Alteration in biochemical constituents and nutrients partitioning of *Asparagus racemosus* in response to elevated atmospheric CO$_2$ concentration

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Abstract

Understanding the response of medicinal plants to elevated CO$_2$ concentrations is crucial to evaluate the climate change impacts on medicinal plant’s productivity together with the accumulation of biochemical constituents counting nutrients wealth. The present study investigated the effect of elevated CO$_2$ concentrations (ambient—~400±4, 600±12, and 800±16 μmol CO$_2$ mol$^{-1}$) on the biochemical constituents (viz. chlorophyll, carotenoids, ascorbic acid, protein, total sugars, and carbon partitioning) and accumulation of mineral nutrients (viz. potassium, phosphorus, and magnesium) in different plant parts (viz. leaf, stem, and root) of *Asparagus racemosus* Willd., an endangered medicinal plant species. The results confirmed that the elevated CO$_2$ concentration significantly ($p \leq 0.05$) enhanced the leaves biochemical constituents, viz. chlorophyll, protein, total sugars, and carbon content while conversely diminishes the ascorbic acid content in leaf. The accumulation of nutrients especially potassium and magnesium were significantly ($p \leq 0.05$) improved while it is reverse in case of phosphorus under the elevated CO$_2$ concentration. Moreover, elevated CO$_2$ notably altered protein, sugars, carbon, and nutrients partitioning in leaf, stem, and root tissues. This study will be helpful in anticipating the effect of rising atmospheric CO$_2$ concentration on medicinal and threatened plants and require further intensive studies to comprehend the effects of elevated CO$_2$ concentration.

Keywords *Asparagus racemosus* · Biochemical response · Nutrients · Carbon partitioning · Elevated CO$_2$ concentration

Introduction

*Asparagus racemosus* Willd. (Shatavari) is an important medicinal plant species distributed across India from tropical to subtropical parts, including Andamans and ascending in the Himalayas up to an altitude of 1500 masl (Sachan et al. 2012). It has been prioritized as top eight medicinal plant species by the National Medicinal Plant Board, Government of India. IUCN has categorized the species as endangered in India due to overexploitation, deforestation, and degradation besides climate change impacts.

The fertilization effect of risen atmospheric CO$_2$ concentration on physiology, growth, morphology, biochemical compounds, carbon allocation, and productivity of various plant species owing to increased carbon assimilation under elevated CO$_2$ has been accounted globally (Cha et al. 2017; Singh et al. 2018; Sharma et al. 2018; Yadav et al. 2019; Ahammed et al. 2020). It is also stated that atmospheric CO$_2$ concentration will have a profound impact on medicinal plants at various levels such as growth, physiology, productivity, and biochemical constituents, including nutrients and health-promoting substances or primary and secondary metabolites (Ghasemzadeh and Jaafar 2011; Jaafar et al. 2012; Saldanha et al. 2014; Al Jaouni et al. 2018; Kaundal et al. 2018). Due to this reason, various researchers have stressed a strong need to investigate the impact of elevated CO$_2$ concentrations on partitioning or allocation of biochemical compounds and nutrients to the plant’s parts such as the leaf, stem, and root tissues (Aranjuelo et al. 2013; Aljazairi et al. 2014; Butterly et al. 2015; Thompson et al. 2017; Wang et al. 2019). Increased CO$_2$ concentrations have immediate impacts on physiological processes, viz. change in photosynthesis, transpiration, stomatal conductance, and water use efficiency (Singh et al. 2020). Besides, rising CO$_2$ concentration also
induces changes in the biochemical constitutes, including the nutrients status of plant tissues. Nonstructural carbohydrates such as sugars and starches have been reported to be enhanced by 30 to 40% under elevated CO2 concentrations (Ainsworth 2008; Ainsworth and Long 2005).

