

Ultraviolet-B induced responses of *Psoralea corylifolia* L. with special emphasis on: growth, anatomy, physiology, yield, essential oil content and composition

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Research Article

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Abstract

Psoralea corylifolia L. is a traditionally and medicinally important, endangered plant of the family Fabaceae. Seeds obtained from it widely used in treatment of skin diseases like leprosy, psoriasis, leucoderma, vitiligo. The present study was aimed to assess the growth, physiology, anatomy, yield and essential oil content and composition of *P. corylifolia* in response to elevated UV-B (eUV-B; ambient + 7.2 kJm⁻²day⁻¹). The results showed reductions in the growth and physiological parameters under eUV-B treatment (except chlorophyll a/b ratio, carotenoids content and water use efficiency which showed increment) while reverse trend was observed for UV-B absorbing compounds. The total thickness of leaves decreased by 13.8% under eUV-B exposure. Due to eUV-B exposure number of racemes, flowers and seeds, and the length of racemes significantly reduced whereas length of flowers, seed size and seed mass (thousand seeds weight) showed non-significant variations. The essential oil content of seeds showed increment by 46.4% under eUV-B treatment. The GC-MS analysis of essential oil revealed 28 major compounds from control and eUV-B exposed plants. Overall the monoterpenes showed reduction whereas sesquiterpenes and meroterpene showed increment. The metabolites caryophyllene, caryophyllene oxide and bakuchiol (possess anti-cancerous, anti-inflammatory activities) were identified as major metabolites of essential oil, which showed increment under eUV-B treatment. The study displayed that eUV-B enhanced the content of essential oil with improvement in the quality of seeds in terms of medicinally important compounds of seeds.

Introduction

Although ultraviolet-B (UV-B) radiation is a minor component of the solar spectrum, it has the ability to significantly affect all living organisms on the Earth' surface. Upcoming UV-B radiation is affected by many factors including seasonal, diurnal, meteorological factors, altitude, latitude and atmospheric pollution (Jenkins 2017). Recently it has been reported by various groups of researchers that the changes in incident surface UV-B radiation is highly contributed by climate change, irrespective of the changes in level of stratospheric ozone (Barnes et al. 2019; Bomman et al. 2019). As plants are sessile and photoautotrophic organisms, they constantly exposed to high energy UV-B radiation in their environments that may influence development, growth and overall physiology and metabolisms of plants. UV-B radiation directly or indirectly, through the enhanced generation of reactive oxygen species (ROS) imposes damaging effects on nucleic acids, proteins, membrane lipids and chloroplasts (Takshak and Agrawal 2019). In addition to indirect effect via inhibition of photosynthesis and thus availability of photo assimilates, UV-B may also directly perturb the reproductive growth. UV-B affects a variety of floral structures or it may also lead to abortion of floral buds and reduction in number of flowers, leading to decreased reproductive success (Del Valle et al. 2020). However, nowadays the perspective of UV-B radiation studies have changed from stressor to regulator of plant growth' and as a physic tool to improve the nutraceutical and pharmaceutical qualities of fruits and medicinal plants (Coffey et al. 2017; Mannucci et al. 2020). Hideg et al. (2013) reported that balance between distress and eustress i.e. damaging and regulating effects of UV-B on a plant species depends on several factors including time and dose of UV-B exposure, genetic setup, environmental variables, acclimation strategy and developmental stages of plants.

Psoralea corylifolia L. (syn. *Cullen corylifolium* L.) commonly known as bakuchi, babchi is a medicinally important, endangered plant species of the family Fabaceae, which is widely distributed in subtropical and tropical region of the world. It has been used for a long time in traditional Chinese and Ayurvedic system of medicine. It is also known as Kushtanashini due to its frequent use since ages in the treatment of skin diseases like leprosy, leucoderma and psoriasis (Khushboo et al. 2010). Whole plant parts, especially seeds and volatile oils obtain from seeds are rich source of several bioactive constituents such as psoralen, bakuchiol, isopsoralen, bavachalcone, isobavachalcone, psoralidin, bavachinin, bavachin, psoralidin, caryophyllene, daidzein, genistein, which belongs to various classes such as furanocoumarins, coumarins, terpenes, meroterpenes, flavones and isoflavonoids. Due to presence of these important constituents it possess several pharmaceutical activities such as anticancer, anti-inflammatory, anti-HIV, anti-vitiligo, anti-psoriatic, antidepressant, estrogenic, neuroprotective, immunomodulatory and anti-Alzheimer's activities and is widely used in the treatment of various diseases (Agrawal and Pandey 2019; Koul et al. 2019). Its bioactive constituent psoralen along with UV-A radiation used in PUVA therapy for the cure of several skin diseases (Khushboo et al. 2010). Taking into account the medicinal importance of *P. corylifolia*, there are only few reports available on abiotic stress response of *P. corylifolia* including, gamma radiation (Bhat et al. 2015; Jan et al. 2015) cadmium (Satdive et al. 2014) SO₂ (Ali et al. 2008) and salt stress (Katare et al. 2012), however there are no reports yet available on the effect of UV-B radiation on *P. corylifolia*. Considering the literature lacunae, the present study was conducted to unravel the response of *P. corylifolia* against elevated UV-B radiation with the following objectives: (1) to evaluate the effect of eUV-B on growth, reproductive and anatomical features (ii) to estimate the effect of eUV-B on physiological parameters along with UV-B absorbing compounds under eUV-B (iii) to study the effect of eUV-B on medicinally important plant part i.e seeds and its essential content and composition.

Material And Methods

Experimental area

The pot study was performed at the Botanical Garden, Department of Botany, Institute of Science, Banaras Hindu University, Varanasi (25° 16' 14.1" N, 82° 59' 20.9" E) during the period July 2018-February 2019. The experimental area soil was alluvial, sandy loam (silt 28%, clay 27% and sand 45%) pale brown in color and slightly alkaline in pH with range 7.2–7.4.

Experimental setup, plant material and sampling

An important medicinal plant *Psoralea corylifolia* L. (Fabaceae) was selected for the study. The seeds of *P. corylifolia* were provided by Indian Council of Agricultural Research-National Bureau of Plant Genetic Resources (ICAR-NBPGR), Indian Agricultural Research Institute (IARI), New Delhi, India. The soil was taken from the botanical garden (i.e. experimental site) upto the depth of 25 cm and after removal of plant material and stone particles manually, soil was filled in pots. A total of 54 earthen pots (diameter 30 cm; height 40 cm) were filled with 10 kg soil and left for a week for the stabilization of soil. Earthen pots were then transferred to six, 1m × 1m experimental plots (9 earthen pots per plot at equal distance). Seeds were sown in garden soil and prior to sowing, seeds were soaked in double distilled water for overnight to moisten the seed coat, as it was reported in literature that the seed coat of *P. corylifolia* is very hard (Khushboo et al. 2010). After germination of seeds, two seedlings each of 10 cm were transplanted in a pot. At a regular interval watering was done in equal amount to maintain the level of moisture. Once plants were established in pot, they were subjected to eUV-B radiation. The UV-B (elevated dose) was provided by Q Panel UV-B 313 40 W fluorescent lamps (Q panel Inc., Cleveland, OH, USA) covered with cellulose diacetate filters (0.13 mm; Cadillac Plastic Co., Baltimore, MD, USA). The lamps were covered with 0.13 mm polyester filters for control plots, the filters of both control and treatment plots were replaced in a week. The distance of 45 cm between UV-B lamp and plant canopy was maintained constantly throughout the experiment. Control plots (3 plots) receive only ambient UV-B while the treatment plots (3 plots) receive ambient +7.2 kJ m⁻²d⁻¹ elevated UV-B. An elevated dose of UV-B (eUV-B) was provided for 3 h (11:00 – 14:00) up to the experiment completion. The elevated dose of UV-B was selected as per reported by Jaiswal et al. (2020). The monitoring of ambient UV-B radiation was performed through PMA2100 Solar Radiometer (100 East Glenside Avenue, Glenside, PA 19038, USA). Sampling was done at 60DAT, 120DAT and 180 DAT (days after transplant), that represent the vegetative, reproductive and post-reproductive stages of *P.corylifolia*, respectively.

Growth parameters and Anatomical features

Growth parameters were analyzed at three growth stages on five randomly selected plants from both control and treatment plots with respect to root and shoot length, number of nodules, number of leaves, leaf area and number of branches. Leaf area was calculated using leaf area meter ((Model Li-3100, Li-COR, Inc., USA). For estimation of above and below ground biomass, plant parts were oven dried at 70°C for 72 h and then weighed. For anatomical studies, transverse sectioning of leaves was carried out and further processed as reported by Ansari et al. (2021). The sections were observed and photographs were taken by digital camera attached to a binocular microscope (Olympus CX21i, India). The thickness of adaxial epidermis, palisade parenchyma, spongy parenchyma, abaxial epidermis and the proportion taken by intercellular spaces were measured by using Fiji-Image J software (National Institute of Health, Maryland, USA).

Gas exchange and chlorophyll fluorescence

All gas exchange measurements such as photosynthetic rate (Ps), stomatal conductance (gs), internal CO₂ (Ci), transpiration rate (Tr) were measured between 8:00 to 11:00 h by using LI-6400XT portable photosynthesis system (LI-6400XT; LI-COR, Inc., Lincoln, NE, USA), on the third or fourth fully expanded leaf from the top of plants, from both control and treatment plots. Water use efficiency (WUE) was calculated as ratio of Ps and Tr. Chlorophyll fluorescence parameter (Fv/Fm) was measured using Plant Efficiency Analyzer (PEA, MK2, 9414, Hansatech Instruments Ltd. Norfolk, UK) on the same leaves on which gas exchange parameters were measured as described by Takshak and Agrawal (2018).

