



Exogenous Strigolactone (GR24) Positively Regulates Growth, Photosynthesis, and Improves Glandular Trichome Attributes for Enhanced Artemisinin Production in *Artemisia annua*

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Received: 16 November 2021 / Accepted: 24 March 2022 / Published online: 8 April 2022
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Abstract

Artemisia annua is a medicinal plant particularly known for the production of a sesquiterpene lactone artemisinin; a specialty metabolite known for its efficacy in the treatment of malaria by killing different strains of *Plasmodium falciparum* due to radicals released upon the cleavage of its endoperoxide motif. Considering these facts and the immense medicinal value of artemisinin, the enhancement of *in planta* production of artemisinin is highly desirable. As strigolactones are known to regulate various aspects of plant growth and development, the effects of foliar spray of different concentrations of synthetic strigolactone analog GR24 (0, 0.5, 1, 2, 4, and 8 μM) on *A. annua* were studied. As compared to the control group, the foliar application of GR24 had a positive impact on general growth, photosynthesis, and other physiological indices with 4 μM GR24 showing the best results. The results indicate that GR24 application increased the plant biomass and various attributes related to photosynthesis, like total chlorophyll content, chlorophyll fluorescence, stomatal conductance, internal CO_2 , and net photosynthetic rate. Moreover, the activity of various enzymes related to photosynthesis like carbonic anhydrase, nitrate reductase, and RuBisCO was escalated. The GR24 also improved certain attributes related to glandular trichomes, with a significant enhancement in content and yield of artemisinin as compared to untreated plants.

Keywords Artemisinin · Carotenoid-derived phytohormone · Glandular Trichomes · Strigolactones

Introduction

Malaria is a global infectious disease caused by different species of *Plasmodium* parasite and remains a primary cause of mortality and morbidity in the developing world. According to World Health Organization (WHO) Malaria report 2020, there were an estimated 229 million malaria cases in 2019 in 87 malaria-endemic countries and about 409,000 malarial deaths worldwide during this period. Malaria is a curable and preventable disease, provided that the WHO-recommended guidelines given in the *Global technical*

strategy for malaria 2016–2030 are thoroughly followed (WHO 2021).

Artemisinin, an antimalarial phytochemical synthesized in the glandular trichomes of *Artemisia annua* L. (Family: Asteraceae) and characterized by the existence of an endoperoxide bridge (Luo and Shen 1987), is very effective against the chloroquine resistant strains of *Plasmodium falciparum* (Liu et al. 2006). Artemisinin based combination therapies (ACTs), a combination of an artemisinin derivative and a structurally unrelated synthetic antimalarial partner drug, are endorsed by WHO as the first and second line of treatment for chloroquine-resistant *P. vivax* malaria as well as for uncomplicated *P. falciparum* malarial strains (WHO 2019). Besides malaria, ACTs are also effective against certain types of cancers (Efferth 2017), viral infections (Efferth 2018), and several parasitic diseases, like schistosomiasis (Munyangi et al. 2018). Moreover, the extract obtained from the dried leaves of *A. annua* plants has also shown efficacy in inhibiting the replication of SARS-COV-2 and its two variants B.1.1.7 and B.1.351 under in vitro conditions (Nair

Handling Editor: Parvaiz Ahmad.

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et al. 2021). However, this anti-viral effect did correlate with artemisinin content and need further study under in vivo conditions as well. Despite the immense potential of artemisinin in combating malaria, drugs based on this are still not available to millions of people in developing countries, particularly Africa and South-East Asia due to low artemisinin content *in planta* (Wallaart et al. 1999; Wani et al. 2021). Considering the importance of this plant, substantial research has been carried out to find ways of escalating the *in planta* artemisinin production, as its chemical synthesis is quite complex and costly. Exogenous application of several phytohormones has been found to enhance the terpenoid biosynthesis in many aromatic plants (Shukla et al. 1992), and numerous studies have shown the impact of exogenous application of different phytohormones like salicylic acid (Pu et al. 2009; Aftab et al. 2011a; Kumari et al. 2018), gibberellic acid (Aftab et al. 2010; Banyai et al. 2011; Chen et al. 2021), abscisic acid (Zehra et al. 2020), and methyl jasmonate (Aftab et al. 2011b; Zhou and Memelink 2016) in artemisinin enhancement. Therefore, this study was carried out to investigate the impact of exogenous GR24 treatment on growth, physiology, and artemisinin biosynthesis in *A. annua*.

Strigolactones are a group of apocarotenoid compounds synthesized by enzymatic modifications of carotenoid precursors, which were first found to induce seed germination of some parasitic plants (Kohlen et al. 2011). Besides working as allelochemicals in the rhizosphere, these multifunctional plant metabolites were later on recognized as a novel class of terpenoid phytohormones involved in suppression of shoot branching by two groups of independent researchers in 2008 (Gomez-Roldan et al. 2008; Umehara et al. 2008). Besides their role in the regulation of shoot architecture and stimulating seed germination of some root parasitic weeds, they regulate a range of other growth and developmental processes in plants including modulation of root architecture and adaptive responses of plants to different environmental stressors (Kapulnik et al. 2011; Hu et al. 2014). Due to their vast diversity and involvement in various growth and developmental processes, they can potentially be used for improving plant agronomic and physiological traits (Wani et al. 2020). For example, CRISPR/Cas9-mediated targeted disruption of *CCD7*, which is involved in strigolactone biosynthesis, resulted in increased yield in rice plants (Butt et al. 2018). Strigolactone production has been demonstrated in several plant species, particularly in their roots (Brewer et al. 2013), through the action of various oxygenases via the carotenoid pathway (Matusova et al. 2005; Gomez-Roldan et al. 2008). Due to low concentrations of endogenous strigolactones (15–30 pg plant⁻¹ day⁻¹) and their instability, a number of strigolactone analogs like GR5, GR7, and GR24 have been synthesized chemically, with GR24 showing the highest activity (Sato et al. 2003; Koltai and Prandi. 2014).

GR24 is widely used to study multiple pathways by which strigolactones affect various aspects of plant growth and development during normal and stressful conditions.

Considering the immense medicinal value of *A. annua* plants and the intriguing biological properties of strigolactones, the present study was conducted to investigate the effect of exogenous strigolactone (GR24) on growth, photosynthesis, its related physio-biochemical parameters, and artemisinin production in *A. annua*. The correlation between exogenous GR24 spray and the growth status of *A. annua* plants and related parameters of photosynthesis, enzymatic activity, and artemisinin production were investigated to determine the optimal GR24 concentration for improved growth of *A. annua* plants. This study may open new avenues for future research to explore the potential of this novel family of bioactive molecules in improving the agronomic traits of plants.