Different effect of elevated CO2 on individual nutrient accumulation was observed in plants. Nitrogen content in plant tissues has demonstrated a reduced trend under high CO2 concentration (Cotrufo et al. 1998; Taub and Wang 2008). The decline nitrogen content might be due to the various factors such as nitrogen dilution from increased carbohydrate concentrations, reduced minerals uptake from the soil system, reduction in stomatal conductance and less water uptake by the plants, and declined nitrogen assimilation into the organic compound of the plant’s systems (Loladze 2002; Taub and Wang 2008; Bloom et al. 2010). The influence of elevated CO2 on the phosphorus accumulation in plants has always been more changeable than nitrogen with the confirmation for declined (Teng et al. 2006), increased (Liu et al. 2012), and no effects on plant phosphorus (Johnson et al. 2004). Duval et al. (2012) reported that the response of phosphorus in plants system under elevated CO2 concentration varied, which depends on plant functional groups and other climatic and soil conditions, besides high CO2 concentration (Huang et al. 2015). Elevated CO2 induces accumulation of biochemical constituents such as chlorophyll, protein, total sugars, carbon, and other biochemicals in plant tissues. Dong et al. (2018) reported an increasing and decreasing chlorophyll content, ascorbic acid, total sugars, etc., in various plant tissues. It has been investigated that elevated CO2 can diminish the photorespiration process of the plant system. Reduced photorespiration might subsequently lessen the formation of free oxygen radicals, hence dipping antioxidant metabolism (Pérez-López et al. 2018). Wu et al. (2017) reported that elevated CO2 concentration may influence the accumulation of antioxidants, especially ascorbic acid, via a complex mechanism considering the synthesis, recycling, and ascorbic acid’s degradation, hence decreasing ascorbic acid content in plant tissues. Thus, it is clear that rising elevated atmospheric CO2 medicinal plants require much more exploration for food and health security and livelihood support in the wake of predicted climate change. The effect of climate change on the growth and production of secondary chemicals, biochemical constituents, and nutrients in medicinal plants is not well understood and needs further investigation (Gairola et al. 2010).

The alteration in biochemical compounds/constituents and nutrients partitioning in plant tissues of A. racemosus has not been investigated yet for understanding response to future climate change, mainly elevated CO2 concentrations. To address this gap, the present study was performed to investigate the effect of elevated CO2 concentration on biochemical and nutritional parameters in leaf, stem, and root parts of A. racemosus.

Materials and methods

Brief account of open-top chamber (OTC) facility

Open top chambers (OTCs) facility of Forest Research Institute, Dehradun, Uttarakhand (32° 20′ 44.2172″ N, 78° 0′ 41.6185″ E and 668 masl) was utilized to expose the seedlings of A. racemosus to elevated CO2 concentrations. High-quality multilayer polycarbonate sheet with 80–85% transmittance was used to construct open-top chambers with the dimension of 3×3×4 m (width× length× height), i.e. chamber floor area of 9.0 m² and height of 4.0 m (Singh et al. 2018; Sharma et al. 2018). Pure (100%) CO2 gas of commercial grade was supplied continuously between 09:00 and 17:00 h from the CO2 gas cylinder to the respective OTCs which was regulated by PC linked Program Logic Control (PLC) system and Supervisory Control and Data Acquisition (SCADA) system.

Seedlings preparation and exposure to CO2 treatments

The 5-month-old seedlings of A. racemosus were obtained from Non-Wood Forest Product Division, Forest Research Institute, Dehradun. The seedlings were transplanted in earthen pots with growing medium of soil:sand:manure (2:2:1). The potted seedlings were left within open-top chambers (OTCs) without CO2 treatments for 1 week to acclimate the pot and chamber conditions. The experiment was laid out within OTCs in a completely randomized design with CO2 treatments 600±12 and 800±16 μmol CO2 mol⁻¹ in addition to ambient, i.e. ~400±4 μmol CO2 mol⁻¹. Five replications were considered with a set of eight seedlings per replication. The seedlings were grown for 6 months with proper watering and weeding of pots.

