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Alteration in Biochemical Constituents and Nutrients Partitioning of Asparagus Racemosus in Response to Elevated Atmospheric CO2 Concentration

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Research Article

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Alteration in biochemical constituents and nutrients partitioning of *Asparagus racemosus* in response to elevated atmospheric CO_2 concentration

Abstract

Human-induced CO_2 emissions since the preindustrial era have accumulated CO_2 in the atmosphere which has influenced the plant structure and function including bio-chemical constituents of the plant system. The Himalayan vegetation has been predicted to be more vulnerable and sensitive to climate change. However, it is still not well documented that how atmospheric CO_2 concentration will change the biochemical constituents considering nutrients status of Himalayan endangered plants in future climate change. Hence, we examined the impacts of elevated CO_2 concentrations (ambient- ~ 400, 600, and 800 μmol CO_2 mol^{-1}) on biochemical constituents (chlorophyll, carotenoids, ascorbic acid, protein, and total sugars and carbon partitioning) and nutrients response (potassium, phosphorus, and magnesium) in leaf, stem and root tissue of *Asparagus racemosus* Willd. (an endangered medicinal plant species of Himalayas). The results showed that the elevated CO_2 concentration significantly ($p \leq 0.05$) enhanced the chlorophyll, protein, total sugars, and carbon accumulation conversely diminished ascorbic acid in leaf tissues. The nutrients accumulation especially potassium and magnesium were significantly ($p \leq 0.05$) improved while phosphorus accumulation suppressed under elevated CO_2 concentration. Moreover, elevated CO_2 notably altered protein, sugars, carbon, and nutrients partitioning in plant tissues *viz.* leaf, stem, and root of *A. racemosus*. The fate of rising atmospheric CO_2 concentrations beyond 800 μmol CO_2 mol^{-1} will require much more study. Further studies are needed to understand the impacts of elevated CO_2 concentration as well as a combination with other associated climatic variables on biochemical response particularly bioactive ingredients/health-promoting substances and nutrient profiling of this and other endangered medicinal plant species for improving livelihood support of the society.

Keywords: *Asparagus racemosus,* biochemical response, nutrients, and carbon partitioning, elevated CO_2 concentration

Introduction

Climate change, a global phenomenon has got attention amongst scientific as well as political communities worldwide. It has already impacted the structure and function of the Earth's

terrestrial system resulting in biodiversity change across the globe. Indian Himalayan Region (IHR), a biodiversity hotspot and repository of medicinal plants (Gangwar et al., 2010), is the most vulnerable and sensitive ecosystem to climate change (IPCC, 2007; Rana et al., 2015). Rising atmospheric CO_2 concentration is an important climatic variable coupled to global climate change (IPCC, 2001). Atmospheric CO_2 concentrations have been projected to reach ~475-1313 μmol CO_2 mol^{-1} by end of the 2100 (IPCC, 2013). The fertilization effect of risen atmospheric CO_2 concentration on physiology, growth, and morphology ensuing productivity of the diverse crops species has been advocated by various researchers globally due to increased carbon assimilation (Cha et al., 2017; Singh et al., 2018; Sharma et al., 2018; Yadav et al., 2019; Ahammed et al., 2020). However, literature pointed out that the response of medicinal plants towards projected climatic variables particularly elevated CO_2 concentrations has not been explored much more for the understanding response – feedback mechanism under climate change scenario (Applequist et al., 2020). Although, few experiments on medicinal plants suggested a profound impact on growth, physiology, productivity, and chemical substances including health-promoting substances or primary and secondary metabolites within elevated CO_2 concentration (Zobayed and Saxena, 2004; Ziska et al., 2008; Moghaddam et al., 2011; Ghasemzadeh and Jaafar 2011a; Ghasemzadeh and Jaafar 2011b; Jaafar et al., 2012; Saldanha et al., 2014; Al Jaouni et al., 2018; Kaundal et al., 2018). Moreover, small accounts of the investigation focused on change in biochemical constituents and nutrients in leaf tissues of medicinal plants (Myers et al., 2014; Fernando et al., 2015; Broberg et al., 2017; Jayawardena et al., 2017; Kumar et al., 2020), conversably partitioning or allocation of biochemical compounds and nutrients to the plant's parts such as leaf, stem and root tissues in response to elevated CO_2 concentration has neglected (Sutter et al., 2002; Aranjuelo et al., 2013; Aljazairi et al., 2014; Butterly, 2015; Thompson et al., 2017; Wang et al., 2019). Notably increased productivity of the foxglove was accounted for the plants grown under elevated CO_2 concentration (Stuhlfauth et al., (1987; Stuhlfauth and Fock, 1990). After a decade, the plants of spider lily grown at 400 and 700 ppm produced 40% and 56% more above and belowground biomass, respectively (Idso et al., 2000). Later, an experiment performed on the influence of CO_2 concentration on growth and productivity of *Hypericum perforatum,* a medicinal plant species to Europe and West Asia was witnessed to a significant increment in biomass production (Zobayed and Saxena, 2004; Mosaleeyanon et al., 2005). Further studies conducted with few medicinal plant species viz.

Nicotiana species and *Datura stramonium* (Ziska et al., 2005), *Panax ginseng* (Ali et al., 2005), *Hizikia fusiforme* (Zou, 2005), *Papaver setigerum* (Ziska et al., 2008), *Thymus vulgaris*(Vurro et al., 2009), *Podophyllum hexandrum* (Chaturvedi et al., 2009), *Vernonia herbacea* (Oliveira et al., 2010), *Zingiber officinale* (Ghasemzadeh and Jaafar, 2011a), *Centella asiatica* (Moghaddam et al., 2011), *Elaeis guineensis* (Ibrahim and Jaafar, 2012), *Labisia pumila* (Ibrahim et al., 2014), *Catharanthus roseus* (Saravanan and Karthi, 2014; Thinh et al., 2017), *Withania sominifera* (Sharma et al., 2018) have revealed a significant improvement in productivity and secondary metabolites of the plant system. In these studies, partitioning of biochemical ingredients and nutrients in plant tissues in response to elevated concentration of atmospheric CO_2 has not studied. Although, few above studied medicinal plant species belong to the Himalayan region.

Hence, in a wake of predicted climate change especially rising elevated atmospheric CO_2 medicinal plants of the Himalayan region requires much more exploration for food and health security and livelihood support of the community. The effect of climate change on the growth and production of secondary chemicals, biochemical constituents, and nutrients medicinal plants of IHR is still unclear and needs assessment (Gairola et al., 2010).