Pigments and UV-B absorbing compounds

Chlorophyll and carotenoids content were estimated as per the methodologies described by Takshak and Agrawal (2015). Leaf tissue (0.1 g) was crushed in 80% acetone (10 mL) and centrifuged at 5000 × g for 15 min, and then final volume of supernatant was maintained to 25 mL with 80% acetone. The absorbance of supernatant was recorded at 480 nm and 510 nm for carotenoids content and at 645nm and 663 nm for chlorophyll content. Anthocyanins content was analyzed following the methodology of Gitelson et al. (2007). 0.1 g of leaf tissue was macerated in methanol (10 mL) containing conc. hydrochloric acid (a drop) and calcium carbonate (1%). The homogenate was centrifuged and the absorbance was recorded at 535 and 650 nm and the content of anthocyanins was calculated following Deikman et al. (1995). Flavonoids content was estimated as per the protocol of Flint et al. (1985), by using standard curve of quercetin. Estimation of total phenol was performed following the methodology of Bray and Thorpe (1954). The concentration of phenols was calculated by using standards of gallic acid.

Reproductive parameters and yield attributes

Ten plants per plot were selected randomly and tagged for further observations. The number of racemes and flowers were counted. The length of racemes and flowers were measured on ten randomly selected flowers from separate plants per plot. Ten tagged plants per plot were harvested for assessing yield parameters including number of seeds, diameter and length of seeds and the test weight (1000 seeds weight).

Essential oil extraction, GC-MS analysis and identification of compounds

The extraction of essential oil from seeds was performed following the methodologies of Takshak and Agrawal (2018) with few modifications. Fresh seeds (250 g) were collected in triplicate from both control and treatment plots at 180 DAT. Samples of seeds were homogenized finely using a grinding machine and then subjected to oil extraction using the hydrodistillation technique in a Clevenger apparatus for 6 hours. The essential oil was collected and stored in a dark glass vials at 4 °C after drying with anhydrous sodium sulfate (Gautam and Agrawal 2017). The essential oil obtained was measured and expressed as μL of essential oil per 250 g fresh seeds.

The essential oil obtained from seeds was subjected to GC-MS (QP-2010 Plus Shimadzu), having column (Rtx-5Ms capillary column) of 0.25 mm diameter, 30 mm in length and 0.25 μm film thickness. Initially the temperature of column oven was kept at 50 °C for 2 min, followed by the increment in temperature to 210 °C at a rate of 3 °C min^{-1} and holding time of 2 min. The temperature was again increased upto 280 °C at a rate of 8 °C min^{-1} with a hold time 4 min, which was kept constant till the end of program. Essential oil (1.0 μL) was injected into the system at 260 °C with flow rate of 1.21 mL min^{-1} and helium as a carrier gas. The pressure of carrier gas (helium) was 69 kPa with total flow (16.3 mL min^{-1}), column flow (1.21 mL min^{-1}), purge flow (3.0 mL min^{-1}) and linear velocity (39.9 cm s^{-1}). The interface and ion source temperature of MS were 270 °C and 220 °C, respectively and the solvent cut time was 3.5 min. The mass scan start at 40 amu and end at 650 amu with scan speed 1428 amu s^{-1} and event time 0.50 s. The total running time of program was 70.08 min. The metabolites of essential of seeds were identified by comparison of their retention indices and mass spectra fragmentation pattern with those of available in libraries (NIST14, NIST14s, WILEY8, FFNSC, and SZTERP) of GC-MS data system.

Statistical analysis

The student's t-test (confidence level of 95%) was performed at each growth stages to find out the difference between the means of two groups (i.e. control and eUV-B treatment). A two-way analysis of variance (ANOVA) was carried out to evaluate the significant effect of ages, treatment, and their interaction. A bivariate Pearson's correlation was performed to identify the correlation between growth, physiological parameters and UV-B absorbing compounds. Further, the identified metabolites of essential oil from two groups (control and eUV-B treatment) were subjected to principal component analysis (PCA). PCA was performed by varimax rotation and Kaiser's normalization. All the statistical analysis was conducted by SPSS Inc. version 20.0 (IBM Corp, Armonk, NY).

Results

Growth parameters

Under eUV-B exposure shoot length and root length significantly decreased at three growth stages with maximum reduction in shoot length at 120 DAT (11.71%, $p \leq 0.01$) and root length at 60 DAT (38%, $p \leq 0.01$) as compared to control. The number of leaves and leaf area also reduced under eUV-B exposure with significant reduction in number of leaves at 120 DAT (16.48%, $p \leq 0.05$) and 180 DAT (14.78%, $p \leq 0.01$) and in leaf area by 24.4, 6.2 and 6.6% at 60, 120 and 180 DAT, respectively (Fig. 1). The number of branch and number of nodules showed a significant reduction by 20 and 21.1% at 120 DAT and 18.9 and 19.8% at 180 DAT respectively when exposed to eUV-B. Under eUV-B exposure below and above ground biomass significantly reduced at all the growth stages with maximum reduction at 60 DAT by 48 ($p \leq 0.001$) and 44.3% ($p \leq 0.001$) respectively in below and above ground biomass (Fig. 2). As per the result of two way ANOVA all the growth parameters were significantly affected by age, treatment and their interaction except root length and leaf area which only varied with the individual factors of age and treatment (Table 1).

Anatomical features

Under eUV-B exposure, total thickness of leaves was significantly reduced by 13.8% compared to control ones. The thickness of adaxial and abaxial epidermis also decreased by 41.1 and 34.7%, respectively due to eUV-B exposure (Table 2). In control plants the thickness of spongy parenchyma was higher as compared to palisade parenchyma whereas opposite trend was observed for eUV-B exposed plants. Due to eUV-B exposure, thickness of palisade parenchyma increased and the cells of palisade parenchyma appear compressed as the proportion of intercellular cavities in palisade parenchyma decreased. However the proportion of intercellular cavities in spongy parenchyma was non-significantly affected by eUV-B exposure (Table 2). Exposure of plants to eUV-B also increased the number of oil glands as compared to control (Fig. 3)

Gas exchange and chlorophyll fluorescence

Due to eUV-B exposure photosynthetic rate (Ps), stomatal conductance (gs) and transpiration rate (Tr) reduced with maximum reduction at 60 DAT by 33.9 ($p \leq 0.001$), 40.78 ($p \leq 0.001$) and 54.6 ($p \leq 0.001$), respectively in Ps, gs and Tr. Internal CO₂ concentration (Ci) was only significantly reduced by 6.2 ($p \leq 0.01$) and 8.5% ($p \leq 0.05$) at 60 and 180 DAT respectively. Under eUV-B exposure water use efficiency (WUE) significantly increased by 47.18% at 60 DAT whereas this increase was non-significant ($p \geq 0.05$) at two later growth stages. There was no significant variations ($p \geq 0.05$) noticed in Fv/ Fm at any of the growth stages of plants under eUV-B exposure (Fig. 4). Ps, gs, Tr and WUE were significantly affected by age, treatment and their interaction while Ci and Fv/ Fm only affected by individual factor of age and treatment (Table 1).

Pigments and UV-B absorbing compounds

Total chlorophyll content significantly reduced by 10.6 ($p \leq 0.001$), 2.6 ($p \leq 0.01$) and 13 % ($p \leq 0.001$) at 60, 120 and 180 DAT, respectively under eUV-B exposure as compared to control. Chlorophyll a/b ratio and carotenoids content was found to increase under eUV-B exposure with maximum increase of 32.4 ($p \leq 0.001$) and 35.2% ($p \leq 0.001$) in Chlorophyll a/b ratio and carotenoids content respectively at 60 DAT, however this increase was non-significant ($p \geq 0.05$) for Chlorophyll a/b ratio and carotenoids content at 120 and 180 DAT, respectively (Fig. 5).

UV-B absorbing compounds like flavonoids, anthocyanins and phenols showed significant increase under eUV-B exposure at all growth stages with maximum increment in anthocyanins (16.3 %; $p \leq 0.01$) and flavonoids (15.95%; $p \leq 0.001$) at 60 DAT whereas in phenols (41.4; $p \leq 0.001$) at 180 DAT (Fig. 5). All the pigments and UV-B absorbing compounds were affected by treatment, age and its interaction except flavonoids which was only affected by age and treatment (Table 1).

Reproductive parameters and yield attributes

The number of racemes per plant and the number of flowers per plant were significantly reduced under eUV-B treatment by 28.2 ($p \leq 0.001$) and 8 % ($p \leq 0.001$). Further, the length of racemes also significantly reduced (14.5 %; $p \leq 0.001$) under eUV-B, while the length of flowers was not significantly affected ($p > 0.05$) by eUV-B as compared to control (Table 3).

The number of seeds per plant decreased by 9.3 % ($p \leq 0.01$), whereas the length and diameter of seeds showed a non-significant reduction ($p > 0.05$). Test weight also showed non-significant variation ($p > 0.05$) under eUV-B exposure as compared to control (Table 3).

Essential oil content and composition

The essential oil of seeds of *P. corylifolia* was yellowish in color with a pleasant odor and stickiness. The essential oils content increased by 46.4% under eUV-B as compared to control (Fig. 6). The GC-MS analysis of essential oil of seeds displayed a total of 58 compounds (including trace and major compounds of control and eUV-B exposed plant), among which 28 major compounds were identified and shown here (Table 4). The identified compounds were categorized in different classes such as monoterpenes, sesquiterpenes, meroterpene and others, that overall contributes to 4.43, 74.37, 20.23 and 0.95%, respectively. Overall the monoterpenes showed reduction (32%), whereas sesquiterpenes (6.8%) and meroterpene (14.2%) showed increment under eUV-B exposure, compared to control ones. Among identified metabolites, β -caryophyllene, caryophyllene oxide and bakuchiol were responsible for the major proportion of essential oil and showed increment under eUV-B treatment compared to control (Table 4). The metabolites such as β -myrcene, β -limonene, tetradecynol and cyclosativene were not detected under eUV-B treatment whereas nonadienol and α -amorphene were only detected in eUV-B exposed plants. The eUV-B exposure leads to non-significant changes in some metabolites such as myrtanol, patchoulane, α - and γ -muurolene and cis-calamenene while reduction in β -linalool, β -geraniol and α -pinene oxide. Further some of the metabolites of sesquiterpene class (farnesol, α -humulene, γ -gurjunene, β -selinene and humulene oxide) showed reduction whereas some metabolites (α -copaene, δ -cadinene, aromadendrene, caryophylladienol, caryophyllene epoxide) showed increment under eUV-B treatment as compared to control (Table 4).

