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Research article

# Seasonal variation in biochemical responses of bamboo clones in the sub-tropical climate of Indian Himalayan foothills

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ABSTRACT

Seasonal variations affect the rate of biomass accumulation in plants which is internally governed by biochemical metabolites. Studying the impact of atmospheric seasonal changes on biochemical parameters can improve our understanding of various plant species' physiological plasticity. Bamboos are a fast-growing group of woody grass species, widely distributed across tropical and sub-tropical regions of the world, and are an important species of the Indian subcontinent. Nevertheless, limited information is available on the seasonal response of biochemical's in bamboo species growing in ambient atmospheric circumstances. Therefore, we investigated the seasonal biochemical responses of *Dendrocalamus strictus* clones *viz.* Pantnagar (PNT) and Dhampur (DHM) to seasonal ambient atmospheric conditions. The concentrations of chlorophyll, protein, carbon, nitrogen, phosphorus, potassium, and magnesium in bamboo leaves were increased significantly ($p < 0.025$) in monsoon compared to summer and winter seasons. Carotenoid, total sugar and ascorbic acid contents were highest during winters and reduced significantly during monsoon. Proline content was highest in summer and reduced by 97% during monsoon, indicating effective adaptation to both clones' water-limited conditions. It was inferred that seasonal variation in atmospheric conditions significantly influenced the biochemical constituents of plants. This study provides a biochemical approach for screening potential bamboo species with adaptive nature for plantation purposes intended to mitigate climate change.

## 1. Introduction

In recent years, bamboo has emerged as one of the most promising alternatives to forest crops for achieving sustainable development goals. In India, bamboo is distributed naturally in the majority of its sub-climatic regions. Hence, it has found its way in numerous cultural and indigenous practices, ranging from culinary uses to art and craft to construction material (Sawarkar et al., 2020). Due to its widespread benefits and suitable climatic conditions in the country, bamboo is grown extensively in India, and the bamboo forest area increased by 3,229 sq. km in 2019 compared to 2017. Bamboo grows almost naturally across the country except in the Kashmir region (FSI, 2019). Fast growth rate resulting in adequate sequestration of atmospheric carbon dioxide for mitigating the climate change impacts is another merit of bamboo species has been accoladed with (Lou et al., 2010). It can survive in a wide variety of climatic and edaphic conditions (Tewari et al., 2015). Various factors decide the natural distribution of bamboo, particularly rainfall, temperature (8 °C–36 °C), altitude, and soil. A minimum of 100 cm annual rainfall and a high atmospheric humidity promotes luxuriant growth (Clark et al., 2015). The multiple uses of bamboo are dependent upon individual species' characteristics such as culms strength, flexibility, and size. Their contribution to an area's ecology derives from their ability to recycle nutrients efficiently (Rao and Ramakrishnan, 1989). Bamboo culms with high tensile strength can easily be used as a replacement for wood and plastics. Bamboos play an essential role in rural people's daily lives, mainly tribal in numerous ways. The old and mature culms are generally harvested after three years and are used for building houses and farming tools. The young tender culms emerging during the rainy season are widely used as an indigenous delicacy in the tribal communities' parts of the country. People are now finding ways into the urban population's culinary demands and many health benefits of culms. It has been reported that the edible parts of bamboo, i.e., shoot, have high vitamins, carbohydrates, minerals, and proteins that meet the nutritional requirements of poor people (Satya et al., 2010).

Bamboo leaves are an alternative source of food for cattle in the dry season (Scurlock et al., 2000). Recently, bamboo is being used to make

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clothes because it is much softer than cotton (Cheng et al., 2006; Prakash et al., 2012; Solorio-Ferrales et al., 2019; Othman, 2020). Bamboo fiber is relatively long (1.5–3.2 mm) and ideal for paper production (Bassam, 2013; Nayak and Mishra, 2016; Saxena and Gowri, 2003; Sugesty et al., 2015). About 40 % material of bamboo is used in the paper industry. Due to the multipurpose use of bamboo in India, benefits have been described extensively by Sawarkar et al. (2020).

*Dendrocalamus strictus* is the most widespread and commonly used bamboo species among all the natural and cultivated bamboo species in India. It comprises 53% of the country's total bamboo resources (Pathak, 1988; Tewari et al., 2015). Generally, it is found in tropical conditions, which survive well in a range of 750–1000 mm rainfall and is drought resistant. Besides, it can properly grow in a temperature range from -5° to 45° (Pathak, 1988).

Plant responses at the metabolic/biochemical level have been used as important criteria to understand the extent of plant and atmospheric interaction under various micro-climatic conditions (Singh et al., 2010, 2014). The metabolites, protein, sugars, and carbon, are directly related to plant growth and biomass accumulation (Singh et al., 2017; Singh et al., 2018). Also, biochemical traits like proline and ascorbic acid signify the plant's ability to cope with various stresses (Singh et al., 2020; Sharma et al., 2018). The nutrients, especially phosphorous, potassium, and nitrogen, are essential macro-nutrients that regulate different growth and development stages, leading to biomass production and yield (Uchimura 1985; Kinhal 1985; Huang et al., 1994).

The seasonal variations in biochemical parameters can help understand bamboo's adaptive capacity to changes in ambient atmospheric temperature, rainfall, humidity, vapor pressure, etc. Studying the correlation within the biochemical parameters can also determine their mutual role in the growth and impact of on nutrient's concentration. In the past, the biochemical study of *D. strictus* germplasms has been performed to understand the suitability of India's various agro-climatic regions (Malik et al., 2017). Nirmala et al. (2014) studied the biochemical accumulation in bamboo culms of the edible variety. Bamboo species used as food giant and have been studied for their seasonal biochemical accumulation (Wang et al., 2017; Jiang et al., 2018). Studies focused on the seasonal changes in the biochemical constituents of bamboo plants are severely lacking. Besides, studies on seasonal carbon sequestration and climate change mitigation potential of bamboos are limited. Therefore, the present study was designed to test the hypothesis that the accumulation of biochemical metabolites in leaves of *D. strictus* differed significantly with seasonal changes in ambient atmospheric conditions. The study aimed to elucidate the seasonal biochemical responses of two clones (PNT-Pantnagar and DHM-Dhampur) of *D. strictus* grown in sub-tropical climates of foothills of the Indian Himalaya.

## 2. Materials and methods

### 2.1. Study site

The study was performed in the Doon valley of the Indian Himalayan foothills. The valley experiences a humid subtropical climate with an annual rainfall of 2209.8 mm. Maximum rainfall (81%) is concentrated in the monsoon season from mid-June to mid-September (Kaushal et al., 2016). The winter temperature ranges from 23.4 °C to 5.2 °C and extends from November to February. The summer temperature ranges from 36 °C to 16.7 °C.

The climatic data for the study period (i.e., from July 2017 to December 2018) was obtained from the Forest Research Institute's Forest Meteorological Observatory, Dehradun. During the study, the observed value of mean temperature was highest during June (27 °C), which decreased from July onwards with an increase in rainfall. The highest amount of rainfall was recorded, in August (810 mm). It is noteworthy that during the monsoon, the mean temperature decreased slightly, and the gap between the maximum and minimum temperatures narrowed appreciably. The lowest mean temperature was recorded in January (11.9 °C), and also the gap between the minimum and maximum temperature was widest during this period. It is also noted that 95% of the total annual rainfall occurred in June, July, August, and September. Of this, 70% of the rainfall occurred in just two months of July and August. The rainfall was almost negligible in October and November, while experienced some winter rain between December and January (Figure 1).