Biochemical analysis of plant tissues

The biochemical analysis of plant tissues, particularly leaf and root, was performed at the reproductive and maturity stage. Leaf protein (hereafter referred to as LPROT) and root protein (hereafter referred to as RPROT) were estimated using the Bradford method (1976). Fresh tissues were brought to the laboratory in the icebox for further analysis. Leaf sample (100 mg) was homogenized in 1ml phosphate buffer over an ice tray. The homogenate was then centrifuged at 13000 rpm at 4°C for 20 min. The supernatant was removed in a tube and added phosphate buffer (5 ml) to it. A volume (0.5 ml) of this mixture was taken and made to 1 ml with phosphate buffer and added Bradford dye (3 ml). This mixture was kept at room temperature for 25 min to complete the reaction. Further, optical density of this mixture was measured at 595 nm against a blank containing 3 ml Bradford reagent and 1ml phosphate buffer.
buffer. Bovine serum albumin (BSA) with different concentrations was taken to develop the standard curve. The tissues’ protein concentration was estimated using a linear equation obtained from the BSA standard curve.

Total sugars in leaf (TSL) and total sugars in root (TSR) were determined as per the method of Dubois et al. (1956). A solution (contains 5% phenol and 80% ethanol) was prepared using distilled water. The fresh leaves (100 mg) and roots were chopped separately into small pieces. The chopped tissues were placed in the separate graduated test tube prefilled with 5 ml of 80% ethanol and subsequently incubated at 80°C for 1 h in the oven. The sample extract volume (0.5 ml) was taken in another test tube and added 0.5 ml distilled water (DW). A volume of 5% phenol (1 ml) was supplemented to this solution and immediately incubated at room temperature for an hour. A volume of 98% concentrated H2SO4 (2.5 ml) was added to this solution by placing the tubes on an ice tray. This reaction mixture was shaken using an orbital shaker for few seconds. After that, the optical density was read at 490 nm against a blank containing 0.5 ml ethanol, 0.5 ml DW, 1 ml 5% phenol, and 2.5 ml concentrated H2SO4 (98%). The standard curve was obtained using different concentrations of dextrose. The total sugar in the plant tissues was computed from the linear equation of the standard curve. The value obtained was multiplied by the dilution factor to obtain the total sugar content in plant tissues.

Ascorbic acid (hereafter referred to as ASC) leaves was analyzed by Harris and Ray’s method (1953). Fresh leaves (200 mg) were homogenized in 2 ml of 4% Trichloroacetic acid (TCA) and then centrifuged at 20,000 rpm for 10 min. In centrifuged tube, a pinch of activated charcoal was added and once more centrifuged at 12,000 rpm for 10 min to convert ascorbate to dehydroascorbate. A volume of supernatant (0.5 ml) was taken into a test tube and immediately added 1.5 ml of 2% molybdate solution (DNPH) and 2 drops of 10% thiourea. This mixture was set aside at 37°C for 3 h to complete the reaction. The reaction was terminated by placing the test tubes on an ice tray and subsequently added 2.5 ml of concentrated H2SO4 (98%). This mixer was left at room temperature for 30 min to produce orange colour. The optical density of orange colour mixture was read at 540 nm against a blank containing 0.5 ml of 2% molybdate solution, 2 drops thiourea, and 2.5 ml concentrated H2SO4 (98%). The ascorbic acid content was determined by the standard curve equation obtained from different concentrations of pure ascorbic acid. The values obtained were multiplied by the dilution factor to acquire ascorbic acid content in leaf tissues.

Leaf chlorophyll content was estimated as per the method of Hiscox and Israelstam (1979). Fresh leaves (50 mg) were chopped into the tiny pieces and then transferred into the test tubes containing 8 ml of dimethyl sulfoxide (DMSO). Further, the tubes were incubated at 65°C for 3 h in the oven. The samples were filtered into a graduated test tube and made the volume to 10 ml using DMSO. The absorbance of the samples was read at 663 and 645 nm against pure DMSO as a blank using spectrophotometer (Systronics Visiscan 167). Total leaf chlorophyll content (mg g⁻¹ as fresh weight (F.W.)) was calculated using the following equation:

\[
\text{Chlorophyll } a, \text{Chla (mg g}^{-1}\text{ as F.W.}) = \frac{12.7 A_{665}-2.69 A_{645}}{w \times 1000}
\]

\[
\text{Chlorophyll } b, \text{Chlb (mg g}^{-1}\text{ as F.W.}) = \frac{22.9 A_{665}-4.68 A_{645}}{w \times 1000}
\]

