ORIGINAL ARTICLE

Adaptive physiological response, carbon partitioning, and biomass production of *Withania somnifera* (L.) Dunal grown under elevated CO_2 regimes

Rupali Sharma[1] · Hukum Singh[1] · Monica Kaushik[1] · Raman Nautiyal[2] · Ombir Singh[1]

Abstract
Winter cherry or Ashwagandha (*Withania somnifera*) is an important medicinal plant used in traditional and herbal medicine system. Yet, there is no information available on response of this plant to changing climatic conditions particularly elevated atmospheric CO_2 concentrations. Therefore, we conducted an experiment to examine the effect of elevated CO_2 concentrations (ECs) on *Withania somnifera*. The variations in traits of physiological adaptation, net primary productivity, carbon partitioning, morphology, and biomass in response to elevated CO_2 concentrations (ambient, 600 and 800 μmol mol^{-1}) during one growth cycle were investigated within the open top chamber (OTC) facility in the foothill of the Himalayas, Dehardun, India. ECs significantly increased photosynthetic rate, transpiration rate, stomatal conductance, water use efficiency, soil respiration, net primary productivity and the carbon content of plant tissues (leaf, stem, and root), and soil carbon. Furthermore, ECs significantly enhanced biomass production (root and shoot), although declined night leaf respiration. Overall, it was summarized that photosynthesis, stomatal conductance, water use efficiency, leaf, and soil carbon and biomass increased under ECs rendering the physiological adaptation to the plant. Increased net primary productivity might facilitate mitigation effects by sequestering elevated levels of carbon dioxide. We advocate further studies to investigate the effects of ECs on the accumulation of secondary metabolites and health-promoting substances of this as well as other medicinal plants.

Keywords Elevated CO_2 · Physiological adaptations · Net primary productivity · Carbon partitioning · Biomass production · Leaf and soil respiration · Medicinal plants · *Withania somnifera*

Introduction

The atmospheric CO_2 has changed from its preindustrial concentration of 280 μmol CO_2 mol^{-1} to the current level of approximately 400 μmol CO_2 mol^{-1} largely due to anthropogenic activities. If atmospheric CO_2 levels continue to increase at the current level (2.11 μmol CO_2 mol^{-1}), it is projected to reach 720–1000 μmol CO_2 mol^{-1} causing increased air temperature (2.6–5.4 °C) before the end of this century (IPCC 2007; Dlugokencky and Tans 2017).

Increasing atmospheric CO_2 concentration is also increasing the uncertainty in precipitation which could decline growth and biomass of crops and influence the performance, adaptation, and survival of vegetation system globally (Thompson et al. 2005). Elevated CO_2 (EC) directly affects the species growth performance by shifting their physiological tolerance limits to cope with modified climatic conditions. EC is already forcing living organisms and ecosystems to adapt either through shifting habitat, changing life cycles and the development of new physical and/or physiological traits (CBD 2014; Zari 2014; Tilman and Lehman 2001). Species that are unable to adapt might face extinction (Thomas et al. 2004; Urban 2015). According to predictions, approximately one million species may extinct due to climate change (Gopalakrishna et al. 2011). However, impacts of climate change are expected to vary across regions (Parry et al. 2007). Studies indicate that lately in addition to shifting phenology, plant species have started to conform to latest climate adjustments through alteration at the metabolic

✉ Hukum Singh
  hukumsingh97@yahoo.com

1 Ecology, Climate Change and Forest Influence Division, Forest Research Institute, P.O. New Forest, Dehradun, Uttarakhand 248006, India

2 Indian Council of Forestry Research and Education, P. O. New Forest, Dehradun, Uttarakhand 248006, India

Springer

and physiological levels of the species (Buckley et al. 2013) or through shifting species ranges (Parmesan 2006). The impact of increased CO_2 concentrations on the adaptation of agricultural species has been understood widely (Bunn et al. 2015; Saadi et al. 2015). However, there is dearth of knowledge regarding the physiological response and adaptations of medicinal plants to increasing CO_2 concentrations. This information is urgently required for understanding the adaptive behavior, ultimately biomass, and yield in future conditions.

Winter cherry or Ashwagandha (*Withania somnifera*) is one of the important ancient medicinal plants utilized for curing various diseases. It is an important plant species used since ancient era and has been mentioned in various pieces of literature particularly Ayurvedic, Unani and African traditional medicines (Patwardhan et al. 1998; Singh et al. 2015). Due to it is medicinal properties leading to huge demand in the domestic and international markets, World Health Organization (WHO) and National Medicinal Plant Board (NMPB) of India are continuously focusing on cultivation, conservation, sustainable exploration and its management for future demand (Mirjalili et al. 2009; Prajapati et al. 2003; NMPB; WMO). Effects of climate change particularly EC on medicinal plants could be detrimental for the economy of local communities and medical industries. Studies on medicinal plant's response to EC have been carried out on species such as *Digitalis lanata* (Stuhlfauth and Fock 1990), *Mentha spicata, Thymus vulgaris, Mentha aquatica* (Tisserat 2002), *Hymenocallis littoralis* (Idso et al. 2000), *Ocimum basilicum* (Singh et al. 2016), *Hizikia fusiforme* (Zou and Gao 2005), *Podophyllum hexandrum* (Chaturvedi et al. 2009), *Papaver setigerum* (Ziska et al. 2008), *Scutellaria barbata* and *Scutellaria lateriflora* (Stutt et al. 2008), *Thymus vulgaris* (Vurro et al. 2009), *Halia bentong, Halia bara* (Ghasemzadeh et al. 2010), *Vernonia herbacea* (Oliveira et al. 2010), and *Catharanthus roseus* (Saravanan and Karthi 2014). The near tripling of EC increased the dry biomass production of the woolly foxglove (*Digitalis purpurea*) plant by 63 and 83% under dry and well-watered conditions, respectively. Stuhlfauth et al. (1987) also obtained similar results when near tripling of EC enhanced plant dry weight biomass by 75% with 15% increase in digoxin per unit dry weight of plant material. Later, Idso et al. (2000) investigated the response of spider lily plants grew at EC of 400 or 700 ppm and reported 48 and 56% increment in above and below ground plant biomass, respectively. Zobayed et al. (2003) reported an increase of net photosynthetic rates of herb *Hypericum perforatum* by 124 and 107% greater dry weight under EC (1000 ppm). Above-ground biomass of the plants at elevated CO_2 increased too (Ziska et al. 2008). Ibrahim et al. (2012) grew oil palm *Elaeis guineensis* at ECs 400, 800, and 1200 μmol CO_2 mol^{-1} and reported increase

in flavonoid contents by 86 and 132%, and total phenolics by 52 and 91% at 800 and 1200 μmol CO_2 mol^{-1}.