The effect of elevated CO_2 on nutrients contents of plants is contradictory with increased, decreased or no effects. Nitrogen content in plant tissues has demonstrated decreased trend under elevated CO_2 concentration (Cotrufo et al., 1998; Taub and Wang 2008) is the result of the carbohydrate dilution (Loladze, 2002) and the inhibition of nitrate assimilation in plant systems (Bloom et al., 2010). The response of nutrients accumulation in plants under elevated CO_2 conditions has always been a debatable equation. The influence of elevated CO_2 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) as well as no effects on plant phosphorus (Johnson et al., 2004). Based on the meta-analysis, Duval et al (2012) reported that the response of phosphorus in plants system under elevated CO_2 concentration varied which depends on plant functional groups and other climatic as well as soil conditions, besides elevated CO_2 concentration (Huang et al., 2015).

Elevated CO_2 generally induced accumulation of biochemical constituents such as chlorophyll, protein, total sugars, and carbon in plant tissues along with little evidence for decreased or neutral or no effects on these bio-chemicals. Dong et al (2018) in his meta-analysis showed

increasing and decreasing content of chlorophyll, ascorbic acid, total sugars, etc in various plant tissues. It has been investigated that elevated CO_2 can diminish the photorespiration process of the plant system. Reduced photorespiration might subsequently diminish the formation of oxygen radicals, hence dipping 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.

Asparagus racemosus Willd. (Shatavari), an important medicinal plant species are used for curing various diseases since the ancient era. Due to higher medicinal value, it has occupied an important place in various parts of literature mainly Ayurvedic, Unani, and African traditional medicines. Because of importance in the literature, *A. racemosus* is leading a huge demand amongst local, national, and international markets (Mirjalili et al., 2009; Prajapati et al., 2003). *A. racemosus* has got endangered status by IUCN in India due to overexploitation, deforestation, and degradation in addition to climate change. It is one of the important medicinal plants of IHRwhich has been prioritized top eight medicinal plant species by the National Medicinal Plant Board, Government of India.

The alteration in biochemical compounds/constituents and nutrients partitioning in plant tissues of *A. racemosus* has not been investigated yet to understand how *A. racemosus* respond to climate change mainly elevated CO_2 concentrations. To address this gap, the present study was to investigate the variation in biochemical constituents and nutrients partitioning in leaf, stem, and root parts of *Asparagus racemosus* in response to elevated atmospheric CO_2 concentrations. We hypothesized what the climate change could make possible changes in the plants wealth in relation to primary metabolites, biochemical constituents and nutrients partitioning.

Materials and Methods

Brief of open-top chamber (OTC) facility

We experimented within identical open-top chambers (OTCs) constructed at Forest Research Institute, Dehradun, Uttarakhand (32°20′44.2172″N, 78°0′41.6185″E and 668 m.a.s.l.). High-quality multilayer polycarbonate sheet with 80-85% transmittance was used to construct open-top chambers with a dimension of 3w×4l×4l (Singh et al., 2018; Sharma et al., 2018). Pure (100%) CO_2 gas of commercial-grade was supplied from the CO_2 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. The CO_2 was supplied from 09:00 am to 05:00 pm during the study period by considering enough sunlight is available during between this duration.

Seedlings preparation of *Asparagus racemosus*

The five-month-old seedlings of *A. racemosus* gained obtained from Non-Wood Forest Product Division, Forest Research Institute, Dehradun. The healthy seedlings were transplanted in earthen pots with a proper growing medium of soil: sand: manure (2:2:1). The potted seedlings were left within and outside open-top chambers (OTCs) for one week to acclimatize the conditions. The experiment was laid out in Completely Randomized Design with four CO_2 treatments viz. 600, 800, 1000, 1200 µmol mol^{-1} CO_2 in addition to control. Five replications were considered with eight seedlings per replication (N=40).

CO_2 treatments

After a week, a set of eight potted seedlings were placed within OTCs with replication to grow under elevated CO_2 concentration (600±12 and 800±16 µmol mol^{-1}) in addition to ambient OTCs conditions without CO_2 treatments. The proper watering and weeding of pots were done during the study period of the six months.

Measurement of biochemical constituents and biomass estimation of plant tissues

The biochemical constituents were analyzed from leaf and root tissues at reproductive as well as the maturity phase. The healthy leaves and root tissues were preferred for analyzing biochemical parameters from the upper, middle, and lower parts of the plants. Leaf (LPROT) and root protein (RPROT) estimation was done by the method of Bradford (1976). Fresh tissues were taken from each plant and kept in icebox to prevent denaturation of protein while bringing to the laboratory. Leaf sample (100 mg) was homogenized with 1ml phosphate buffer over an ice tray. The homogenate was then centrifuged at 13000 rpm at 4°C for 20 minutes. Then, phosphate buffer (5ml) was added to the supernatant. After that, 0.5ml of this mixture was taken and made to 1ml with phosphate buffer. Bradford dye (3 ml) was added to the above solution and it was kept at room temperature for 25 minutes for the completion of the reaction. Optical density was measured at 595 nm against a blank containing 3ml Bradford reagent and 1ml phosphate buffer. Different concentrations of bovine serum albumin (BSA) were taken for obtaining the standard

curve. Protein concentration in the sample was then estimated using a linear equation of the BSA standard curve.

Total sugars from leaf (TLS) and root (RLS) tissues were determined as per the method of Dubios et al. (1956). Separate solutions of 5% phenol and 80% ethanol were prepared using distilled water. Separate samples (100 mg) of fresh and fully expanded leaves and healthy root tissues from each plant were chopped into small pieces and placed in a graduated test tube prefilled with 5ml of 80% ethanol and subsequently incubated for one hour at 80^{o}C. Afterward, 0.5 ml of this sample extract was taken in another test tube and further added 0.5 ml distilled water (DW). After that, 5% phenol (1 ml) was added to the above solution and incubated for an hour at room temperature. After an hour, 2.5 ml of concentrated H_2SO_4 was added by keeping the samples on ice-tray and the reaction mixture was shaken well in an orbital shaker for a few seconds. The optical density was read at 490 nm against a blank containing 0.5ml ethanol, 0.5ml DW, 1ml 5% phenol, and 2.5 ml concentrated H_2SO_4. The standard curve was obtained by using different concentrations of Dextrose. The amount of total sugar in the sample was determined from the linear equation of the standard curve. The value obtained was multiplied by the dilution factor to obtain the total sugar concentration in terms of µg/mg fresh weight of the sample.