**Total leaf Chlorophyll, TChl (mg g}^{-1}\text{ as F.W.})**

\[
= \left( \frac{20.2 \times A_{665} + (8.02 \times A_{660}) \times V}{a \times 1000 \times w} \right)
\]

where \( A_{665} \) and \( A_{663} \) are the O.D. values measured at 645 nm and 663 nm, respectively, \( V \) is the final volume of extract, \( a \) is the path length of the cells (1 cm), and \( w \) is the weight of the leaf tissues taken.

For estimating total leaf carotenoids content, the absorbance of the same samples was taken at 470 nm using a spectrophotometer. The below equation was adopted for calculating total carotenoids (Car) in leaf tissues (\( \mu g \text{ g}^{-1}\text{ as fresh weight (F.W.))} \):

\[
\text{Carotenoids content, CAR (\mu g g}^{-1}\text{ as F.W.}) = \frac{1000 A_{470}-1.9 (\text{Chla-Chlb})}{214}
\]

**Nutrients analysis in plant tissues**

Nutrients, viz. phosphorus (P), potassium (K), and magnesium (Mg) in leaf, stem, and root tissues, were analyzed after harvesting of the plants. The tri-acid solution was prepared using nitric acid (HNO₃), perchloric acid (HClO₄), and sulphuric acid (98%H₂SO₄) in the ratio 10:4:1 for digestion of plant tissues.

Phosphorus content was estimated as per the method of Holman (1943). The reagents, i.e. molybdate solution, hydrazine sulphate solution, and sodium hydroxide solution, were used during phosphorus estimation. Molybdate solution was prepared by adding 12.5 gm of ammonium molybdate in 150 ml of DW. A volume (140ml) of concentrated H₂SO₄ (98%) was mixed in 150ml of DW and added to the above solution. Hydrazine sulphate (HS) solution was prepared by adding 0.15 gm of HS to 100 ml of DW. Forty-five grams of NaOH (99%) pellets were mixed in 100 ml DW to make NaOH solution. Then, 2 ml of tri-acid solution was mixed with 10 ml DW in a 100-ml flask. After that, 1 to 2 drops of phenolphthalein were added to it and this mixture was titrated against NaOH solution till the completion of the reaction and until the appearance of pink colour. Further, ammonium molybdate solution (10 ml) was added followed by a 2-ml HS
solution. The volume of the mixture was made to 100 ml by adding DW. The flasks were incubated in a boiling water bath for 15 min to complete the reaction. The optical density of this solution was measured at 830 nm for calculating phosphorus content in plant tissues.

$$\text{Phosphorus} \ (\%) = \frac{\text{O.D.} \times \text{Volume} \times 100}{10^6 \times \text{Weight of plant tissues}}$$

Potassium content was determined using the method of Vogel (1961). For potassium content, 2 ml of stock solution was mixed in 100 ml of DW in a 100-ml conical flask. The flame photometer (Systronics Flame Photometer 128) was calibrated with DW at 100 ppm and 40 ppm standard potassium solutions. The readings were taken for each sample and the calculations were done as follows:

$$\text{Potassium} \ (\%) = \frac{\text{O.D.} \times \text{Volume} \times 100}{10^6 \times \text{Weight of plant tissues}}$$

For magnesium estimation, 2 ml of the tri-acidic solution was taken in a 50-ml volume of flask (Young and Gill 1951). Further, DW (10 ml) was added to it followed by the compensatory solution (2 ml), 2% polyvinyl alcohol (2 ml), hydroxylamine hydrochloride solution (1 ml), titan yellow solution (1 ml), and 45% sodium hydroxide. The net volume was made to 50 ml with DW and light orange colour appeared. The absorbance of this mixture along with blank was measured at 540 nm using the spectrophotometer (Systronics Visiscan 167).

Organic carbon (OC) was estimated in leaf (LOC), stem (SOC), and root (ROC) after harvesting the plants as per the procedure of Walkley and Black (1934).

