

Original Research Article

Effect of different shade levels on growth, physiology and biochemical characteristics of small cardamom (*Elettaria cardamomum* Maton)

Abstract

Elettaria cardamomum was grown under shade levels (75% shade, 50% shade and open irradiance) to evaluate its photosynthetic characteristics, physiology and biochemical characters. The highest net photosynthetic rates and stomatal conductance were observed under 75% shade, followed in descending order by 50% and open condition. The highest quantum yield of photosystem II was observed under 50% shade. As shade level increased, Chl a, Chl b and total Chl contents also increased significantly. Total number of opened stomata generally displayed the best activity in leaves subjected to 75% shade. The highest total phenol and epicuticular wax contents were observed in open irradiance treatment and highest proline level was observed in plants subjected to the 50% shade treatment. The results indicate that increased plant height, chlorophyll and proline contents and higher quantum yield of Photosystem II and stomatal activity may play an important role in shade adapting mechanism in small cardamom.

Keywords: Shade; Photosynthesis; Stomatal behaviour; Proline; Phenolic acid; Epicuticular wax; Small cardamom

Introduction

Small cardamom (*Elettaria cardamomum* (L.) Maton, Zingiberaceae), a shade loving understory crop of herbaceous non-woody shrub of Indian origin, is cultivated extensively in the high-altitude hilly areas of the Western Ghats of Kerala, Tamil Nadu and Karnataka states of peninsular India for its spicy and aromatic fruits (capsules). Cardamom has C₃ photosynthetic pathway (Murugan *et al.*, 2008).

The practice of shade regulation in cardamom has been practiced since last many decades. Therefore the much more complex tropical forest has been converted to a more open, simple and uniform system and hence leading to degradation of cardamom agroforestry system in India. Reduced soil water content can decrease the yield of cardamom significantly during summer months (April and May), the number of flowers and capsules formed as well as the percentage of capsule set per plant (30% in summer and 80% in August or September). Synchronous warming (both soil and atmospheric air warming) in cardamom hills (Murugan *et al.*, 2008) is evident which can adversely impact soil nutrient dynamics particularly major nutrients (nitrogen, phosphorus and potassium) leading to yield reduction which is unavoidable.

Light has long been known to be the most important factor influencing plant growth, morphology, and anatomy, various aspects of physiology and cellular biochemistry, and, ultimately, flowering time and plant productivity (Dai *et al.*, 2009; Favaretto *et al.*, 2011; Deng *et al.*, 2012). In the light reactions of photosynthesis, light energy is used to produce ATP and NADPH, which are then used for carbon fixation and production of oxygen during the light-independent phase. Shading effect has a major impact on plant photosynthesis. Normal plant growth needs optimal light irradiance because excessively high and low irradiances would result in photoinhibition and light deficiency respectively, resulting in restricted plant growth. Under high irradiance conditions, photoinhibition takes place: the photosynthetic apparatus absorbs excessive light energy, resulting in the inactivation or impairment of the chlorophyll-containing reaction centers of chloroplasts and consequent depression of photosynthetic activity (Bertaminia *et al.*, 2006; Chen and Murata, 2011) In contrast, under low irradiance conditions, insufficient ATP is produced to allow for carbon fixation and carbohydrate biosynthesis. This leads to a reduction in plant growth.

Therefore, study is needed to understand the physiology of cardamom yield under various shade level. Under the fast changing cardamom growing areas in western Ghats climate such ecophysiological studies would be of immense use to manage and to safe guard the cardamom agroforestry system in a rational way. The objectives of the present study were to quantify the influence of different shade levels on photosynthetic characteristics, stomatal activity, and physiology of small cardamom, to determine optimum light intensity for plant growth, and to consequently expand our understanding on its shade-adaptation mechanism. This may help in establishing a sound foundation for improving the cultivation and breeding of this important plant species.