Principal component analysis

Two principal components (PCs) were extracted which explained 85.52 % of total variance. PC1 with eigenvalue 21.24 was responsible for 75.85 % of total variance and had high negative loadings for β -caryophyllene, caryophyllene oxide, bakuchiol, nonadienol, α -amorphene, caryophyllene epoxide, aromadenderene and caryophylladienol whereas high positive loadings for tetradecynol, cyclosativene, α -pinene oxide, β -linalool, humulene oxide, farnesol, α -humulene, β -myrcene, β -limonene and β -geraniol. Further PC2 with eigenvalue 2.70 was accountable for 9.6 % of total variance and had high loading for patchoulane, α - and γ -muurolene (Fig. 7).

Discussion

The adverse effects of UV-B on the growth, biochemistry and physiology of plants are well known however the extent of damage differ among the plant species or the cultivars of same species (Reddy et al. 2013; Choudhary and Agrawal 2014). The results of present study

demonstrated that eUV-B negatively affected all the studied growth parameters under eUV-B exposure. Alterations in the plant growth under eUV-B exposure might be due to disturbance in the process of cell elongation and cell division as a consequence of imbalance in the endogenous level of growth regulators (through direct photodegradation by UV-B or by the activation of oxidases) or the alteration in transport of growth regulators (Hopkins et al. 2002; Takshak and Agrawal 2018). In present study number of leaves and leaf area showed reduction under eUV-B exposure and was positively correlated (Fig. 8; $r = 0.989$), which might be seen as an adaptive response of plants to reduce the absorption of UV-B radiation (Choudhary and Agrawal 2014; Tripathi et al. 2019). Chen et al. (2016) revealed that, the enhanced UV-B radiation significantly decreased the shoot height, basal diameter, total leaf area and total dry mass of male and female *Morus alba* saplings. Takshak and Agrawal. (2018) also reported reduction in root length, shoot length, number of leaves, leaf area and total biomass of *Coleus forskohlii* under eUV-B condition. The process of nodulation and activation of *Nod* gene depends on specific composition of flavonoids, which may alter following UV-B exposure (Chimphango et al. 2004). However reduction in number of nodules as noticed in our study might be due to UV-B induced inhibition of photosynthesis that may alter the availability of resources to micro-symbionts, which leads to reduced number and activity of nodules or it might be due to UV-B induced increment in flavonoids and phenols content in leaves. This hypothesis was supported by the positive correlation between number of nodules and photosynthetic rate (Ps) (Fig. 8; $r = 0.770$) and the negative correlation of number of nodules with phenols (Fig. 8; $r = -0.543$). Similarly Choudhary and Agrawal (2014) reported reduction in number and fresh weight of nodules in *Pisum sativum* under UV-B stress.

The biomass of plants represents the long term integration of all the growth, physiological and biochemical aspects (Teramura 1983). The reduction in biomass as observed in present study was corroborated with several other studies on *Cymbopogon citratus* (Kumari and Agrawal 2010) *Coleus forskohlii* (Takshak and Agrawal 2015) and *Morus alba* (Chen et al. 2016) under UV-B stress condition. Tevini and Teramura (1989) reported that reduction in total biomass under UV-B was correlated with reduced plant height and leaf area of plant which was also noticed in our study as reduction in above and below ground biomass of test plant was correlated well with the reduced leaf area (Fig. 8; $r = 0.972$ and 0.965) and shoot length (Fig. 8; $r = 0.965$ and 0.969). More reduction in below ground biomass and root length as compared to above ground biomass and shoot length under eUV-B exposure in present study suggest the lower allocation of photo-assimilates towards the belowground plant parts. Further the higher reduction in above and belowground biomass of eUV-B exposed plants at initial growth stage showed the plant was more sensitive at early growth stage. This finding was corroborated well with the results of Nazari and Zarinkamar (2020), who reported that *Mentha aquatica* exposed to UV-B radiation was more sensitive at early vegetative growth stage as compared to later growth stage. The anatomical studies showed the reduction in total thickness of leaves under eUV-B which was associated with reduction in adaxial epidermis, spongy parenchyma and abaxial epidermis. Similarly, Kakani et al. (2003) and Romanatti et al. (2019) also reported reduction in leaf thickness in cotton and eggplant, respectively. The adaxial surface of leaves, that was directly exposed to eUV-B showed highest reduction of 41.16%. The decrease in the thickness of epidermal cells might be related to reduction in division and expansion of cell. Inostroza-Blancheteau et al. (2014) reported that the slower division of cells provides additional time for repair of DNA, which is one of the important strategies adopted by plants to protect against UV-B induced damage. The increased thickness of palisade parenchyma with short, multilayered and compact cells could be seen as a protective strategy of test plant to reduce the penetration of UV-B radiation to spongy parenchyma.

The major photosynthetic processes including photochemical reactions in the thylakoid membranes, enzymatic reactions of the CO₂ fixation in Calvin cycle, and the stomatal control of CO₂ diffusion get altered following the exposure of plants to UV-B radiation. However the photosynthetic responses of plants under enhanced UV-B condition varies and depends on plants species, cultivar, UV-B dose and other environmental factors (Zhao et al. 2004). In the present study exposure of plants to eUV-B decreased the Ps at all the growth stages, which was mainly attributed to reduced gs and Ci that was further supported by positive correlation of Ps with gs (Fig. 8; $r = 0.728$) and Ci (Fig. 8; $r = 0.856$). Under eUV-B exposure reduction in Tr could also be seen as a strategy of plants to conserve water for later use as shown by increased WUE. This hypothesis was strengthened by the negative correlation between Tr and WUE (Fig. 8; $r = -0.637$). Our results were in agreement with previous findings on *Curcuma* species (Jaiswal and Agrawal 2021) and *Cymbopogon citratus* (Kumari and Agrawal 2010). However contrasting results (reduction in both Tr and WUE) were reported by Rai and Agrawal (2020) in *E. alba* under intermittent and continuous UV-B treatment.

Fv/Fm (maximum quantum yield of PSII) is a measurement ratio that represents the efficiency and stability of PSII, a major component of the photosynthetic apparatus. Any alteration in Fv/Fm, reflects the changes in the photochemical conversion efficiency of PSII, due to which Fv/Fm can be seen as a good indicator of photo-inhibition of photosynthesis (Ranjbarfordoei et al. 2011). However in our study non significant variations in Fv/Fm under eUV-B treatment was noticed at all the growth stages of plants that reflect the absence of PSII photo-inhibition and the consequent photodamage. Martínez-lüscher et al. (2013) reported that both the short and long term exposure of 5.9 and 9.6 kJ m⁻² d⁻¹ UV-B radiation did not cause any alteration in Fv/Fm in *Vitis vinifera*.

Our study displayed that under eUV-B exposure, chlorophyll content reduced that might be the result of inhibition of enzymes involved in chlorophyll biosynthesis pathway or it might be due to destruction of chlorophyll molecule and its precursors (Kataria et al. 2014). The

reduction in chlorophyll content might be taken as one of the reasons (in addition to stomatal limitation) for reduction in Ps under eUV-B exposure in our study as both the parameters (i.e. chlorophyll content and Ps) showed positive correlation (Fig. 8; $r = 0.696$). An increased chlorophyll a/b ratio under eUV-B exposure as observed in the present study reflects the content of chlorophyll b was affected more than chlorophyll a. Tevini et al. (1981) reported that biosynthesis of chlorophyll b was inhibited more than the biosynthesis of chlorophyll a under UV-B exposure that result in enhanced chlorophyll a/b ratio in bean, radish, barley and corn. Carotenoids act as a quencher that dissipate excess excitation energy and protect chlorophyll molecule from photo-oxidative damage. The increase in carotenoids content in present study with concomitant increase in chlorophyll a/b might have offered protection to *P. corylifolia* against eUV-B. The reduced content of chlorophyll under eUV-B treatment could also be seen as a strategy of plants to reduce light absorbance that contributes to its photo-protection (Machado et al. 2017), which was evidenced in the present study as absence of any significant changes in photochemical efficiency of PSII (Fv/Fm).

The secondary metabolites such as flavonoids, anthocyanins and phenols, which are also known as UV-B absorbing compounds accumulate mainly in epidermal and mesophyll cells and help in reducing the penetration of UV-B deep inside the leaves. Further, it may also scavenge the free radicals and provide protection against oxidative damage (Agati et al. 2012; Pandey and Agrawal 2020). In the present study all the UV-B absorbing compounds increased differentially at different growth stages of *P. corylifolia* which was corroborated well with the results of Jaiswal et al. (2020) observed in *Curcuma* sp.