Materials and Methods

Plant Material and Treatments

This study was performed on *A. annua* (CIM-Arogya) plants under natural net house conditions following CRBD. Healthy seeds with uniform size and texture were selected, surface sterilized with 0.2% HgCl₂, and then washed repeatedly with deionized water. These seeds were then sown in seedbeds for germination. The experimental pots were filled with soil and farmyard manure (4:1 ratio), with the final weight of the homogeneous mixture reaching 5 kg each. Four weeks after the sowing of seeds, seedlings of uniform size were selected and one seedling was transplanted into each earthen pot. Four weeks after transplantation, five GR24 treatments (0, 0.5, 1, 2, 4, and 8 μM) were sprayed on plant leaves, with five replicates conducted for each treatment and one set of plants was kept as control and sprayed with water only.

Determination of Shoot Height and Root Length

The plants were carefully uprooted from the pots and a ruler was used to take the measurements of shoot height and root length. The average value of three replicates was obtained and used as the measured value of shoot height and root length.

Determination of Plant Biomass

The roots of the plants were separated from the above-ground portion to determine the fresh weight of roots and shoots. The roots and shoots were then dried at 80 °C for 48 h for the measurement of their respective dry weights.

Determination of Carbonic Anhydrase (CA) and Nitrate Reductase (NR) Activity

Randomly selected fresh leaves were used to estimate the CA activity using the protocol given by Dwivedi and Randhawa (1974). Leaf samples weighing 200 mg were transferred into petri dishes and leaf pieces were then dipped into 10 ml 0.2 M cysteine hydrochloride solution for 20 min at 0–4 °C. After that, 0.2 ml of 0.022% bromothymol blue and 4 ml of 0.2 M sodium bicarbonate solution were added to the leaf material. It was kept as such for 20 min and leaf material was poured out; the remaining solution added with a few drops of methyl red as an indicator was titrated against 0.05 N HCl. Finally, the CA activity was expressed as $\mu\text{M CO}_2 \text{ kg leaf FW}^{-1} \text{ s}^{-1}$.

NR activity was estimated by the method of Jaworski (1971). Freshly collected leaves weighing 200 mg were sliced into small pieces and transferred into plastic vials. A 5.5 ml reaction mixture consisting of 2.5 ml 0.1 M PO_4 buffer, 2.5 ml 5% isopropanol, and 0.5 ml of 0.2 M potassium nitrate was added into vials containing leaf material and incubated for 2 h at 30 °C. The nitrate formed was calorimetrically estimated at 540 nm after azocoupling with 0.3 ml each of sulfanilamide and naphthalene diamine dihydrochloride. The enzyme activity was expressed as $\text{nM NO}_2 \text{ g FW}^{-1} \text{ h}^{-1}$.

Estimation of Photosynthetic Parameters and Pigments

The net photosynthetic rate (P_N), stomatal conductance (G_s), and internal carbon dioxide (C_i) were estimated in fully expanded leaves on sunny day at 10 AM with an Infra-Red Gas Analyzer (IRGA, Li-Cor 6400, Lincoln, Nebraska, USA). Before recording these parameters, the IRGA was calibrated and zero was adjusted every 30 min during the measurement period. Each randomly selected leaf for the estimation of these parameters was enclosed in a gas exchange cuvette for 60 s during measurement. These parameters were recorded three times for each treatment under optimum temperature (25 ± 1.5 °C). CO_2 concentration and photosynthetic photo-flux density (PPFD) were maintained at 600 ppm and $800 \mu\text{mol mol}^{-2} \text{ s}^{-1}$, respectively. Chlorophyll fluorescence was determined with the help of a saturation pulse fluorometer PAM-2000 (Walz, Effeltrich, Germany). Chlorophyll and carotenoid contents were estimated using the protocol given by Lichtenthaler and Buschmann (2001).

Estimation of RuBisCO Enzyme Activity

The RuBisCO enzyme activity was determined spectrophotometrically by monitoring NADH oxidation at 30°C at 340 nm following the protocol of Usuda (1985). 1 g of leaf

tissue was homogenized in a chilled mortar and pestle with ice cold extraction buffer containing 0.25 M Tris-HCl (pH 7.8), 0.0025 M EDTA, 0.05 M MgCl_2 , and 37.5 mg DTT. It was then centrifuged at $10,000 \times g$ for 10 min at 4 °C and the resulting supernatant was then used for enzyme assay. The reaction mixture contained 40 mM NaHCO_3 , 100 mM Tris-HCl (pH 8.0), 0.2 mM NADH, 10 mM MgCl_2 , 0.2 mM EDTA, 5 mM DTT, 4 mM ATP, and 1 U of 3-phosphoglycerate kinase. The enzyme activity was estimated after the addition of 0.2 mM RuBP and enzyme extract.

Scanning Electron Microscopy (SEM)

SEM analysis was carried out for visualizing leaf ultrastructure including stomata and trichomes present on leaves. For this purpose, the leaves were kept in 4% glutaraldehyde solution for 2 h and then washed with 0.1 M sodium cacodylate buffer solution having pH 7.2. The leaf material was passed through a sequential ethanol series as described by Duke and Paul (1993). After that SEM analysis was performed using a scanning electron microscope (JSM 6510 LV, JEOL, Japan).

Extraction and Estimation of Artemisinin

For leaf artemisinin estimation, High-performance liquid chromatography (HPLC) was employed following the protocol given by Zhao and Zeng (1986) as described in our earlier publication Zehra et al (2020).

Statistical Analysis

Descriptive statistics were presented as mean \pm standard error of replicates. The variation among different treatments was tested by One-way ANOVA. Duncan's Multiple Range Test ($\text{DMRT} \leq 0.05\%$) was also used to identify different treatments. The statistical significance level was considered as 0.05%. All the statistical calculations were carried out using SPSS (ver: 22) statistical program.

Results

Growth and Biomass of Attributes

In the present study, the foliar application of strigolactone (GR24) significantly increased the key growth parameters of *A. annua* plants as compared to the control (Table 1). The plant height, root length, fresh, and dry weight of shoot and root increased in response to GR24 treatment, with 4 μM GR24 giving the best results. These attributes increased with an increase in GR24 concentration; however, at 8 μM concentration, there was a slight decrease in these attributes as compared to its influence at 4 μM . It resulted in 28.45

Table 1 Effects of different concentrations of GR24 on growth attributes of *Artemisia annua*