Materials and Methods

Plant Materials and Growth Conditions

Small cardamom plants (Variety: Appangala-1) were obtained from ICAR-Indian Institute of Spices Research, Regional station and were maintained in an open field, Appangala, Kodagu district, Karnataka (12°23'19"N 75°42'59"E), at an altitude of 920 meters above mean sea level and the average rainfall is 2783 mm and annual average temperature was 20.5°C during 2016-2018. Plants were subjected to three different shade treatments for 18 months, starting from August, 2016. Treatments consisted of 75% and 50% shade levels and open condition (natural irradiance), and shades were created by using green colour commercial plastic shading nets. Each treatment involved seven replications (Figure 1). All plants were well-irrigated and protected from pathogens, pests and weed competition.

Growth characteristics

Plants were allowed to grow up to 18 months, after which observations on number of shoots, leaf length and breadth and specific leaf weight were recorded.

Physiological observations

Photosynthetic Parameters: Photosynthetic parameters were recorded using a portable photosynthetic system (LCpro-SD Advanced Photosynthesis Measurement System, England). The parameters measured were net photosynthetic rate (P_n , $\mu\text{mol m}^{-2} \text{s}^{-1}$), stomatal conductance (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$), Photosynthetic photon flux density (PPFD, $\text{mmol m}^{-2} \text{s}^{-1}$), intercellular CO_2 concentration (C_i , $\mu\text{mol mol}^{-1}$) and transpiration rate. During the treatment period, data were recorded between 9:30 and 11:30 am. The measurements were recorded when both P_n and g_s were stable. Five representative plants were randomly selected from each treatment for recording the above parameters.

Chlorophyll fluorescence: Chlorophyll fluorescence of the same leaves used for determination of photosynthetic parameters was measured with a chlorophyll flurometer (Os-30p) in 10-15 minutes dark adapted leaves. The maximum PS II quantum yield (F_v/F_m) was determined in dark-adapted leaves between 9:30 and 11:30 am local time according to Strasser *et al.* (1995). Gas exchange and chlorophyll fluorescence were analyzed on a typical sunny day. All measurements were performed on the attached fully expanded leaves with five plants per treatment.

Biochemical Assays

Total chlorophyll and carotenoid contents were estimated by adopting the method of Yang *et al.* (2014) and expressed as mg g^{-1} of fresh weight. The total phenolic content of the leaves extract was determined by the Folin–Ciocalteu method (Kaur and Kapoor, 2002). The total phenolic content was calculated from the calibration curve, and expressed as $\mu\text{g g}^{-1}$ of fresh weight. Proline content of the leaf sample was estimated by the method of Bates *et al.* (1973) and expressed as $\mu\text{g g}^{-1}$ of fresh weight. Leaf epicuticular wax (EW) content was determined in leaves using the method described by Ebercon *et al.* (1977) and the content was expressed in $\mu\text{g dm}^{-2}$.

Statistical analysis:

Data was subjected to analysis of variance and Duncan's multiple range tests was used to differentiate means as described by Duncan (1955). Values were considered at a significance level of 95% ($p < 0.05$). Statistical analyses were performed using WASP-Web Agri Stat Package 2.0.

Result and Discussion

Shade treatment significantly enhanced plant height in 75% shade (Fig 1B); however, shade treatment had no significant effect on leaf length and width (Fig 1B). Shade treatment significantly reduced specific leaf weight in 75% shade (Fig 1C), total number of tillers (Fig 1D) but had no significant effect on the number of productive tillers (Fig 1D). These results suggest that open treatment significantly reduced plant fitness, suggesting that high light intensity might not be a suitable habitat for small cardamom. This may explain why the main distribution habitats of small cardamom in Western Ghats are in shaded environment, rather than habitats with open environment.