Sexual reproduction is a sophisticated process of a plant species' life cycle, and the various stages of reproductive development were noticed to be sensitive against UV-B radiation (Tripathi et al. 2019). However some studies also documented stimulatory effects of UV-B on the reproductive parameters (Grammatikopoulos et al. 1998; Petropoulou et al. 2001). In present investigation, number of seeds reduced under eUV-B exposure and was directly linked to reduce number of racemes and flowers, resulting from UV-B induced negative impact on vegetative growth of plants as observed by reduced plant height and number of branches. Gan et al. (2013) reported that branches directly influence seeds output and reproductive allocation of a plant species, as the branches accounts for 'assurance of material' for the reproduction in varied environments. Our results were in accordance with study of Jan et al. (2011), who noticed reduction in these reproductive parameters of *P. corylifolia* at higher doses of gamma radiation. Tripathi et al. (2019) also reported reduction in number of heads and achenes due to eUV-B exposure in *Helianthus annuus*. In present study the length of racemes reduced under eUV-B condition that might be due to disturbance in the architecture of inflorescence under stressful condition (Park et al. 2016). Seed size is one of the potential traits of plants' life history and is an important factor that determines the fitness of a plant species. It has been reported that there is a trade-off between number and size of seeds, which enable plants to adapt and survive in their varied habitat (Gan et al. 2013). Abeli et al. (2017) reported that under stressful environment plants invest more photosynthates towards reproduction and enhance the provisioning and quality of seeds as an adaptive response to ensure survival of seedlings. This might be happened in our case, where the number of seeds reduced while seeds size and mass did not show any significant variation as an adaptive response under stressful environment of UV-B. Similarly Yao et al. (2006) reported reduction in seed yield while test weight was unaffected, at lower dose of enhanced UV-B radiation in autumn buckwheat.

Essential oils are formed by plants as a complex mixture of secondary metabolites and are volatile in nature and have a strong odor. Essential oils are known to possess strong potential against several diseases and are use in the preservation of foods from the deteriorating effects of oxidants (Hajlaoui et al. 2009). In present study content of essential oil of seeds increased under eUV-B treatment that might be related with reduced leaf area and higher density of oil glands (Fig. 3). Jaiswal and Agrawal (2021) reported that exposure of plants to eUV-B causes induction of pathway related to essential oil biosynthesis which may leads to increased production and varied composition of essential oils. It has been reported by several authors that the production and composition of essential oil depend on several factors, including environmental conditions, genetic setup of plants, developmental stages, plant tissues and analytical conditions (Ebrahimi et al. 2008; Hajlaoui et al. 2009; Pandey et al. 2021). This might be the probable reason for the observed difference between our study (where caryophyllene, caryophyllene oxide and bakuchiol were observed as major component) with those of the studies of Jan et al. (2015), who reported, α -pinene, psoralen, bakuchiol and caryophyllene as major components of *P. corylifolia* seeds' essential oils.

Bakuchiol which is one of the major components of *P. corylifolia*, belongs to meroterpene class of compound and is derived from the phenylpropane and isoprenoid units. Mehta et al. (1966) extracted bakuchiol for the first time from *P. corylifolia*. It possesses several pharmacological properties such as anticancer, anti-inflammatory, antidepressant, hypoglycemic, neuroprotective, estrogenic, anti-aging activities (Agrawal and Pandey 2019; Xin et

al. 2019). Due to eUV-B exposure content of bakuchiol increased, so this trend of increase in major active compounds of *P. corylifolia* was significant, considering the pharmaceutical perspectives. Jan et al. (2015) also reported increased content of bakuchiol in *P. corylifolia* exposed to variable doses of gamma radiation. Further, the reduction and disappearance of most of the monoterpenes under eUV-B treatment, in our study was might be due to impairment in the chlorophyll containing machinery and thus disturbance in the biosynthesis pathway of monoterpenes, as monoterpenes are mainly synthesized in plastids via methylerythritol pathway (MEP), by using geranyl pyrophosphate (GPP) as a precursor (Rai and Agrawal 2020).

Sesquiterpenes are synthesized in cytosol via mevalonate (MVA) pathway by utilizing farnesyl pyrophosphate (FPP) as precursor. The metabolites α -humulene and β -caryophyllene are the widely occurring and medicinally important sesquiterpenes which possess anti-inflammatory, anticarcinogenic activities. Both of these sesquiterpenes utilize a common humulyl intermediate, that itself generated by FPP (Cane 1999). An increment in β -caryophyllene and its derivatives (caryophyllene oxide, caryophyllene epoxide and caryophylladienol) whereas reduction in α -humulene and its derivative (humulene oxide) suggest the involvement of humulyl intermediate towards the caryophyllene and its derivatives synthesis, and might be the reason for increment of β -caryophyllene and its derivatives under eUV-B in present study. This hypothesis was confirmed by the results of PCA where the metabolites β -caryophyllene, caryophyllene oxide, caryophyllene epoxide and caryophylladienol grouped in one cluster (cluster 1) whereas α -humulene and humulene oxide grouped in another cluster (cluster 2) and also the metabolites of two clusters showed negative correlation (Fig. 7).

Further the farnesol represents the simplest acyclic sesquiterpene and is formed by action of phosphatases on the terminal phosphate moiety of FPP. Farnesol together with FPP provides the homeostatic control of carbon flux in the MVA pathway (Chappell and Coates 2010). In present study reduction in farnesol might be explained by involvement of farnesol in the synthesis of others sesquiterpenes metabolites, resulting in its reduced content under eUV-B. It was also observed that some metabolites (γ -gurjunene, β -selinene) decreased while others such as α -copaene, δ -cadinene, aromadendrene increased indicating differential responses of different enzymes or precursors involved in their synthesis against UV-B radiation. PCA results also showed that the metabolites which showed increasing trend or only detected under eUV-B, clustered together and form the cluster 1 whereas the metabolites which grouped together in cluster 2 showed reduction or not detected under eUV-B treatment.

Conclusions

Our study highlighted the negative effect of eUV-B on most of the growth and physiological parameters with a concomitant increase in UV-B absorbing compounds. The non-significant variations in Fv/Fm suggest, the reduction in photosynthesis was mainly associated with stomatal limitation and chlorophyll reduction, rather than the limitation of PSII. The negative effect of eUV-B on vegetative growth was also reflected by reproductive parameters (as showed by reduction in number of racemes and flowers), as the branches provide maternal assurance for reproduction. Further reduction in number of seeds whereas non-significant variation in seeds mass and seeds size could be seen as an adaptive strategy of plant under stressful environment of eUV-B. The content of essential oil of seeds increased by 46.4 % due to eUV-B treatment. Under eUV-B exposure some metabolites such as α -copaene, δ -cadinene, aromadendrene, caryophylladienol, caryophyllene epoxide showed increment whereas farnesol, α -humulene, humulene oxide, β -linalool, β -geraniol and α -pinene oxide showed reduction; however the major metabolites like caryophyllene, caryophyllene oxide and bakuchiol which possess anticancerous and anti-inflammatory activities increased under eUV-B condition. Overall the study concludes that eUV-B enhanced the quality of seeds in terms of essential oil content and major active metabolites of essential oil obtained from seeds at the expense of growth, physiology and quantity of seeds. So if we aim to obtain a large quantity of seeds, UV-B may not be preferable but if the objective is to improve the medicinal quality of seeds of *P. corylifolia*, UV-B may be seen as a promising one. However for better understanding the detailed mechanism adopted by *P. corylifolia* under UV-B stress condition the study needs to be further explored.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing Interests

The authors declare that they have no competing interests.

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Authors' contributions

Avantika pandey: Conceptualization, Validation, Formal analysis, Investigation, Data curation, Writing-Original draft. Madhoolika Agrawal: Visualization, Formal analysis. Shashi Bhushan Agrawal: Conceptualization, Validation, Supervision. All authors read and approved the final manuscript

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Tables

Table 1 Two way ANOVA test to find the effect of eUV-B treatment (T) plant growth stage (A) and their interaction (T×A) on different growth, physiological parameters and UV-B absorbing compounds of *P. corylifolia* (F ratios and level of significance; ns – non-significant, *p < 0.05, **p < 0.01, ***p < 0.001)

Parameters	A	T	T×A
Leaves	1719.9***	67.8***	14.1***
Leaf area	5946.2***	60.6***	1.5ns
Shoot length	3459.0***	99.5***	14.91***
Root length	291.6***	97.6***	0.92ns
No. of branch	504.8***	39.2***	4.9*
Above ground biomass	2904.7***	50.5***	5.4*
Below ground biomass	896.6***	86.0***	9.9**
Nodules	110.0***	27.8***	5.6*
Photosynthetic rate (Ps)	323.3***	147.8***	29.28***
Stomatal conductance (gs)	150.4***	49.9***	10.4**
Internal CO ₂ (Ci)	1316.2***	39.1***	1.4ns
Transpiration rate (Tr)	377.0***	147.4***	17.3***
Water use efficiency (WUE)	97.7***	10.2**	24.3***
Maximum quantum yield of PSII (Fv/Fm)	10.07**	12.0**	0.823ns
Total chlorophyll	4902.2***	708.0**	103.5***
Chlorophyll a/b	230.1***	75.2***	11.7***
Carotenoids	418.4***	125.4***	45.0***
Flavonoids	5044.7***	407.1***	3.2ns
Phenols	9.6**	97.8***	14.2***
Anthocyanins	3914.6***	56.7***	3.6*

Table 2 Variations in anatomical features of *P. corylifolia* under control and eUV-B conditions along with percent change. Values are mean \pm S.E. Level of significance between control and eUV-B plants; ns – non-significant, *p < 0.05, **p < 0.01, ***p < 0.001

	Control	eUV-B	% Change
Total leaf thickness (μ m)	70.1 \pm 0.36	60.38 \pm 0.22**	-13.86
Adaxial epidermis thickness (μ m)	3.73 \pm 0.02	2.19 \pm 0.13**	-41.16
Abaxial epidermis thickness (μ m)	3.12 \pm 0.03	2.04 \pm 0.03**	-34.75
Pallisade parenchyma thickness (μ m)	27.7 \pm 0.19	28.8 \pm 0.19*	3.94
Spongy parenchyma thickness (μ m)	29.4 \pm 0.27	25.7 \pm 0.49*	-12.55
Proportion taken by intercellular spaces in PP	12.2 \pm 0.32	10.3 \pm 0.10*	-14.95
Proportion taken by intercellular spaces in SP	39.4 \pm 0.66	35.9 \pm 1.0 ^{ns}	-8.9