Treatments/ Parameters	Plant height (cm)	Root length (cm)	Fresh weight of shoot (g)	Dry weight of shoot (g)	Fresh weight of root (g)	Dry weight of root (g)
Control	93.40 ± 0.88 ^d	31.92 ± 0.35 ^d	427.24 ± 5.11 ^e	79.83 ± 0.66 ^d	9.28 ± 0.28 ^d	4.55 ± 0.33 ^d
0.5 μM	98.73 ± 1.13 ^c	33.90 ± 1.06 ^{c,d}	449.95 ± 5.75 ^{d,e}	83.91 ± 1.03 ^{c,d}	10.28 ± 0.54 ^{c,d}	4.96 ± 0.31 ^d
1 μM	103.16 ± 1.11 ^c	36.42 ± 1.15 ^c	473.93 ± 6.93 ^{c,d}	87.29 ± 1.48 ^{b,c}	11.29 ± 0.68 ^{b,c}	5.23 ± 0.19 ^{c,d}
2 μM	108.96 ± 1.30 ^b	40.25 ± 1.09 ^b	491.95 ± 9.10 ^{b,c}	91.76 ± 1.64 ^b	12.00 ± 0.58 ^{b,c}	6.00 ± 0.20 ^{b,c}
4 μM	119.97 ± 2.49 ^a	46.53 ± 1.80 ^a	536.23 ± 10.52 ^a	98.65 ± 2.29 ^a	14.34 ± 0.51 ^a	7.11 ± 0.38 ^a
8 μM	112.79 ± 1.87 ^b	42.23 ± 0.51 ^b	501.52 ± 8.03 ^b	92.13 ± 1.67 ^b	12.22 ± 0.53 ^b	6.47 ± 0.40 ^{a,b}

Data represent the mean value ± SE from five replicates ($n=5$). The different lower case letters represent statistically significant differences between treatments ($p < 0.05$) according to the Duncan's multiple range test

Table 2 Effects of different concentrations of GR24 on CA, NR activities, and chlorophyll fluorescence in *Artemisia annua*

Treatments/ Parameters	CA activity ($\mu\text{mol CO}_2 \text{ kg}^{-1}$ FW s^{-1})	NR activity ($\mu\text{mol NO}_2 \text{ g}^{-1}$ FW h^{-1})	Chlorophyll fluorescence (F_v/F_m)
Control	229.23 ± 0.95 ^e	304.40 ± 2.36 ^e	0.740 ± 0.005 ^c
0.5 μM	236.97 ± 1.02 ^d	311.20 ± 3.61 ^d	0.762 ± 0.007 ^{b,c}
1 μM	245.63 ± 1.17 ^c	320.40 ± 5.92 ^c	0.772 ± 0.005 ^b
2 μM	251.15 ± 1.30 ^b	332.60 ± 4.94 ^b	0.778 ± 0.007 ^b
4 μM	259.45 ± 1.58 ^a	344.80 ± 3.49 ^a	0.833 ± 0.029 ^a
8 μM	254.22 ± 1.33 ^b	339.30 ± 1.13 ^{a,b}	0.790 ± 0.015 ^{a,b}

Data represent the mean value ± SE from five replicates ($n=5$). The different lower case letters represent statistically significant differences between treatments ($p < 0.05$) according to the Duncan's multiple range test

and 46.53% increase in shoot height and root length; 25.51 and 54.53% increase in fresh weight of shoot and root; and 23.58 and 56.26% increase in dry weight of shoot and root, as compared to the control (Table 1).

Carbonic Anhydrase (CA) and Nitrate Reductase (NR) Activity

The foliar application of GR24 increased the activity of photosynthesis-related enzyme CA (Zinc-containing metalloenzyme), and nitrogen flux-related enzyme NR (Table 2). The activity of both of these enzymes increased with increasing concentration of exogenous GR24, with a maximum increase of 13.18 and 13.27% at 4 μM concentration, as compared to their respective controls.

Gas Exchange Parameters and Photosynthetic Pigments

Upon the exogenous application of GR24, there was a significant increase in key photosynthetic parameters of the leaf, as compared to the control values (Fig. 1). GR24 (4 μM)

application enhanced photosynthetic characteristics including net photosynthetic rate, stomatal conductance, and internal CO_2 concentration increased up to 30.67%, 26.55%, and 21.65%, respectively, as compared to their respective controls. Moreover, it also resulted in increased total chlorophyll content by 30.52% as compared to the control, with carotenoid content showing an increase of 31.80% (Fig. 1). Furthermore, it also enhanced the chlorophyll fluorescence by 12.57% as compared to the control (Table 2).

RuBisCO Activity

There was an increase in RuBisCO activity with an increase in the concentration of GR24, with 4 μM GR24 showing an increase of 14.14% as compared to the control (Fig. 1).

Glandular Trichome Development

The exogenous GR24 application had a positive impact on certain parameters related to glandular trichomes (Fig. 2). There was a significant increase in the trichome density, length, width, and area of trichomes, with 4 μM GR24 giving best results, increasing these parameters by 21.59%, 9.08%, 14.53%, and 24.93%, respectively (Table 3).

Artemisinin Content and Yield

In this study, the exogenous GR24 application had a positive impact on artemisinin production in *A. annua* plants (Table 3). The artemisinin was maximally enhanced at 4 μM GR24 concentration, showing an increase of 29.98% and 51.11% in artemisinin content and yield, respectively, as compared to the control plants.

Discussion

In the current study, the understanding of plant responses to exogenous strigolactone (GR24) application is being investigated, with a demonstration that strigolactones

have a positive impact on growth, physio-biochemical parameters, and artemisinin production in *A. annua*. Phytohormones act as key chemical messengers that regulate various aspects of plant development by influencing different physiological processes and coordinating cellular activities at very low concentrations (Fleet and Williams 2011). Strigolactones are the most recently discovered group of phytohormones, which act both exogenously, e.g., promoting symbiosis with arbuscular mycorrhizal fungi and endogenously, e.g., induce stem thickening and regulate root and shoot architecture (Akiyama et al. 2005; Gomez-Roldan et al. 2008; Koltai et al. 2010; Brewer et al. 2013). Strigolactones regulate the growth and phenotypic traits of different plant species under normal (Agusti et al. 2011; Laressergues et al. 2015) as well as stressed conditions, like drought and salt stress (Ha et al. 2014; Qiu et al. 2021). In the present study, the exogenous application of GR24 resulted in increased growth and biomass of *A. annua* plants (Table 1). The increased root length, shoot length, and biomass could be due to the ability of SLs to interact in a range of developmental processes and photomorphogenesis, including vegetative and reproductive growth (Urquhart et al. 2015; Kramna et al. 2019). Upon GR24 spray, the increased root system was conducive for absorbing more water and nutrients, whereas the suppression of lateral buds due to increased plant height is conducive for full photosynthesis, which is also reported in an earlier study conducted on rice (Ling et al. 2020).