### 2.2. Experiment material

The two clones of *D. strictus*, namely Pantnagar (PNT) and Dhampur (DHM) named after their respective areas of origin, were selected for the study. Pantnagar is situated at an altitude of 215 m above the mean sea level in the Tarai belt of the Himalayas. The climate of Pantnagar is temperate, with severe cold winter and hot summer. Annual rainfall varies from 1200-1500 mm with an average yearly temperature of 24 °C (Kumar et al., 2014). Dhampur is a city in the Bijnor district of Uttar Pradesh, India. It has an average yearly temperature of 24 °C and 1, 118 mm rainfall (climate-data.org). One-year-old ramets of PNT and DHM clones were obtained from the Genetics and Tree Propagation Division of Forest Research Institute, Dehradun. The plants were transferred to pots containing a proper growing mixture of soil: sand: manure (2:2:1). The soil used was alfisol type (as per the USDA classification). Alfisols are soils with an argillic horizon, moderate to high base content developing in humid and sub-humid climates with annual precipitation of 500–1300 mm and are reported to be suitable for the growth of bamboo plants (Muthukumar and Udaiyan, 2006). The manure used was cow dung manure composed of 1% nitrogen, 0.2% phosphorus, and 0.7% potassium. The concrete pots used for planting the bamboo plants were 35cm in diameter at the top, 27cm in diameter at the base, and 30cm in height. The pot was filled with 30kg of soil: sand: manure mixture. The experiment was laid out in a complete randomized design with 15 replications for each clone.

### 2.3. Biochemical analysis

The biochemical analysis was determined seasonally, *i.e.,* winter (December–January), summer (April–May), and monsoon (July–August), to study the seasonal variations with 15 replications for each clone. The analysis was done on the leaves of the bamboo plants. Fresh and fully expanded leaves from the top, middle, and bottom of the plant were collected from all four plant directions and then chopped into small pieces of approximately $2 \times 2$ mm$^2$. The required amount of sample was taken from this mixture for every analysis, and the remaining sample was stored at -4 °C for further use in the next experiment.

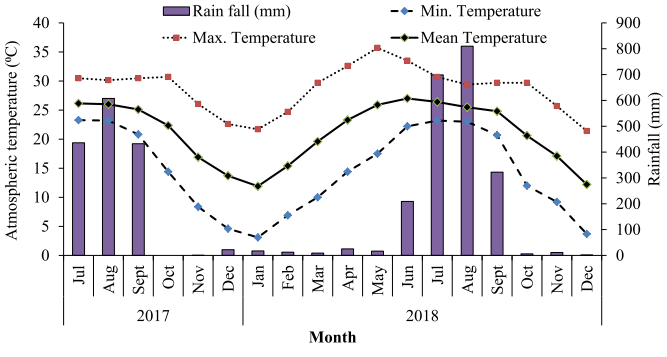


**Figure 1.** Seasonal variations in climatic conditions during the current study period.

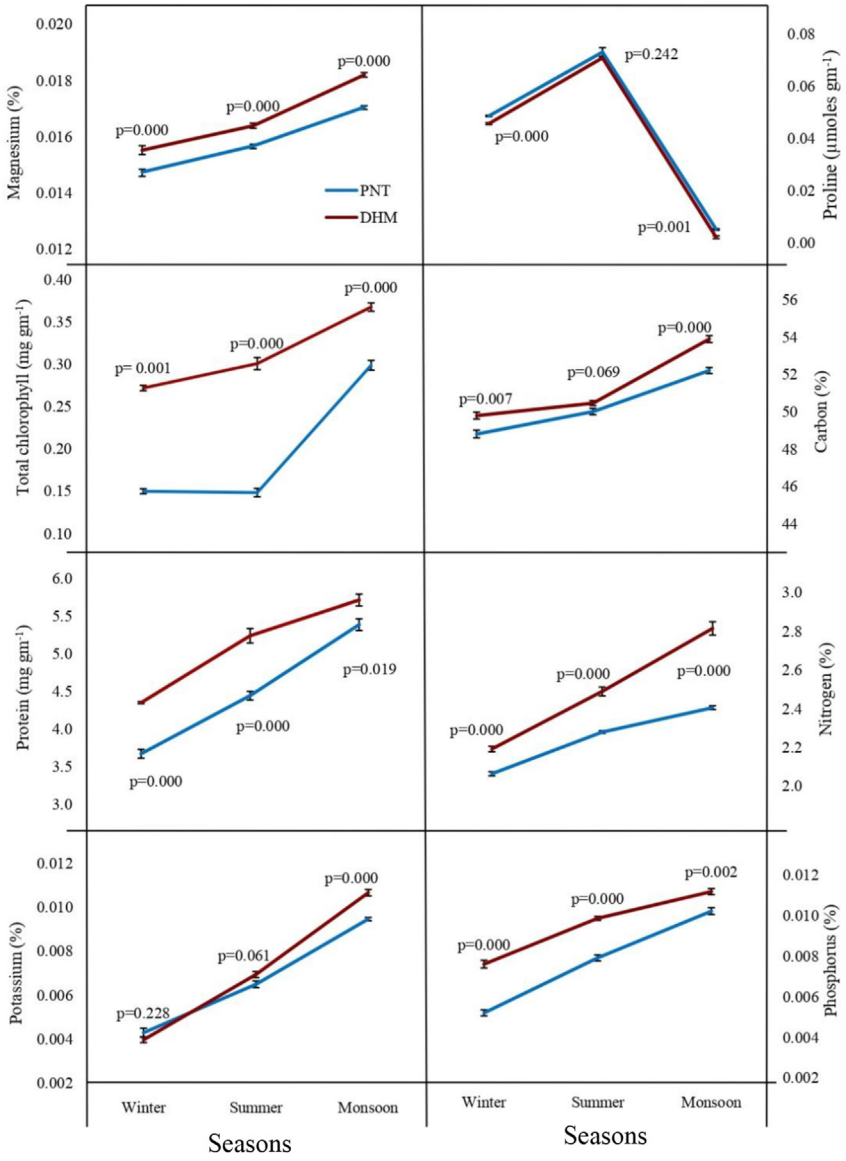


**Figure 2.** Seasonal variation of Total chlorophyll, carbon, protein, proline, magnesium, nitrogen, potassium, and phosphorus in leaves of *D. strictus* clones. All values are mean ± standard error of mean (SEM). N = 15. Means with $p < 0.05$ are significantly different at alpha = 0.05.

### 2.4. Pigments

Chlorophyll and carotenoid content were determined in bamboo leaves by dimethylsulphoxide (DMSO) method (Hiscox and Israelstam, 1979). Leaf sample (50mg) was used for the analysis. The reaction mixture's optical density was recorded at 663, 645, and 470 nm using a multiple wavelength mode spectrophotometer (UV-VIS Spectrophotometer 3000$^{+}$, LABINDIA ANALYTICAL, India). Hiscox and Israelstam (1979) established that Arnon's (1949) equations for calculation of chlorophyll extraction using 90% acetone holds equally good for extraction in DMSO. Hence, chlorophyll and carotenoid was calculated as follows. The amount of chlorophyll and carotene in the leaf samples was expressed in mg gm$^{-1}$ fresh weight.

$$Chl_a\ (mg\ g^{-1}) = 12.7\ A_{663} - 2.69\ A_{645}\ /\ (wt.\ of\ sample\ in\ gm\ x\ 1000) \quad (1)$$

$$Chl_b\ (mg\ g^{-1}) = 22.9\ A_{663} - 4.68\ A_{645}\ /\ (wt.\ of\ sample\ in\ gm\ x\ 1000) \quad (2)$$

$$Total\ Chl\ (mg/g) = 0.0202\ A_{663} + 0.00802\ A_{645}/\ (wt.\ of\ sample\ in\ gm\ x\ 1000) \quad (3)$$

For calculating carotenoid content following equation was used:

$$(C_{x+c}) = (100t0\ A_{470} - 1.9\ (Chl_a - Chl_b))/\ 214 \quad (4)$$

### 2.5. Protein

Protein was estimated by the method of Bradford (1976). Fresh and fully expanded leaves (100 mg) were homogenized with 1ml phosphate buffer over an ice tray. 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 used to obtain the standard curve. Protein concentration in the sample was then estimated using a linear equation of the BSA standard curve. The amount of protein in the leaf samples was expressed in mg gm$^{-1}$ fresh weight.

