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

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23 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. The 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 essential role in shade adapting mechanism in small cardamom.

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

39 **Introduction**

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

45 The principle of shade regulation in cardamom has been in practice for decades. The
46 complex tropical forest has been converted to a more open, simple and uniform system and
47 hence leading to degradation of cardamom agroforestry system in India (Muruganet *al.*,
48 2009). Reduced soil water content can decrease the yield of cardamom significantly during
49 summer in the months of April and May. The number of flowers and capsules formed and the
50 percentage of capsule set per plant were also reduced to 30% in summer and 80% in August
51 or September. Synchronous soil and atmospheric warming of cardamom hills (Murugan *et*
52 *al.*, 2008) is evident which can adversely impact soil nutrient dynamics particularly major
53 nutrients (nitrogen, phosphorus and potassium) leading to unavoidable yield reduction.

54 Light is a most important factor influencing plant growth, morphology, anatomy,
55 various aspects of physiology, cellular biochemistry, flowering time and plant productivity
56 (Dai *et al.*, 2009; Favaretto *et al.*, 2011; Deng *et al.*, 2012). In the light reactions of
57 photosynthesis, light energy is used to produce ATP and NADPH, which are then used for
58 carbon fixation and production of oxygen during the light-independent phase. Shading has a
59 significant impact on plant photosynthesis. Normal plant growth needs optimal light
60 irradiance because excessively high and low irradiance would result in photoinhibition and
61 light deficiency respectively, resulting in restricted plant growth. Under high irradiance
62 conditions, the photosynthetic apparatus absorbs excessive light energy, resulting in the
63 inactivation or impairment of the chlorophyll-containing reaction centers of chloroplasts and
64 consequent reduction of photosynthetic activity (Bertaminiaet *al.*, 2006; Chen and Murata,
65 2011).In contrast, under low irradiance conditions, insufficient ATP is produced to allow for
66 carbon fixation and carbohydrate biosynthesis. This leads to a reduction in plant growth.

67 Therefore, an investigation is necessary to understand the physiology of cardamom
68 yield under various shade level. Under the fast changing climate conditions of cardamom
69 growing areas in Western Ghats, ecophysiological studies would be of immense use to
70 manage and safeguard the cardamom agroforestry systems. The objectives of the present
71 study were 1) to quantify the influence of different shade levels on photosynthetic
72 characteristics, stomatal activity, and physiology of small cardamom, 2) to determine
73 optimum light intensity required for plant growth, and 3) to understand cardamom plant's
74 shade-adaptation mechanism. The present investigation may also help in establishing
75 improved cultivation practices for cardamom and future crop improvement practices of this
76 economically viable condiment.

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80 **Materials and Methods**

81 **1. Plant Materials and Growth Conditions**

82 Small cardamom plants (Variety: Appangala-1) were obtained from ICAR-Indian
83 Institute of Spices Research, Regional station and were maintained in an open field,
84 Appangala, Kodagu district, Karnataka (12°23'19"N 75°42'59"E), at an altitude of 920
85 meters above mean sea level. The observed average rainfall in experimental location was
86 accounted to 2783mm with an average temperature of 20.5°C during 2016-2018. Plants were
87 subjected to three different shade treatments for 18 months, starting from August, 2016.
88 Treatments consisted of 75% and 50% shade levels and open condition (natural irradiance),
89 and shades were created by using green colour commercial plastic shading nets (Prathu Agro
90 company, Coimbatore, India). Per cent interception of light in the canopy was calculated
91 (middle point of a slope) by comparison with a Photometer (Luxor, USA) placed in the open
92 field situation. The measurement was made between mid-day and 2.00 pm as per the method
93 suggested by Nelli *et al.*, (1974).

94 Light interception = (Mean of light intensity at the middle of the canopy and ground
95 level / Light intensity in the open) X 100

96 Each treatment involved seven replications. All plants were well-irrigated and protected
97 from pathogens, pests and weed competition.