Carbonic anhydrases are ubiquitous metalloenzymes which mostly contain zinc ligands and play a vital role in a number of physiological processes in plants like acid–base balance, gas and ion exchange, facilitation of bicarbonate for anaplerotic reactions, and carboxylation/decarboxylation reactions (Georgios et al. 2004; Tetu et al. 2007; Wei-Hong et al. 2014). Phytohormones including strigolactones are known to have a stimulatory effect on various enzymes involved in photosynthesis, as reported by many researchers (Shen et al. 2020). The foliar spray of GR24 resulted in increased CA activity, which may facilitate increased CO₂ fixation via photosynthesis, leading to improved plant growth. This response of plants to exogenous strigolactones may be due to increased stomatal conductance (Fig. 1), facilitating increased diffusion of CO₂ into stomata, which is then acted upon by CA and finally reduced by RuBisCO in the stroma of chloroplasts. The increased CA anhydrase activity upon GR24 application has also been reported in green alga *Chlorella vulgaris* (Shen et al. 2020). Nitrate reductase, a key enzyme involved in nitrate assimilation and an important enzymatic source of nitric oxide in plants, showed increased activity upon GR24 spray. The increased nitrate assimilation might have a positive impact on general growth attributes of plants due to increased conversion rate of nitrate into organic

nitrogenous compounds (Heidari et al. 2011). Moreover, the production of NO by nitrate reductase might also have a beneficial impact on growth of plants.

As evident from the data (Fig. 1), exogenous strigolactone application maintained a better photosynthetic rate in *A. annua* plants. The chlorophyll content in chloroplasts is used as an indicator of photosynthetic performance and also an indicator of chloroplast development. In our study, we observed increased total chlorophyll content upon GR24 application, which may be due to inhibition of chlorophyllase (EC 3. 1. 1. 14; chlorophyll degrading enzyme), which is in agreement with the earlier reports by Naeem et al. 2012 and Lu et al. 2019. Moreover, in earlier studies involving *ORT₁* mutants of tomato, deficient in strigolactones, lower chlorophyll level was detected in these mutants as compared to wild-type plants (Mayzlish-Gati et al. 2010). In another study by Ma et al. (2017) exogenous GR24 application has been shown to increase chlorophyll content in *Brassica napus* under salinity stress. Furthermore, GR24 could maintain the stability of thylakoid membranes by regulating the combination of chlorophyll and membrane proteins, thereby enhancing photosynthetic efficiency (Lu et al. 2019). GR24-pretreated plants have shown increased chlorophyll content as well as increased stomatal conductance and photosynthetic efficiency as compared to non-GR24-pretreated cucumber seedlings under salinity stress (Zhang et al. 2022). The positive influence of exogenous strigolactone treatment on chlorophyll content and photosynthetic rate is also in conformity with Sedaghat et al. (2017), Zheng et al. (2021), and Sattar et al. (2021) as observed in wheat, apple, and maize, respectively. Therefore, the regulation of photosynthesis and chlorophyll pigments in a positive direction could be one of the strategies for GR24 to improve growth and other physio-biochemical characteristics of *A. annua*, which may be even more important under stressful growth conditions.

Out of the total light energy absorbed by the leaves, only 50% is transferred to the photosystems, and this energy conversion occurs via a series of electron carriers on the thylakoid membrane and finally to NADP⁺ in stroma (Ralph and Gademann. 2005; Yamori et al. 2011). Chlorophyll fluorescence (Fv/Fm) is an important technique used to get detailed information of the state of PSII and is generally used as an indicator of photonic energy conversion by PSII, which is used in photochemical processes (Murchie and Lawson. 2013; Shu et al. 2016). During the current study, the Fv/Fm increased upon the application of GR24, which suggests an increase in conversion of absorbed photons into chemical energy and thus less loss of quanta as heat and fluorescence. These results may be due to the ability of GR24 in maintaining PSII complex stability and improving electron transport for assimilatory power (ATP and NADPH) (Lu et al. 2017; 2019). In an earlier study conducted on tomato by Lu et al. (2019), the expression analysis of genes *psbA*, *psbB*, *psbD*,