Table 3 Reproductive and yield parameters of *P. corylifolia* under control and eUV-B conditions with percent change. Values are mean \pm S.E. Level of significance between control and eUV-B plants; ns – non-significant, *p < 0.05, **p < 0.01, ***p < 0.001

Parameter	Control	eUV-B	%Change
No. of racemes plant ⁻¹	51.1±0.9	36.7±0.7 ^{***}	-28.2
No. of flowers plant ⁻¹	874.2± 5.5	803.4±6.5 ^{***}	-8.0
No. of seeds plant ⁻¹	851.4±5.9	771.8±24.0 ^{**}	-9.35
Raceme length (cm)	2.59±0.04	2.21±0.02 ^{***}	-14.54
Flower length (cm)	0.64±0.01	0.6±0.01 ^{ns}	-5.7
Seed length (cm)	0.36±0.00	0.35±0.00 ^{ns}	-1.2
Seed diameter (cm)	0.25±0.00	0.255±0.0 ^{ns}	-0.5
Test weight (g)	18.77±0.03	18.69±0.03 ^{ns}	-0.4

Table 4 Major identified metabolites of *P. corylifolia* seeds' essential oil with their respective retention time (R.T), classes, %area under control, and eUV-B treatment alongwith percent change and importance. C and E represent control and eUV-B treatment, respectively. Values are mean ± SE; nd, not detected. Level of significance between control and eUV-B treated plants: ns, non-significant, *p < 0.05, **p < 0.01, ***p < 0.001

SN	R.T	Compounds	Class	% Area		% Change	Importance	References
				C	E			
1	8.845	β - myrcene	monoterpene	0.07 \pm 0.01	nd	-	Sedative	Hartsel et al. (2016)
2	10.4	β - limonene	monoterpene	0.053 \pm 0.02	nd	-	Used in fragrance	Elisabetsky (2002)
3	12.775	Nonadienol	-	nd	0.44 \pm 0.05	-	-	-
4	13.709	β - linalool	monoterpene	0.29 \pm 0.00	0.12 \pm 0.01**	-57.52	Anticonvulsant	Elisabetsky (2002)
5	13.907	Myrtnanol	monoterpenes	0.52 \pm 0.04	0.24 \pm 0.02ns	-52.56	Insect repellent	Zhao et al. (2019)
6	16.473	β - geraniol	monoterpenes	0.1 \pm 0.01	0.04 \pm 0.00*	-53.33	Flavor and fragrance	Chen and Viljoen (2010)
7	17.257	α -pinene oxide	monoterpenes	4.15 \pm 0.06	3.12 \pm 0.05*	-24.71	Anticogulant, antitumor	Salehi et al. (2019)
8	17.651	Tetradecynol	-	0.23 \pm 0.03	nd	-	-	-
9	20.612	Farnesol	sesquiterpene	0.64 \pm 0.04	0.30 \pm 0.02*	-52.6	Antifungal	Buckle et al. (2015)
10	20.875	Patchoulane	sesquiterpene	0.11 \pm 0.01	0.09 \pm 0.01ns	-12.12	Anti-inflammatory	Buckle et al. (2015)
11	25.258	α - amorphene	sesquiterpene	nd	0.09 \pm 0.02	-	Antimicrobial	Khubeiz and Mansour (2016)
12	25.325	Cyclosativene	sesquiterpene	0.12 \pm 0.02	nd	-	Antioxidant	Turkez et al. (2015)
13	25.665	α - copaene	sesquiterpene	0.64 \pm 0.02	0.81 \pm 0.04*	26.56	Antioxidant, antigenotoxic	Turkez et al. (2014)
14	28.129	β - caryophyllene	sesquiterpene	30.06 \pm 0.05	33.28 \pm 0.04***	10.68	Anticancerous, analgesic	Fidy et al. (2016)
15	29.163	α - humulene	sesquiterpene	3.27 \pm 0.08	2.44 \pm 0.08*	-25.43	Anti-inflammatory	Fernandes et al. (2007)
16	29.258	γ - gurjunene	sesquiterpene	0.29 \pm 0.01	0.20 \pm 0.02*	-30.33	Antibacterial	Bittencourt et al. (2015)
17	29.864	γ - muurolene	sesquiterpene	0.34 \pm 0.02	0.33 \pm 0.00ns	-0.98	Antibacterial(Sellapan et al. 2018)	Bittencourt et al. (2015)
18	30.82	α - muurolene	sesquiterpene	0.23 \pm 0.01	0.20 \pm 0.01ns	-11.59	Antioxidant	Sellapan et al. (2018)
19	31.676	δ - cadinene	sesquiterpene	0.76 \pm 0.02	0.89 \pm 0.05*	17.98	Antibacterial	Pérez-López et al. (2011)
20	31.783	Cis-calamenene	sesquiterpene	0.18 \pm 0.01	0.24 \pm 0.02ns	34.54	-	-
21	32.306	Aromadendrene	sesquiterpene	0.10 \pm 0.00	0.15 \pm 0.01**	40.62	Antimicrobial	Mulyaningsih et al. (2010)
22	34.706	Caryophyllene oxide	sesquiterpene	28.99 \pm 0.06	32.43 \pm 0.04***	11.87	Anticancer, analgesic	Fidy et al. (2018)
23	34.902	β -selinene	sesquiterpene	0.27 \pm 0.02	0.17 \pm 0.02*	-36.14	Antioxidant	Chandra et al. (2017)
24	35.333	Humulene oxide	sesquiterpene	2.69 \pm 0.1	1.65 \pm 0.11***	-38.53	Anti-inflammatory	Fernandes et

25	36.144	Caryophylladienol	sesquiterpene	0.87±0.01	0.94±0.02**	8.42	-	
26	37.065	Caryophyllene epoxide	sesquiterpene	0.93±0.04	1.13±0.04*	20.99	Antitermite activity	Ashitani et al. (2013)
27	37.688	Germacrene-4(15),5,10(14)-trien-1- α -ol	-	1.19±0.09	nd	-	-	-
28	53.078	Bakuchiol	Meroterpene	18.52±0.06	21.15±0.04***	14.19	Anticancer, estrogenic, neuroprotective	Xin et al. (2019)

Figures

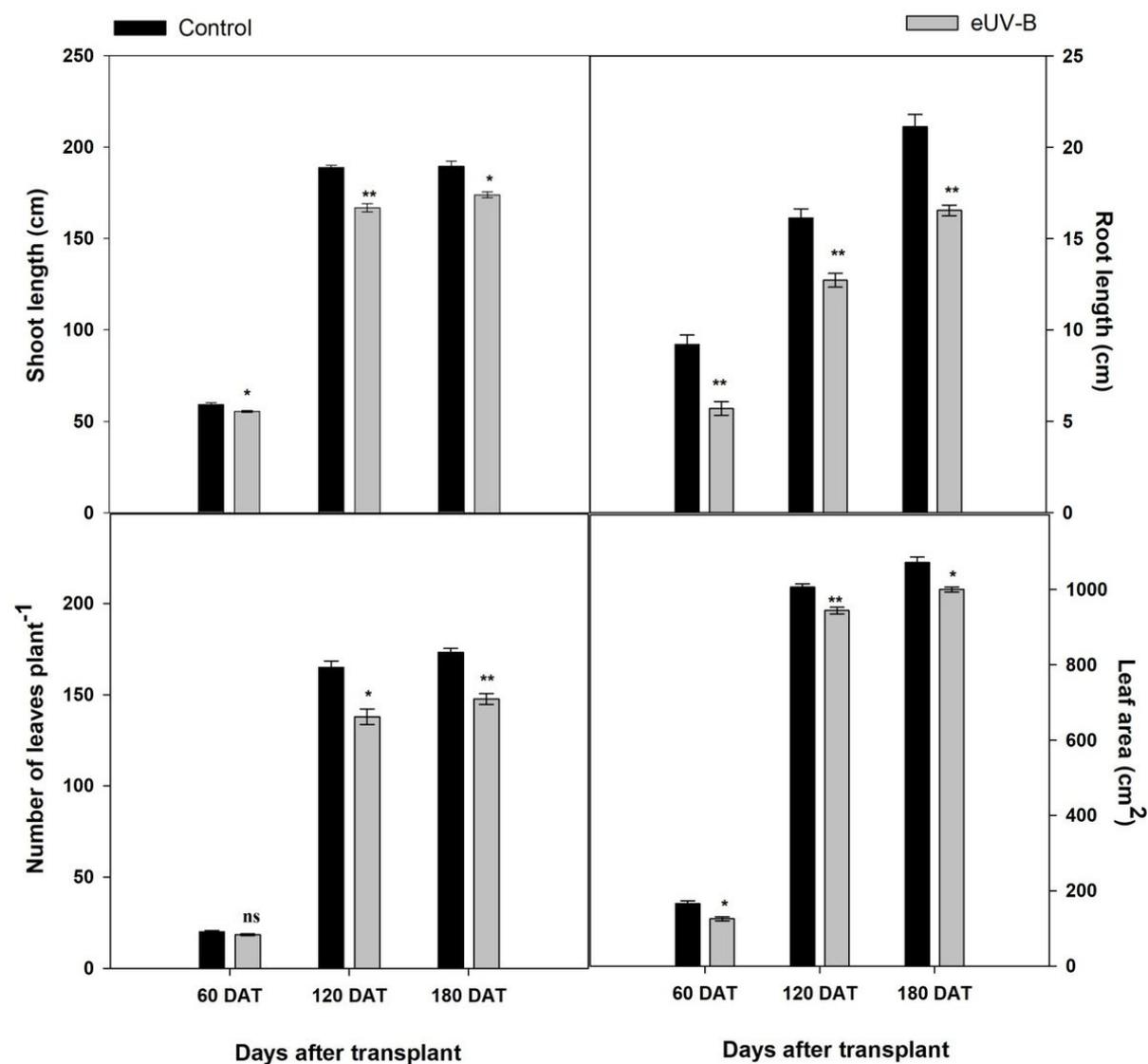


Figure 1

Variation in shoot length, root length, number of leaves and leaf area of *P. corylifolia* at three growth stages under control and eUV-B treatment. Values of bars represent mean \pm SE; Level of significance between control and eUV-B treated plants: ns, non-significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