### 2.6. Total sugar

Total sugar from the leaves was determined by the method of Dubois et al. (1956). Chopped leaf tissue (100 mg) was used for the analysis. The

reaction mixture's optical density was recorded at 490 nm against a blank sample (0.5 ml ethanol+0.5ml DW+ 1ml 5% phenol+ 2.5ml) concentrated $H_2SO_4$. The standard curve was obtained using a different concentration of D-glucose. The amount of total sugar in the sample was determined from the linear equation of the standard curve. The value received was then multiplied by the dilution factor to obtain the total sugar concentration in the leaf tissues, and it was expressed as mg $gm^{-1}$ fresh weight.

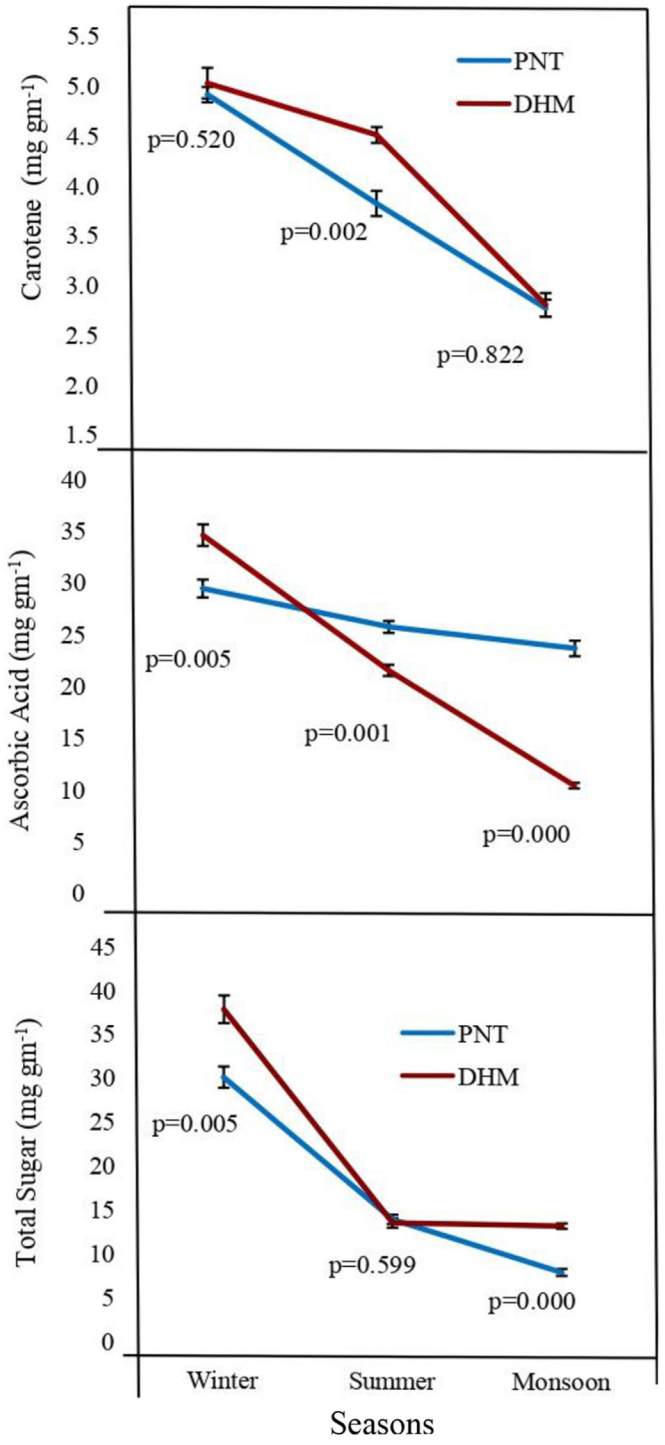


**Figure 3.** Seasonal variation of carotenoid, ascorbic acid, and total sugar content in leaves of *D. strictus* clones. All values are mean ± standard error of mean (SEM). N = 15. Means with $p < 0.05$ are significantly different at alpha = 0.05.

### 2.7. Proline

Proline concentration is one of the key indicators of physiological adaptation in plants. Under this study, proline concentration in leaf tissue was estimated by the method explained by Bates et al. (1973). Five gram of the chopped leaf sample was used for the analyses. The optical density of the red to pinkish chromatophore was recorded at 520 nm using a spectrophotometer. A solution of 4ml toluene mixed with 2ml glacial acetic acid and 2ml acid ninhydrin was used as blank. A standard curve was prepared using different concentrations of pure proline. The proline concentration in the bamboo leaves was determined using the standard curve and expressed in μmol/gm fresh weight. The proline content in leaf tissues was computed using the following equation:

$$\text{Proline (μmol/gm fresh weight)} = [(\text{μg proline/ml}) \times (\text{ml toluene})/115.5\ \text{μg/μmol}] / [(\text{g sample})/5]$$

### 2.8. Ascorbic acid

Ascorbic acid, another indicator of stress in plants, was estimated using the method developed by Harris and Ray's (1935). Leaf sample (1 gm) was used for the analyses. The optical density 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 pure ascorbic acid concentrations. The values obtained were multiplied by the dilution factor to get ascorbic acid content in leaf tissues and expressed as μg/100mg fresh weight.

### 2.9. Nutrients analysis

Nutrients, especially nitrogen (N), potassium (K), phosphorous (P), and magnesium (Mg) were determined from the leaf tissues. Oven-dried leaves (1 gm) were grounded and placed into a conical flask containing 10ml of tri-acid solution ($HNO_3$:$H_2SO_4$:$HClO_4$::10:4:1) and left overnight. The next day, the mixture was entirely digested using the hot plate method until the solution turned white. This solution was cooled and filtered using 100 ml of Distilled Water (DW) and stored in glass bottles. This nutrients stock solution was used to determine K, P, and Mg in leaf tissues.