98 **2. Observations on plant growth characteristics**

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

101 **3. Physiological observations**

102 **i. Photosynthetic Parameters:**

103 Photosynthetic parameters were recorded using a portable photosynthetic system
104 (LCpro-SD Advanced Photosynthesis Measurement System, England). The parameters
105 measured were net photosynthetic rate (P_n , $\mu\text{mol m}^{-2} \text{s}^{-1}$), stomatal conductance (g_s , mol m^{-2}
106 s^{-1}), Photosynthetic photon flux density (PPFD, $\mu\text{mol m}^{-2} \text{s}^{-1}$), intercellular CO_2 concentration
107 (C_i , $\mu\text{mol CO}_2 \text{mol}^{-1}$) and transpiration rate (T_r , $\text{mmolH}_2\text{O m}^{-2} \text{s}^{-1}$). During the treatment
108 period, data were recorded between 9:30 and 11:30 am. The measurements were recorded
109 when both P_n and g_s were stable. Five representative plants were randomly selected from
110 each treatment to record the above parameters.

112 **ii. Chlorophyll fluorescence:**

113 Chlorophyll fluorescence, of the same leaves that were used for determination of
114 photosynthetic parameters, was measured with a chlorophyll fluorometer (Os-30p) in 10-15
115 minutes dark adapted leaves. The maximum PS II quantum yield (F_v/F_m) was determined in
116 dark-adapted leaves between 9:30 and 11:30 am local time according to Strasser *et al.* (1995).
117 Gas exchange and chlorophyll fluorescence were analyzed on a typical sunny day. All

measurements were performed on the attached fully expanded leaves of five plants per treatment.

iii. Biochemical Assays

Total chlorophyll and carotenoid contents were estimated by adopting the method of Arnon, 1949 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 $\mu\text{g dm}^{-2}$.

4. Statistical analysis:

Data was subjected to analysis of variance and Duncan's multiple range test 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 1A); 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), a total number of tillers (Fig 1D) but had no significant effect on the number of productive tillers (Fig 1D) (Table 1). These observed 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 the Western Ghats are in the shaded environment, rather than habitats with an open environment.

Stomata have a dramatic effect on photosynthesis (Pan *et al.*, 2008). The present results demonstrated that a 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 (Taye Kufa and Jurgen Burkhardt, 2011).

Leaf chlorophyll content is an important determinant of photosynthetic rate (Mao *et al.*, 2007) and dry matter production (Ghoshet *al.*, 2004). Naidu *et al.* (1984) 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). A decrease in Chl a and Chl b contents may thus reflect the destruction of pigments by excessive irradiance under high irradiance open 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 75% shade treatments (Fig 3A and 3B and Table 2). 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 grown in 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 daytime, 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). Net photosynthetic rate (Pn) varied significantly among shade treatments (Figure 4 and 5A). The Pn 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). Stomatal conductance (g_s) values of plants grown under open condition were lower and displayed a slight decrease in 50% shade. The highest g_s values were observed under 75% shade. The intracellular carbon dioxide concentration (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). The present study indicated that the C_i and Pn in leaves showed the negative relationship in open and 50% shade, while it showed positive relationship in 75% shade suggesting that contribution of C_i on Pn may vary in different shade levels (Alagupalamuthirsolai *et al.*, 2018) (Fig 5A, 5C and Table 3). 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 , indicate that stomatal closure was due to light saturation and resulting in reduced water loss.

Transpiration rate (Tr) and leaf temperature were varied significantly among shade levels (Fig 5D and 7A). The highest Tr 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 an increase in PPFD (Schapendonk *et al.*, 1997). Thus, under light limitation ($<300 \text{ mols m}^{-2} \text{sec}^{-1}$) plants also exhibit a similar behavior of adaptation to escape from adverse climatic conditions as that in high light condition ($>900 \text{ mols m}^{-2} \text{sec}^{-1}$), by closing stomata due to water saturation, thereby adapting to a low light situation (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 Rascheret *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 utilise the energy absorbed by PSII antenna pigments (Guo *et al.*, 2006 and Shao *et al.*, 2014).