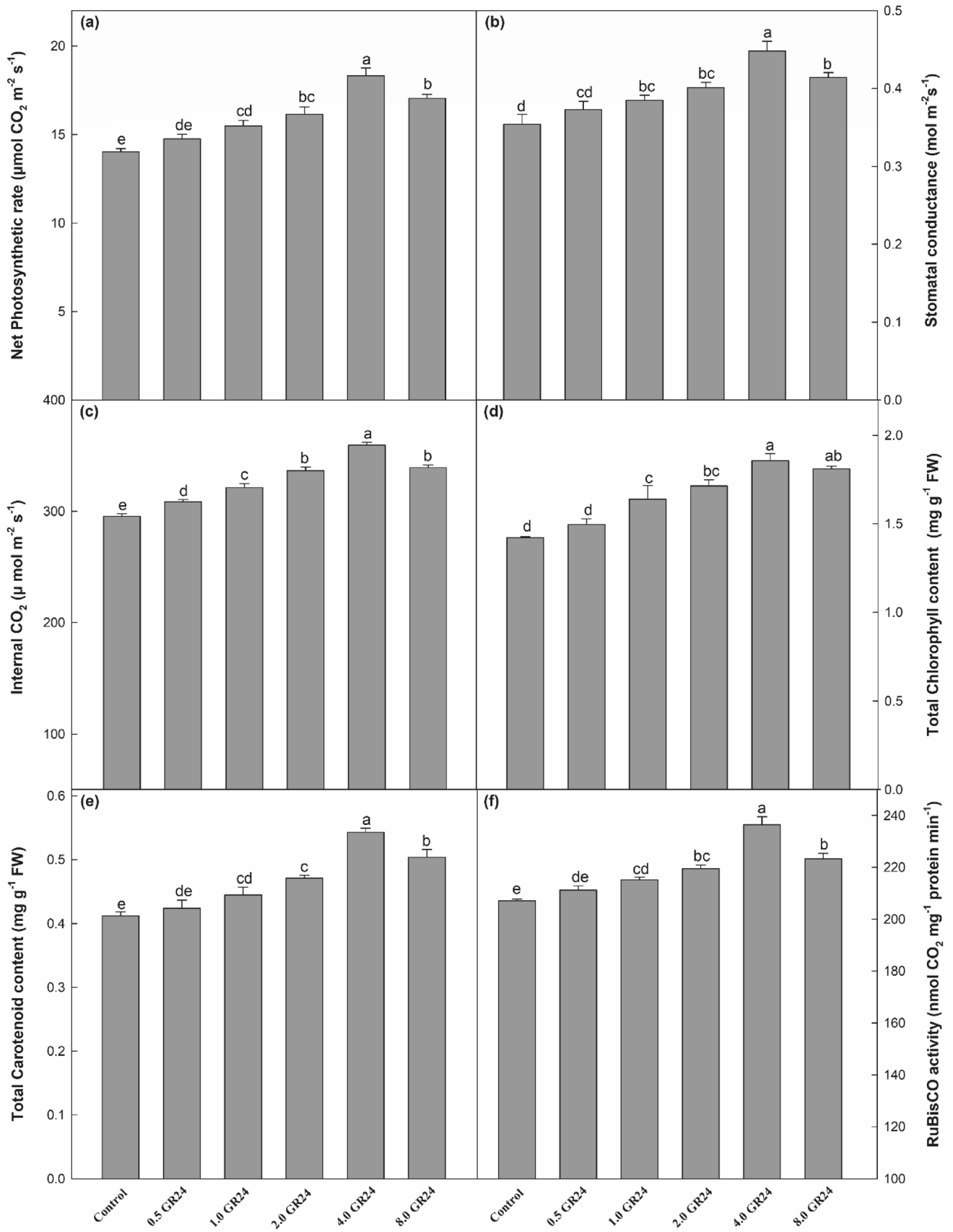


Fig. 1 Effects of different concentrations of GR24 on net photosynthetic rate, stomatal conductance, internal CO₂, total chlorophyll content, total carotenoid content, and RuBisCO activity of *Artemisia annua*. Bars ($n=5$) showing the different letters are significantly different at $p \leq 0.05$ as determined by Duncan's multiple range test. Error bars show SE

psbP, and *cab* associated with PSII showed that these genes were significantly up-regulated upon GR24 application, suggesting that PSII proteins are protected effectively at the transcriptional level. Moreover, they also reported the enhanced expression of *psaA* and *psbB* genes of PSI upon GR24 application, indicating the positive impact of strigolactones on PSI reaction center proteins in preventing PSI photo-inhibition and maintaining its stability (Florian et al., 2009; Mayzlish-Gati et al. 2010; Lu et al. 2019). In the present study, the RuBisCO activity was also enhanced with increased concentration of GR24 (Fig. 1), which was also reported earlier by Mayzlish-Gati et al. (2010), who also showed that exogenous GR24 spray on leaves of tomato also up-regulates transcription of certain other light harvesting-related genes. These results show the positive impact of exogenous GR24 on electron transport chain, energy distribution, and gene expression, which enhances the light

harvesting potential of plants, thereby improving the overall growth of plants.

In *A. annua*, glandular secretory trichomes are the biofactories of artemisinin and produce other secondary metabolites as well, which defend the plants from biotic agents by acting as protective barriers (Wang et al. 2009; Xiao et al. 2016). The glandular secretory trichomes are 10 celled structures consisting of stalk cells, basal cells, sub-apical cells, and apical cells and a sub-cuticular space in which artemisinin is stored after being produced in apical and sub-apical cells (Olsson et al. 2009; Xiao et al. 2016). The morphology and population of these trichomes in *A. annua* are often correlated with artemisinin, with more glandular secretory trichomes related to more artemisinin (Nguyen et al. 2013). In the present investigation, the exogenous application of GR24 resulted in the increased length, width, as well as density of trichomes (Table 3). As increased glandular trichome size and density are associated with increased artemisinin production, likewise increased artemisinin was also detected upon GR24 application. These results suggest that GR24 could be used for glandular trichome protection and increased artemisinin production in *A. annua* plants under normal and stress conditions as well.

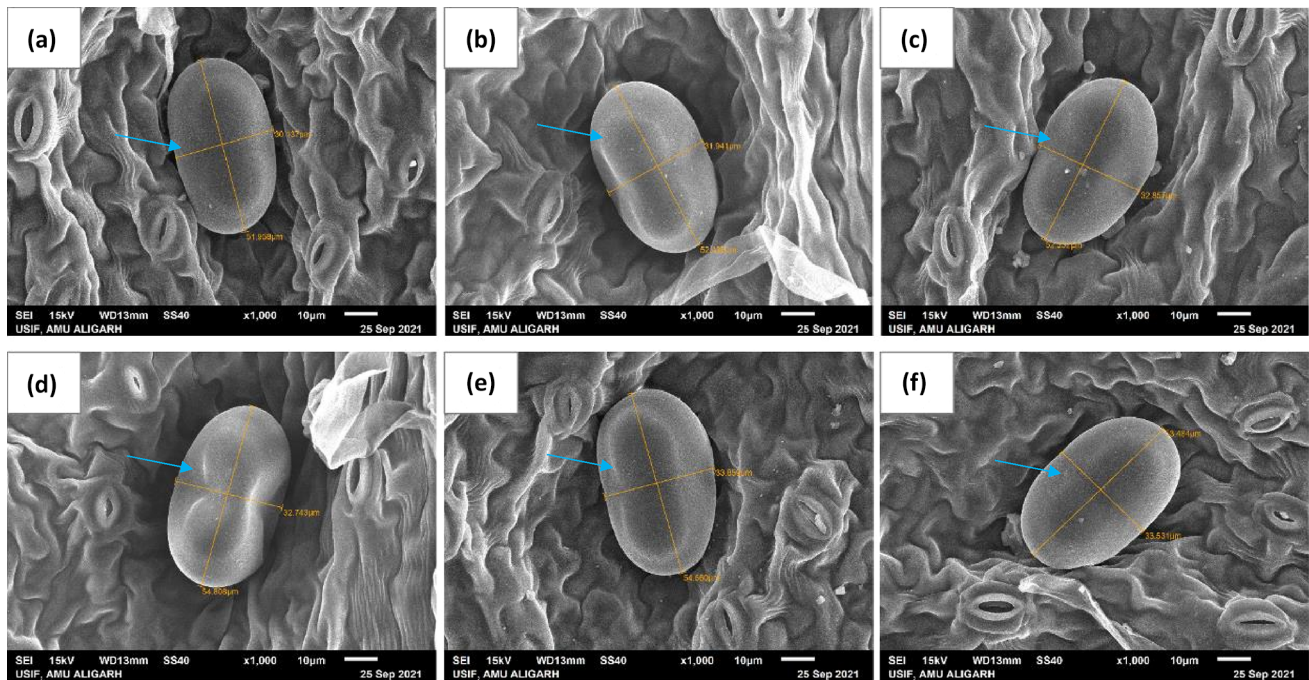


Fig. 2 Effects of different concentrations of GR24 on glandular trichomes as observed under scanning electron microscopy. **a** Control, **b** 0.5 µM, **c** 1 µM, **d** 2 µM, **e** 4 µM, and **f** 8 µM

Table 3 Effects of different concentrations of GR24 on glandular trichome attributes, artemisinin content, and yield in *Artemisia annua*