However, we do not have sufficient knowledge of *Withania somnifera* in respect of physiological adaptation, carbon partitioning, net primary productivity, and biomass production to EC. The magnitude of the direct and indirect effect of EC on stimulation of traits related to physiological adaptation especially photosynthesis, water use efficiency, growth, and biomass production of medicinal plants has significant economic and social bearing. Thus, the study was performed to understand the adaptive physiological response, carbon partitioning, net primary productivity, growth, morphology, and biomass production of *Withania somnifera* grew at various levels of EC, i.e., ambient, 600 and 800 μmol CO_2 mol^{-1}.

Materials and methods

Description of open top chamber (OTC) facility

The experiment was conducted in the open top chamber (OTC) facility of Ecology, Climate Change and Forest Influence Division, Forest Research Institute—Dehradun, Uttarakhand—India (32°20′44.2172″N, 78°0′41.6185″E and 668 m.a.s.l.). Each OTC (3.0 m, width × 3.0 m, length × 4.0 m height) was constructed using high-quality multilayer polycarbonate sheet of 3.0–4.0 mm thick and 80–85% transmittance (Singh et al. 2018). CO_2 gas of commercial grade (100%) was released into the chambers at the required concentration. One OTC was used as control chamber. Another two OTCs, EC (600 and 800 μmol mol^{-1}) was maintained during whole growth cycle of *Withania somnifera*, from seedling to harvest stage. The CO_2 concentration was measured by sensors installed in each chamber. The EC in each chamber was maintained using the solenoid valves of the corresponding chamber.

Seedlings preparation of *Withania somnifera*

The seeds of *Withania somnifera* acquired from Non-wood Forest Product Division, Forest Research Institute—Dehradun, Uttarakhand, India. Seeds were sown in plastic trays with growing media (2:1:1; soil:sand:manure). One-month-old seedlings were transferred to pots and kept for 4 days outside of OTC for acclimatization in pot conditions. Subsequently, seedlings were placed in OTCs and left for 4 days to acclimatize in OTCs conditions. Finally, the required concentration of CO_2 was supplied into each OTC, except control. CO_2 treatment was given during whole one growth cycle of plants. Regular watering and weeding were carried to maintain the uniform soil moisture.

Measurement of adaptive physiological traits

The adaptive physiological traits, i.e., photosynthetic rate (μmol CO_2 m^{-2} s^{-1}), transpiration rate (mmol H_2O m^{-2} s^{-1}), stomatal conductance (mol H_2O m^{-2} s^{-1}), and intercellular CO_2 concentration (μmol CO_2 mol^{-1}), were measured during vegetative phase using portable photosynthetic system (Model 6400 XT- LICOR, Incl, USA). The youngest and fully expended leaves were preferred for measuring physiological parameters between 09:30 am and 12:30 pm in clear sky condition. Forty observations for each parameter were recorded from eight plants (five replicates from each plant). The instantaneous water use efficiency (A/E) was computed as the ratio of CO_2 photosynthetic rate (A) and transpiration (E). Intrinsic water use efficiency (A/gs) was estimated as the ratio of photosynthetic rate (A) to stomatal conductance (gs), whereas intrinsic carboxylation efficiency (A/Ci) was calculated using ratio of photosynthetic rate (A) to intercellular CO_2 concentration (Ci) furthermore ratio of intercellular CO_2 concentration (Ci) to stomatal conductance (gs) was calculated for obtaining the intrinsic mesophyll efficiency (Ci/gs) (Warrier et al. 2013; Singh et al. 2015, 2018).

Estimation of leaf and soil respiration, and net primary productivity

Night leaf respiration (μmol CO_2 m^{-2} s^{-1}) was measured from fully developed leaf during vegetative stage between 4:30 to 5:00 am and soil respiration was measured between 09:30 am and 12:30 pm with help of portable photosynthesis system (Model 6400 XT- LICOR, Incl, USA). For measuring soil respiration from pot soil, a chamber of 10 cm × 10 cm × 10 cm was constructed using polyvinyl chloride sheet with 80–85% transmission level of natural light. The chamber was placed on the top of the pot soil, made air tight by pressing it into soil about 2 cm deep and sealing it with soil from outside to break the contact of inside condition to the outer atmosphere. The packed chamber was connected to reference pipe of portable photosynthesis system to visualize variations in CO_2 concentration on a digital screen. Before measuring the soil CO_2 respiration, we recorded the initial CO_2 concentration and left for 1 h interval to observe the change in CO_2 concentration. After 1 h interval, the final CO_2 concentration was recorded. The soil CO_2 respiration (mg CO_2 m^{-2} h^{-1}) was then calculated by subtracting the initial value of CO_2 from the final value using following equation (Singh et al. 2017):

$$CO_2 \text{ flux} = \frac{\Delta X \times \text{ECV(STP)} \times \text{MW} \times 1000 \times 60}{1000000 \times 22400 \times T \times A}$$

where ΔX = Difference in flux value between 60 and 0 min (converted to ppm based on the standard CO_2 values), ECV (STP) = effective chamber volume at standard temperature and pressure, MW = molecular weight of CO_2, T flux time (min), and A area of the chamber.

Moreover, net primary productivity (NPP; μmol CO_2 m^{-2} s^{-1} = GPP − respiration) was calculated by subtracting the carbon lost through plant respiration from the carbon assimilated during the photosynthesis (Gross Primary Production or GPP) (Singh et al. 2017).

Biomass and carbon estimation

Biomass allocation in plant parts was calculated by carefully uprooting the plants and segregating into leaves, stems, and root parts. The roots were cleaned with distilled water to remove soil particles sticking to root hairs. The fresh plant parts were weighed and subsequently oven dried at 65 °C until a constant weight reached and weighed again (Wu et al. 2013; Singh et al. 2018). Organic carbon was estimated following Walkley and Black (1934) method. In 500 ml conical flask, the plant tissue (0.1 gm) was first mixed with 30 ml of 1N potassium dichromate solution followed by 20 ml of concentrated sulphuric acid. After swirling for few minutes, the solution was incubated at room temperature for 40 min. Then, 200 ml of distilled and 10 ml orthophosphoric acid was added followed by five drops of diphenylamine indicator. At last, the solution was titrated against ferrous ammonium sulphate solution to reach an end point of brilliant bright green color solution. The following equation was used to calculate organic carbon in plant parts:

$$\%C = \frac{(B - T) \times S \times 0.003 \times 1.33 \times 100}{W}$$

where B amount of ferrous ammonium sulphate required in blank titration, T amount of ferrous ammonium sulphate required in sample titration, S strength of ferrous ammonium sulphate (from blank titration), and W weight of plant sample.

Statistical analysis

We used the SPSS 16.0 software to carry out the multivariate general linear model to observe the existence of significant mean difference in response of physiological parameters, biomass, and carbon accumulation at different CO_2 levels. Further post hoc Tukey test was performed to identify the homogeneous subsets.