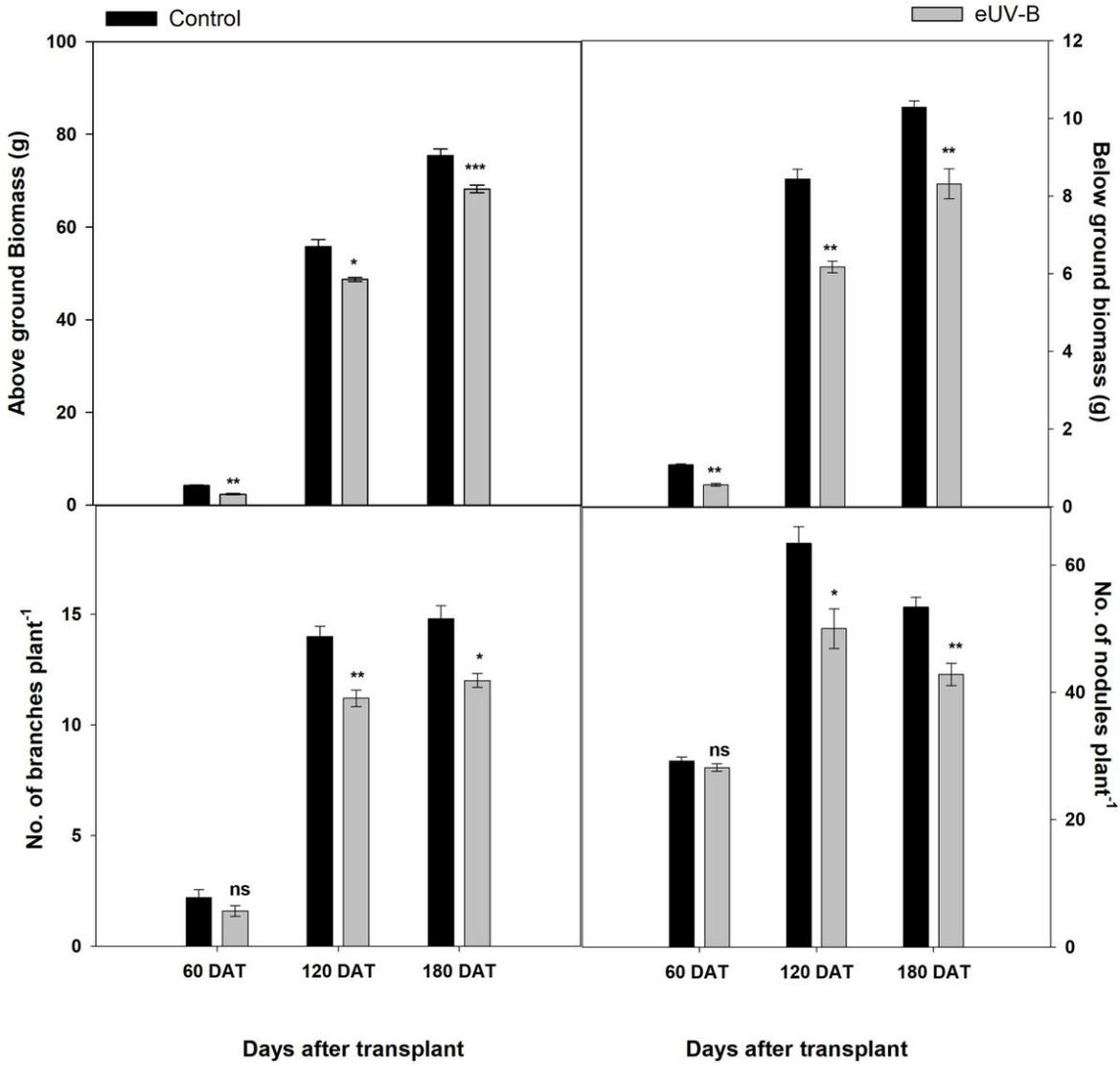


Figure 2

Variation in above and below ground biomass, number of branches and number of nodules of *P. corylifolia* at three growth stages under control and eUV-B treatment. Values of bars represent mean \pm SE; Level of significance between control and eUV-B treated plants: ns, non-significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

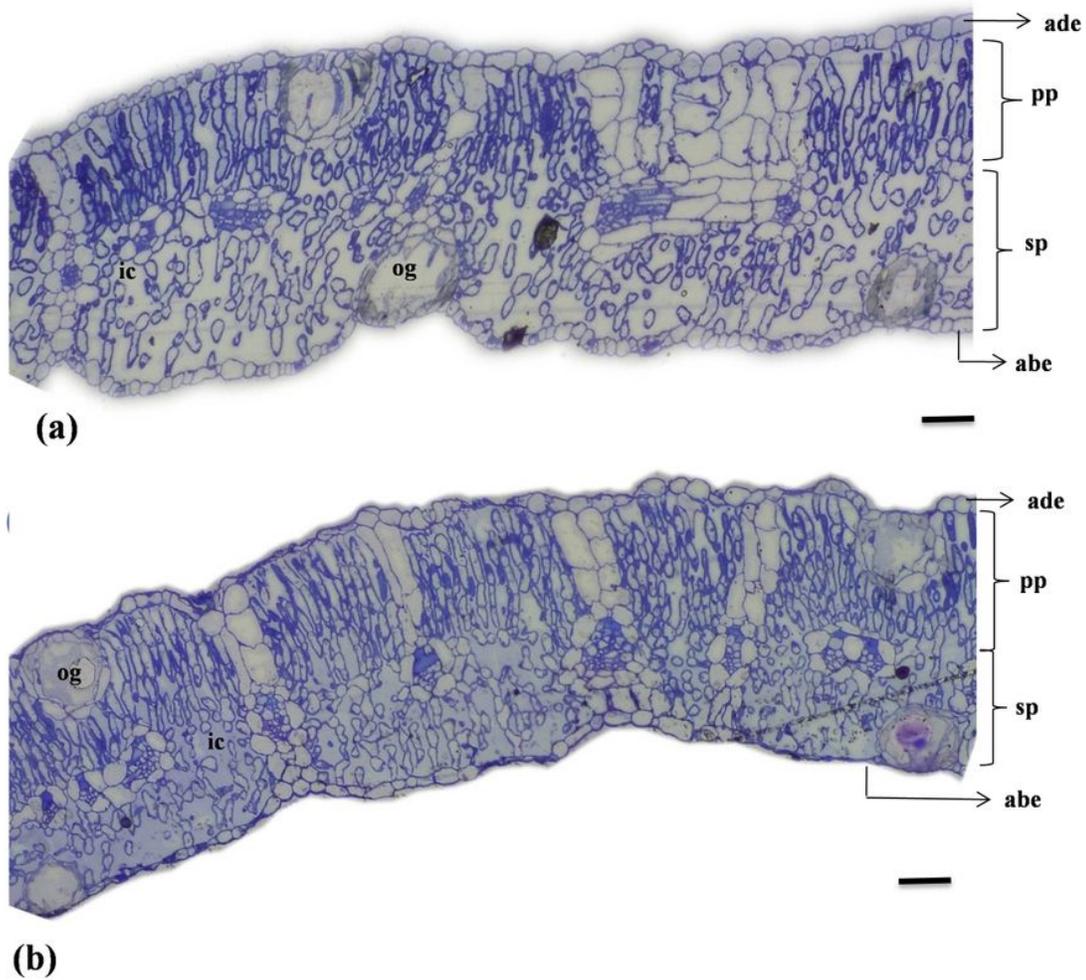


Figure 3

Anatomy of the leaf limb of *P. corylifolia* under control (a) and eUV-B (b) conditions. Abbreviations: ade- adaxial epidermis, pp- palisade parenchyma, sp- spongy parenchyma, abe- abaxial epidermis, ic- intercellular cavities, og- oil glands. Bars-10 μ m