Stomata have dramatic effect on photosynthesis (Pan *et al.*, 2008). The present results demonstrated that number of opened stomata in plants grown under 75% shade treatment was

greater than that of the plants grown under open condition (Fig. 2A). In the present study, high photon flux densities might limit photosynthesis through high irradiance and thermal damage resulting in stomatal closure. Stomatal pore features were significantly affected by irradiance. Plant stomata developed under high irradiance had better closing ability when stressed than plants grown under low irradiance, thus allowing them to retain more water.

Leaf chlorophyll content is an important determinant of photosynthetic rate (Mao *et al.*, 2007) and dry matter production (Ghosh *et al.*, 2004). Naidu *et al.* (1984) have suggested that reduced rates of photosynthesis may be due to reduced levels of chlorophyll, particularly Chl a, which is more directly involved in determining photosynthetic activity (Sestak, 1996). Decreases in Chl a and Chl b contents may thus reflect destruction of pigments by excessive irradiance under high irradiance condition. We observed significant variation in chlorophyll content under different shade levels. Chl a, Chl b and total Chl contents increased and total phenol content decreased in shade treatments (Fig 3A and 3B). The highest Chl a, Chl b, and total Chl contents were observed in plants grown under 75% shade and highest total phenol content was observed in plants under open condition. Shade significantly increased proline content and decreased epicuticular wax content (Fig 3C and 3D). The highest proline was observed in 50% shade.

During day time, the Photosynthetic photon flux density (PPFD) ranged from 900 to 1700, 500 to 850 and 250 to 600 $\text{mmols m}^{-2} \text{sec}^{-1}$ under open, 50% shade and 75% shade level respectively (Fig 4). Photosynthetic rate (P_n) varied significantly among shade treatments (Figure 4 and 5A). The P_n value was highest in 75% shade treatment, followed (in descending order) by 50% shade treatment. Stomatal conductance (g_s) also varied significantly among various shade levels (Fig 5B). g_s values of plants grown under open condition were lower and displayed slight decrease in 50% shade. The highest g_s values were observed under 75% shade. C_i decreased significantly in 50% shade compared to other treatments and there was no significant difference in C_i between open and 75% shade treatment (Fig 5C). Present study indicated that the C_i and P_n in leaves showed negative relationship in open and 50% shade, while it showed positive relationship in 75% shade suggesting that contribution of C_i on P_n may vary in different shade levels (Fig 5A and 5C) (Alagupalamuthirsolai *et al.*, 2018). At the same time, g_s decreased significantly in plants grown under open condition compared to other treatments (Figure 5B). Under the high light environment, the observed reductions in g_s . G_s indicate that stomatal closure was due to light saturation and functioned to decrease water loss.

Transpiration rate (T_r) and leaf temperature were varied significantly among shade levels (Fig 5D and 7A). The highest T_r and leaf temperature were observed in 75% shade and 50% shade treatments respectively. When net CO_2 assimilation became light saturated, transpiration constantly decreased with the increased photosynthetic photon flux density (Schapendonk *et al.*, 1997). Thus under light limitation ($<300 \text{ mols m}^{-2} \text{sec}^{-1}$) plants also exhibit a similar behavior as that in high light condition ($>900 \text{ mols m}^{-2} \text{sec}^{-1}$), that is, closing stomata due to water saturation to adapt to low light (Fig 6).

Chlorophyll fluorescence measurement is a mainstay of studies of photosynthetic regulation and plant responses to the environment because of its sensitivity, convenience, and nonintrusive characteristics (Schreiber *et al.*, 1995 and Rascher *et al.*, 2000). In present study, the plants under open condition showed a significant reduction in maximum PSII efficiency (Fv/Fm) (Fig. 7B). Highest Fv/Fm was observed under 50% shade indicates that these plants were able to effectively reduce irradiance heat and efficiently utilize the energy absorbed by PSII antenna pigments (Guo *et al.*, 2006 and Shao *et al.*, 2014).