Potassium content in leaf tissues was analyzed using the method described by Vogel (1961). Two milliliters of nutrient stock solution was mixed with 100ml of DW in a 200ml conical flask. The flame photometer (Flame photometer 128, Systronics - India) was calibrated with DW at 100ppm and 400ppm standard potassium solutions. The readings were observed for each sample, and the calculations were done as follows:

$$\text{Potassium (\%)} = (\text{K concentration in ppm} \times \text{volume} \times 100)/(10^6 \times \text{leaf weight})$$

Phosphorus (P) was determined using the molybdate blue method (Holman, 1943), which requires three reagents, *i.e.* molybdate solution, hydrazine sulphate solution, and sodium hydroxide solution. Two-milliliter nutrients stock solution was mixed with 10ml DW in a 100ml flask. One to two drops of phenolphthalein were used as an indicator and this was titrated against NaOH solution till the completion of the reaction. Now, 10 ml of ammonium molybdate solution was added, followed by 2 ml hydrazine sulphate solution. The final volume of the mixture was made to 100ml by adding DW. The flasks were incubated in a boiling water bath for 15 min to complete the reaction. Subsequently, the optical density (OD) of this solution was measured at 830nm. Further, phosphorus (%) was calculated with the help of the formula:

$$\text{Phosphorous (\%)} = (\text{OD} \times \text{volume} \times 100)/(10^6 \times \text{leaf weight})$$

Magnesium analysis in the leaves was performed using Thiazol yellow method (Young and Gill, 1951). This method requires four

reagents, i.e. polyvinyl alcohol (2%), titan yellow, hydroxylamine hydrochloride (5%), and compensatory solution. Nutrient stock solution (2 ml) was taken in a 50ml flask and added 10ml DW. Compensatory solution (2ml), 2% polyvinyl alcohol (2 ml), 5% Hydroxylamine hydrochloride solution (1ml), and titan yellow solution (1ml) were added in the sequence, followed by the addition of 2ml of 45% NaOH. Then, the final volume was made up to 50 ml with DW for reading optical density at 540nm.

### 2.10. Nitrogen

Nitrogen (N) was determined using the Kjeldahl Digestion Method (1883). The sample was digested in 3.33% salicylic acid, salt mixture ($K_2SO_4$, $FeSO_4$, $CuSO_4$:: 10:1:0.5), and 5 gm of $Na_2S_2O_3$. Sodium hydroxide (43%) was used for distillation. N/10 $H_2SO_4$ with methyl red as the indicator was used to collect the distillate. After completion of distillation, indicated by the appearance of pink color, the samples were titrated against N/10 NaOH. The volume of NaOH used in titration was noted, and the percentage of nitrogen in bamboo leaves was calculated as follows:

$$\text{Nitrogen}(\%) = (\text{Vol. of } H_2SO_4 - \text{Vol. of NaOH used}) \times 0.14 \times 100/\text{leaf weight} \times 10$$

### 2.11. Carbon estimation in leaf tissues

Leaf carbon content was analyzed using methods of Walkley and Black (1934). Oven-dried leaf sample (0.1 gm) was added in a conical flask followed by 30ml of 1N $K_2Cr_2O_7$ and 20 ml of $H_2SO_4$ and then allowed to incubate at room temperature for 45 minutes. Further, distilled water (200 ml) added to this solution followed by 10ml of orthophosphoric acid and 5 drops of Diphenylamine as an indicator. Subsequently, the solution was titrated against the N/2 ferrous ammonium sulphate (FAS) solution. The amount of carbon present in leaf tissues was calculated as follows:

$$\text{Carbon}(\%) = \text{Amount of FAS used} \times 0.003 \times 1.33 \text{ x } 100 \times 0.5/\text{leaf weight (gm)}$$

### 2.12. Statistical analysis

All the parameters were analysed by SYSTAT Ver.13. The observations on biochemical variables were taken seasonally for three seasons to test the two-tailed alternative hypothesis for total chlorophyll, protein, carbon, NPK, and Mg when $H_1$:$\mu_w < \mu_s < \mu_m$, where $\mu_w$, $\mu_s$, $\mu_m$ is the mean of the concerned biochemical parameter during winters, summers, and monsoon respectively. For carotenoid, ascorbic acid, and total sugar the $H_1$:$\mu_w > \mu_s > \mu_m$, whereas for proline the $H_1$:$\mu_w < \mu_s > \mu_m$. The data were checked for normality by the Shapiro-wilk test, and it was considered normally distributed when $p \geq 0.05$. Since the observations were made on equal intervals of time on the same set of plants, the repeated measures ANOVA was done to determine the effect of time on the measured variables. More precisely, the repeated measures ANOVA was used to see the significance of the impact of the within-subject factors (observations taken in different seasons), i.e., whether the seasons affect the biochemical composition of *D. strictus* plants and also if the means of the various biochemical parameters of the PNT and DHM clones differ significantly from each other. The GG correction to F statistic was used to test the significance if the GG coefficient's value was near to one. When the within-subject factors had a significant effect on the variables, a post hoc test was carried out by applying Bonferroni corrections to compare the means during different seasons. Correlations between all the parameters were determined using Karl 'Pearson's correlation coefficient. The differences in means and correlations were considered statistically significant at a 5% level of significance.

## 3. Results

### 3.1. Leaf chlorophyll content

It was observed that the total leaf chlorophyll content doubled from winter to the monsoon for the PNT clone. For DHM, the leaf chlorophyll content was found to have increased by 35% from winter to the monsoon (Figure 2). The mean of total chlorophyll content was significantly higher in the DHM clone for all seasons compared to the PNT clone (Table 1). The clones differ in mean chlorophyll content taken as a vector of all seasons ($F = 150.66$, $p < 0.001$) (Table 2).

The effect of seasonal changes in climatic conditions on chlorophyll content was also significant ($p < 0.001$). The total chlorophyll content was lowest during the winters and highest during the monsoons. The result of pair-wise comparisons between the mean chlorophyll content in three seasons after applying Bonferroni correction for multiple comparisons showed that it did not increase significantly from winters to summers ($p > 0.025$) while, during monsoons, it rose as compared to that in winters and summers (Table 1).

### 3.2. Leaf carotenoid content

The carotenoid content in both the clones was highest during winters and lowest during monsoons. It was reduced by 50% during the monsoons (Figure 3). The carotenoid content was significantly higher in the DHM clone for all seasons than the PNT clone (Table 1). The clones differ in mean carotenoid content taken as a vector of all seasons ($F = 22.46$, $p = 0.001$) (Table 2).

The effect of seasonal changes in climatic conditions on carotenoid content was also significant ($p < 0.001$). The result of pair-wise comparisons between mean carotenoid content in three seasons after applying Bonferroni correction for multiple comparisons showed that the carotenoid content decreased significantly during summers ($p < 0.001$) as compared to winters, and again reduced significantly during monsoons as compared to summer and winter ($p < 0.001$) (Table 1).

The interaction between the seasons and the mean carotenoid content of each clone is significant, indicating that the two clones' carotenoid content is significantly different in different seasons with an F-value of 4.04 and significance <0.05 (Table 2).

### 3.3. Leaf protein content

Protein content in the leaf tissues was significantly higher ($p < 0.001$) from winter to monsoon season. The protein increment was approximately 46% in the PNT clone, whereas for DHM, nearly 32% (Figure 2). The total protein content was significantly higher in the DHM clone for all seasons than the PNT clone (Table 1). The clones differ in mean protein content taken as a vector of all seasons ($F = 124.26$, $p < 0.001$) (Table 2).

The effect of seasonal changes in climatic conditions on protein content was also significant ($p < 0.001$). The mean protein content was lowest during the winters and highest during the monsoons. The result of pair-wise comparisons between the mean protein content in three seasons after applying Bonferroni correction for multiple comparisons showed that it was significantly higher in summer ($p < 0.001$) as compared to winter and monsoon as compared to winter ($p < 0.001$) and summer ($p < 0.001$) (Table 1).

The interaction between the seasons and clones indicates that the two clones' protein content is significantly different in different seasons with an F-value of 6.007 and significance <0.025 (Table 2).