Ascorbic acid (ASC), another indicator of stress in plants was estimated using the method developed by Harris and Ray's (1953). Fresh and fully expanded flag leaves from each plant were homogenized with 2 ml of 4% TCA and then centrifuged at 20,000 rpm for 10 minutes. In each centrifuged tube, a pinch of activated charcoal was added and again centrifuged at 12,000 rpm for 10 minutes to convert ascorbate to dehydroascorbate. A volume of supernatant (0.5ml0 was taken into a test tube and then 1.5ml of 4% TCA was added immediately, followed by the addition of 0.5ml 2% DNPH and 2 drops of 10% thiourea. The mixture was kept at 37^{o}C for three hours for completion of the reaction. The reaction was terminated by placing the test tubes on an ice tray and then added 2.5ml of H_2SO_4 and left it at room temperature for 30 minutes, and the mixture produced orange colour. The optical density of orange colour mixture was read at 540 nm against a blank (0.5ml DNPH, 2 drops thiourea, and 2.5 ml concentrated H_2SO_4). The Ascorbic acid content in the leaf tissue was determined by the standard curve equation obtained using different concentrations of pure ascorbic acid. The values obtained were multiplied by the dilution factor to get ascorbic acid content in leaf tissues and expressed as µg/100mg fresh weight.

196 Leaf chlorophyll content was estimated from fresh and healthy leaves as per the method
197 described by Hiscox and Israelstam (1979). Fresh leaf tissues (50 mg) were chopped into the tiny
198 pieces and then transferred into the test tubes containing 8 ml of Dimethyl Sulfoxide (DMSO).
199 Further, the tubes were incubated at 65 ^{o}C for three hours in the oven. The sample was afterward
200 filtered into a graduated test tube and the volume was made to 10 ml using DMSO. The
201 absorbance of the samples was read at 663 and 645 nm against pure DMSO as a blank using
202 spectrophotometer (Systronics Visiscan 167). Total leaf chlorophyll content (mg g^{-1} as fresh
203 weight (F.W.)) was calculated using the following equation;

204 $$Chlorophyll\ \ a, Chl\ a\ (mg\ g^{-1}\ as\ F.W.) = \frac{12.7\ A_{663} - 2.69\ A_{645}}{w \times 1000}$$

205 $$Chlorophyll\ \ b, Chl\ b\ (mg\ g^{-1}\ as\ F.W.) = \frac{22.9\ A_{663} - 4.68\ A_{645}}{w \times 1000}$$

206 $$Total\ leaf\ Chlorophyll, TChl\ (mg\ g^{-1}\ as\ F.W.) = \frac{[(20.2 \times A_{645}) + (8.02 \times A_{663}) \times V]}{a \times 1000 \times w}$$

207 Where; A_{645} and A_{663} - O. D. values measured at 645 nm and 663 nm, respectively. V- Final
208 volume of extract, a - path length of the cells (1cm), and w - the weight of the leaf tissues taken.
209 For estimating total leaf carotenoids content, the absorbance of samples was taken at 470 nm
210 using a spectrophotometer. The below equation was adopted for calculating total carotenoids
211 (Car) in leaf tissues (µg g^{-1} as fresh weight (F.W.));

212 $$Carotenoids\ content, CAR\ (\mu g\ g^{-1}\ as\ F.W.) = \frac{1000\ A_{470}\ - 1.9\ (Chl_a - Chl_b)}{214}$$

213 Biomass allocation in plant parts was calculated by carefully uprooting the plants and
214 segregating into different parts leaves, stems, and root. The roots were cleaned with distilled
215 water to remove particles sticking to root hairs. The fresh plant parts were weighed and
216 subsequently oven dried at 65 °C until a constant weight reached and weighed again (Wu et al.,
217 2013; Singh et al., 2018).

218 **Estimation of nutrients accumulation**

219 Estimation of phosphorus (P), potassium (K), and magnesium (Mg) in plant tissues especially
220 leaf, stem, and root tissues was done after harvesting of the plants. The considered nutrients were
221 estimated as per the procedure of Holman (1943), Morwin and Peach (1951), Young, and Gill
222 (1951), respectively. The tri-acid solution, nitric acid (HNO_3), perchloric acid ($HClO_4$) and

sulphuric acid (H_2SO_4) in the ratio10:4:1 was used for preparing a stock solution. This stock solution was further used for estimating selected nutrients in plant tissues.

Phosphorus content was estimated as per the method described by Holman (1943). The reagents i.e. molybdate solution, hydrazine sulfate solution, and sodium hydroxide solution were used for further process of phosphorus estimation. Molybdate solution was prepared by adding 12.5gm of ammonium molybdate in 150 ml of DW. H_2SO_4 (140ml) was mixed in 150ml of DW and added to the above solution. Hydrazine sulfate (HS) solution was prepared by adding 0.15gm of HS to 100 ml of DW. NaOH (45gm) was mixed in 100ml DW to make NaOH solution. Then, 2ml of stock solution was mixed with 10ml DW in a 100ml flask. After that, 1 to 2 drops of phenolphthalein were added and the mixture was titrated against NaOH solution till the reaction is completed and until the appearance of pink color. Now, 10ml of ammonium molybdate solution was added followed by a 2ml HS solution. The volume of the mixture was made to 100ml by adding DW. The flasks were incubated in a boiling water bath for 15 minutes to complete the reaction. The optical density of this solution was measured at 830nm.

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

Potassium content in plant tissues was determined using the method described by Vogel, (1961). For potassium content, 2 ml of stock solution was mixed in 100ml of DW in a 100ml conical flask. The flame photometer (Systronics flame photometer 128) was calibrated with DW at 100ppm and 40ppm 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, 2ml of the stock solution was added in a 50 ml flask**.** Then, 10 ml of distilled water was added to it followed by 2ml of the compensatory solution, 2ml of 2% polyvinyl alcohol (PVA), 1 ml of hydroxylamine hydrochloride, 1 ml titan yellow solution and 45% sodium hydroxide**.** The net volume was made 50 ml with distilled water (light orange colour appeared). The absorbance of the sample with blank was measured at 540 nm using the spectrophotometer (Systronics Visiscan 167). Organic carbon (OC) was estimated after harvesting of *Asparagus racemosus* from leaf (LOC), stem (SOC), and root (ROC) as per the standard procedure described by Walkley and Black (1934).