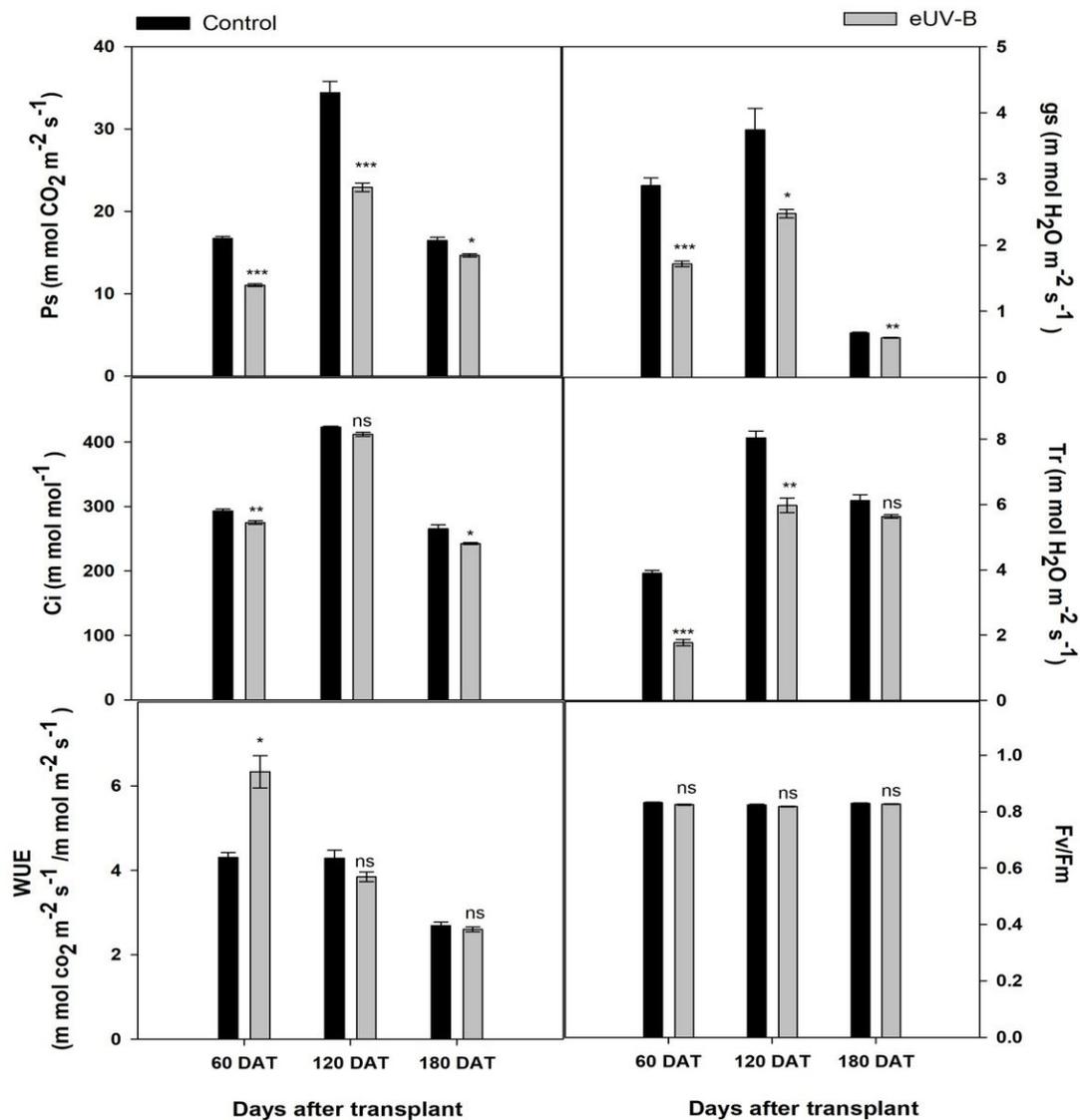


Figure 4

Variation in photosynthetic rate (Ps), stomatal conductance (gs), internal CO₂ (Ci), transpiration rate (Tr), water use efficiency (WUE) and maximum quantum yield of PSII (Fv/Fm) of *P. corylifolia* at three growth stages under control and eUV-B treatment. Values of bars represent mean \pm SE; Level of significance between control and eUV-B treated plants: ns, non-significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

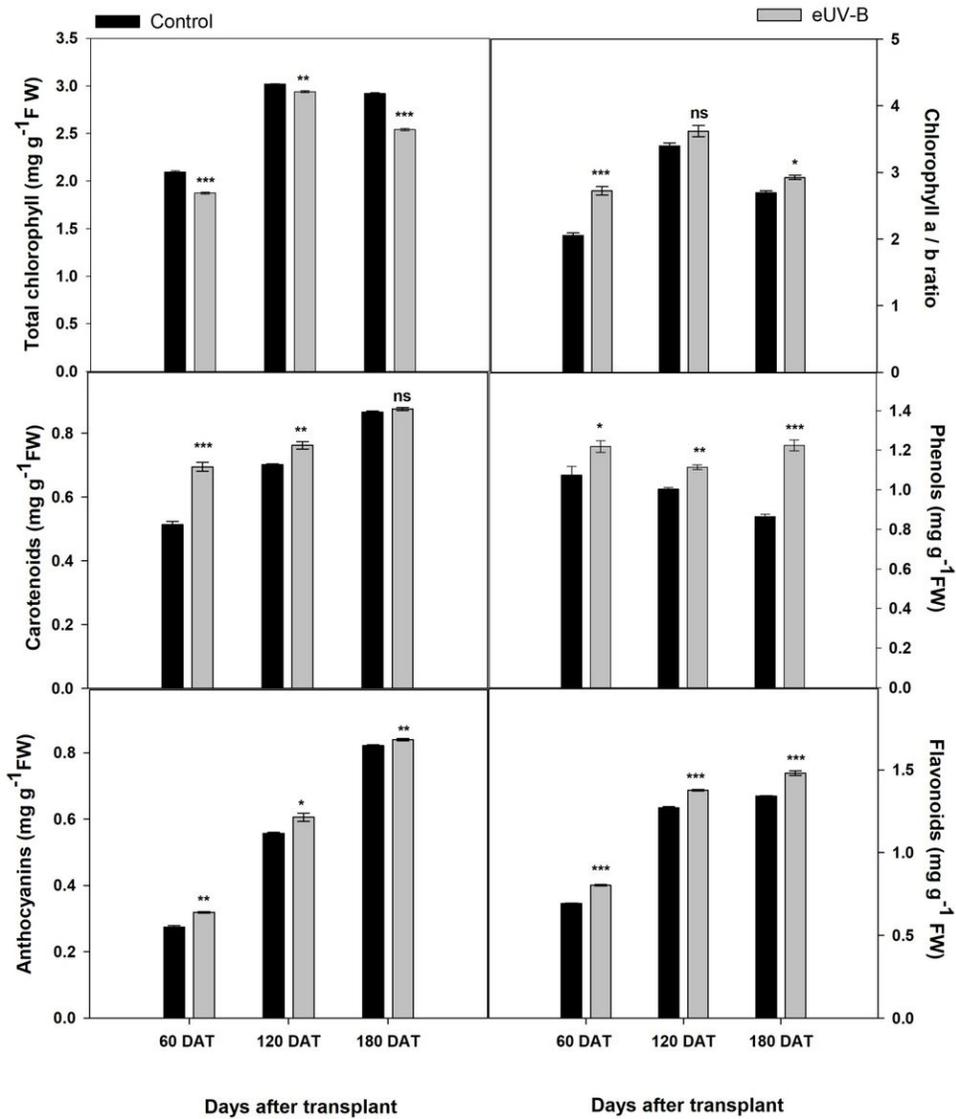


Figure 5

Variation in Total chlorophyll, chlorophyll a/b, carotenoids, phenols, antocyanins and flavonoids content of *P. corylifolia* at three growth stages under control and eUV-B treatment. Values of bars represent mean \pm SE; Level of significance between control and eUV-B treated plants: ns, non-significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

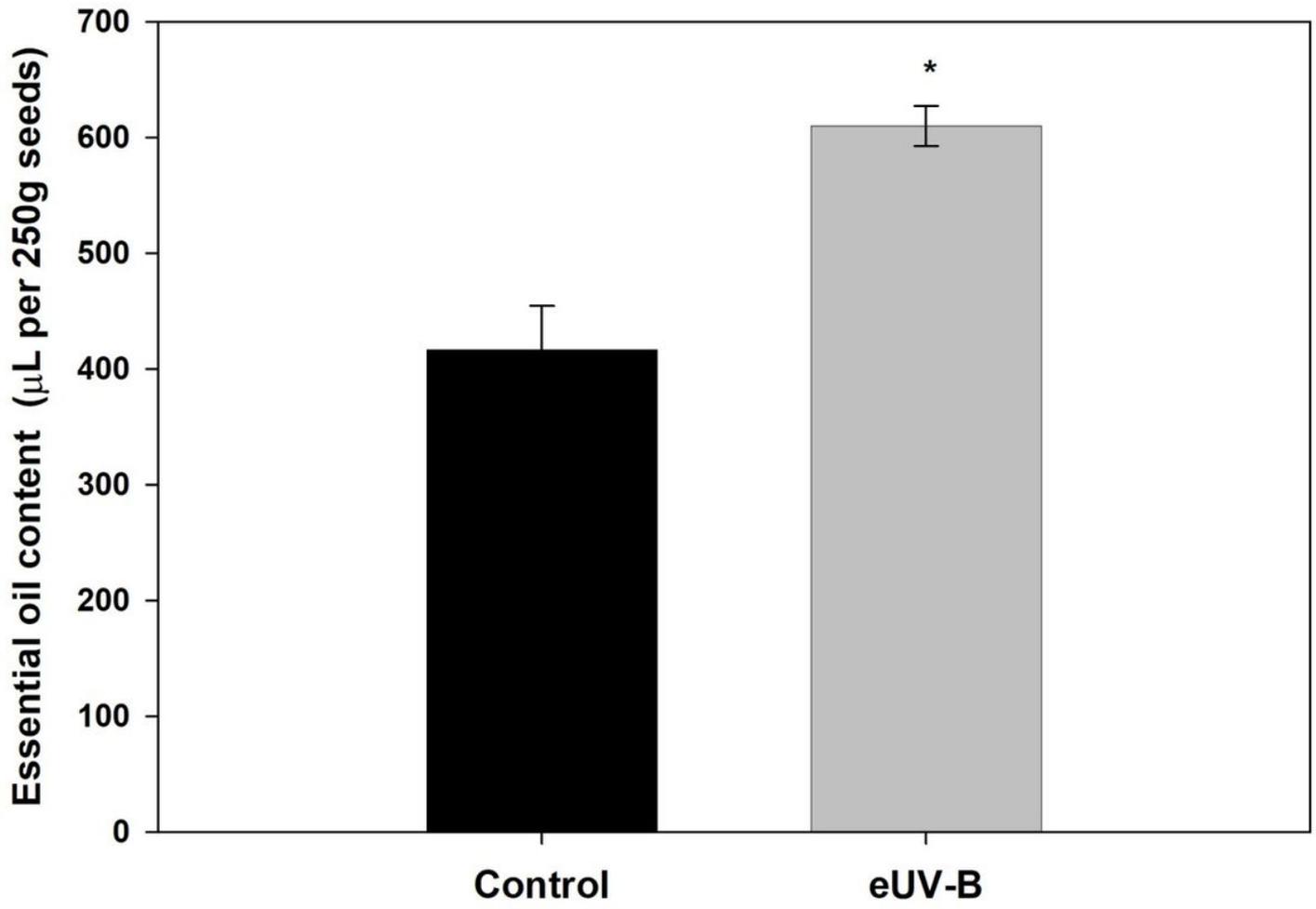


Figure 6

The essential oil content of seeds of *P. corylifolia* under control and eUV-B treatment. Values of bars represent mean \pm SE; Level of significance between control and eUV-B treated plants: *p < 0.05