Conclusions

The observed photosynthetic characteristics associated with light stress sensitivity in small cardamom, has contributed to an understanding of *E. cardamomum* species shade tolerance. The results clearly 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 and 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 received PPFD less than 250 mmol m⁻² s⁻¹, they suffered 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 an ecological niche for cardamom is limited. The future line of research is to continue study reproductive biology and yield of small cardamom under different shade level to understand the response of small cardamom to shade.

Consent & Ethical: NA

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Treatments	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	SLW (g cm ⁻²)	Total Tillers plant ⁻¹	Panicle bearing tillers plant ⁻¹
Open irradiance	224.4 ^b	51.8 ^{ns}	8.61 ^{ns}	0.016 ^a	52.1 ^a	35.2 ^{ns}
50% shade	227.6 ^b	53.1 ^{ns}	8.46 ^{ns}	0.015 ^b	44.0 ^b	31.2 ^{ns}
75% shade	252.0 ^a	52.6 ^{ns}	8.69 ^{ns}	0.015 ^b	43.0 ^b	31.7 ^{ns}
CV%	8.477	6.07	5.44	4.125	14.16	19.37

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Table 1: The effects of different shade on growth characters of small cardamom.

Values represent mean of seven replications. Same letter within each column indicate no significant differences among the treatments (P≤0.05).

Treatments	Chl. 'a' (mg g ⁻¹)	Chl. 'b' (mg g ⁻¹)	Total chl. (mg g ⁻¹)	Total phenol (mg g ⁻¹)	Proline content (μg g ⁻¹)	ECW (μg cm ⁻²)

effects of different shade on biochemical characters of small cardamom.

Open irradiance	0.345 ^c	0.183 ^b	0.566 ^b	4.67 ^a	1.97 ^b	466.8 ^a
50% shade	0.448 ^b	0.260 ^b	0.772 ^b	3.44 ^b	12.2 ^a	392.9 ^b
75% shade	0.527 ^a	0.415 ^a	1.08 ^a	2.61 ^c	9.55 ^a	345.8 ^b
CV%	11.57	18.09	21.78	12.38	34.67	10.63

Values represent mean of seven replications. Same letter within each column indicate no significant differences among the treatments ($P \leq 0.05$).

Table 3: The effects of different shade on photosynthetic gas exchange of small cardamom.

Treatments	Pn ($\mu\text{mol CO}_2$ $\text{m}^{-2} \text{s}^{-1}$)	gs ($\text{mol H}_2\text{O}$ $\text{m}^{-2} \text{s}^{-1}$)	Ci ($\mu\text{mol mol}^{-1}$)	Tr ($\text{mol m}^{-2} \text{s}^{-1}$)	Leaf temperature ($^{\circ}\text{C}$)	Chlorophyll fluorescence (Fv/Fm)
Open irradiance	6.20 ^b	0.089 ^b	267.7 ^a	2.04 ^c	33.4 ^c	0.649 ^c
50% shade	6.74 ^b	0.099 ^b	244.4 ^b	3.41 ^b	39.3 ^a	0.760 ^a
75% shade	8.56 ^a	0.161 ^a	267.1 ^a	3.99 ^a	36.7 ^b	0.746 ^b
CV%	14.81	15.97	5.11	15.43	5.16	4.45

Values represent mean of seven replications. Same letter within each column indicate no significant differences among the treatments ($P \leq 0.05$).

