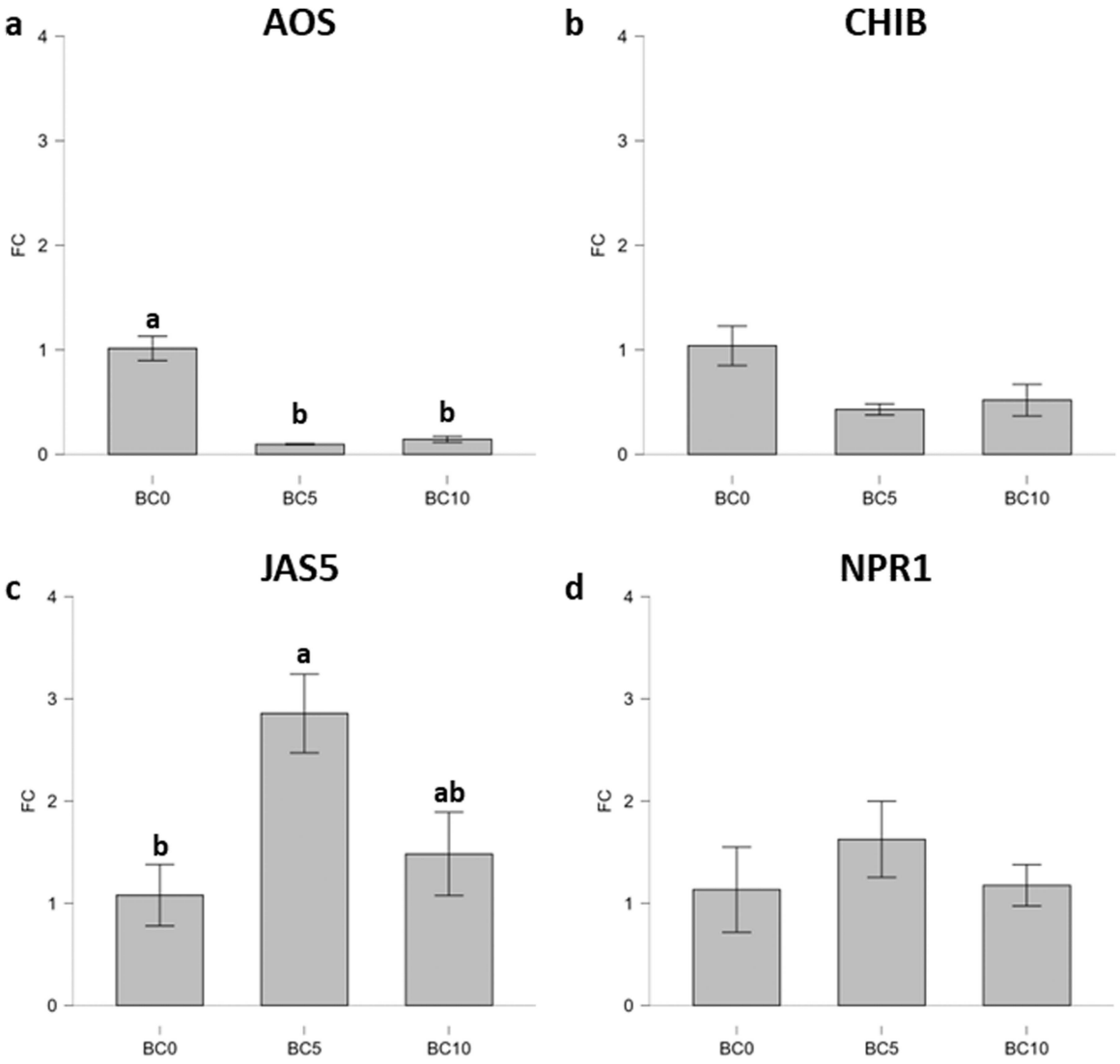


FIGURE 4 | Fold change (FC) in transcript abundance of defence-related genes in blueberry plants grown with different biochar rates: (a) AOS, (b) CHIB, (c) JAS5, and (d) NPR1. Gene expression was quantified using the ΔΔCt method, normalized to the reference gene GAPDH2, and expressed relative to the control treatment (BC0). Bars represent mean values ± standard error (SE). Treatments were BC0 (0% biochar), BC5 (5% biochar), and BC10 (10% biochar, v/v). Different letters indicate statistically significant differences among treatments (one-way ANOVA followed by Tukey's test, $p < 0.05$).

4 | Conclusion

This study provides a preliminary assessment of the short-term effects of biochar as partial substrate replacement on young blueberry plants, combining physiological and molecular responses.

At the physiological level, BC10-treated plants exhibited adjustments in light-response traits, including reduced dark respiration and a lower light-compensation point, without changes in the intrinsic biochemical capacities of the photosynthetic machinery. At the molecular level, biochar treatment was associated with the upregulation of growth-related genes, while defence-related genes displayed variable and dose-dependent responses.

Within the tested range, these results indicate that partial substitution of the substrate with biochar (5–10% v/v) does not improve short-term plant growth and may induce dose-dependent physiological and transcriptional adjustments during the establishment phase. However, given the short duration of the study and the absence of substrate chemical characterization, these findings should be interpreted as preliminary and hypothesis-generating, and no conclusions can be drawn regarding underlying substrate-mediated mechanisms.

Some physiological traits observed in the present study may reflect early adjustments in plant carbon balance, whereas others are likely to change over time due to seasonal dynamics, substrate buffering capacity, and biochar aging. Future long-term studies integrating substrate chemistry, plant physiology, and yield-related traits are required to determine whether these early responses translate into agronomically relevant outcomes across multiple growing seasons.

Author Contributions

Conceptualization: Andrea Vannini, Benedetta Chiancone. Methodology: Anna Agosti, Benedetta Chiancone. Validation: Anna Agosti, Benedetta Chiancone. Formal Analysis: Anna Agosti, Benedetta Chiancone. Investigation: Anna Agosti; Raul Huertas, Raymond

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Campbell, Kyriakos Varypatakis. Resources: Benedetta Chiancone. Data curation: Anna Agosti. Writing – original draft preparation: Anna Agosti, Andrea Vannini, Benedetta Chiancone. Writing – review and editing: Anna Agosti, Andrea Vannini, Benedetta Chiancone, Robert Hancock. Visualization, Anna Agosti, Benedetta Chiancone. Supervision: Benedetta Chiancone, Robert Hancock. Project administration: Benedetta Chiancone. Funding acquisition: Benedetta Chiancone.

Data Availability Statement

Data sharing is available on request.

Conflicts of Interest

The authors declare no conflicts of interest.

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