Treatments/ Parameters	Average length of trichomes (μm)	Average width of trichomes (μm)	Average trichome area (μm^2)	Trichome density (mm^{-2})	Artemisinin con- tent ($\mu\text{g g}^{-1}$ DW)	Artemisinin yield (g plant $^{-1}$ DW)
Control	51.11 \pm 0.19 ^e	30.14 \pm 0.16 ^d	1540.73 \pm 12.8 ^e	31.12 \pm 0.60 ^d	560.84 \pm 1.88 ^e	0.045 \pm 0.001 ^d
0.5 μM	52.64 \pm 0.37 ^d	31.31 \pm 0.44 ^{c,d}	1648.15 \pm 16.5 ^d	33.20 \pm 0.71 ^c	677.23 \pm 2.46 ^d	0.047 \pm 0.003 ^d
1 μM	53.52 \pm 0.58 ^{c,d}	32.14 \pm 0.53 ^{b,c}	1720.13 \pm 47.4 ^{c,d}	34.93 \pm 0.95 ^b	694.54 \pm 2.84 ^e	0.054 \pm 0.005 ^e
2 μM	54.36 \pm 0.51 ^{b,c}	32.76 \pm 0.27 ^b	1780.83 \pm 31.3 ^{b,c}	35.86 \pm 1.05 ^b	711.67 \pm 3.66 ^b	0.058 \pm 0.005 ^b
4 μM	55.75 \pm 0.40 ^a	34.52 \pm 0.57 ^a	1924.49 \pm 28.3 ^a	37.84 \pm 1.22 ^a	728.96 \pm 6.02 ^a	0.068 \pm 0.007 ^a
8 μM	55.18 \pm 0.38 ^{a,b}	33.25 \pm 0.52 ^{a,b}	1834.73 \pm 36.4 ^{a,b}	36.46 \pm 0.76 ^{a,b}	727.19 \pm 3.72 ^a	0.060 \pm 0.004 ^b

Data represent the mean value \pm SE from five replicates ($n=5$). The different lower case letters represent statistically significant differences between treatments ($p < 0.05$) according to the Duncan's multiple range test

Conclusion

Exogenous strigolactone-mediated regulation of different growth and physio-biochemical attributes have not been investigated in *A. annua*, and considering the immense medicinal value of these plants mainly in the treatment of malaria, this study was carried out to elucidate the impact of strigolactone (GR24) on *A. annua* plants. This study revealed that exogenous GR24 application had a positive impact on various morphological and physio-biochemical processes in *A. annua*. The exogenous GR24 also improved various attributes related to glandular trichomes resulting in improved artemisinin production. Further studies need to be carried out to elucidate the role of GR24 in regulating various aspects of growth and development in *A. annua* under normal as well as stressful conditions, with a focus on strigolactone-mediated gene regulation and its crosstalk with other signaling molecules especially karrikins as they have structural similarity.

Acknowledgements Mr. Kaiser Iqbal Wani would like to thank University Grants Commission (UGC), New Delhi, India for providing financial support in the form of fellowship (JRF) to conduct this research.

Author Contributions KIW: contributed to data curation, formal analysis, investigation, methodology, software, AZ: contributed to data curation, investigation, methodology. SC: contributed to data curation, formal analysis, investigation. MN: contributed to validation, visualization, software. MMAK: contributed to project administration, resources. RK: contributed to data curation, formal analysis, investigation. TA: contributed to conceptualization, project administration, resources, supervision, validation, visualization, writing—review & editing of the manuscript.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- Aftab T, Khan MMA, Idrees M, Naeem M, Singh M, Ram M (2010) Stimulation of crop productivity, photosynthesis and artemisinin production in *Artemisia annua* L. by triacontanol and gibberellic acid application. *J Plant Interact* 5:273–281
- Aftab T, Khan MMA, da Silva JAT, Idrees M, Naeem M (2011a) Role of salicylic acid in promoting salt stress tolerance and enhanced artemisinin production in *Artemisia annua* L. *J Plant Growth Regul* 30:425–435. <https://doi.org/10.1007/s00344-011-9205-0>
- Aftab T, Khan MMA, Idrees M, Naeem M, Hashmi N (2011b) Methyl jasmonate counteracts boron toxicity by preventing oxidative stress and regulating antioxidant enzyme activities and artemisinin biosynthesis in *Artemisia annua* L. *Protoplasma* 248:601–612
- Agusti J, Herold S, Schwarz M, Sanchez P, Ljung K, Dun EA, Brewer PB, Beveridge CA, Sieberer T, Sehr EM, Greb T (2011) Strigolactone signaling is required for auxin-dependent stimulation of secondary growth in plants. *Proc Natl Acad Sci USA* 108:20242–20247
- Akiyama K, Matsuzaki K, Hayashi H (2005) Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* 435:824–827
- Banyai W, Mii M, Supaibulwatana K (2011) Enhancement of artemisinin content and biomass in *Artemisia annua* by exogenous GA3 treatment. *Plant Growth Regul* 63:45–54. <https://doi.org/10.1007/s10725-010-9510-9>
- Brewer PB, Koltai H, Beveridge CA (2013) Diverse roles of strigolactones in plant development. *Mol Plant* 6:18–28
- Butt H, Jamil M, Wang JY, Al-Babili S, Mahfouz M (2018) Engineering plant architecture via CRISPR/Cas9-mediated alteration of strigolactone biosynthesis. *BMC Plant Biol* 18:1–9
- Chen R, Bu Y, Ren J, Pelot KA, Hu X, Diao Y, Zhang L (2021) Discovery and modulation of diterpenoid metabolism improves glandular trichome formation, artemisinin production and stress resilience in *Artemisia annua*. *New Phytol* 230:2387–2403
- Duke SO, Paul RN (1993) Development and fine structure of the glandular trichomes of *Artemisia annua* L. *Int J Plant Sci* 154:107–118
- Dwivedi RS, Randhawa NS (1974) Evaluation of a rapid test for the hidden hunger of zinc in plants. *Plant Soil* 40:445–451
- Efferth T (2017) From ancient herb to modern drug: *Artemisia annua* and artemisinin for cancer therapy. *Seminars in cancer biology*, vol 46. Academic Press, Cambridge, pp 65–83
- Efferth T (2018) Beyond malaria: the inhibition of viruses by artemisinin-type compounds. *Biotechnol Adv* 36:1730–2173
- Fleet C, Williams M (2011) Gibberellins. *Teaching tools in plant biology: lecture notes*. *Plant Cell*. 110. www.plantcell.org/cgi/doi/10.1105/tpc. Accessed 05 Aug 2021