Statistical analysis

Descriptive analysis (mean, median, standard deviation and standard error of mean) were performed in Microsoft excel. Statistical software SPSS 16.0 was used with a multivariate general linear model to observe the existence of significant mean difference in response of biochemical parameters and nutrients accumulation at set levels CO_2 concentrations. Further post hoc Tukey test was performed to identify the homogeneous subsets. Coefficient of determination (R^2) was provided in supplementary table for all the studied parameters (Table 1). CO_2 was considered as an independent variable and all studied parameters such as protein, total sugar, magnesium and so on were considered as dependent variables. Correlation analysis and Principal Component Analysis (PCA) was done through R studio statistical software. In the text, the term significant is used to indicate p-value ≤ 0.05.

Results

Effect of elevated CO_2 on the biochemical response of plants

Elevated CO_2 significantly promoted leaf chlorophyll, total sugar, and protein accumulation whereas impeded the ascorbic acid and carotenoid content in response to elevated CO_2 concentration (Fig. 1and 2).The study 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 grown at 600 and 800 µmol CO_2 mol^{-1}, respectively when compared to ambient grown plants (Fig. 1). Another studied important pigment i.e. carotenoid concentration significantly diminished along with the elevated CO_2 concentration, which a reduction ~2.97 and 8.04% under elevated concentrations (600 and 800 µmol CO_2 mol^{-1}) compared to counterparts (Fig. 1). Besides, elevated CO_2 concentration had also enhanced leaf total sugar significantly by ~35.21% and ~46.67 % of plants exposed to 600 and 800 µmol CO_2 mol^{-1}), respectively (Fig. 2). Similarly, total sugars in root tissues enhanced by 105.49% (600 µmol CO_2 mol^{-1}) and 320.44% (800 µmol CO_2 mol^{-1}) than ambient (Fig. 2). It was very interesting to account that protein accumulation in leaf and root significantly improved under elevated CO_2 concentration (Fig. 2). Leaf protein increased by 36.21% and 85.53% at 600 µmol CO_2 $mol^{-1}$and 85.53 at 800 µmol CO_2 mol^{-1}, respectively. The root protein content boosted by 55.08% and 91.68% at 600 and 800 µmol CO_2 mol^{-1} grown plants (Fig. 2). Elevated CO_2suppressed ascorbic acid significantly in leaf tissues by 3.57 and 29.32% at 600 and 800 µmol CO_2 mol^{-1}, respectively compared to counterparts i.e. ambient (Fig. 2).

Nutrients accumulation in leaf, stem and root tissue of plants under elevated CO_2 concentration

Nutrients viz. phosphorus (P), potassium (K), and magnesium (Mg) showed different responses towards increased CO_2 concentrations. K and Mg, macro, and micronutrient respectively significantly increased while P (macro) decreased in response to elevated CO_2 conditions than ambient grown plants (Fig. 3). The allocation of K to root, stem, and leaf tissues were profoundly altered by elevated CO_2 with more allocation towards stem followed by leaf and root tissues. K exhibited variation in different plant tissues more allocation in all the tissues. Leaf K was significantly increased by 26.02% and 34.15% at 600 and 800 µmol $CO_2 mol^{-1}$, respectively compared to ambient plants (Fig. 3). Simultaneously, K content in stem tissues was significantly enhanced by 8.16 and 14.29% for plants grown under CO_2 concentration of 600 and 800 µmol CO_2 mol^{-1}, correspondingly rather than and root K enhanced by 18.41 and 34.58% at 600 and 800 µmol CO_2 mol^{-1}, respectively (Fig. 3). Mg, an important micronutrient of photosynthesis process accumulated more in leaves by 64.96, and 69.03% of plants grown at 600 and 800 µmol CO_2 mol^{-1}, respectively compared to ambient (Fig. 3). Likewise, magnesium (Mg) in the stem was demonstrated better improvement of 39.89 and 182.03% at elevated CO_2 of 600 and 800 µmol CO_2 mol^{-1}, respectively, whilst compared to ambient (Fig. 3). Furthermore, Mg content in root tissues was higher than leaves and stem, with an increment of 60.62 and 155.04% at 600 and 800 µmol CO_2 mol^{-1} and 155.04 rather than ambient (Fig. 3). Also, the magnesium was allocated to root followed by stem and leaf, however, phosphorus appeared to be the opposite with the above trend where the higher proportion was partitioned in leaf followed by root and stem tissues (Fig. 3). Leaf P was found to be decreased significantly by 29.79% at 600 µmol CO_2 mol^{-1} and 39.89 % at 800 µmol CO_2 mol^{-1}, likewise stem P reduced by 23.50% and 34.97% at 600 and 800 µmol CO_2 mol^{-1}, respectively compared to ambient CO_2 conditions (Fig. 3). A similar trend has existed for root P with an increment of 43.69 and 55.31% at 600 and 800 µmol CO_2 mol^{-1}, respectively (Fig. 3).

The response of organic carbon and biomass content in leaf, stem, and tissues under elevated CO_2 concentration

Carbon is a vital element for fostering plant growth and development. Under the present study, elevated CO_2 had significantly impacted carbon content and allocation in plant tissues such as

leaf, stem, and root. The organic carbon (OC) content was reported to be enhanced across the tissues (leaf, stem and root) under elevated CO_2 circumstances (Fig. 2). Elevated CO_2 induced allocations of more carbon to the leaf tissues followed by stem and root for the plants grown at 800 μmol CO_2 mol^{-1} in contrast, at 600 μmol CO_2 mol^{-1} more organic carbon allocation was documented in stem followed by root and leaf (Fig. 2). Leaf and stem organic carbon was reported significantly higher under elevated CO_2 concentration compared to ambient grown plants (Fig. 3). Similarly, a significant increment in root organic carbon was reported at elevated CO_2 concentration when compared to ambient grown plants (Fig. 3).

concentrations

There was a uniform accumulation of dry matter grown under elevated CO_2 concentration recorded for all plants. The magnitude of percent increase in dry biomass was 28.27 (%) and 50.48(%) at 600 and 800 μmol CO_2 mol^{-1}, respectively when compared to ambient. A significant enhancement was noted in the stem, leaf and root dry biomass when exposed to elevated CO_2 concentrations (Fig. 4).

The relationship amongst biochemical constituents and nutrients of plant tissues

More than half of the traits studies represented a significant correlation between biochemical parameters and nutrients (Fig. 5). 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). Interestingly, root organic carbon and root protein were significantly correlated (r = 0.92). Principal component analysis (PCA) demonstrated more contribution of two principal components viz. PC1 and PC2 which contributed approximately 70 and 7%, respectively through analyzing the magnitude and directions of the coefficients (Fig. 6).