Results

Adaptive physiological response to EC

The traits of adaptive physiological response showed significant difference across control and elevated CO_2 concentrations (Table 1). The photosynthetic rate increased to 38.63 and 86.77% at EC of 600 and 800 μmol mol^{-1}, respectively, to ambient CO_2 level. It was reported that the increased CO_2 concentration induced higher water lost form leaf through the process of transpiration. Approximately, transpiration rate was 20.16 and 36.18% at EC (600 and 800 μmol mol^{-1}) level when compared to control. The stomatal opening and closing in terms of stomatal conductance was found to be stimulated significantly under EC (800 μmol mol^{-1}), but not for 600 μmol mol^{-1} compared to ambient (Table 1). Stomatal conductance was 45.19% higher in EC 800 μmol mol^{-1} condition as compared to ambient. The study also reported a significant increase in intercellular CO_2 concentration (Table 1) and the increase was 69% higher at 800 μmol mol^{-1} and 26.52% higher at 600 μmol mol^{-1}, whereas lowest in ambient conditions. Instantaneous (A/E) and intrinsic (A/gs) water use efficiency had the same trend as seen in stomatal conductance (Table 1). The percentage increase of A/E was 36.31% higher and for A/gs around 28.5% at EC 800 μmol mol^{-1} condition than ambient. The carboxylation efficiency showed significant increment at elevated CO_2 concentrations than ambient (Table 1). Furthermore, a tendency of higher value was observed for the intrinsic mesophyll efficiency under 800 μmol mol^{-1}

CO_2 level when compared to the plant grown at ambient condition (Table 1). Mesophyll efficiency increased to 16.29% at 800 μmol mol^{-1} CO_2 compared to ambient. It can be seen from the results that there was an enhancement of mesophyll efficiency around 10% at 600 μmol mol^{-1} and 16.20% at 800 μmol mol^{-1} CO_2 level (Table 1).

Response of leaf and soil respiration, and net primary productivity to EC

The response of leaf and soil respiration and net primary productivity (NPP) against EC is presented in Table 1. Night leaf respiration significantly reduced in plants treated with EC than the one in ambient. The EC had negative impact on night leaf respiration by suppressing 16.80 and 29.38% significantly for the plant grown at 600 and 800 μmol mol^{-1} compared to ambient. Soil respiration, a major component in the global carbon cycle, is affected by elevated atmospheric CO_2 concentrations. The soil respiration was higher by 48.24 and 148.88% under 600 and 800 μmol CO_2 mol^{-1}, respectively, compared to ambient CO_2. The NPP showed a positive response to rising CO_2 by showing elevated values by 36.16 and 70.47% for the plants exposed to 600 and 800 μmol mol^{-1} CO_2 to ambient condition.

Response of carbon and biomass partitioning to EC

Interestingly, the EC showed substantial effects on leaf, stem, and root organic carbon (Table 2). The leaf organic

Table 1 Effects of elevated CO_2 concentrations (EC) on physiological parameters of *Withania somnifera*

Physiological traits	CO_2 concentration (μmol CO_2 mol^{-1})			P value
	Ambient	EC (600)	EC (800)	
A (μmol CO_2 m^{-2} s^{-1})	15.03[a] ± 0.23	20.83[b] ± 0.08	28.06[c] ± 0.31	0.000
E (mmol H_2O m^{-2} s^{-1})	7.04[a] ± 0.27	8.45[b] ± 0.34	9.581[c] ± 0.22	0.000
Gs (mol H_2O m^{-2} s^{-1})	0.35[a] ± 0.01	0.40[a] ± 0.02	0.51[b] ± 0.02	0.000
A/E	2.16[a] ± 0.09	2.49[a] ± 0.10	2.94[b] ± 0.09	0.000
A/gs	43.29[a] ± 1.90	52.17[b] ± 2.26	55.65[b] ± 2.38	0.002
Ci (μmol CO_2 mol^{-1})	284.17[a] ± 7.52	359.52[b] ± 8.76	480.25[c] ± 6.22	0.000
A/Ci	0.053[a] ± 0.001	0.058[b] ± 0.001	0.0585[b] ± 0.001	0.011
Ci/gs	816.63[a] ± 34.93	897.82[ab] ± 34.31	949.62[b] ± 30.31	0.032
NLR (μmol CO_2 m^{-2} s^{-1})	5.65[c] ± 0.10	4.70[b] ± 0.04	3.99[a] ± 0.11	0.000
SR (mg CO_2 m^{-2} h^{-1})	3.13[a] ± 0.19	4.64[b] ± 0.37	7.79[c] ± 0.36	0.000
NPP (μmol CO_2 m^{-2} s^{-1})	11.89[a] ± 0.35	16.19[b] ± 0.35	20.27[c] ± 0.62	0.000

Means not sharing a common letter are significantly different at $p \le 0.05$

A photosynthetic rate, *E* transpiration rate, *gs* stomatal conductance, *A/E* instantaneous water use efficiency, *A/gs* intrinsic water use efficiency, *Ci* intercellular CO_2 concentration, *A/Ci* intrinsic carboxylation efficiency, *Ci/gs* intrinsic mesophyll efficiency, *NLR* night leaf respiration, *SR* soil respiration, *NPP* net primary productivity, ± the standard error of the mean (SEM). $N = 8$.

carbon of the plant increased by 8.3% at 600 μmol mol^{-1} and 14.2% at 800 μmol mol^{-1} than the ambient. The parameters investigated after harvesting of the plant are presented in Table 2. The stem organic carbon increased by 5.63% at 600 μmol mol^{-1} and 8.90% at 800 μmol mol^{-1} to the ambient. Accumulation of root organic carbon also increased under EC both at 600 and 800 μmol mol^{-1} concentrations by 10.4 and 15.8% (Table 2). There was a uniform accumulation of dry matter of *W. somnifera* grown under elevated CO_2 concentration recorded for all plants (Table 2). Dry weight of leaves was too increased by 53.2 and 90.7% at 600 and 800 μmol mol^{-1}, respectively. Stem dry weight was also enhanced when plants were exposed to elevated CO_2 with maximum value at 800 μmol mol^{-1} and lowest under controlled condition. Simultaneously, root dry weight was also maximum at 800 μmol mol^{-1} (Table 2). The magnitude of percent increase in leaves dry biomass accumulation was 90.66 and 53.19% at 800 and 600 μmol mol^{-1}, respectively. Similar trend was observed in stem and root dry biomass accumulation, i.e., 93.06 and 32.59% in stems and 14.24 and 8.75% in roots when exposed to 800 and 600 μmol mol^{-1} CO_2 concentrations.