- Florian B, Normanpa HN, Ingo E (2009) Biochemical constrains limit the potential of the photochemical reflectance index as a predictor of effective quantum efficiency of photosynthesis during the winter spring transition in Jack pine seedlings. *Funct Plant Biol* 36:115–119. <https://doi.org/10.1071/FP08043>
- Georgios A, Dimou M, Flemetakis E, Plati F, Katinakis P, Drossopoulos JB (2004) Immunolocalization of carbonic anhydrase and phosphoenolpyruvate carboxylase in developing seeds of *Medicago sativa*. *Plant Physiol Biochem* 42:181–186
- Gomez-Roldan V, Fermas S, Brewer PB et al (2008) Strigolactone inhibition of shoot branching. *Nature* 455:189–194
- Heidari B, Matre P, Nemie-Feyissa D, Meyer C, Rognli OA, Møller SG, Lillo C (2011) Protein phosphatase 2A B55 and A regulatory subunits interact with nitrate reductase and are essential for nitrate reductase activation. *Plant Physiol* 156:165–172
- Hu Z, Yamauchi T, Yang J, Jikumaru Y, Tsuchida-Mayama T, Ichikawa H, Nakazono M (2014) Strigolactone and cytokinin act antagonistically in regulating rice mesocotyl elongation in darkness. *Plant Cell Physiol* 55:30–41
- Jaworski EG (1971) Nitrate reductase assay in intact plant tissues. *Biochem Biophys Res Commun* 43:1274–1279
- Kapulnik Y, Resnick N, Mayzlish-Gati E, Kaplan Y, Winer S, Hershenhorn J, Koltai H (2011) Strigolactones interact with ethylene and auxin in regulating root-hair elongation in *Arabidopsis*. *J Exp Bot* 62:2915–2924
- Kohlen W, Charnikhova T, Liu Q, Bours R, Domagalska MA, Beguerie S, Ruyter-Spira C (2011) Strigolactones are transported through the xylem and play a key role in shoot architectural response to phosphate deficiency in nonarbuscular mycorrhizal host *Arabidopsis*. *Plant Physiol* 155:974–987
- Koltai H, Prandi C (2014) Strigolactones: biosynthesis, synthesis and functions in plant growth and stress responses. *Phytohormones: A window to metabolism, signaling and biotechnological applications*. Springer, New York, pp 265–288
- Koltai H, Dor E, Hershenhorn J, Joel DM, Weininger S, Lekalla S, Kapulnik Y (2010) Strigolactones' effect on root growth and root-hair elongation may be mediated by auxin-efflux carriers. *J Plant Growth Regul* 29:129–136
- Kramna B, Prerostova S, Vankova R (2019) Strigolactones in an experimental context. *Plant Growth Regul* 88:113–128
- Kumari A, Pandey N, Pandey-Rai S (2018) Exogenous salicylic acid-mediated modulation of arsenic stress tolerance with enhanced accumulation of secondary metabolites and improved size of glandular trichomes in *Artemisia annua* L. *Protoplasma* 255:139–152
- Lauressergues D, André O, Peng J, Wen J, Chen R, Ratet P, Rochange SF (2015) Strigolactones contribute to shoot elongation and to the formation of leaf margin serrations in *Medicago truncatula* R108. *J Exp Bot* 66:1237–1244
- Lichtenthaler HK, Buschmann C (2001) Extraction of photosynthetic tissues: chlorophylls and carotenoids. *Curr Protoc Food Anal Chem* 1:F4
- Ling F, Su Q, Jiang H, Cui J, He X, Wu Z, Zhao Y (2020) Effects of strigolactone on photosynthetic and physiological characteristics in salt-stressed rice seedlings. *Sci Rep* 10:1–8
- Liu C, Zhao Y, Wang Y (2006) Artemisinin: current state and perspectives for biotechnological production of an antimalarial drug. *Appl Microbiol Biot* 72:11–20
- Lu T, Meng Z, Zhang G, Qi M, Sun Z, Liu Y et al (2017) Sub-high temperature and high light intensity induced irreversible inhibition on photosynthesis system of tomato plant (*Solanum lycopersicum* L.). *Front Plant Sci* 8:365
- Lu T, Yu H, Li Q, Chai L, Jiang W (2019) Improving plant growth and alleviating photosynthetic inhibition and oxidative stress from low-light stress with exogenous GR24 in tomato (*Solanum lycopersicum* L.) seedlings. *Front Plant Sci* 10:490
- Luo XD, Shen CC (1987) The chemistry, pharmacology, and clinical applications of qinghaosu (artemisinin) and its derivatives. *Med Res Rev* 7:29–52
- Ma N, Hu C, Wan L, Hu Q, Xiong J, Zhang C (2017) Strigolactones improve plant growth, photosynthesis, and alleviate oxidative stress under salinity in rapeseed (*Brassica napus* L.) by regulating gene expression. *Front Plant Sci* 8:1671
- Matusova R, Rani K, Verstappen FWA, Franssen MCR, Beale MH, Bouwmeester HJ (2005) The strigolactone germination stimulants of the plant-parasitic *Striga* and *Orobanche* spp. are derived from the carotenoid pathway. *Plant Physiol* 139:920–934
- Mayzlish-Gati E, LekKala SP, Resnick N, Winer S, Bhattacharya C, Lemcoff JH, Koltai H (2010) Strigolactones are positive regulators of light-harvesting genes in tomato. *J Exp Bot* 61:3129–3136
- Munyangi J, Cornet-Vernet L, Idumbo M, Chen L, Lutgen P, Perronne C, Ngombe N, Bianga J, Mupenda B, Lalulala P, Mergeai G, Mumba D, Towler MJ, Weathers PJ (2018) Effect of *Artemisia annua* and *Artemisia afra* tea infusions on schistosomiasis in a large clinical trial. *Phytomedicine* 51:233–240. <https://doi.org/10.1016/j.phymed.2018.10.014>
- Murchie EH, Lawson T (2013) Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. *J Exp Bot* 64:3983–3998
- Naeem MS, Warusawitharana H, Liu H, Liu D, Ahmad R, Waraich EA et al (2012) 5-aminolevulinic acid alleviates the salinity-induced changes in *Brassica napus* as revealed by the ultrastructural study of chloroplast. *Plant Physiol Biochem* 57:84–92. <https://doi.org/10.1016/j.plaphy.2012.05.018>
- Nair MS, Huang Y, Fidock DA, Polyak SJ, Wagoner J, Towler MJ, Weathers PJ (2021) *Artemisia annua* L. extracts inhibit the in vitro replication of SARS-CoV-2 and two of its variants. *J Ethnopharmacol* 274:114016
- Nguyen KT, Towler MJ, Weathers PJ (2013) The effect of roots and media constituents on trichomes and artemisinin production in *Artemisia annua* L. *Plant Cell Rep* 32:207–218
- Olsson ME, Olofsson LM, Lindahl AL et al (2009) Localization of enzymes of artemisinin biosynthesis to the apical cells of glandular secretory trichomes of *Artemisia annua* L. *Phyto-Chemistry* 70:1123–1128
- Pu GB, Ma DM, Chen JL, Ma LQ, Wang H, Li GF, Liu BY (2009) Salicylic acid activates artemisinin biosynthesis in *Artemisia annua* L. *Plant Cell Rep* 28:1127–1135. <https://doi.org/10.1007/s00299-009-0713-3>
- Qiu CW, Zhang C, Wang NH, Mao W, Wu F (2021) Strigolactone GR24 improves cadmium tolerance by regulating cadmium uptake, nitric oxide signaling and antioxidant metabolism in barley (*Hordeum vulgare* L.). *Environ Pollut* 273:116486
- Ralph PJ, Gademann R (2005) Rapid light curves: a powerful tool to assess photosynthetic activity. *Aqua Bot* 82:222–237. <https://doi.org/10.1016/j.aquabot.2005.02.006>
- Sato D, Awad AA, Chae SH, Yokota T, Sugimoto Y, Takeuchi Y, Yoneyama K (2003) Analysis of strigolactones, germination stimulants for striga and orobanche, by high-performance liquid chromatography/tandem mass spectrometry. *J Agric Food Chem* 51:1162–1168
- Sattar A, Ul-Allah S, Ijaz M, Sher A, Butt M, Abbas T, Alharbi SA (2021) Exogenous application of strigolactone alleviates drought stress in maize seedlings by regulating the physiological and antioxidant defense mechanisms. *Cereal Res Commun*. <https://doi.org/10.1007/s42976-021-00171-z>
- Sedaghat M, Tahmasebisarvestani Z, Emam Y, Mokhtassibidgoli A (2017) Physiological and antioxidant responses of winter wheat cultivars to strigolactone and salicylic acid in drought. *Plant Physiol Biochem* 119:59–69