Discussion

Effect of elevated CO_2 on the biochemical response of plants

During external stress the carbon fixation is not allocated to growth function despite it is directed to secondary metabolites production since growth is inhibited to photosynthesis (Mooney et al., 1991). Thus, elevated CO_2 modified the biochemical accumulation in plants to adjust with

present conditions. Under the present study, chlorophyll content was increased under elevated carbon dioxide up to 800 µmol CO_2 $mol^{-1}$when compared to ambient. Increased chlorophyll is associated with the 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 growth of the plant system. The increase in total chlorophyll content under elevated carbon dioxide was reported in previous studies conducted with *Medicago sativa* (Sgherri et al., 1998), x Mokara species (Gouk et al., 1999), *Gycine max*, *Pennisetum glaucum*, *Chenopodium album*, and *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, total sugar, etc contributed towards secondary metabolite production by providing building blocks and biosynthetic enzymes that were procured from primary metabolites. The study reported an increase in total sugar content which directly proportional to an increase in physiological parameters (Fig. 5). It has been reported that elevated CO_2 concentration induced to increase triose phosphate of leaves that further contribute to the formation of carbohydrates resulting in more sugar accumulation, as we reported in the present study. Various studies are witnessed to increase total sugar content in plants such as viz. *Labisia pumila* (Ibrahim and Jaafar, 2011), *Labisia pumila*, and *Catharanthus roseus* (Saravanan and Karthi, 2014). Protein helps to support plant structure which found to be increased under this study in response to elevated CO_2 concentration. In support, increased protein content under elevated CO_2 was reported by researchers in *Oryza sativa* and *Chaetoceros gracilis* (Liu et al., 2017; 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 linkage to nitrogen content (Hocking and Meyer, 1991). Ascorbic acid was reduced under exposure to elevated carbon dioxide and such suppression supported by Azam et al. (2017) on *Capsicum annuum* grown within elevated carbon dioxide concentration up to 1000 µmol CO_2 mol^{-1}**.** Contradictory, few studies reported increased ascorbic acid under higher CO_2 concentrations, yet 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). The meta-analysis performed by Dong et al. (2018) suggested an increment or decline in chlorophyll, ascorbic acid, total sugars, in plant tissues. Elevated CO_2 generally declines the photorespiration mechanism of the plants 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.

Nutrients accumulation in leaf, stem and root tissue of plants under elevated CO_2 concentration

Essential nutrients, whose limitation hinders the process of growth and survival since they are paramount of 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 a heart of chlorophyll and it helps in plants capturing more sunlight for performing photosynthesis process. K and Mg content was increased in both species studied under elevated carbon dioxide concentrations which led to enhancement physiological processes like transpiration rate and stomatal conductance might be the reason for an increase in K and Mg under elevated CO_2 concentration which increases uptake rate of nutrients from the soil system. Such a trend was in *Picea abies* (Sallas et al., 2003) and *O. sativa* (Seneweera, 2011) for those plants exposed to high CO_2 levels. The study depicted a decrease in P content in plant tissues under elevated CO_2 concentrations. The reduction in P might be due to high carbohydrate in plant tissues which results in more biomass production and further contributes to the mechanism of dilution effect (Dong et al., 2018). Such a decrease in P content was reported in *Lactuca sativa* and *Spinacia oleracea* under elevated CO_2 concentration (Giri et al., 2016). The effect of increased CO_2 concentration on nutrients of plant tissues has been always considered as the debatable research question. Nutrients concentration was reported to be increasing, decreasing, and no effect in response to elevated CO_2 concentration in plant species. Nitrogen content in plant tissues has demonstrated decreased trend under elevated CO_2 concentration (Cotrufo et al., 1998; Taub and Wang, 2008) is the result of the carbohydrate dilution (Loladze, 2002) and the inhibition of nitrate assimilation in plant systems (Bloom et al., 2010). The influence of elevated CO_2 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) as well as no effects on plant P (Johnson et al., 2004). A study performed using meta-analysis found reported decreased P in plants in

response to elevated CO_2 concentration which depends on plant functional groups and other climatic circumstances (Duval et al., 2012), besides elevated CO_2 concentration (Huang et al., 2015).

The response of organic carbon and biomass accumulation in leaf, stem, and tissues under elevated CO_2 concentration

In the present study, elevated CO_2 had altered carbon allocation to the plant's tissues such as leaf, stem, and root under elevated CO_2 concentration. The plants grown at 600 μmol CO_2 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 μmol CO_2 mol^{-1}. This trend explained that 800 μmol CO_2 mol^{-1} slows the partitioning mechanism of carbon. The transfer of carbon to root tissues may require extended duration, besides other causes, it would be the long time required for the root kinetics mechanism of sequestering nutrients. However, the exact mechanism needs to be studied in the future to illustrate the mechanism of carbon allocation concerning time. Increase in carbon allocation to leaves, stem and root tissues under elevated CO_2 condition was supported by Jeong et al. (2018) who performed a study on the same aspect with *Hibiscus hambo*, *Paliurus ramosissimus, Cicuta virosa*, *Bupleurum latissimum*, *Viola raddeana*, *Iris dichotoma* and Lavanya et al. (2017) on *Morus* species. The increase and decrease in biomass content varies from species to species. During the process of photosynthesis the plants absorb carbon dioxide and results in increase the biomass content in *Asparagus racemosus*. Such biomass production and increase in leaf biomass stem biomass, root biomass and dry biomass under elevated levels of CO_2 concentrations were also reported (Thinh et al., 2017; Saravanan and Karthi, 2014).

Conclusion

It is concluded that rising CO_2 concentration is 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 CO_2 concentration. Further, protein, sugars, carbon, and nutrients allocation in plant tissues altered profoundly at elevated CO_2 concentrations. In a nutshell, the *Asparagus racemosus* will

adapt in future climate change particularly in rising atmospheric CO_2 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, investigation on the accumulation of bioactive ingredients/health-promoting substances and nutrient profiling of endangered medicinal plant species of the Himalayan ecosystem for sustainable food and health security.

Declarations

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

Consent for publication: Not applicable

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

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions: 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.