Relationship between functional traits

Variation in selected adaptive physiological traits (*viz.* photosynthetic rate, transpiration rate, leaf respiration, soil respiration, net primary productivity, and biomass production is depicted graphically (Fig. 1). The relationship between functional traits is illustrated in Figs. 2 and 3. As per our expectations, we observed a strong correlation between photosynthetic rate ($r = 0.99$), stomatal conductance ($r = 0.81$), transpiration rate ($r = 0.81$), soil respiration ($r = 0.90$), leaf respiration ($r = -0.94$), and net primary productivity ($r = 0.94$). Root organic carbon and root biomass production were also strongly correlated ($r = 0.84$).

Discussion

Adaptive physiological response to EC

Our study showed that all the physiological parameters of *Withania somnifera* increased at EC except leaf respiration. The increase in photosynthetic rate potentially related to rising in the activity of ribulose 1, 5-bisphosphate carboxylase enzyme (Rubisco) that can change the growth and even secondary metabolites (Ibrahim and Jaafar 2011). With an increase in CO_2 concentration, there is an increase in the rate at which carbon is subsumed into carbohydrate and the rate continues to grow until one of the factors becomes limiting. Increase in carbon dioxide cause rapid development of leaf area, which increases availability of transpiring surface subsequently enhancing transpiration rate (Morison and Gifford 1984). Stomatal conductance increased at EC which could be attributed to rapid opening of stomatal guard cells to the elevated CO_2 concentration. Water use efficiency increased by improving plant water relationship, i.e., by increasing plant turgor pressure which is important for the growth processes. Mechanism for the observed response could potentially be as follows. CO_2 helps in accumulation of more carbohydrate concentration that enhances the leaf osmotic potential and ultimately helps to maintain adequate leaf water contents for other phenomena of photosynthesis and growth. Similarly, the growing concentration of climatic carbon dioxide influences the mechanisms of plant photosynthesis, metabolism, and development (Nowak et al. 2004; Ainsworth and Long 2005). The other adaptive physiological process of *W. somnifera* such as intrinsic carboxylation efficiency (A/Ci) and intrinsic mesophyll efficiency (Ci/gs) exhibited increased trends at EC compared to plants grown at ambient condition. These physiological observations were in congruence to earlier studies (Singh et al. 2018; Warrier et al. 2013; Saravanan 2014). The response of physiological processes of *W. somnifera* to EC (600 and 800 μmol mol^{-1}) is represented in Table 1 and Fig. 1.

Table 2 Effects of elevated CO_2 concentrations (EC) on carbon/biomass partitioning parameters of *Withania somnifera*

Carbon/biomass partitioning	CO$_2$ concentration (μmol CO$_2$ mol^{-1})			*P* value
	Ambient	EC (600)	EC (800)	
Organic carbon leaves (%)	49.59[a] ± 0.30	53.62[b] ± 0.34	56.61[c] ± 0.32	0.000
Organic carbon stem (%)	54.60[a] ± 0.29	57.67[b] ± 0.28	59.46[c] ± 0.24	0.000
Organic carbon root (%)	57.46[a] ± 0.29	63.44[b] ± 0.34	66.56[c] ± 0.44	0.000
Leaves biomass (gm plant^{-1})	7.74[a] ± 0.32	11.86[b] ± 0.26	14.76[c] ± 0.26	0.000
Stem biomass (gm plant^{-1})	14.03[a] ± 0.40	18.60[b] ± 0.24	27.08[c] ± 0.64	0.000
Root biomass (gm plant^{-1})	7.88[a] ± 0.07	8.57[b] ± 0.08	9.02[c] ± 0.14	0.000