Conclusions

Our study, which observed photosynthetic characteristics associated with light stress sensitivity in small cardamom, has contributed to an understanding of this species shade tolerance. Our results explained that shading is essential for its normal growth, although different degrees and durations of shading treatments significantly influence photosynthetic activity, chlorophyll and epicuticular wax content, chlorophyll fluorescence, biochemical traits. Plants under open condition (PPFD, 900 to 1600 mmol m⁻² s⁻¹) suffer photoinhibition because of excess light exposure, whereas those grown under 50 to 75% shade (300 to 900 mmol m⁻² s⁻¹) showed higher photosynthetic activity and when plants receive PPFD less than 250 mmol m⁻² s⁻¹, they suffer from light deficiency. This study indicated that small cardamom may adapt to shade conditions through increased plant height, chlorophyll and proline contents and higher Photosystem II and stomatal activity. This information will be of immense use for cardamom growing ecosystem as the present knowledge level on ecological niche for cardamom is limited.

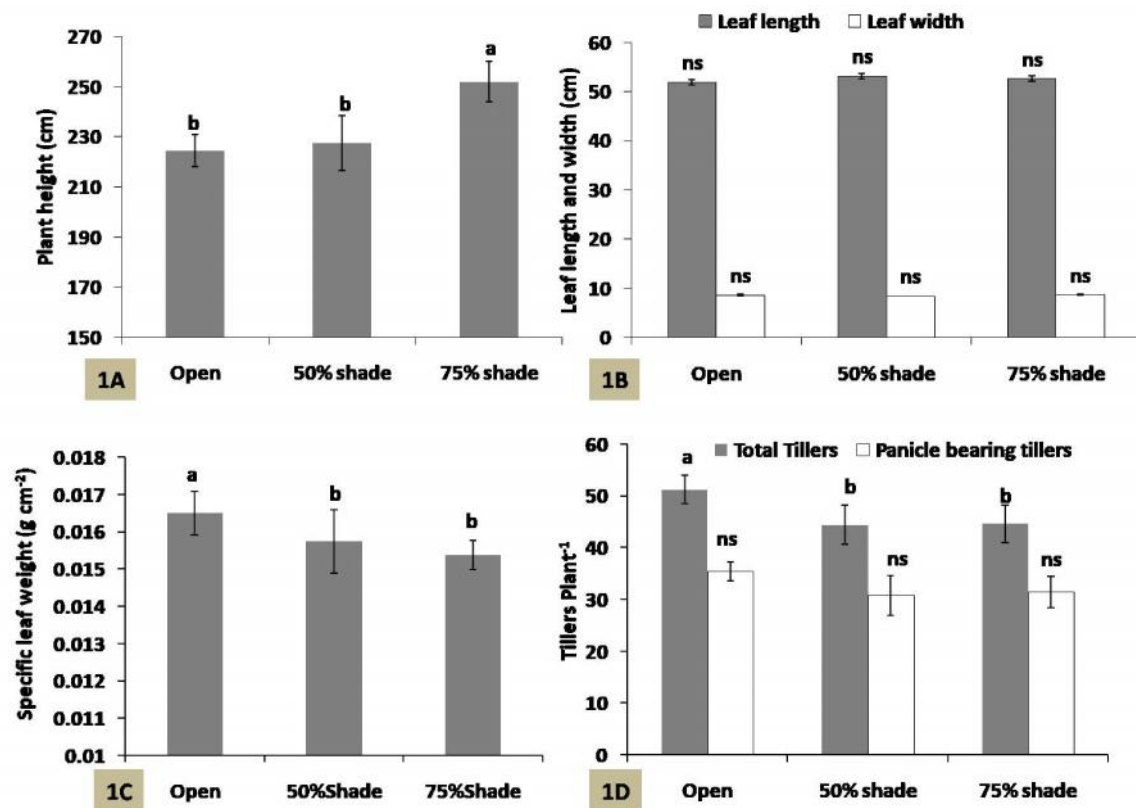
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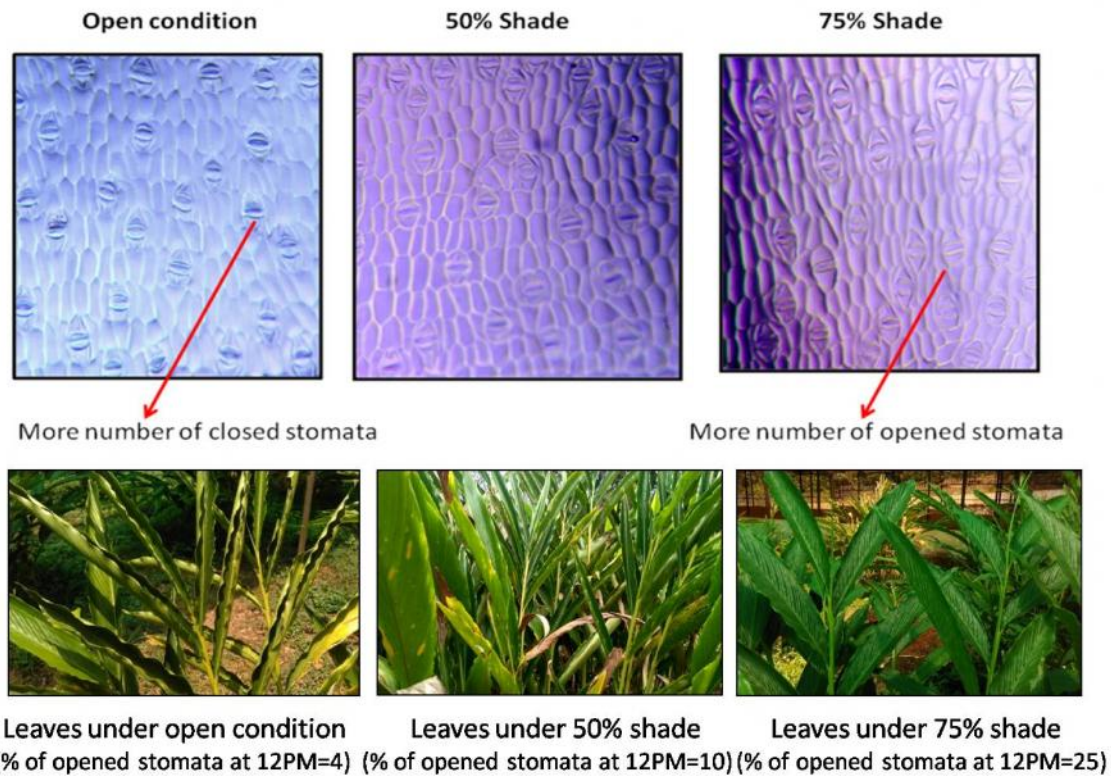
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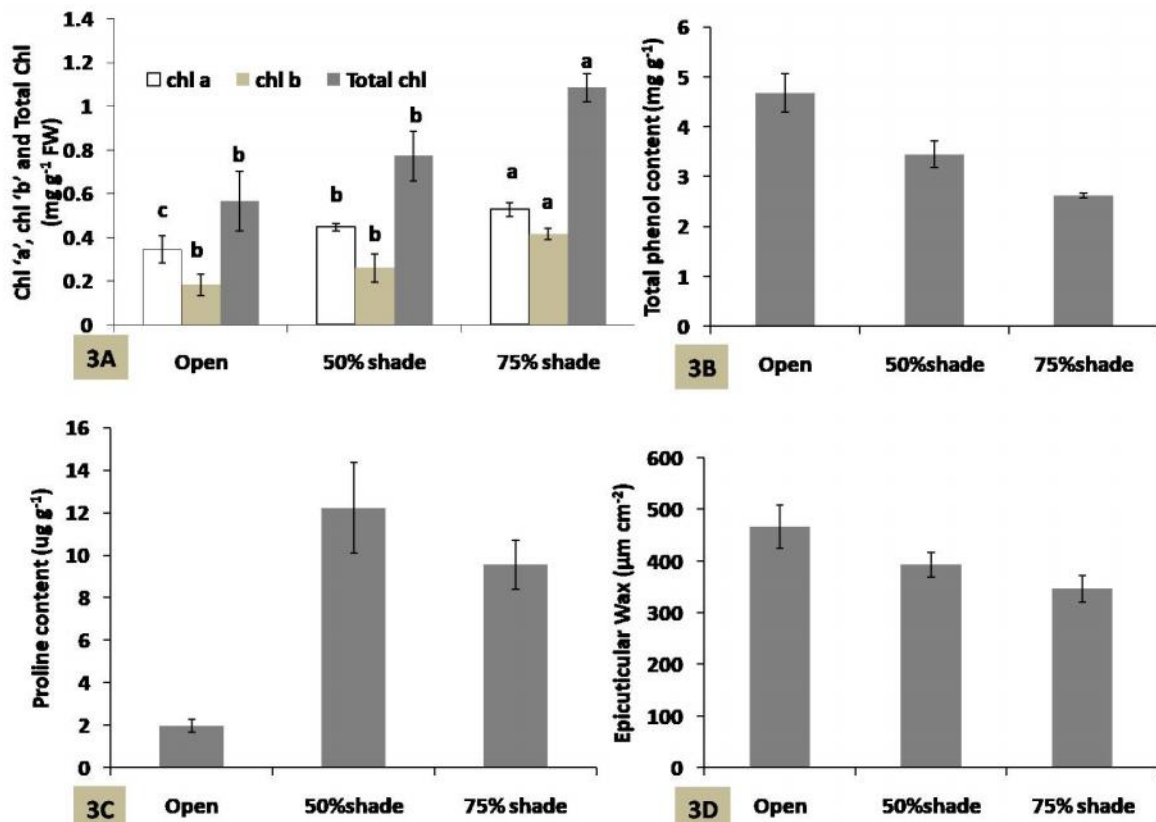
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2A Status of stomata under different shade level during Mid day