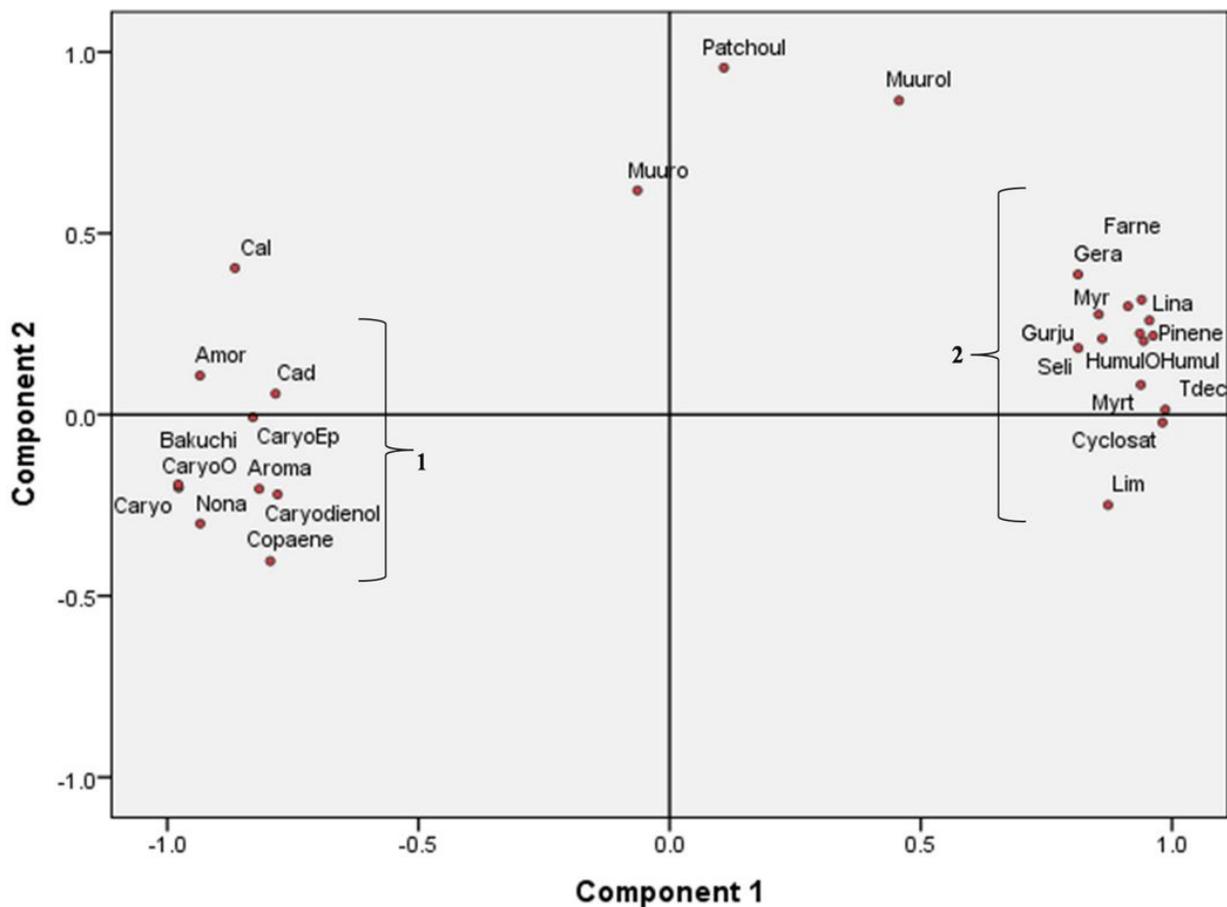


Figure 7

Principal component analysis (PCA): bi-plot representing the relationship between identified metabolites of essential oil of seeds of *P. corylifolia*. Abbreviations: Myr- β -myrcene; Lim- β -limonene; Nona- Nonadienol; Lina- β -linalool; Myrt- Myrtanol; Gera- β -geraniol; Pinene- α -pinene oxide; Tdec- Tetradecynol; Farne- Farnesol; Patchoul- Patchoulane; Amor- α -amorphene; Cyclosat-Cyclosativene; Copaene- α -copaene; Caryo- β -caryophyllene; Humul- α -humulene; Gurju- gammaGurjunene; Muuro- gammaMuuroleone; MuuroI- Muuroleonealpha; Cad- δ -cadinene; Cal- cisCalamenene; Aroma- Aromadendrene; CaryoO-Caryophylleneoxide; Seli- β -selinene; HumulO-Humuleneoxide; Caryodienol- Caryophylladienol; CaryoEp- Caryophyllene epoxide; Ger- Germacra-4(15),5,10(14)-trien-1-alpha-ol; Bakuchi- Bakuchiol

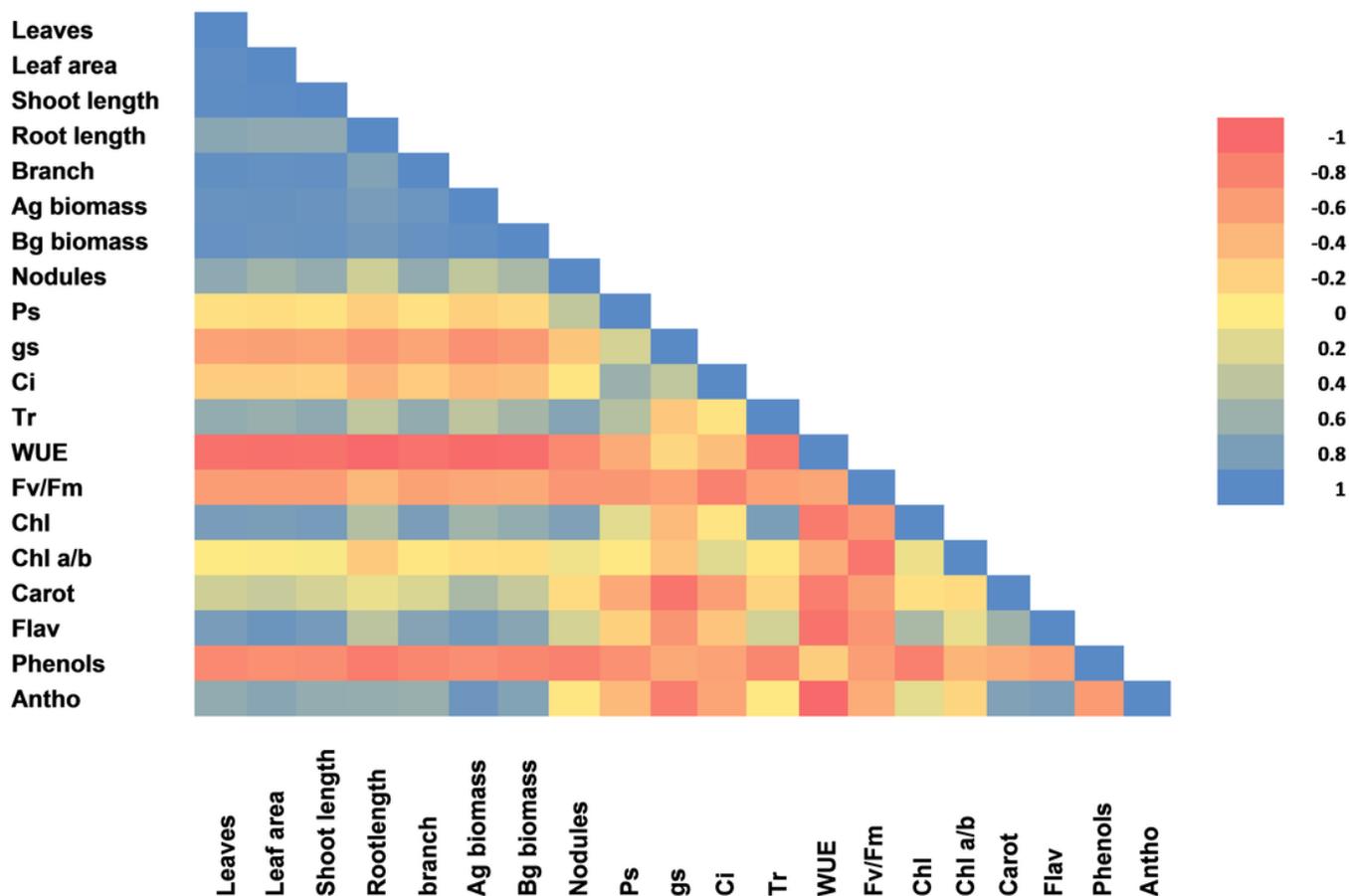


Figure 8

Correlation matrices based on Pearson's correlation coefficient between growth, physiological parameters and UV-B absorbing compounds of *P. corylifolia*. A Pearson's coefficient closer to 1 indicates a positive correlation, closer to 0 means no correlation and closer to -1 showed negative correlation. Abbreviations- Ag biomass- above ground biomass, Bg Biomass- belowground biomass, Ps- photosynthetic rate, gs- stomatal conductance, Ci- internal CO₂, Tr- transpiration rate, WUE- water use efficiency, Fv/Fm- maximum quantum yield of PSII, Chl- Total chlorophyll, Chl a/b- chlorophyll a/b, Carot- carotenoids, Flav- flavonoids, Antho- anthocyanins