± Indicates standard error of the mean (SEM). $N = 40$. Means not sharing a common letter are significantly different at $p \leq 0.05$
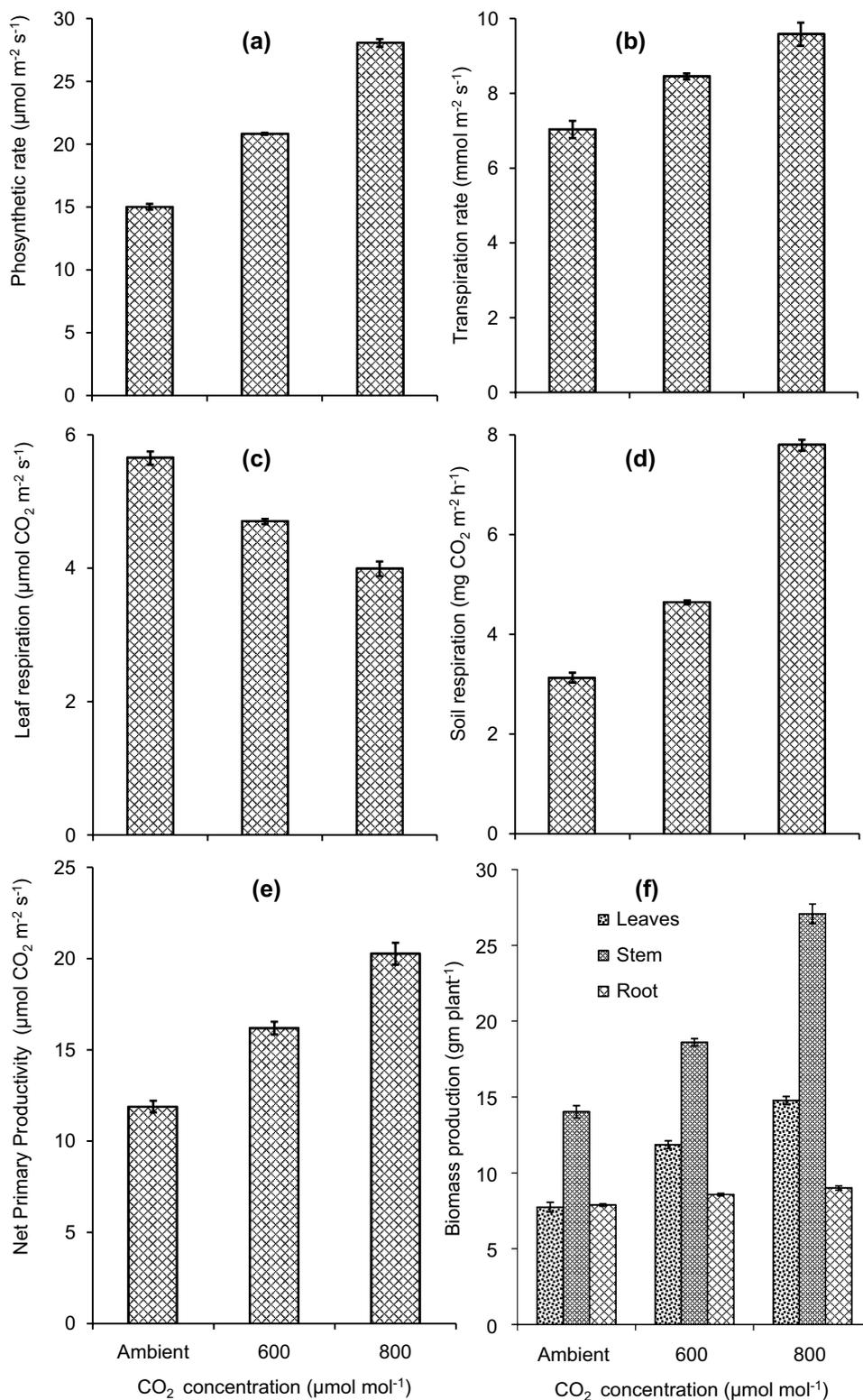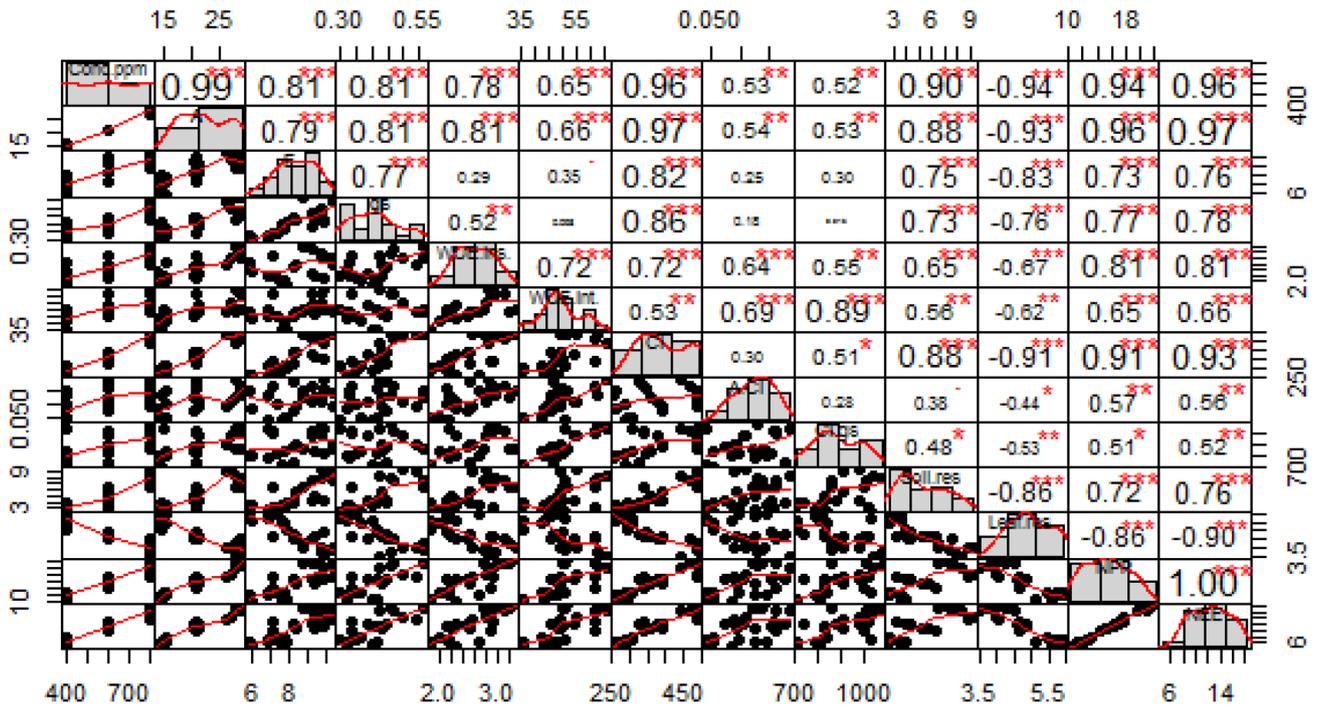
Fig. 2 Correlation between various physiological parameters. Where, *A* photosynthetic rate, *E* transpiration rate, *gs* stomatal conductance, *WUEins* instantaneous water use efficiency, *WUEintr* intrinsic water use efficiency, *Ci* intercellular CO_2 concentration, *A/ci* carboxylation efficiency, *Ci/gs* mesophyll efficiency, *SR* soil respiration, *LR* leaf respiration, *NPP* net primary productivity, *NEE* net ecosystem exchange (at pot conditions)

Fig. 3 Correlation between plant dry biomass and plant organic carbon (where *OC. Lvs* organic carbon leaves, *OC. Stem* organic carbon stem, *OC. Root* organic carbon root, *Lvs. Bms* leaves biomass, *Stem.bms* stem biomass, *Root.Bms* root biomass, *Dry.Bms* dry biomass)
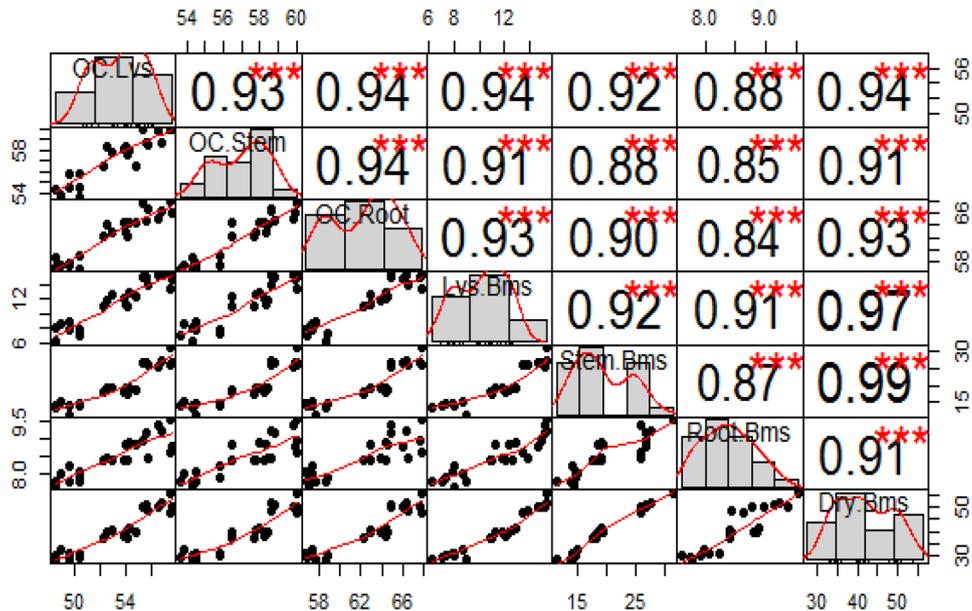
Response of leaf and soil respiration, and net primary productivity to EC

The CO_2 efflux (net respiratory CO_2 evolution originating from respiratory substrates minus the CO_2 being re-fixed by carboxylases) reduced in leaves of C_3 plants (Pinelli and Loreto 2003). This can be explained by stimulation of dark CO_2 fixation catalyzed by phosphoenolpyruvate carboxylase (PEPC), evolved in an evident reduction of CO_2 efflux (Amthor 1997; Drake et al. 1999). Griffin et al. (1996) investigated a reduction of 20–4% in leaf respiration of plant grown under increased CO_2 concentration. The response of

leaf respiration of study was also reported in other studies (Hamilton et al. 2001; Tan et al. 2013).