### Statistical analysis

We performed descriptive analysis (mean, median, standard deviation, and standard error of the mean) in Microsoft Excel. Statistical software SPSS 16.0 was used with a multivariate general linear model to observe the significant mean difference in response of biochemical parameters and nutrients accumulation at set levels CO2 concentrations. Further post hoc Tukey test was performed to identify the homogeneous subsets (groups having similar and dissimilar means). The coefficient of determination ($R^2$) is provided in the supplementary table for all the studied parameters. During this analysis, CO2 was considered an independent variable, and all studied parameters were deemed dependent variables. Correlation analysis and principal component analysis (PCA) were performed through R studio statistical software. In the result, the term significant is used to indicate a $p$-value $\leq 0.05$.

### Results

#### Alteration in plant biochemical constituents under elevated CO2 concentrations

Elevated CO2 significantly promoted leaf chlorophyll, total sugar, and protein accumulation whereas impeded the ascorbic acid and carotenoid content (Figs. 1 and 2). We reported an increment of ~5.12 and ~9.14 (Chl $a$), ~3.01 and ~5.57 (Chl $b$), and ~3.20 and ~6.81 (TChl) for the plants exposed to 600±12 and 800±16 μmol CO2 mol$^{-1}$, respectively, compared to ambient (Fig. 1). The pigment i.e. carotenoid content significantly diminished by ~2.97 and 8.04% under 600±12 and 800±16 μmol CO2 mol$^{-1}$, respectively, than their counterparts (Fig. 1). Besides, elevated CO2 had significantly enhanced total sugar in leaf (TSL) by ~35.21% and ~46.67% of plants treated with 600±12 and 800±16 μmol CO2 mol$^{-1}$, respectively (Fig. 2). Similarly, total sugars in root (TSR) were improved by 105.49% and 320.44% under 600±12 and 800±16 μmol CO2 mol$^{-1}$, respectively, compared to ambient (Fig. 2). It was very interesting to account that leaf protein (LPROT) and root protein (RPROT) significantly superior within elevated CO2 concentration than their counterparts, i.e. ambient (Fig. 2). LPROT increased by 36.21% and 85.53% at 600±12 μmol CO2 mol$^{-1}$ and 85.53 at 800±16 μmol CO2 mol$^{-1}$, respectively. RPROT boosted by 55.08% and 91.68% at 600 and 800 μmol CO2 mol$^{-1}$ grown plants than ambient (Fig. 2). Moreover, elevated CO2 was found to significantly suppress ascorbic acid in leaf (ASC) by 3.57 and 29.32% under 600 and 800 μmol CO2 mol$^{-1}$, respectively, compared to ambient (Fig. 2).

#### Alteration in plant nutrients under elevated CO2 concentration

In the present study, nutrients, viz. phosphorus (P), potassium (K), and magnesium (Mg), showed different responses towards increased CO2 concentrations. Nutrients K and Mg significantly increased while P decreased in response to elevated CO2 conditions than ambient grown plants (Fig. 3). The allocation of K to root, stem, and leaf tissues were profoundly altered by elevated CO2 with more allocation in stem followed by leaf and root tissues. Leaf K was significantly increased by 26.02% and 34.15% at 600±12 and 800±16 μmol CO2 mol$^{-1}$, respectively, compared to ambient (Fig. 3). Simultaneously, K content in stem was significantly found more by 8.16 and 14.29% under CO2 concentration of 600±12 and 800±16 μmol CO2 mol$^{-1}$, correspondingly rather than ambient. Root K is enhanced by 18.41 and 34.58% at 600±12 and 800±16 μmol CO2 mol$^{-1}$, respectively (Fig. 3). Mg was found to accumulate more in leaves by 64.96 and 69.03% of plants grown under 600±12 and 800±16 μmol CO2 mol$^{-1}$, respectively, than ambient (Fig. 3). Likewise, Mg demonstrated
better improvement in the stem by 39.89 and 182.03% at elevated CO2 of 600±12 and 800±16 μmol CO2 mol−1, respectively, while lower in ambient (Fig. 3). Overall, higher Mg was reported more in root followed by stem and leaf; however, P exhibited opposite trend to Mg, and higher proportion was partitioned to leaf followed by root and stem tissues (Fig. 3). Leaf P was found to decrease significantly by 29.79% at 600±12 μmol CO2 mol−1 and 39.89% at 800±16 μmol CO2 mol−1; likewise, stem P reduced by 23.50% and 34.97% at 600±16 and 800±16 μmol CO2 mol−1, respectively, compared to ambient CO2 conditions (Fig. 3). A similar trend has existed for root P with an increment of 43.69 and 55.31% under 600±12 and 800±16 μmol CO2 mol−1, respectively (Fig. 3).