**Table 1.** Seasonal variability of biochemical traits in bamboo leaves.

| Biochemical Parameter | Clone –PNT | | | Clone –DHM | | |
|---|---|---|---|---|---|---|
| | Seasons | | | Seasons | | |
| | Winter | Summer | Monsoon | Winter | Summer | Monsoon |
| Total chlorophyll (mg $gm^{-1}$ FW) | $0.15^{a} \pm 0.003$ | $0.15^{a} \pm 0.005$ | $0.30^{b} \pm 0.006$ | $0.27^{a} \pm 0.003$ | $0.30^{b} \pm 0.007$ | $0.37^{c} \pm 0.005$ |
| Carotene (mg $gm^{-1}$ FW) | $4.92^{c} \pm 0.07$ | $3.83^{b} \pm 0.13$ | $2.79^{a} \pm 0.08$ | $5.04^{c} \pm 0.15$ | $4.52^{b} \pm 0.08$ | $2.83^{a} \pm 0.12$ |
| Protein (mg $gm^{-1}$ FW) | $3.67^{a} \pm 0.06$ | $4.44^{b} \pm 0.05$ | $5.38^{c} \pm 0.07$ | $4.34^{a} \pm 0.01$ | $5.24^{b} \pm 0.10$ | $5.71^{c} \pm 0.07$ |
| Ascorbic (mg $gm^{-1}$ FW) | $29.51^{c} \pm 0.86$ | $25.86^{b} \pm 0.58$ | $23.85^{a} \pm 0.75$ | $34.67^{c} \pm 1.04$ | $21.66^{b} \pm 0.54$ | $10.59^{a} \pm 0.26$ |
| Total sugar (mg $gm^{-1}$ FW) | $30.16^{c} \pm 1.21$ | $14.04^{b} \pm 0.52$ | $8.09^{a} \pm 0.39$ | $37.88^{b} \pm 1.57$ | $13.63^{a} \pm 0.53$ | $13.36^{a} \pm 0.31$ |
| Proline (μmoles $gm^{-1}$) | $0.049^{b} \pm 0.003$ | $0.073^{c} \pm 0.002$ | $0.005^{a} \pm 0.003$ | $0.046^{b} \pm 0.004$ | $0.07^{c} \pm 0.004$ | $0.002^{a} \pm 0.001$ |
| Carbon (%) | $48.79^{a} \pm 0.21$ | $49.99^{a} \pm 0.17$ | $52.19^{b} \pm 0.16$ | $49.78^{a} \pm 0.18$ | $50.45^{a} \pm 0.13$ | $53.87^{b} \pm 0.18$ |
| Nitrogen (%) | $2.06^{a} \pm 0.01$ | $2.28^{b} \pm 0.01$ | $2.40^{c} \pm 0.01$ | $2.19^{a} \pm 0.01$ | $2.49^{b} \pm 0.02$ | $2.81^{c} \pm 0.035$ |
| Potassium (%) | $0.004^{a} \pm 0.0002$ | $0.007^{b} \pm 0.0002$ | $0.009^{c} \pm 0.0001$ | $0.004^{a} \pm 0.0001$ | $0.007^{b} \pm 0.0001$ | $0.011^{c} \pm 0.0002$ |
| Phosphorus (%) | $0.005^{a} \pm 0.0002$ | $0.008^{b} \pm 0.0002$ | $0.010^{c} \pm 0.0002$ | $0.008^{a} \pm 0.0002$ | $0.010^{b} \pm 0.0001$ | $0.011^{c} \pm 0.0002$ |
| Magnesium (%) | $0.015^{a} \pm 0.0001$ | $0.016^{b} \pm 0.0001$ | $0.017^{c} \pm 0.0001$ | $0.015^{a} \pm 0.0002$ | $0.016^{b} \pm 0.0001$ | $0.020^{c} \pm 0.0001$ |

All values are mean ± standard error of the mean (SEM). N = 15.
Means (observations taken in different seasons) with different alphabets across the columns for each clone are significantly different at alpha = 0.05. Results are based on post hoc test done by applying Bonferroni corrections for multiple comparisons.

**Table 2.** Results from repeated measure analysis of variance.

| Biochemical Parameter | Between subject Analysis (Between the means of the two clones) | | Within-subject analysis (Between observations taken in different seasons) | | Univariate F- test | | |
|---|---|---|---|---|---|---|---|
| | F ratio | p value | F ratio | p/G-G value | p- Winter | p-Summer | p-Monsoon |
| Chlorophyll | 150.658 | 0.000 | 13.551 | GG = 0.001 | 0.001 | 0.000 | 0.000 |
| Carotenoid | 22.464 | 0.001 | 4.036 | GG = 0.048 | 0.052 | 0.002 | 0.822 |
| Protein | 124.259 | 0.000 | 6.007 | GG = 0.011 | 0.000 | 0.000 | 0.019 |
| Ascorbic acid | 58.640 | 0.000 | 76.225 | GG = 0.000 | 0.005 | 0.001 | 0.000 |
| Total sugar | 29.311 | 0.001 | 11.810 | p = 0.006 | 0.005 | 0.599 | 0.000 |
| Proline | 20.588 | 0.002 | 0.149 | p = 0.863 | 0.000 | 0.242 | 0.001 |
| Carbon | 73.280 | 0.000 | 5.527 | GG = 0.021 | 0.007 | 0.069 | 0.000 |
| Nitrogen | 266.430 | 0.000 | 28.550 | GG = 0.000 | 0.000 | 0.000 | 0.000 |
| Potassium | 10.729 | 0.011 | 15.639 | p = 0.000 | 0.228 | 0.061 | 0.000 |
| Phosphorous | 185.213 | 0.000 | 12.399 | GG = 0.002 | 0.000 | 0.000 | 0.002 |
| Magnesium | 88.055 | 0.000 | 3.750 | GG = 0.056 | 0.003 | 0.000 | 0.000 |

The effects are significant when $p \leq 0.05$.

### 3.4. Total leaf sugar content

The total sugar concentration in the leaves was observed to drop drastically from winter to summer by 73% and 64% for PNT and DHM, respectively, and further reduced mildly during monsoons for both the clones. The total sugar content was significantly higher in the DHM clone during winters and monsoons. In summers, although the total sugar content was higher in the DHM as compared to the PNT clone (Figure 3), this difference was not significant (Table 1). The clones differ in mean total sugar content taken as a vector of all seasons ($F = 29.31$, $p < 0.001$) (Table 2).

The effect of seasonal changes in climatic conditions on total sugar content was also significant ($p < 0.001$). The result of pair-wise comparisons between mean total sugar content in three seasons after applying Bonferroni correction for multiple comparisons showed that the total sugar content decreased significantly during summers ($p < 0.001$) as compared to winters, and again significantly reduced during monsoons as compared to summer and winter ($p < 0.001$) (Table 1).

The interaction between the seasons and the mean total sugar content of each clone is significant, indicating that the two clones' total sugar content is significantly different in different seasons with an F-value of 11.81 and significance <0.025 (Table 2).

### 3.5. Leaf proline content

The leaf proline concentration was highest during summers and decreased significantly ($p < 0.001$) during monsoons in both clones, with a 97% decrease in proline concentration from summer to monsoon. The proline content was higher in the PNT clone than the DHM clone during winters and monsoons (Figure 2). In summers, although the proline content was higher in the PNT, this difference was not significant (Table 1). The clones differ in mean proline content taken as a vector of all seasons ($F = 20.59$, $p < 0.001$) (Table 2).

The effect of seasonal changes in climatic conditions on proline content was also significant ($p < 0.001$). The proline content was lowest during the monsoons and highest during the summers. The result of pair-

wise comparisons between the mean proline content in three seasons after applying Bonferroni correction for multiple comparisons showed that the mean proline content was significantly higher in summer than winter ($p < 0.001$), and it decreased substantially during monsoons ($p < 0.001$), the decrease in proline content from winter to monsoon was also significant ($p < 0.001$) (Table 1).