Soil respiration, a major component in the global carbon cycle, is affected by elevated atmospheric CO_2 concentrations. The soil respiration increased linearly with increased CO_2 concentration under the present study. However, effect of elevated CO_2 on soil respiration has rarely been studied. This study reported an increase in soil respiration under elevated CO_2 condition both at 600 and 800 μmol mol^{-1} than ambient. The maximum return of CO_2 flux from terrestrial ecosystems back to the atmosphere comes from the soil (Raich and Schlesinger 1992). Similar results of stimulation of soil respiration were seen in the wheat field under FACE experiment (Pendall et al. 2001). The increased rate of soil respiration under elevated CO_2 might be due to greater carbon availability for microbial activity through CO_2 induced carbon inputs (Adair et al. 2011). An increment of 20% in soil respiration was reported in a temperate grassland ecosystem by Keidel et al. (2015).

In terrestrial ecosystem, doubling of CO_2 concentrations without change in temperature is predicted increase in Global NPP by 16.3% (Melillo et al. 1993). NPP is a sensitive indicator of climatic and other forms of environmental change (Beer et al. 2010). The exchange of carbon from the atmosphere to the plant is through the stomata. The increase of CO_2 may influence the response of stomata which in turn may affect the plant's NPP. The result was similar to the study that showed elevated CO_2 may increase rates of photosynthesis, increase productivity, and increase biomass in most C_3 Plants (Houghton 2007). Elevated CO_2 concentrations increase the net exchange of CO_2 between the biosphere and the atmosphere, through stimulation of photosynthesis, increased water and nitrogen use efficiency and increase in biomass (Drake et al. 1997).

Response of carbon and biomass partitioning to EC

Despite the fact that analyzing plant development by surveying leaves, stem and root biomass might be an important beginning stage in deciding plant reaction to elevated CO_2 it might turn out to be fairly a heartless pointer of what is really happening to plant development regarding structure and capacity (Stulen and den Hertog 1993; Taylor et al. 1994; Sattler and Rutishauser 1997). The present study exhibited an enhancement of biomass production under elevated CO_2 condition. Carbon accumulation in roots was higher followed by stem and leaf (Singh et al. 2018; Lin and Wang 1998). Dry weight of leaved increased by 90.66% under 800 μmol mol^{-1} and 53.19% at 600 μmol CO_2 mol^{-1} than ambient. Similarly, shoot dry weight increased by 93.06 and 32.59%, root dry weight by 14.24 and 8.75% under 800 and 600 μmol mol^{-1}, respectively. More biomass was allocated to the stem tissues while minimum to the root tissues

for the plant under elevated CO_2 concentration. Besides above, the carbon allocation to leaves, stem, and root parts was also enhanced under an elevated CO_2 condition. The plants become taller and produce maximum biomass under elevated CO_2 condition. It was also observed that rising of atmospheric CO_2 could significantly stimulate leaf photosynthesis and above-ground dry weight biomass production of a C3 species, *Xanthium strumarium* (Ziska 2001). Similar results were reported for biomass and photosynthesis for *Cirsium arvense L.* (Ziska 2002). The biomass production and water use efficiency of the plants exposed to elevated CO_2 condition too increased. The corresponding results were reported by Singh et al. (2018).

Relationship between functional traits

The relationship between functional traits in presented in Figs. 2 and 3. The increased CO_2 concentration had stimulatory effect on photosynthesis rate that positively correlated to stomatal conductance and transpiration rate. More stomatal conductance leads to exchange higher rate of CO_2 and H_2O from the leaf and extended CO_2 atmospheric condition. Stomatal conductance was positively correlated to water use efficiency which produces more carbohydrate and ultimately higher biomass production. Similar relationship was observed by Singh et al. (2018). Night leaf respiration (NLR) suppressed under EC. Amthor et al. (1992) stated that there was an immediate and fully reversible decrease in leaf respiration rates when atmospheric CO_2 partial pressures are increased due to increased CO_2 concentration. This reduction mechanism of respiration has not been elucidated, but may be related to dark CO_2 fixation by phosphoenolpyruvate carboxylase (PEPc), altered cytosolic pH, or direct effects on membranes or enzymes (Amthor et al. 1992). Similar relationship was also reported by other researcher (Griffin et al. 1996; Hamilton et al. 2001; Tan et al. 2005). The photosynthesis was enhanced under EC which eventually increased NPP and total biomass production. The NPP, photosynthesis, and biomass in plant tissues were also correlated with each other. Increasing rate of photosynthesis enhances NPP and biomass under elevated CO_2 condition in this study. Total daily photosynthesis (GPP) and net primary production (NPP) correlated positively and were consistently higher under the elevated CO_2 treatment than the ambient CO_2 treatment (Cheng et al. 2000). This study reported positive correlation between elevated CO_2 and soil respiration. The increased rate of soil respiration under elevated CO_2 might be due to greater carbon availability for microbial activity through CO_2-induced carbon inputs (Adair et al. 2011). A relationship was also observed by Keidel et al. (2015) in a temperate grassland ecosystem, where soil respiration increased by 20%.

Conclusion

The present study concludes that the *W. somnifera* could adapt to changing climatic conditions especially rising of atmospheric CO_2 concentration. In such conditions of elevated CO_2 concentration, the physiological processes likely photosynthetic rate, water use efficiency, transpiration, and stomatal conductance might be enhanced contributing to plant growth and ultimately more biomass production particularly the root biomass in *W. somnifera*. Therefore, this species could be one of the most important medicinal species which will be more suitable to mitigate climate change in the future. Consequently, this species might be more adaptable in near future and can be used in suitable production of *W. Somnifera* and meet the increasing demand of pharmaceutical industries/medical to produce ayurvedic medicine in the country.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest in the publication.