**Alteration in organic carbon under elevated CO2 concentration**

Carbon is a vital element for fostering plant growth and development. Under the present study, elevated CO2 had significantly impacted carbon content and allocation in various plant tissues. The organic carbon (OC) was reported to be enhanced in all plant tissues, viz. leaf, stem, and root under elevated CO2 circumstances (Fig. 2). Elevated CO2 significantly induced more carbon allocation to the leaf tissues followed by stem and root under 800±16 μmol CO2 mol−1. However, in case of plant treated with 600±12 μmol CO2 mol−1, more carbon was found in stem followed by root and leaf (Fig. 2). Leaf and stem carbon was reported significantly higher under elevated CO2 concentration compared to ambient grown plants (Fig. 3). Similarly, a significant increment in carbon content in root was reported at elevated CO2 concentration when compared to ambient grown plants (Fig. 3).
Relationship between biochemical constituents and plants nutrients

In the present study, maximum studied parameters represented a significant correlation with each other (Fig. 4). We observed a strong correlation between total chlorophyll, root protein \( (r = 0.89) \), leaf protein \( (r = 0.94) \), leaf organic carbon \( (r = 0.97) \), root organic carbon \( (r = 0.94) \), root magnesium \( (r = 0.87) \), and root potassium \( (r = 0.75) \) (Fig. 4). Interestingly, root organic carbon and root protein were significantly correlated \( (r = 0.92) \) (Fig. 4). The PCA analysis between biochemical constituents and plant nutrients (Fig. 5) demonstrated that plant’s responses are segregated in a two-dimensional ordination diagram that exhibits the correlation between biochemical components and plant nutrients grown under enriched CO\(_2\) conditions and ambient. PC1 contributed approximately 70 rather than PCA2 which shown 7% contribution. ASC was positively correlated with CAR, LP, SP, and RP while negatively correlated with Chl a, Chl b, Tchl, LPROT, RPROT, LTS, RTS, LOC, SOC, ROC, LK, SK, RK, LM, SM, and RM. CAR exhibited similar trend to the ACS. LP, SP, and RP correlated positively with CAR and ASC and negatively correlated with all the parameters which negatively correlated in case of ASC. Chl a, Chl b, Tchl, LPROT, RPROT, LTS, RTS, LOC, SOC, ROC, LK, SK, RK, LM, SM, and RM are positively correlated with each other while negatively correlated with ASC, CAR, LP, SP, and RP (Fig 5).

Discussion

Alteration in plant biochemical constituents under elevated CO\(_2\) concentrations

During plant exposure to external stress, the carbon fixed is not allocated to growth function, although it is directed to the production of biochemical compounds, including secondary metabolites (Mooney et al. 1991). Thus, elevated CO\(_2\) modified the biochemical accumulation and allocation in plants to adjust the novel environmental circumstances. Under the present study, chlorophyll content was increased under elevated carbon dioxide compared to ambient. Increased chlorophyll is associated with the increased rate of photosynthesis process. During photosynthesis, chlorophyll absorbs energy from sunlight while staying down at the thylakoid membrane of chloroplast which facilitates photosynthesis ultimately carbohydrates production and plant growth. The increased total chlorophyll content under elevated carbon dioxide was reported in *Medicago sativa* (Sgherri et al. 1998), *Gycine max*, *Pennisetum glaucum*, *Chenopodium album*, *Amaranthus retroflexus* (Hamid et al. 2012), *Raphanus raphanistrum* (Urbonaviciute et al. 2006), *Catharanthus roseus* (Singh and Agarwal 2015), and *Acrocomia aculeata* (Rosa et al. 2019).