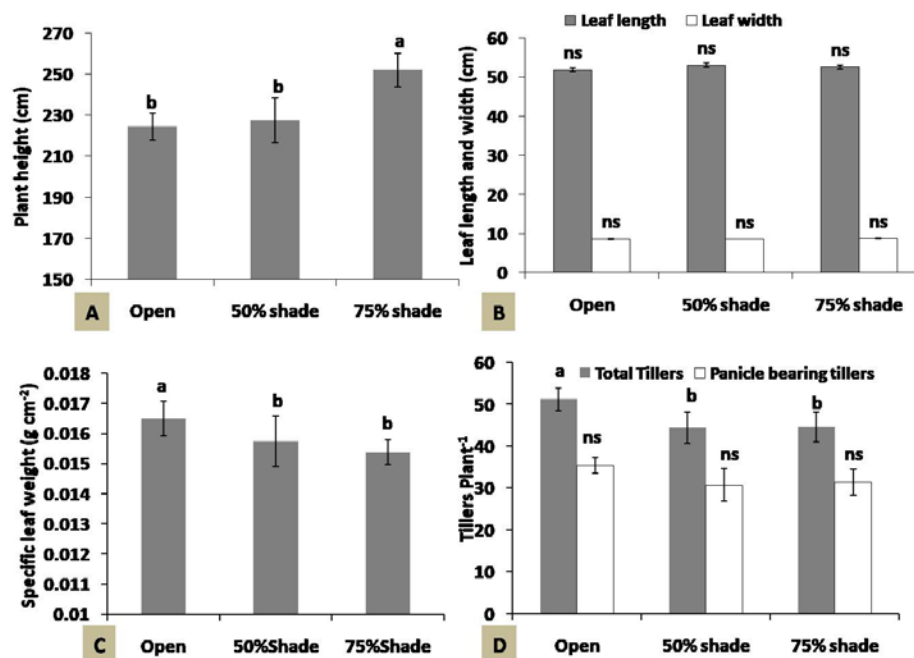


Figure 1. Plant height (A), leaf length and width (B), specific leaf weight (C) and tillers plant⁻¹ (D). The values represented mean $\pm$ SE, and different letters mark significant differences among shade treatments on the same day ($P < 0.05$).