References

Adair EC, Reich PB, Tsost JJ, Hobbie SE (2011) Elevated CO_2 stimulates grassland soil respiration by increasing carbon inputs rather than by enhancing soil moisture. Glob Change Biol 17:3546–3563

Ainsworth EA, Long SP (2005) What have we learned from 15 years of free-air CO_2 enrichment (FACE)—a meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. New Phytol 165:351–372

Amthor JS (1997) Plant respiratory responses to elevated CO_2 partial pressure. In: Allen LH, Kirkham MB, Olszyk DM, Whitman CE (Eds) Advances in carbon dioxide effects research. Madison: ASA, CSSA and SSSA 35–77 American Society of Agronomy Special Publication (proceedings of 1993 ASA Symposium, Cincinnati, OH)

Amthor JS, Koch GW, Bloom AJ (1992) CO_2 Inhibits respiration in leaves of *Rumex crispus* L. Plant Physiol 98:757–760

Beer C, Reichstein M, Tomelleri E, Ciais P, Jung M, Carvalhais N, Rödenbeck C, Arain MA, Baldocchi D, Bonan GB, Bondeau A, Cescatti A, Lasslop G, Lindroth A, Lomas M, Luyssaert S, Margolis H, Oleson KW, Roupsard O, Veenendaal E, Viovy N, Williams C, Woodward FI, Papale D (2010) Terrestrial gross carbon dioxide uptake: global distribution and covariation with climate. Science 32:834–838

Buckley LB, Nufio CR, Kingsolver JG (2013) Phenotypic clines, energy balances and ecological responses to climate change. J Anim Ecol 83:41–50

Bunn C, Laderach P, Ovalle-Rivera O, Kirschke D (2015) A bitter cup: climate change profile of global production of Arabica and Robusta coffee. Clim Change 129:89–101

Chaturvedi AK, Vashistha RK, Rawat N, Prasad P, Nautiyal MC (2009) Effect of CO_2 enrichment on photosynthetic behaviour of *Podophyllum Hexandrum*, an endangered medicinal herb. J Am Sci 5:113–118

Cheng W, Sims, DA, Luo Y, Johnson DW, Ball JT, Coleman JS (2000) Carbon budgeting in plant-soil mesocosms under elevated CO_2: locally missing carbon? Glob Change Biol 6:99–110

Convention on Biological Diversity (CBD) (2014) https://www.cbd.int/climate/intro.shtml. Accessed 3 Mar 2018

Dlugokencky E, Tans P (2017) Trends in atmospheric carbon dioxide. National Oceanic & Atmospheric Administration, Earth System Research Laboratory (NOAA/ESRL). http://www.esrl.noaa.gov/gmd/ccgg/trends/. Accessed 3 Mar 2018

Drake BG, Gonzalez-Meler MA, Long SP (1997) More efficient plants: a consequence of rising atmospheric CO_2. Annu Rev Plant Physiol Plant Mol Biol 48:609–639

Drake BG, Azcón-Bieto J, Berry JA, Bunce J, Dijkstra P, Farrar J, Koch GW, Gifford R, Gonzàlez-Meler MA, Lambers H et al (1999) Does elevated CO_2 inhibit plant mitochondrial respiration in green plants. Plant Cell Environ 22:649–657

Ghasemzadeh A, Jaafar HZE, Rahmat A (2010) Synthesis of phenolics and flavonoids in ginger (*Zingiber officinale* Roscoe) and their effects on photosynthesis rate. Int J Mol Sci 11:4539–4555

Gopalakrishna R, Mathangi JR, Bala G, Ravindranath NH (2011) Climate change and Indian Forest. Curr Sci 101:25–29

Griffin KL, Ball JT, Strain BR (1996) Direct and indirect effects of elevated CO_2 on whole-shoot respiration in ponderosa pine seedlings. Tree Physiol 16:33–41

Hamilton JG, Thomas RB, Delucia EH (2001) Direct and indirect effects of elevated CO_2 on leaf respiration in a forest ecosystem. Plant Cell Environ 24:975–982

Houghton RA (2007) Balancing the global carbon budget. Annu Rev Earth Planet Sci 35:313–347

Ibrahim MH, Jaafar HZ (2011) Increased carbon dioxide concentration improves the antioxidative properties of the Malaysian herb Kacip Fatimah (*Labisia pumila*). Molecules 16:6068–6081

Ibrahim MH, Hawa ZE, Jaafar HZ (2012) Impact of elevated carbon dioxide on primary, secondary metabolites and antioxidant responses of *Eleais guineensis* Jacq. (Oil Palm) seedlings. Molecules 17:5195–5211

Idso SB, Kimball BA, Pettit GR, Garner LC, Backhaus RA (2000) Effects of atmospheric CO_2 enrichment on the growth and development of *Hymenocallis littoralis* (Amaryllidaceae) and the concentrations of several antineoplastic and antiviral constituents of its bulbs. Am J Bot 87:769–773

IPCC (2007) Inter Governmental Panel on Climate Change. Summary Report of the working group of IPCC Paris

Keidel L, Kammann C, Grünhage L, Moser G, Müller C (2015) Positive feedback of elevated CO_2 on soil respiration in late autumn and winter. Biogeosciences 12:1257–1269

Lin W, Wang D (1998) Effects of elevated CO_2 on growth and carbon partitioning in rice. Chin Sci Bull 43:1982–1986

Melillo JM, Mcguire AD, Kicklighter DW, Moore B, Vorosmarty CJ, Schloss AL (1993) Global climate change and terrestrial net primary production. Nature 363:234–240

Mirjalili MH, Moyano E, Bonfill M, Cusido RM, Palazon J (2009) Steroidal lactones from *Withania somnifera*, an ancient plant for novel medicine. Molecules 14:2373–2393

Morison JIL, Gifford RA (1984) Plant growth and water use with limited water supply in high CO_2 concentrations: leaf area, water use, and transpiration. Aust J Plant Physiol 11:361–374

Nowak RS, Ellsworth DS, Smith SD (2004) Functional responses of plants to elevated atmospheric CO_2 do photosynthetic and productivity data from FACE experiments support early predictions. New Phytol 162:253–280

Oliveira VF, Zaidan LBP, Braga MR, Aidar MPM, Carvalho MAM (2010) Elevated CO_2 atmosphere promotes plant growth and inulin production in the cerrado species *Vernonia herbacea*. Funct Plant Biol 37:223–231

Parmesan C (2006) Ecological and evolutionary responses to recent climate change. Annu Rev Ecol Syst 37:637–639

Parry ML, Canziani OF, Palutikof JP, van der Linden PJ, Hanson CE (2007) Technical summary Box TS.6. The main projected impacts for regions, in IPCC AR4 WG2 pp 59–63

Patwardhan B, Panse GT, Kulkarni PH (1998) Ashwagandha a review. J Natl Integr Med Assoc 30:7–11

Pendall E, Leavitt SW, Brooks T, Kimball BA, Pinter PJ, Wall GW, LaMorte RL, Wechsung G, Wechsung F, Adamsen F, Matthias AD, Thompson TL (2001) Elevated CO_2 stimulates soil respiration in a FACE wheat field. Basic Appl Ecol 2:193–201