Increased accumulation of primary metabolites, i.e. protein and total sugar, contributed towards secondary metabolite production by providing building blocks and biosynthetic enzymes that were acquired from primary metabolites. The present study reported an increase in total sugar, which directly proportional to the rise in physiological parameters (Fig. 5). It has been reported that elevated CO\(_2\) induces increment in triose phosphate of leaves that further contribute to the formation of carbohydrates resulting in more sugar accumulation (Ibrahim and Jaafar 2011). Earlier studies are witnessed to increase total sugar content in plants such as *Labisia pumila* (Ibrahim and Jaafar 2011) and *Catharanthus roseus* (Saravanan and Karthi 2014). Protein helps to support plant structure which found to increase in this study under elevated CO\(_2\) concentration. Increased protein content under elevated CO\(_2\) was reported in *Oryza sativa* (Liu et al. 2017) and *Chaetoceros gracilis* (Khairy et al. 2014). There is no satisfactory explanation until now for such an increase nevertheless; such an increase can be justified with the fact of protein...
Ascorbic acid was reduced under exposure to elevated carbon dioxide. Suppression of the formation of ascorbic acid was reported in *Capsicum annuum* grown under elevated CO$_2$ concentration of 1000 μmol CO$_2$ mol$^{-1}$ (Azam et al. 2017). A study on transcript profile on genes of carrot justified the occurrence of a complex process that degrades the ascorbic acid (Dong et al. 2018). However, Dong et al. (2018) reported higher ascorbic acid under higher CO$_2$ concentrations. Dong et al. (2018) reported increment or decline in chlorophyll, ascorbic acid, and total sugars in plant tissues under elevated CO$_2$ concentrations. Elevated CO$_2$ generally declines the photosynthetic mechanism which further reduces the formation of oxygen radicals, consequently reducing antioxidant metabolism (Pérez-López et al. 2018). Wu et al. (2017) stated that elevated CO$_2$ concentration may influence the accumulation of antioxidants especially ascorbic acid via a complex mechanism considering the synthesis, recycling, and ascorbic acid’s degradation hence decreasing ascorbic acid content in plant tissues.

### Alteration in plant nutrients under elevated CO$_2$ concentration

Essential nutrients, whose limitation hinders the process of growth and survival since they are paramount to generating new cells, respond differently under elevated carbon dioxide...
concentrations. K regulates the carbon uptake process, along with photosynthesis, and stomatal conductance and increase of K generally help in better response. Likewise, Mg is the heart of chlorophyll and it helps plants in capturing more sunlight for performing photosynthesis process. K and Mg content were increased in plant tissues under elevated carbon dioxide concentrations. Increment of these nutrients led to enhancement of physiological processes like transpiration rate and stomatal conductance and pulls more nutrients from the soil along with the water (Sallas et al. 2003). This would be the reason for an increase in K and Mg under elevated CO₂ concentration which increases uptake rate of nutrients from the soil system (Sallas et al. 2003). Similar findings were reported in *Picea abies* (Sallas et al. 2003) and *Oryza sativa* (Seneweera 2011) exposed to high CO₂ concentrations. The study depicted a decrease in P content in plant tissues under elevated CO₂ concentrations. The reduction in P may be owing to the high carbohydrate production, which further contributes to the mechanism of the dilution effect (Dong et al. 2018). Giri et al. (2016) reported phosphorus reduction in *Lactuca sativa* and *Spinacia oleracea* under elevated CO₂ concentration. The response of nutrients towards elevated CO₂ concentration has always been a debatable issue stressing further investigations. Dong et al. (2018) reported increasing, decreasing, and no effect of elevated CO₂ on nutrient concentration in plant species. Reduced nitrogen content in plant tissues under elevated CO₂ concentration has been reported due to dilution effects of carbohydrate production (Cotrufo et al. 1998; Taub and Wang 2008; Loladze 2002) and the inhibition of nitrate assimilation under elevated CO₂ concentration (Bloom et al. 2010). The influence of elevated CO₂ on the P accumulation in plants has always been more changeable than nitrogen with the confirmation for declined (Teng et al. 2006), increased (Liu et al. 2012), and no effects on plant P (Johnson et al. 2004). The meta-analysis reported a decreasing trend of P in plant tissues in response to elevated CO₂ concentration, which depends on plant functional groups and other climatic circumstances (Huang et al. 2015).