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Authors' information (optional)

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Figures

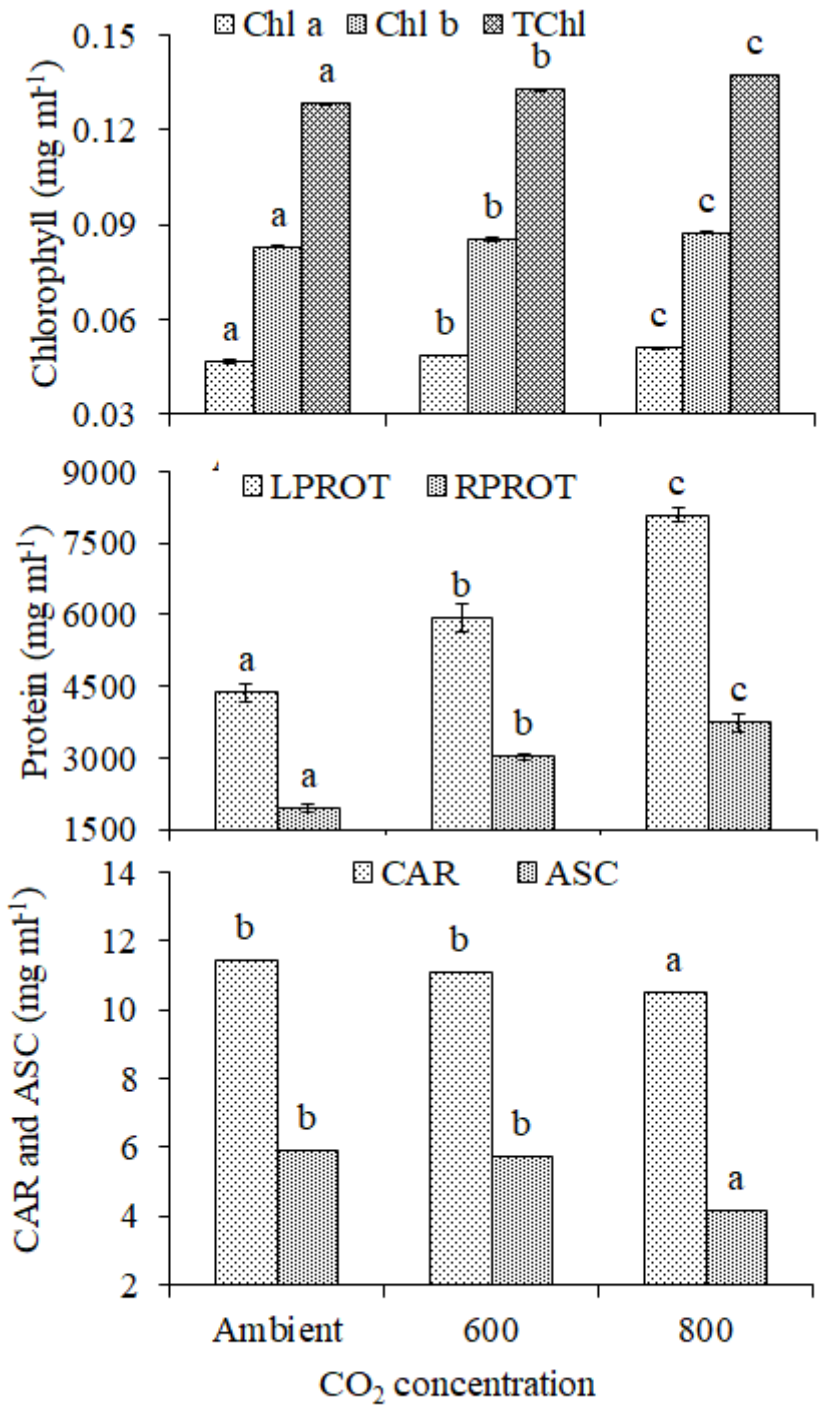

Figure 1

Effect of elevated CO2 concentration on chlorophyll (Chlorophyll a- Chla, Chlorophyll b- Chlb, and Total chlorophyll- TChl), leaf (LPROT) and stem protein (SPROT), carotenoids (CAR), and ascorbic acid (ASC) accumulation in plants of Asparagus racemosus.