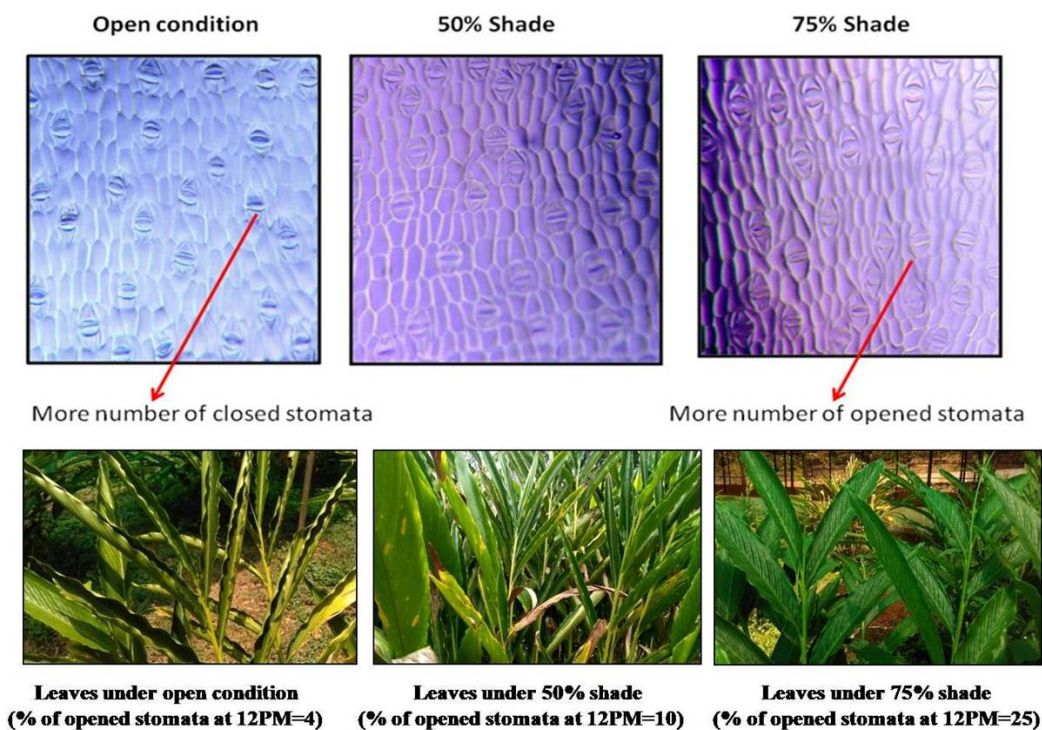


Figure 2: Status of stomata under different shade level during mid day

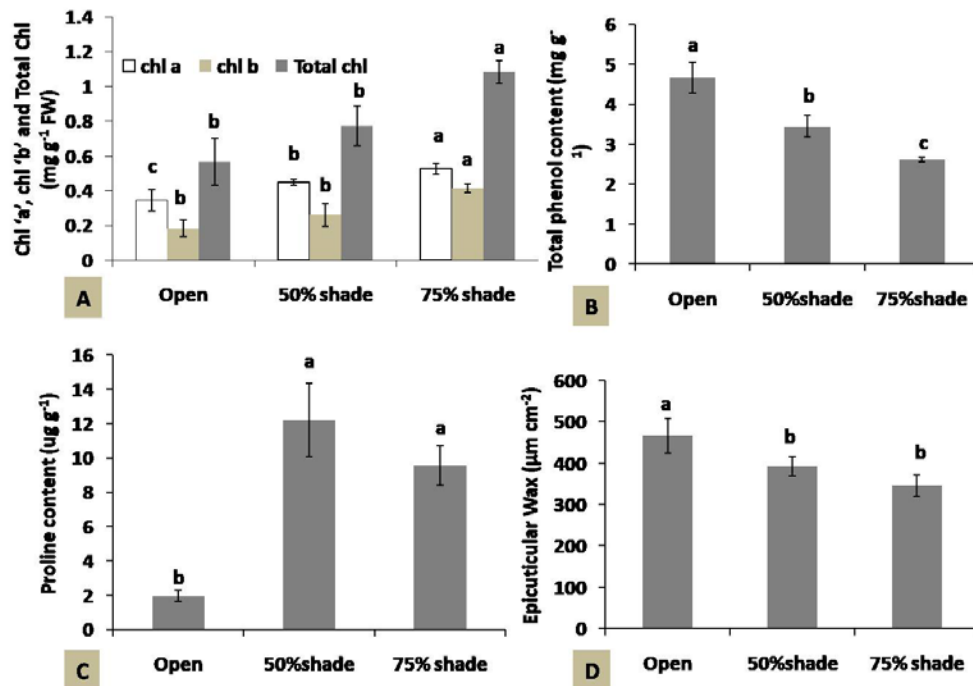


Figure 3. Chlorophyll 'a', 'b' and total chlorophyll content (A), total phenol content (B), proline content (C) and epicuticular wax content (D). The values represented mean $\pm$ SE, and different letters mark significant differences among shade treatments on the same day ($P < 0.05$).