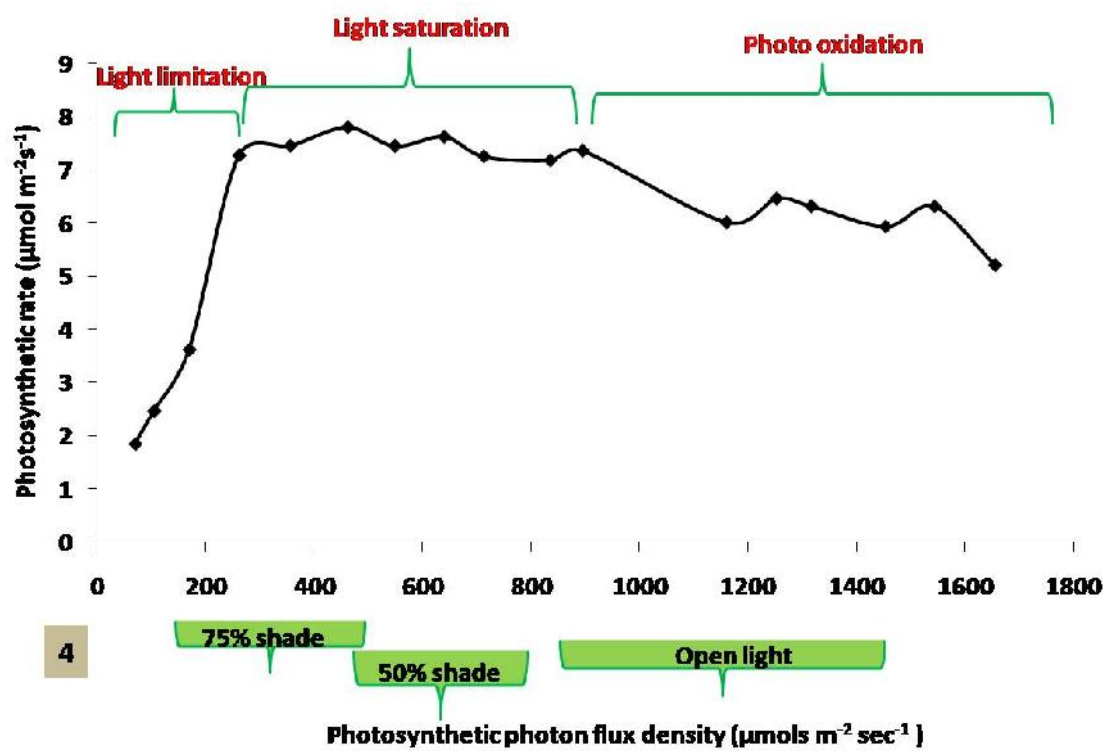
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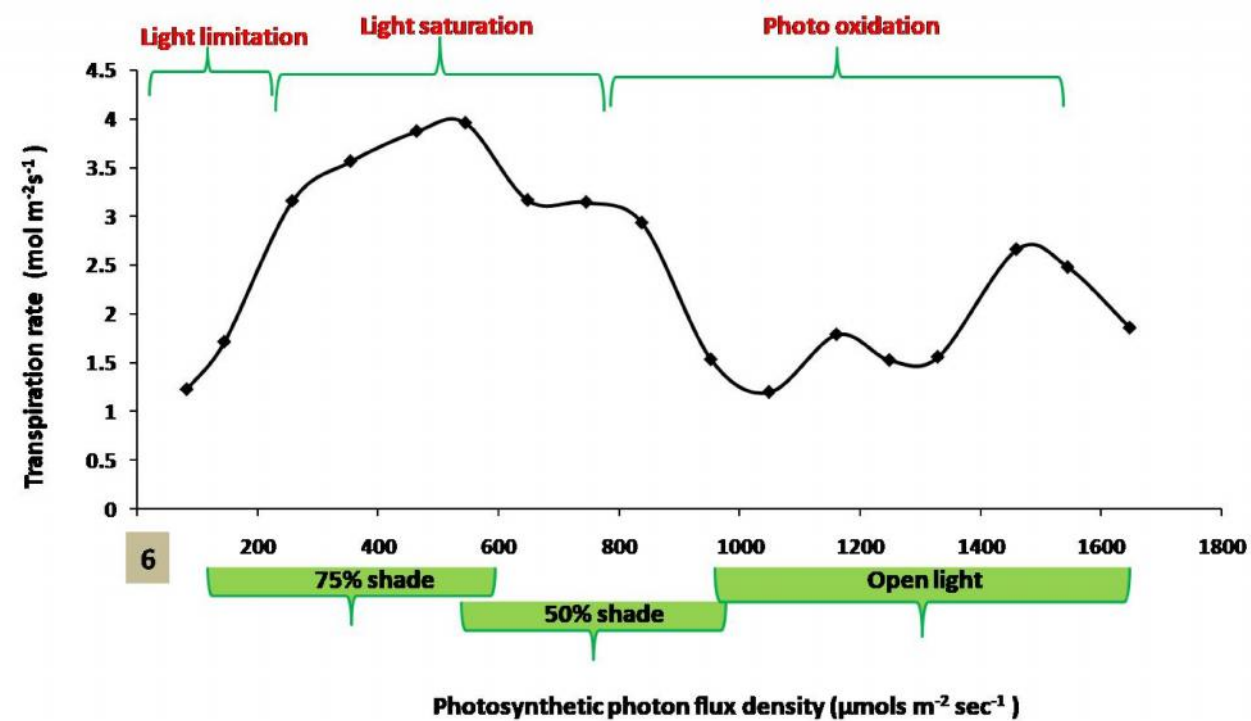
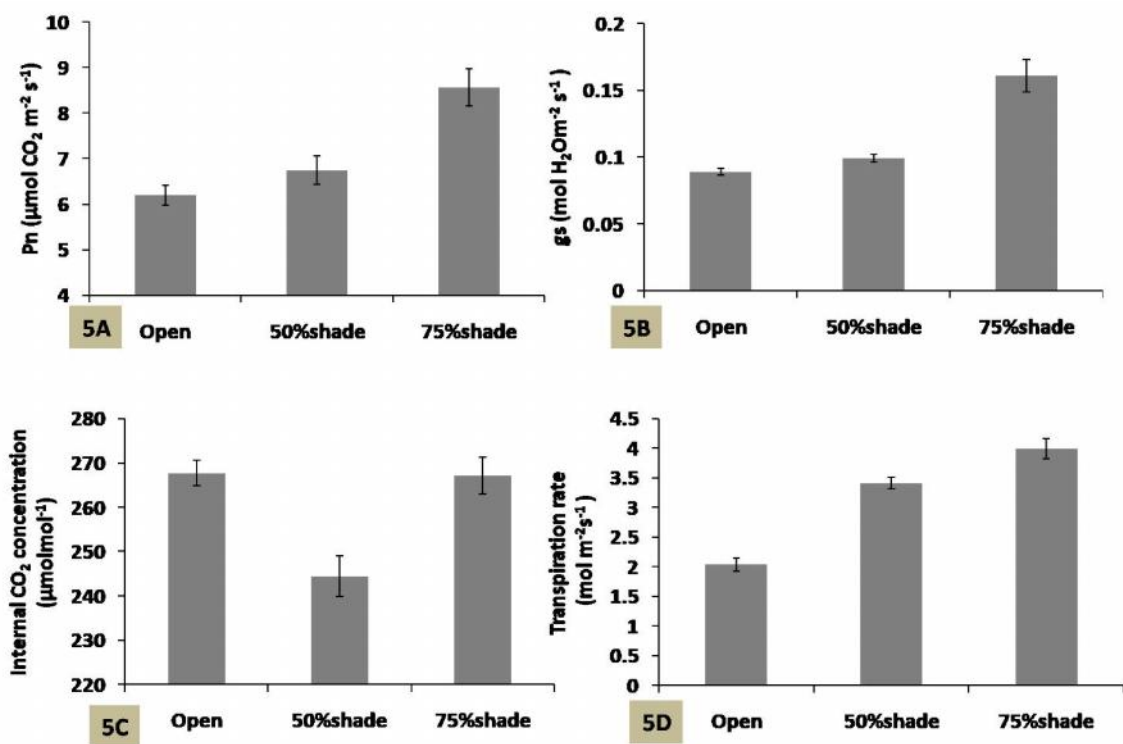
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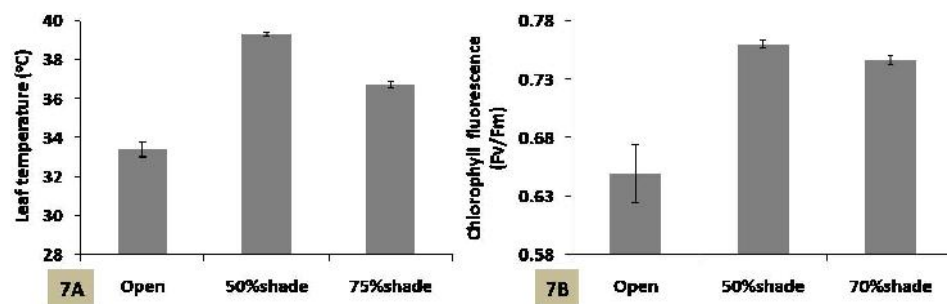
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