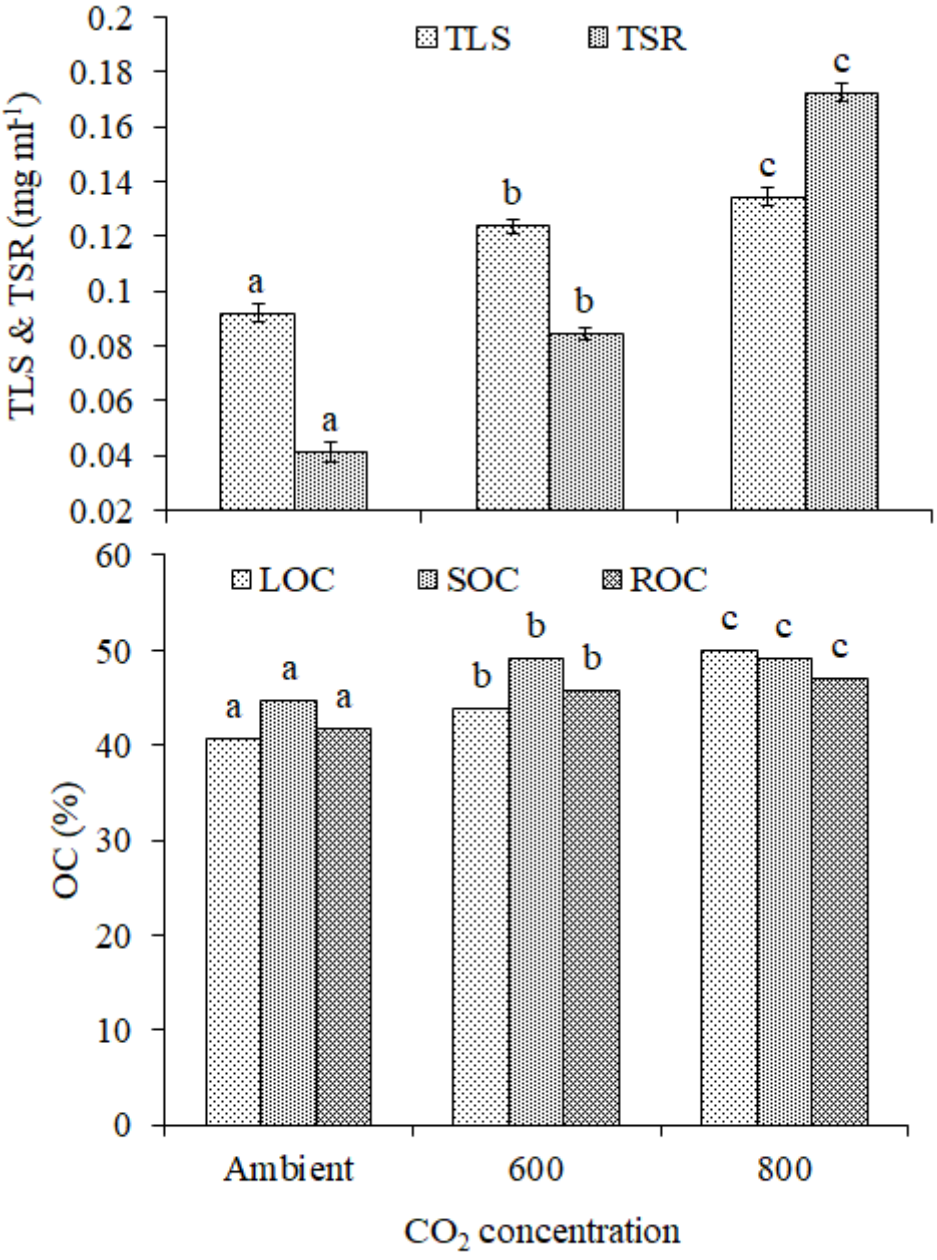

Figure 2

Effect of elevated CO2 concentration on total sugars in stem (TLS) and root (TSR) , and organic carbon content in leaf (LOC), stem (SOC), and root (ROC) tissues of Asparagus racemosus.

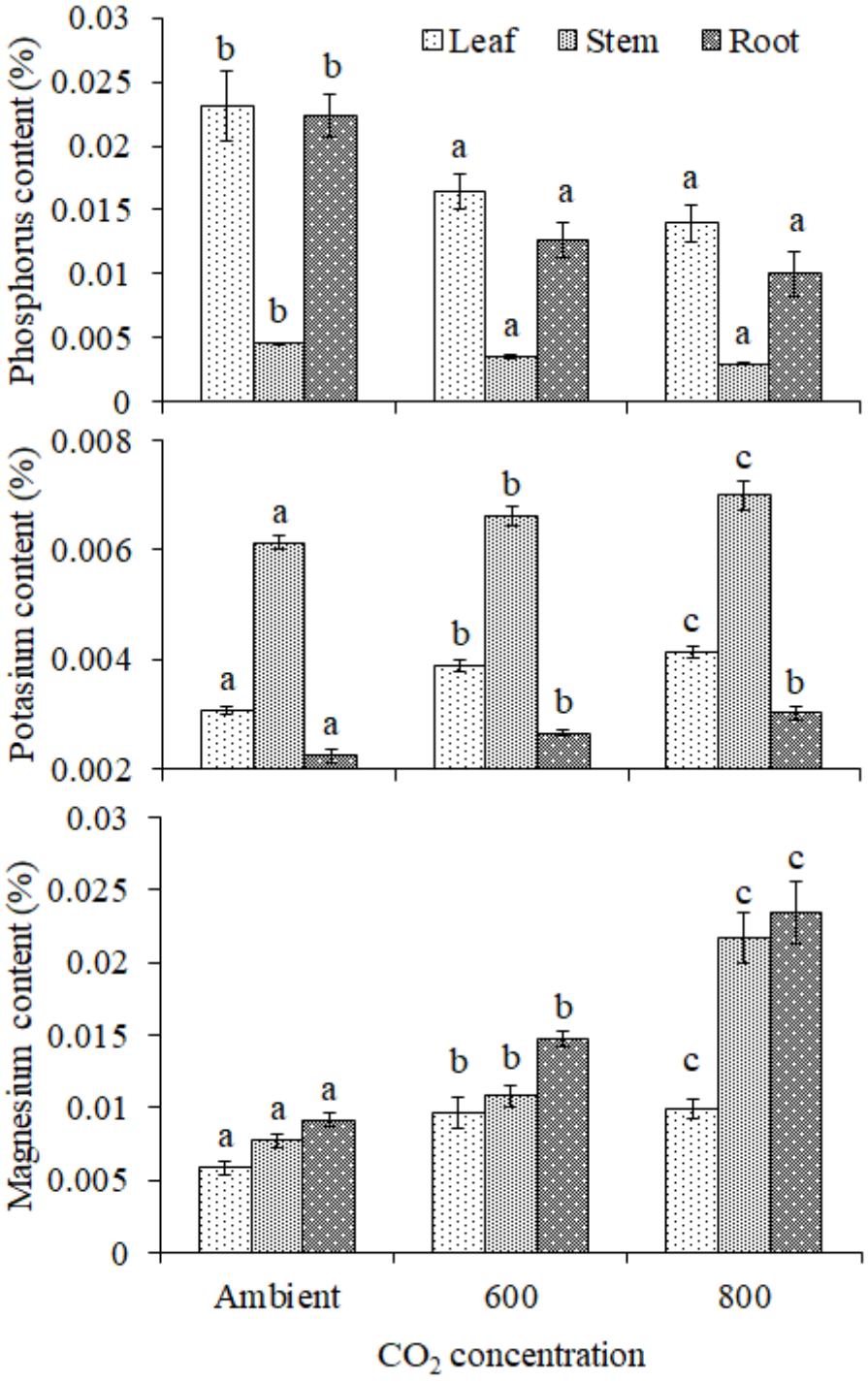

Figure 3

Effect of elevated CO2 concentration on nutrients partitioning n leaf, stem , and root tissues of Asparagus racemosus.