On comparing the two clones' means, it was also observed that the mean proline content in both clones is significantly different from each other in winter and monsoon. In contrast, the means were not significantly different during summers (Table 2).

### 3.6. Ascorbic acid

The ascorbic acid content in leaf tissues was reported to be highest during winter and lowest in monsoon for both the clones (Figure 3). The decrease was 19% for the PNT clone and 69% for the DHM clone. The ascorbic acid content was significantly higher in the DHM clone during winters, while it was higher in the PNT clone during summer and monsoons than the DHM clone (Table 1). The clones differ in mean ascorbic acid content taken as a vector of all seasons ($F = 58.64$, $p < 0.001$) (Table 2).

The effect of seasonal changes in climatic conditions on ascorbic acid content was also significant ($p < 0.001$). The result of pair-wise comparisons between mean ascorbic acid content in three seasons after applying Bonferroni correction for multiple comparisons showed that the ascorbic acid content decreased significantly during summers ($p < 0.001$) as compared to winters, and again considerably reduced during monsoons as compared to summer and winter ($p < 0.001$) (Table 1).

The interaction between the seasons and mean ascorbic acid content of each clone is significant, indicating that the two clones' ascorbic acid content is significantly different in different seasons with an F-value of 76.23 significance <0.025 (Table 2).

### 3.7. Leaf nitrogen content

The nitrogen content was lowest during the winters and highest during the monsoons in both the clones (Figure 2). The total nitrogen content was significantly higher in the DHM clone for all seasons than the PNT clone (Table 1). The clones differ in mean nitrogen content taken as a vector of all seasons ($F = 266.43$, $p < 0.001$) (Table 2).

The effect of seasonal changes in climatic conditions on nitrogen content was also significant ($p < 0.001$). The result of pair-wise comparisons between the mean nitrogen content in three seasons after applying Bonferroni correction for multiple comparisons showed that the nitrogen content increased significantly during summers ($p < 0.001$) as compared to winters, and again markedly increased during monsoons as compared to summer and winter ($p < 0.001$) (Table 1).

The interaction between the seasons and each clone's mean nitrogen content is significant, indicating that the clones' nitrogen content is significantly different in different seasons with an F-value of 28.55 and significance <0.025 (Table 2).

### 3.8. Leaf potassium content

The potassium (K) content in bamboo leaves increased more than twofold from winter to monsoon in both the clones (Figure 2). There was no significant difference in the mean of potassium content of both the clone during winter and summer, while it was significantly higher in the DHM clone during monsoons (Table 1). The clones differ in mean potassium content taken as a vector of all seasons ($F = 10.73$, $p < 0.025$) (Table 2).

The effect of seasonal changes in climatic conditions on potassium content was also significant ($p < 0.001$). The result of pair-wise comparisons between the mean potassium content in three seasons after applying Bonferroni correction for multiple comparisons showed that it increased significantly during summers ($p < 0.001$) as compared to winters, and again markedly increased during monsoons as compared to summer and winter ($p < 0.001$) (Table 1).

The interaction between the seasons and the mean potassium content of each clone is significant, indicating that the two clones' potassium content is significantly different in different seasons with an F-value of 15.64 and significance <0.025 (Table 2).

### 3.9. Leaf phosphorus content

Phosphorus (P) content in leaf tissues was increased by 49% and 32% for PNT and DHM clones, respectively, from winter to monsoon (Figure 2). The total phosphorus content was significantly higher in the DHM clone for all seasons than the PNT clone (Table 1). The clones differ in mean phosphorus content taken as a vector of all seasons ($F = 185.21$, $p < 0.001$) (Table 2).

The effect of seasonal variability on phosphorus content was also significant ($p < 0.001$). The result of pair-wise comparisons between the mean phosphorus content in three seasons after applying Bonferroni correction for multiple comparisons showed that the phosphorus content increased significantly during summers ($p < 0.001$) as compared to winters, and again markedly increased during monsoons as compared to summer and winter ($p < 0.001$) (Table 1).

The interaction between the seasons and the mean phosphorus content of each clone is significant, indicating that the two clones' phosphorus content is significantly different in different seasons with an F-value of 12.4 and significance <0.025 (Table 2).

### 3.10. Leaf magnesium content

The magnesium (Mg) content was modestly improved by 16% for PNT and 17% for DHM clones, with the concentration being the lowest in winter and highest during monsoon (Figure 2). The total magnesium content was significantly higher in the DHM clone for all seasons than the PNT clone (Table 1). The clones differ in mean magnesium content taken as a vector of all seasons ($F = 88.06$, $p < 0.001$) (Table 2).

The effect of seasonal changes in climatic conditions on magnesium content was also significant ($p < 0.001$). The result of pair-wise comparisons between the mean magnesium content in three seasons after applying Bonferroni correction for multiple comparisons showed that the magnesium content increased significantly during summers ($p < 0.001$) as compared to winters, and again increased significantly during monsoons as compared to summer ($p < 0.001$) and winter ($p < 0.001$) (Table 1).

### 3.11. Leaf carbon content

The leaves' carbon percentage was observed to increase from winter to monsoon by 4% for both the clones (Figure 3). It was significantly higher in the DHM clone as compared to the PNT clone during winters and monsoons. In summers, although the carbon content was higher in the DHM, this difference was not significant (Table 1). The clones differ in mean carbon content taken as a vector of all seasons ($F = 73.28$, $p < 0.001$) (Table 2).

The effect of seasonal changes in climatic conditions on carbon content was also significant ($p < 0.001$). The carbon content was lowest during the winters and highest during the monsoons. The result of pair-wise comparisons between the mean carbon content in three seasons after applying Bonferroni correction for multiple comparisons showed that it was significantly higher in summer ($p < 0.001$) as compared to winter and monsoon as compared to winter ($p < 0.001$) and summer ($p < 0.001$) (Table 1).

The interaction between the seasons and mean carbon content of each clone is significant, indicating that the carbon content in the two clones is significantly different in different seasons with an F-value (5.53) and significance <0.025 (Table 2). On comparing the means of the two clones, it was also observed that the mean carbon content in both clones

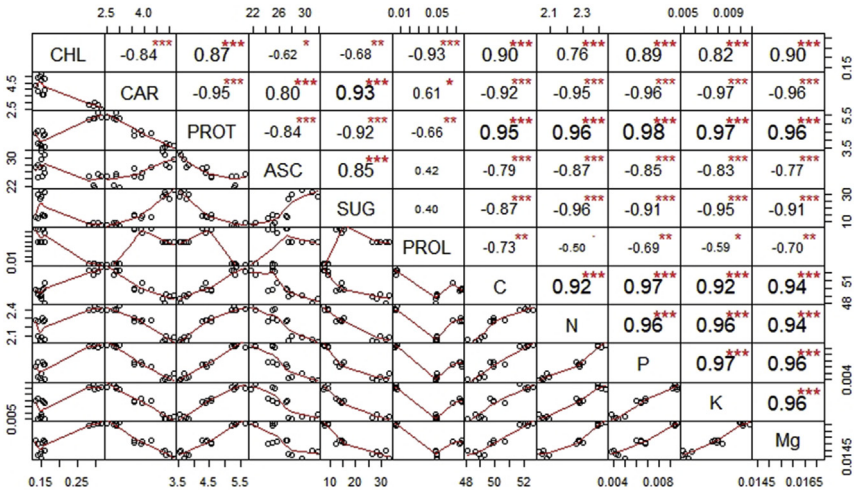


**Figure 4.** Pearson's correlation coefficients for the analyzed biochemical parameters of PNT Clone. CHL = Total chlorophyll, CAR = Carotene, PROT = Protein, ASC = Ascorbic Acid, SUG = Total Sugar, PROL = Proline, C = Carbon, N = Nitrogen, P = Phosphorus, K = Potassium, Mg = Magnesium.

is significantly different from each other in winter and monsoon, while the means were not significantly different during summers (Table 2).