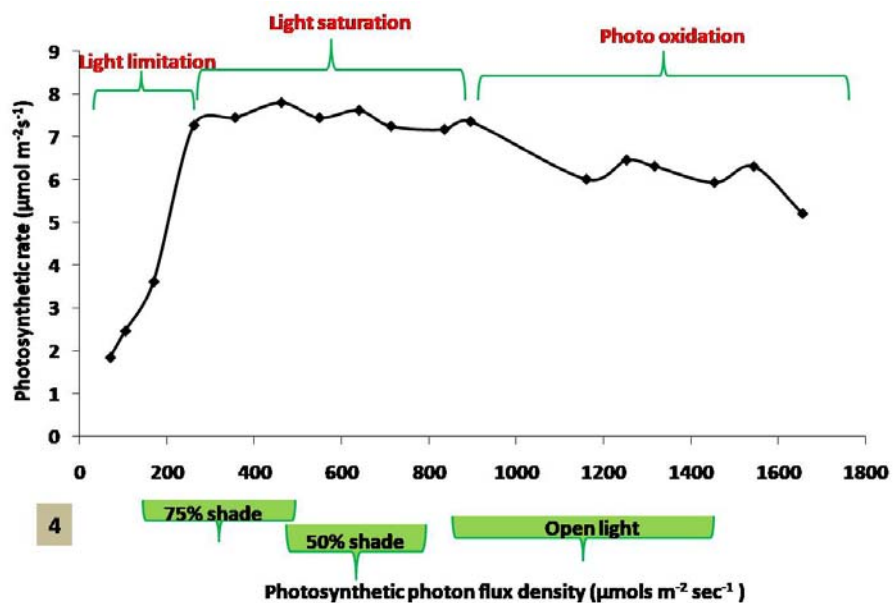


Figure 4. Photosynthesis light responsive curve of small cardamom

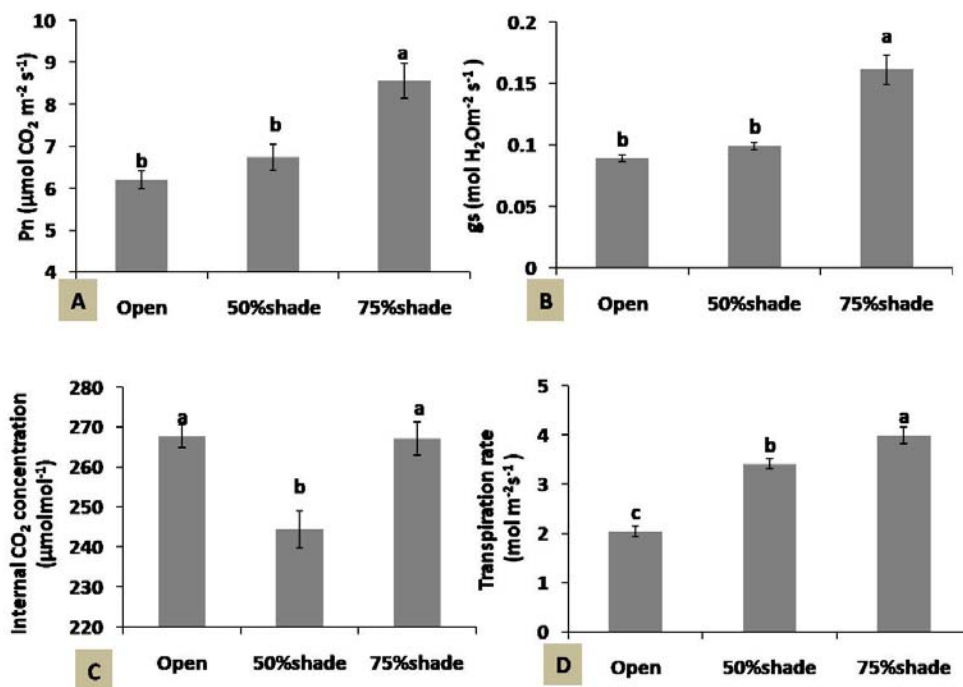


Figure 5. Net photosynthetic rate (Pn) (A), stomatal conductance (gs) (B), intercellular CO₂ concentration (Ci) (C) and transpiration rate (Tr) (D). The values represented mean $\pm$ SE, and different letters mark significant differences among shade treatments on the same day ($P < 0.05$).