- Shen X, Xue Z, Sun L, Zhao C, Sun S, Liu J, Liu J (2020) Effect of GR24 concentrations on biogas upgrade and nutrient removal by microalgae-based technology. *Bioresour Technol* 312:123563
- Shu S, Tang Y, Yuan Y, Sun J, Zhong M, Guo S (2016) The role of 24-epibrassinolide in the regulation of photosynthetic characteristics and nitrogen metabolism of tomato seedlings under a combined low temperature and weak light stress. *Plant Physiol Biochem* 107:344–353. <https://doi.org/10.1016/j.plaphy.2016.06.021>
- Shukla A, Farooqi AA, Shukla YN, Sharma S (1992) Effect of triacontanol and chlormequat on growth, plant hormones and artemisinin yield in *Artemisia annua* L. *Plant Growth Regul* 11:165–171. <https://doi.org/10.1007/BF00024071>
- Tetu SG, Tanz SK, Vella N, Burnell JB, Ludwig M (2007) The *Flaveria bidentis* β -carbonic anhydrase gene family encodes cytosolic and chloroplastic isoforms demonstrating distinct organ-specific expression patterns. *Plant Physiol* 144:1316–1327
- Umehara M, Hanada A, Yoshida S et al (2008) Inhibition of shoot branching by new terpenoid plant hormones. *Nature* 455:195–200
- Urquhart S, Foo E, Reid JB (2015) The role of strigolactones in photomorphogenesis of pea is limited to adventitious rooting. *Physiol Plant* 153(3):392–402
- Usuda H (1985) The activation state of ribulose-1,5-bisphosphate carboxylase in maize leaves in dark and light. *Plant Cell Physiol* 26:1455–1463
- Van Ha C, Leyva-González MA, Osakabe Y, Tran UT, Nishiyama R, Watanabe Y, Tran LSP (2014) Positive regulatory role of strigolactone in plant responses to drought and salt stress. *PNAS* 111:851–856
- Wallaart TE, van Uden W, Lubberink HG, Woerdenbag HJ, Pras N, Quax WJ (1999) Isolation and identification of dihydroartemisinic acid from *Artemisia annua* and its possible role in the biosynthesis of artemisinin. *J Nat Prod* 62:430–433
- Wang W, Wang Y, Zhang Q, Qi Y, Guo D (2009) Global characterization of *Artemisia annua* glandular trichome transcriptome using 454 pyrosequencing. *BMC Genom* 10:465
- Wani KI, Zehra A, Choudhary S, Naeem M, Khan MMA, Castroverde CDM, Aftab T (2020) Mechanistic insights into strigolactone biosynthesis, signaling, and regulation during plant growth and development. *J Plant Growth Regul* 40:1836–1852
- Wani KI, Choudhary S, Zehra A, Naeem M, Weathers P, Aftab T (2021) Enhancing artemisinin content in and delivery from *Artemisia annua*: a review of alternative, classical, and transgenic approaches. *Planta* 254:1–15
- Wei-Hong S, Yan-You W, Zhen-Zhen S, Qiu-Xia W, Xin-Yu W (2014) Enzymatic characteristics of higher plant carbonic anhydrase and its role in photosynthesis. *J Plant Stud* 3:39
- WHO Guidelines for malaria, 16 February (2021) Geneva: World Health Organization; 2021 (WHO/UCN/ GMP/2021.01). Licence: CC BY-NC-SA 3.0 IGO
- WHO (2019) World Malaria Report 2019. World Health Organization, Switzerland, pp. xii–xiii 4–10
- World Health Organization (2020) World malaria report 2020: 20 years of global progress and challenges. In World malaria report 2020: 20 years of global progress and challenges
- Xiao L, Tan H, Zhang L (2016) *Artemisia annua* glandular secretory trichomes: the biofactory of antimalarial agent artemisinin. *Sci Bull* 61:26–36
- Yamori W, Sakata N, Suzuki Y, Shikanai T, Makino A (2011) Cyclic electron flow around photosystem I via chloroplast NAD(P)H dehydrogenase (NDH) complex performs a significant physiological role during photosynthesis and plant growth at low temperature in rice. *Plant J* 68:966–976. <https://doi.org/10.1111/j.1365-313X.2011.04747.x>
- Zehra A, Choudhary S, Wani KI, Naeem M, Khan MMA, Aftab T (2020) Exogenous abscisic acid mediates ROS homeostasis and maintains glandular trichome to enhance artemisinin biosynthesis in *Artemisia annua* under copper toxicity. *Plant Physiol Biochem*. <https://doi.org/10.1016/j.plaphy.2021.02.004>
- Zhang X, Zhang L, Ma C, Su M, Wang J, Zheng S, Zhang T (2022) Exogenous strigolactones alleviate the photosynthetic inhibition and oxidative damage of cucumber seedlings under salt stress. *Sci Horti* 297:110962
- Zhao SS, Zeng MY (1986) Determination of qinghaosu in *Artemisia annua* L. by high performance liquid chromatography. *Chin J Pharma Anal* 6:3–5
- Zheng X, Li Y, Xi X, Ma C, Sun Z, Yang X, Wang C (2021) Exogenous strigolactones alleviate KCl stress by regulating photosynthesis, ROS migration and ion transport in *Malus hupehensis* Rehd. *Plant Physiol Biochem* 159:113–122
- Zhou M, Memelink J (2016) Jasmonate-responsive transcription factors regulating plant secondary metabolism. *Biotechnol Adv* 34:441–449. <https://doi.org/10.1016/j.biotechadv.2016.02.004>

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