### 3.12. Correlation between biochemical parameters

The seasonal accumulation of biochemical parameters was correlated, and the interaction between them was plotted (Figures 4 and 5). For the PNT clone, the total chlorophyll content in bamboo leaves showed a highly significant linear correlation ($r = 0.90$, $p < 0.05$) with leaves' carbon and magnesium content. Phosphorous and protein showed a positive linear correlation with $r = 0.89$, $p < 0.05$ and $r = 0.87$, $p < 0.05$, respectively, with total chlorophyll content. Potassium and nitrogen content of leaves showed a significant positive correlation with total chlorophyll content ($r = 0.82$, $p < 0.05$ and $r = 0.76$, $p < 0.05$, respectively). It was interesting to note that the total sugar content exhibited an insignificant negative correlation with total chlorophyll content. Carotenoid content showed a significant linear correlation with total sugar ($r = 0.93$, $p < 0.05$), ascorbic acid ($r = 0.80$, $p < 0.05$), and proline ($r = 0.61$, $p < 0.05$). Apart from total chlorophyll, protein showed significant ($r = 0.98$, $p < 0.05$) linear correlation with phosphorous, magnesium ($r = 0.96$, $p < 0.05$) and nitrogen ($r = 0.96$, $p < 0.05$), carbon ($r = 0.95$, $p < 0.05$), and potassium ($r = 0.96$, $p < 0.05$). Besides, a significant positive linear correlation was reported between ascorbic acid and total soluble sugar ($r = 0.85$, $p < 0.05$). Another significant correlation to be taken note of was that between carbon content and phosphorous ($r = 0.97$, $p < 0.05$). Carbon content in the leaf tissues showed a positive and significant linear correlation with magnesium ($r = 0.94$, $p < 0.05$), nitrogen ($r = 0.92$, $p < 0.05$), and potassium ($r = 0.92$, $p < 0.05$). Nitrogen showed positive and significant linear correlation with phosphorous ($r = 0.96$, $p < 0.05$), potassium ($r = 0.96$, $p < 0.05$), and magnesium content ($r = 0.94$, $p < 0.05$). Potassium showed significant linear correlation with phosphorous ($r = 0.97$, $p < 0.05$) and magnesium ($r = 0.96$, $p < 0.05$). Phosphorous and magnesium showed significant linear correlation with $r = 0.97$, $p < 0.05$. These results give us an insight into how effectively the concentration of biochemical parameter affects the concentration of another parameter.

For DHM clone, chlorophyll showed a highly significant correlation ($r = 0.95$, $p < 0.05$) with magnesium followed by phosphorous ($r = 0.94$, $p < 0.05$), nitrogen ($r = 0.93$, $p < 0.05$), carbon ($r = 0.92$, $p < 0.05$), protein ($r = 0.88$, $p < 0.05$), and potassium ($r = 0.87$, $p < 0.05$). Carotenoid content showed significant correlation with ascorbic acid ($r = 0.92$, $p < 0.05$) and proline ($r = 0.80$, $p < 0.05$). Unlike PNT clone the

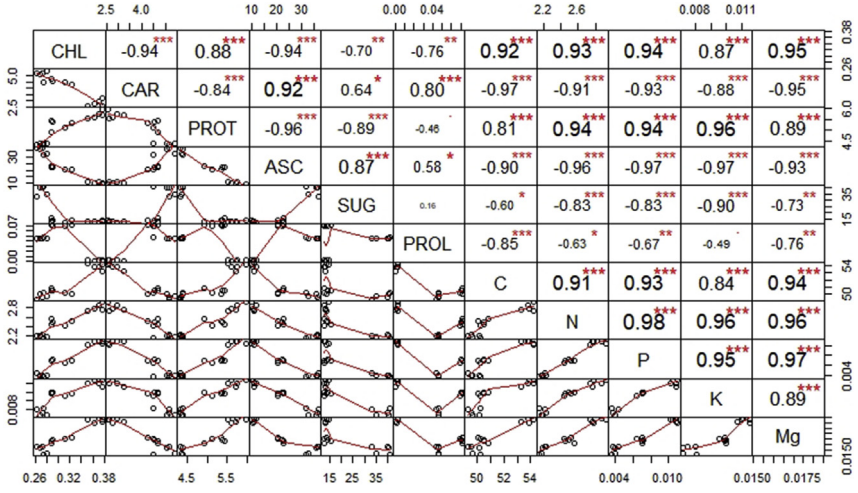


**Figure 5.** Pearson's correlation coefficients for the analyzed biochemical parameters of DHM Clone. CHL = Total chlorophyll, CAR = Carotene, PROT = Protein, ASC = Ascorbic Acid, SUG = Total Sugar, PROL = Proline, C = Carbon, N = Nitrogen, P = Phosphorus, K = Potassium, Mg = Magnesium.

correlation between carotenoid and total sugar was not very significant in the case of DHM clone. The protein content showed the greatest relationship with potassium ($r = 0.96$, $p < 0.05$) and phosphorous and nitrogen ($r = 0.94$, $p < 0.05$). Protein and magnesium showed significant linear correlation with $r = 0.89$ and $p < 0.05$. Ascorbic acid and total sugar showed significant correlation with $r = 0.87$ and $p < 0.05$. Like PNT clone, the leaf nutrients, comprising carbon, nitrogen, potassium, phosphorous, and magnesium, depicted a highly significant positive linear association amongst themselves (Figures 4 and 5).

## 4. Discussion

### 4.1. Seasonal variation in pigments, protein, total sugars, proline, ascorbic acid in the leaf tissue

The clones of *D. strictus* exhibited the lowest total chlorophyll concentration during the winter season. The reduction in chlorophyll concentration in winter can be attributed to short photoperiod coupled with low atmospheric temperature resulting in photoinhibition, which causes a decrease of photochemical activity and decline in production of photosynthetic pigments (Huner et al., 1996). Similar findings were reported in *Phyllostachys humilis* (Van Goethem et al., 2013), montane conifers (Nippert et al., 2004), and seedlings of *Pinus sylvestris* (Strand and Lundmark, 1987). During summers, the air temperature rises, and there are longer photoperiods, leading to an increase in chlorophyll formation (Huner et al., 1998). Monsoon provides optimum hours of sunlight, favorable temperature, and ample precipitation, which further favours chlorophyll synthesis (Malik et al., 2017). Therefore, an increment in the chlorophyll content in the bamboo leaves was observed. Precipitation has been recognized to increase nutrient absorption in plants (Baruah and Baruah, 2000). A higher rate of $Mg^{+2}$ ion uptake, as observed in the results during the rainy season, can also be cited as one of the reasons for increased chlorophyll content in the leaves of *D. strictus*. The trend of chlorophyll content in bamboo leaves in this study witnessed consonance to the findings of Prajapati and Tripathi (2008).

In the present study, the protein content of leaf tissue for both the clones was increasing from winter to summer and was highest in the monsoon season as the plant growth rate increased with favorable atmospheric conditions. This increase in the leaf protein content might be due to plants' nature to accumulate protein during the period of excess availability in the active growth phase to serve as temporary reserves of carbon and nitrogen (Coleman, 1997). Dwarf bamboos like *Sassa nipponica* and Nezasa dwarf bamboo showed high protein accumulation in bamboo leaves during the rainy season (Yokoyama, 1998; Yamamoto et al., 1999). Studies over the years on bamboo and other plant species have reported similar protein concentration patterns in leaf tissue (Blair 1969; von Fircks et al., 2001; Halvorson et al., 2011; Singh et al., 2013).