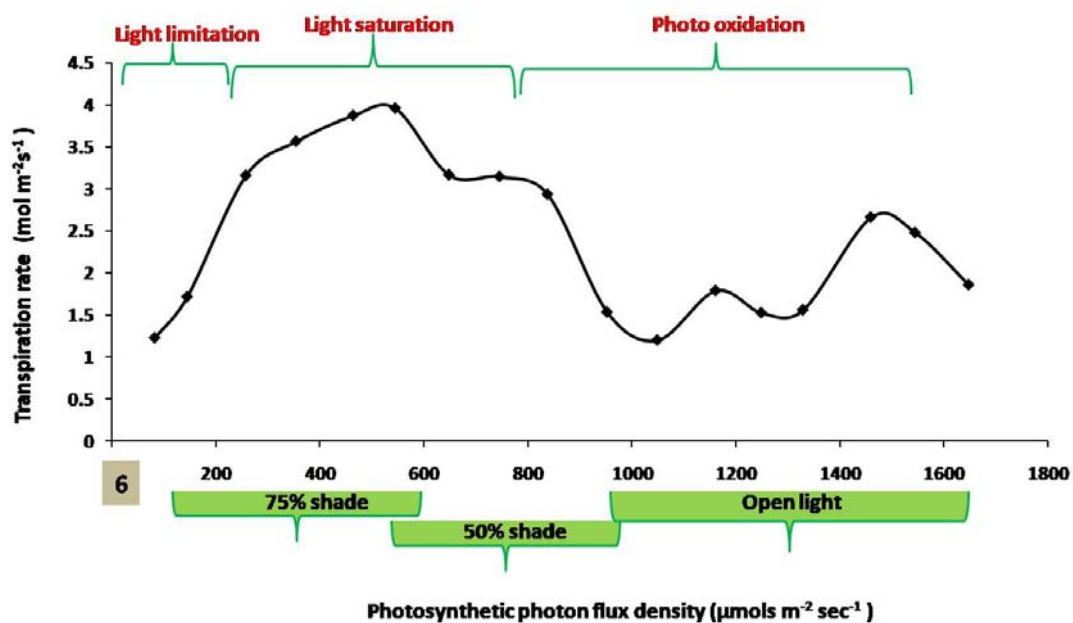


Figure 6. Transpiration light responsive curve of small cardamom

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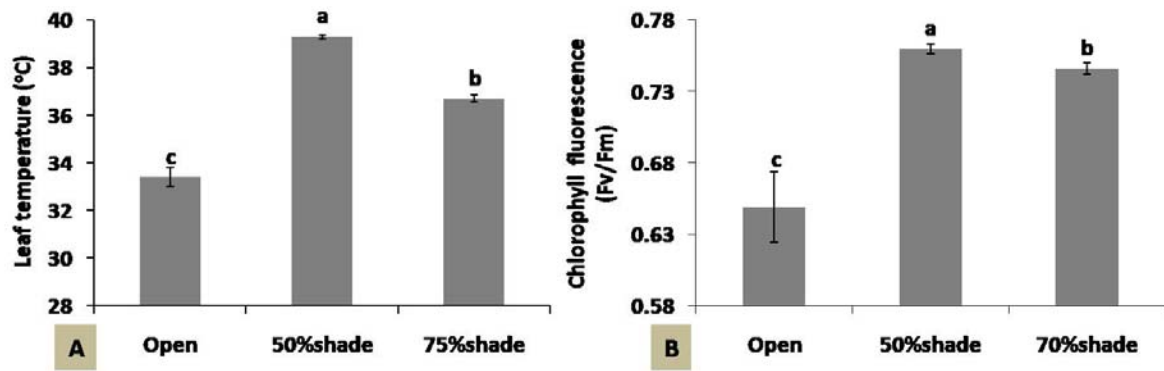


Figure 7. Leaf temperature (A), Chlorophyll fluorescence (B). The values represented mean $\pm$ SE, and different letters mark significant differences among shade treatments on the same day ($P < 0.05$).

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