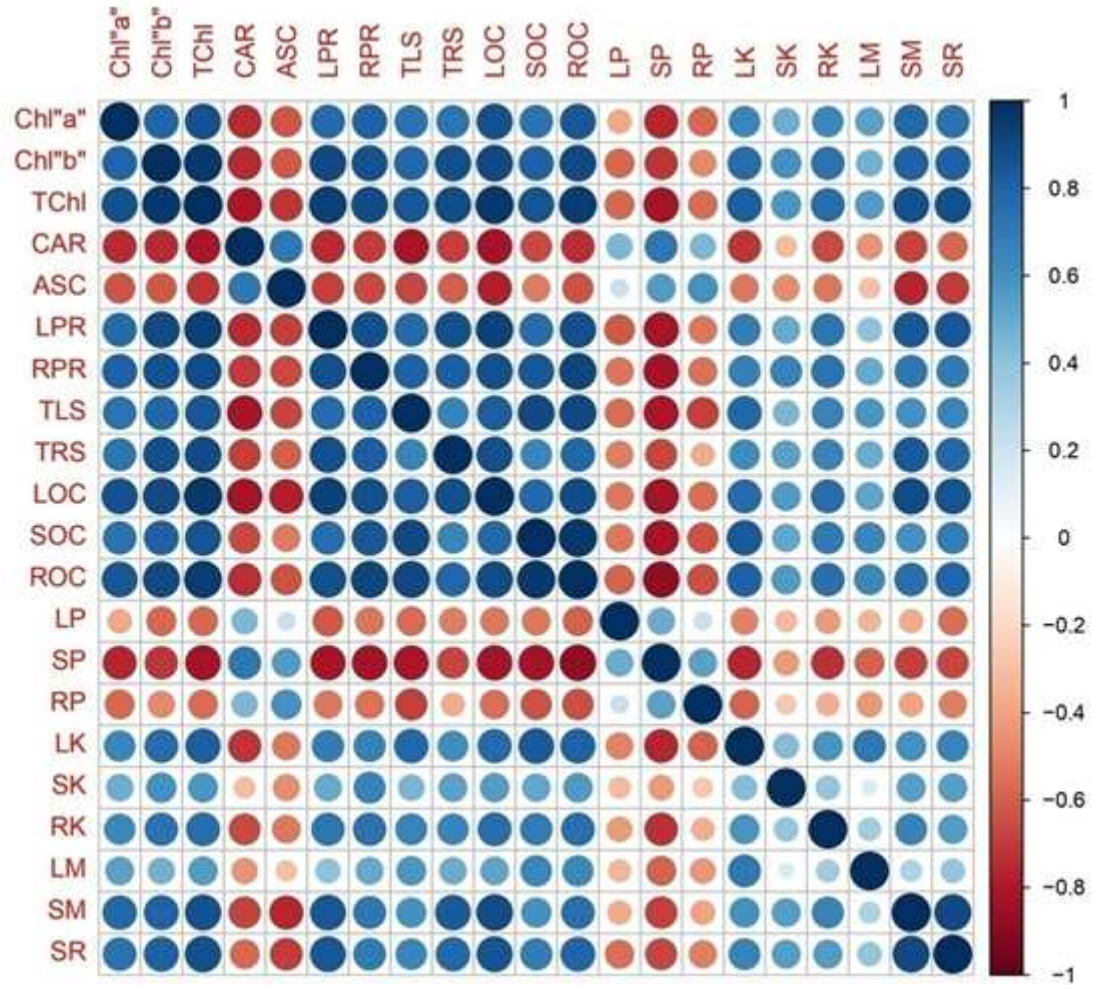

Figure 4

Correlation between biochemical and nutrients traits of Asparagus racemosus.

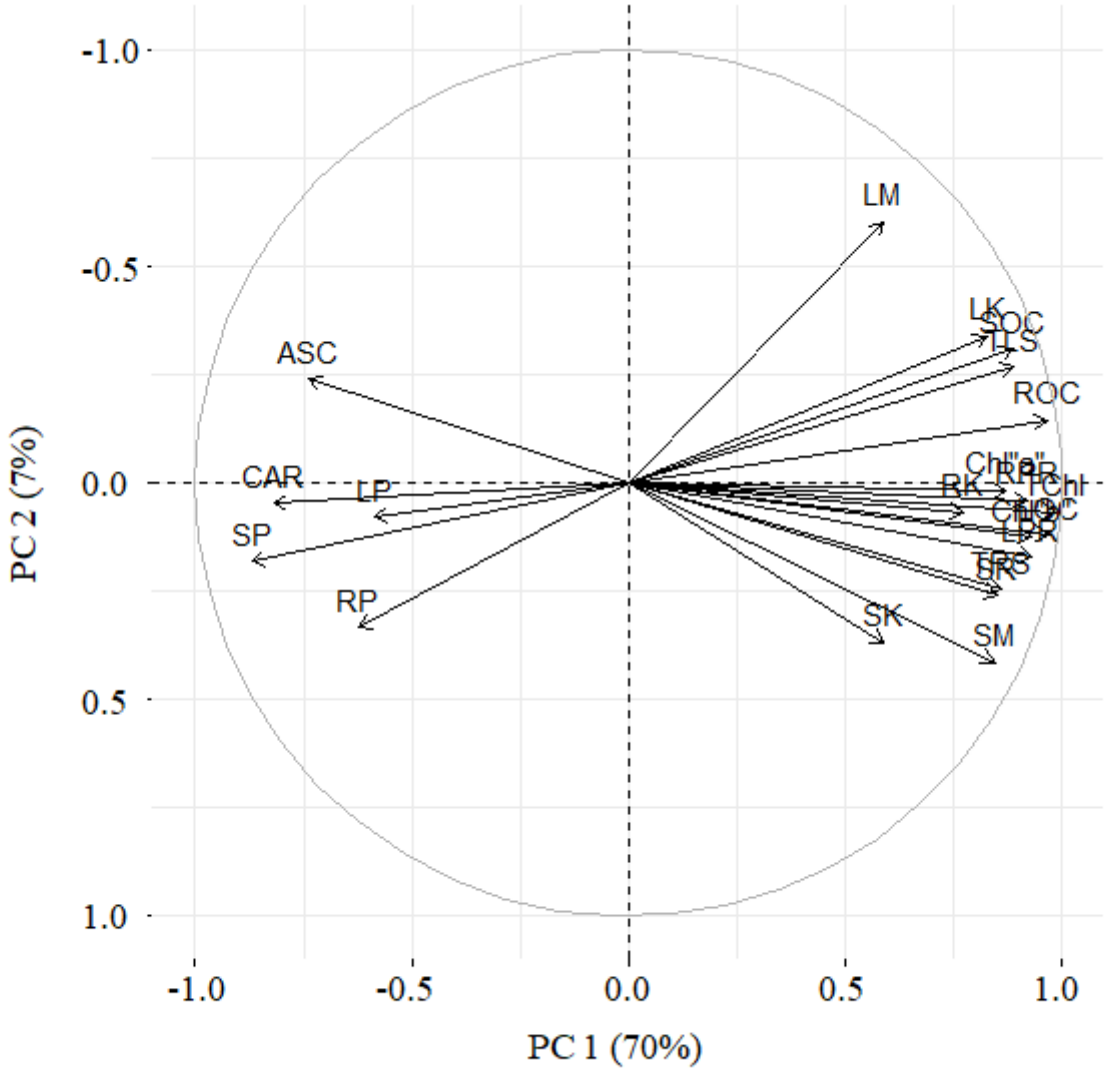

Figure 5

Principapal component analysis of biochemical and nutrients traits of Asparagus racemosus.

Supplementary Files

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- TableS1.docx