Pinelli P, Loreto F (2003) (CO_2)–C-12 emission from different metabolic pathways measured in illuminated and darkened C-3 and C-4 leaves at low, atmospheric and elevated CO_2 concentration. J Exp Bot 54:1761–1769

Prajapati ND, Purohit SS, Sharma AK, Kumar T (2003) A handbook of medicinal plants: a complete source book. Jodhpur: Agrobios India p 756

Raich JW, Schlesinger WH (1992) The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. Tellus B Chem Phys Meteorol 44:81–99

Saadi S, Todorovic M, Tanasijevic L, Pereira LS, Pizzigalli C, Lionello P (2015) Climate change and Mediterranean agriculture: impacts on winter wheat and tomato crop evapotranspiration, irrigation requirements and yield. Agric Water Manag 147:103–115

Saravanan S (2014) Gas exchange characteristics in *Tectona grandis* L. clones under varying concentrations of CO_2 levels. J Stress Physiol Biochem 10:122–133

Saravanan S, Karthi S (2014) Effect of elevated CO_2 on growth and biochemical changes in *Catharanthus roseus*- a valuable medicinal herb. World J Pharm Pharm Sci 3:411–422

Sattler R, Rutishauser R (1997) The fundamental relevance of morphology and morphogenesis to plant research. Ann Bot 80:571–582

Singh P, Guleri R, Singh V, Kaur G, Katari H, Singh B, Kaura G, Kaul SC, Wadhwa R, Pati PK (2015) Biotechnological interventions in *Withania somnifera* (L.) Dunal. Biotechnol Genet Eng Rev 19:1–20

Singh H, Sharma R, Verma A, Kumar M, Kumar S (2016) Can atmospheric CO_2 enrichment alter growth dynamics, structure and functioning of medicinal and aromatic plant (Tulsi)? An approach to understand adaptation and mitigation potential of medicinal and aromatic plants in wake of climate change scenario. In: Annual session of the national academy of sciences India, jointly organized by National Academy of Sciences India and Uttarakhand State Council for Science and Technology (UCOST), Dehradun, 2–4 Dec 2016, pp 94

Singh H, Savita A, Sharma R, Sinha S, Kumar M, Kumar P, Verma A, Sharma SK (2017) Physiological functioning of *Lagerstroemia speciosa* L. under heavy roadside traffic: an approach to screen potential species for abatement of urban air pollution. 3 Biotech 7:1–10

Singh H, Sharma R, Savita A, Singh MP, Kumar M, Verma A, Ansari MW, Sharma SK 2018 adaptive physiological response of *Parthenium hysterophorus* to elevated atmospheric CO_2 concentration. Ind For 144:1–14

Stuhlfauth T, Fock HP (1990) Effects of whole season CO_2 enrichment on the cultivation of a medicinal plant, *Digitalis lanata*. J Agron Crop Sci 164:168–173

Stuhlfauth T, Klug K, Fock HP (1987) The production of secondary metabolites by *Digitalis lanata* during CO_2 enrichment and water stress. Phytochemistry 26:2735–2739

Stulen I, den Hertog J (1993) Root growth and functioning under atmospheric CO_2 enrichment. Plant Ecol 104:99–115

Stutt GW, Eraso I, Rimando AM (2008) Carbon dioxide enrichment enhances growth and flavonoid content of two *Scutellaria* species. J Am Soc Hortic Sci 133:631–638

Tan ZX, Liu S, Johnston CA, Loveland TR, Tieszen LL, Liu J, Kurtz R (2005) Soil organic carbon dynamics as related to land use history in the northwestern Great Plains. Global Biogeochem Cycles 19:GB3011. https://doi.org/10.1029/2005GB002536

Tan KY, Zhou GS, Ren SX (2013) Response of leaf dark respiration of winter wheat to changes in CO_2 concentration and temperature. Chin Sci Bull 58:1795–1800

Taylor G, Ranasinghe S, Bosac C, Gardner SDL, Ferris R (1994) Elevated CO_2 and plant growth: cellular mechanisms and responses of whole plants. J Exp Bot 45:1761–1774

Thomas CD, Cameron A, Green RE, Bakkenes M, Beaumont LJ, Collingham YC et al (2004) Extinction risk from climate change. Nature 427:145–148

Thompson AR, Doelling JH, Suttangkakul A, Vierstra RD (2005) Autophagic nutrient recycling in *Arabidopsis* directed by the ATG8 and ATG12 conjugation pathways. Plant Physiol 138:2097–2110

Tilman D, Lehman C (2001) Human-caused environmental change: impacts on plant diversity and evolution. Proc Natl Acad Sci USA 98:5433–5440

Tisserat B (2002) Influence of ultra-high carbon dioxide levels on growth and morphogenesis of *Lamiaceae* species in soil. J Herbs Spices Med Plants 9:81–89

Urban MC (2015) Accelerating extinction risk from climate change. Science 348:571–573

Vurro E, Bruni R, Bianchi A, Sanità di Toppi L (2009) Elevated CO_2 decreases oxidative stress and increases essential oil yield in leaves of *Thymus vulgaris* grown in a mini-FACE system. Environ Exper Bot 65:99–107

Walkley A, Black IA (1934) An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci 37:29–37

Warrier RR, Jayaraj RS, Balu A (2013) Variation in gas exchange characteristics in clones of *Eucalyptus camaldulensis* under varying conditions of CO_2. J Stress Physiol Biochem 9:333–344

Wu J, Hong J, Wang X, Sun J, Lu X, Fan J, Cai Y (2013) Biomass partitioning and its relationship with the environmental factors at the alpine steppe in northern Tibet. PLoS One 8:12

Zari MP (2014) Ecosystem services analysis in response to biodiversity loss caused by the built environment. Surv Perspect Integr Environ Soc 7:1–14

Ziska LR (2001) Changes in competitive ability between a C4 crop and a C3 weed with elevated carbon dioxide. Weed Sci 49:622–627

Ziska LR (2002) Influence of rising atmospheric CO_2 since 1900 on early growth and photosynthetic response of a noxious invasive weed, Canada thistle (*Cirsium arvense*). Funt Plant Biol 29:1387–1392

Ziska LH, Panicker S, Wojno HL (2008) Recent and projected increases in atmospheric carbon dioxide and the potential impacts on growth and alkaloid production in wild poppy (*Papaver setigerum* DC). Clim Change 91:395–403

Zobayed SMA, Murch SJ, Rupasinghe HPV, Saxena PK (2003) Elevated carbon supply altered hypericin and hyperforin contents of St. John's wort (*Hypericum perforatum*) grown in bioreactors. Plant Cell Tissue Organ Cult 75:143–149

Zou DH, Gao KS (2005) Regulation of gamete release in the economic brown seaweed *Hizikia fusiformis* (Phaeophyta). Biotechnol Lett 27:915–918