**Alteration in organic carbon under elevated CO₂ concentration**

In the present study, elevated CO₂ had altered carbon allocation to the plant’s tissues such as leaf, stem, and root. The plants grown at 600±12 μmol CO₂ mol⁻¹ allocated more carbon towards stem followed by root and leaf whereas more allocation to leaf followed by the stem and root tissues at 800±16 μmol CO₂ mol⁻¹. This trend explained that 800 ±16 μmol CO₂ mol⁻¹ slows the partitioning mechanism of carbon. The transfer of carbon to root tissues may require extended duration, besides other causes. This may also need a long time for the root kinetics mechanism for sequestering nutrients (Jeong et al. 2018). However, the exact mechanism needs to be studied to illustrate the mechanism of carbon allocation concerning time. Increase in carbon allocation to leaves, stem, and root tissues under elevated CO₂ condition was reported by Jeong et al. (2018) in *Hibiscus hambo*, *Paliurus ramosissimus*, *Cicuta virosa*, *Bupleurum latissimum*, *Viola raddeana*, *Iris dichotoma*, and *Morus species* (Lavanya et al. 2017).

**Relationship between biochemical constituents and plants nutrients**

In the present study, we reported a positive correlation between K and Mg, and these nutrients are critically required for physiological functioning. Suppose nutrients’ (K and Mg) availability is insufficient in photosynthetic tissues/organs. In that case, the complex interaction between anatomical, physiological, and biochemical behaviour/responses is altered, resulting in a decline in carbon assimilation (Tränkner et al. 2018). There was a positive correlation existed between Mg and chlorophyll in the present study. Mg is the heart of the chlorophyll molecule, and leaf coloration depends upon Mg availability in leaf tissues. Hence, Mg is directly related to pigmentations (Wilkinson et al. 1990). Most of the studied parameters demonstrated a negative correlation with ascorbic acid content in leaf tissues (Fig 5). Watson and Noggle (1947) reported a relationship between K and Mg in the leaves with ascorbic acid biosynthesis. Singh et al. (2021) reported a negative correlation between leaf tissue ascorbic acid and K and Mg content. Protein content in leaf tissues and K has shown a positive correlation; this relationship might be owing to the high-affinity potassium transport to protein vis-à-vis (Pyo et al. 2010).

**Conclusion**

It is concluded that rising CO₂ concentration significantly induced alteration in biochemical constituents and nutrients allocation in plant tissues such as leaf, stem, and root of *Asparagus racemosus*. The biochemical constituents such as chlorophyll, protein, total sugars, and carbon accumulation increased although ascorbic acid diminished significantly. The nutrients viz. potassium and magnesium improved while phosphorus suppressed significantly against elevated CO₂ concentration. Further, protein, sugars, carbon, and nutrients allocation in plant tissues were altered profoundly under elevated CO₂ concentrations. In a nutshell, the *Asparagus racemosus* will adapt to future climate change, particularly in rising atmospheric CO₂ concentration by modulating biochemical mechanisms and nutrients partitioning. Further studies are required to explore the actual mechanism of accumulation of biochemical ingredients and nutrients allocation in plant tissues in future climate change. Besides, investigations
are required about the impacts of climate change and its variability on the accumulation of bioactive ingredients/health-promoting substances and nutrient profiling of medicinal plant species for the community’s sustainable food and health security.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11356-021-16050-3.

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Availability of data and materials The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Author contribution Rupali Sharma: methodology; data curation and observations; statistical analysis; drafting; Hukum Singh: conceptualization, methodology; data curation; writing and editing, supervision, the original draft; writing and reviewing.

Declarations

Ethics approval and consent to participate The study does not involve any ethical dimension, hence not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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