Total sugar content in leaf tissues of bamboo clones declined from winter onward to monsoon season. The decreasing total sugar content in leaf tissue can be due to the maturing of the plant. Nirmala et al. (2007) reported that juvenile bamboo/younger shoots accumulate more carbohydrates while it decreased in older shoots. Hence aging could be one of the reasons for reducing total sugar content in leaf tissues. According to another theory, carbohydrates are accumulated and stored inside the plant during the growth dormancy period. At the onset of growth favouring conditions like in the rainy season, carbohydrates are metabolized and used as energy to develop new shoots (Rogers et al., 1975; DiPaola and Beard, 1992). This explains the accumulation of total sugar in bamboo leaves during winter, and its utilization in growth resulted in reduced sugar content during the monsoon in the present study.

The proline concentration in both the clones' leaf tissue was highest during summer months; however, lowest in monsoon. During summer, there was less rainfall, high solar intensity, and higher temperature, leading to osmotic stress in plants. Multifold increase in the proline concentration in plants experiencing physiological stress, particularly osmotic stress has been widely reported (Delauney and Verma, 1993; Serraj and Sinclair, 2002; Carillo and Gibon, 2011). Proline concentration may increase to 100 times the normal value during stresses (Barnett and Naylor, 1966). Further, a similar trend of proline accumulation during summer has been reported in various shrub and tree species (Lansac et al., 1994). Zunzunegui et al. (2011) reported that summer leaf water potential is negatively correlated to proline concentration in diverse Mediterranean plant species, and therefore more proline accumulated in leaves during summer. Soni et al. (2015) reported an accumulation of proline during summer in medicinal plants. Proline accumulation could be one indicator of bamboo's adaptive capacity towards physiological and osmotic stress resulting from water scarcity (Meena et al., 2011; Singh and Verma, 2013).

The ascorbic acid content in leaf tissues of both the clones was reduced from winter to monsoon season. The seasonal patterns of accumulation of ascorbic acid might be due to seasonal variation in environmental conditions that generate stress in plants and result in higher ascorbic acid production in leaves (Walia and Bhardwaj, 2017). The higher production of ascorbic acid during the winter season is one of the mechanisms for adapting water-limited or water stress conditions, which *D. strictus* clones would have modulated.

### 4.2. Seasonal variation in nutrients concentration in the leaf tissues

The nutrient content in plants is profoundly affected by the surroundings as well as the growth environment. Understanding the extent to which this interaction controls biosynthesis and accumulation of various plant nutrients would allow a correct selection of the growing environment and guide the species' genetic improvement by traditional breeding and biotechnology (Proietti et al., 2009).

The nitrogen content of the leaf tissue is reported to be translocated to the plant's perennial tissues at the time of leaf senescence (von Fircks et al., 2001). Bollmark et al. (1999) reported that about two-thirds of the leaf nitrogen had been translocated at the time of leaf abscission. The lesser nitrogen concentration in leaves during winter in the present study can be due to leaf nutrients' translocation process. von Fircks et al. (2001) found that about 60% of leaf phosphorous is translocated at the time of leaf abscission, and during the period of active growth of the plant, the phosphorous content in leaves is increased. This result on phosphorus obtained by von Fircks et al. (2001) confirmed with the results of the current study where the phosphorous content was lowest in winters, perhaps due to the process of leaf abscission and increased by 49% and 32% during monsoons for PNT and DHM clones, respectively. Likewise, withdrawal of potassium from leaves during senescence is commonly reported to be in the range of 10%–70% of the original content (Ericsson, 1994). This trend was observed in the present study as the potassium content of leaves increased by 54% and 62% for PNT and DHM, respectively, from winter to monsoon. Since chlorophyll is a magnesium-centric compound, magnesium concentration also improved in leaf tissue from winter to monsoon season. Magnesium is known to be a mobile element of plant tissue, and content as high as 50% of the leaf Mg is reported to be withdrawn from leaves at the time of leaf abscission (Chapin et al., 1990; Ericsson, 1994). Therefore, a dip in the leaf Mg accumulation could be in response to leaf abscission, observed in the plant leaves during winters. During monsoon, the growth rate was highest, and subsequently, the nutrient level in leaf tissue was also highest.

Potassium and magnesium concentrations play a vital role in $CO_2$ fixation (Ericsson and Kähr, 1993, 1995), and hence during the monsoon, when the nutrient content was highest in leaves, the carbon content was increased due to the increased rates of $CO_2$ fixation. This interdependence of plant nutrients in leaves is depicted in a high positive linear correlation amongst the nutrients.

### 4.3. Correlation between biochemical parameters

The nutrients translocated from leaves to perennial plant organs constitute the reserve for early season leaf development and shoot

growth (Tromp, 1983; Millard, 1996). The nutrient reserves are commonly in the form of carbohydrates (sugars) and lipids that are later metabolized at active growth stage (Von Fircks and Sennerby-Forsse, 1998). In the current study, it was exciting to note that the total sugar content in leaf tissue was highest during winters comprising of the nutrient reserve, which was later used in plant growth during summer and monsoon. A drop in total sugar content was noted from winter to monsoon for the clones of *D. strictus*.

It is well understood that the higher the nitrogen content in leaves higher will be the protein accumulation in the form of nitrogen, the building block of protein molecules (Novoa and Loomis, 1981; Yeoh and Wee, 1994). This is reflected in the high correlation between the two parameters in the present study (Figures 4 and 5).

Watson and Noggle (1947) reported that low concentrations of K and Mg in the leaves stimulate ascorbic acid biosynthesis while high concentrations retard this synthesis, and there is a negative correlation between leaf tissue ascorbic acid and K and Mg content (Figures 4 and 5). The results of the current study support this approach. Although, Baruah and Baruah (2000) approach based on better nutrient availability to plants in case of higher rainfall should not be ruled out. According to this approach, the water stress resulted in ascorbic acid accumulation during the dry winter months when lesser nutrients were available to the plants. Further studies on this interaction are needed to pinpoint the basis of this correlation.

## 5. Conclusion

It is concluded that the concentration of biochemical parameters of the leaf tissue of bamboo clones was influenced significantly by the seasonal variations in the microclimatic conditions under this study. The study conferred that seasonal variation in temperature, sunlight intensity, and rainfall could be used as effective treatments to study plant behavior in response to changing microclimatic conditions. It is noted that monsoon is the primary season/period suitable for the better growth and development of *D. strictus*, which was confirmed by various biochemical parameters. The adaptive capability of *D. strictus* to hydrological stress, especially soil water deficiency, is also corroborated through the accumulation of ascorbic acid and proline in winter and summer seasons, respectively. DHM clones indicated a faster growth rate through the higher accumulation of protein and nutrients. The more accumulation of proline and ascorbic acid in the PNT clone means better adaptation potential to water stress than DHM. The study provides a biochemical approach for selecting bamboo clones with better adaptation behavior and a climatically resilient nature for plantation purposes.

## Declarations

### *Author contribution statement*

Hukum Singh: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Saloni Singh: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Satish Kant Sharma: Conceived and designed the experiments.

Raman Nautiyal: Analyzed and interpreted the data.

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### *Data availability statement*

Data will be made available on request.

### *Declaration of interests statement*

The authors declare no conflict of interest.

### *Additional information*

No additional information is available for this paper.

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