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Abstract
Gas exchange and chlorophyll fluorescence quenching analysis were carried out on sunny days during summer to investigate the environmental and physiological factors limiting carbon gain in small cardamom canopies. Leaf net photosynthetic rate showed a unimodal diurnal pattern and midday depression of photosynthesis was observed at 12:00 h. Depression in photosynthesis at midday was mostly attributed to significant reductions in stomatal conductance and increase in intracellular CO_2 concentration. Midday depression in photosynthesis was found to be associated with decrease in maximum PSII efficiency (F_v/F_m) (reversible inactivation of photosystem II reaction centers) and increases of minimal fluorescence (F_o) in response to the high light intensity (PPFD). It is concluded that the decline of leaf stomatal conductance (gs) and reversible inactivation of photosystem II reaction centers under high light intensities in summer is an important factor contributing to midday depression of photosynthesis in small cardamom.
***Keywords**: Elettaria cardamomum Maton; Photoinhibition; Photosynthesis; Chlorophyll fluorescence; Stomatal limitation*

Introduction

Small Cardamom (*Elettaria cardamomum* (L.) Maton, Zingiberaceae), an shade loving understorey 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 fruits. Cardamom has C_3 photosynthetic pathway (Murugan *et al*., 2008).

Agroforestry systems can bring about favorable changes in the microclimatic conditions by influencing light intensities, air temperature and relative humidity all of which will have a significant impact on modifying the rate and duration of photosynthesis, transpiration and stomatal conductance (Monteith *et al*., 1991 and Murugan *et al*., 2009). The knowledge of the physiological characteristics of understorey crop plants is essential to our understanding of the functioning and effective management of agroforestry systems. Much of research, to date, has focused on the impacts of shade and light intensity on annual crops and very little on perennial agroforestry systems which are most unique systems in the world.

Photosynthesis, is the process by which plants use light energy to drive the synthesis organic compounds, is essential for plant growth. Under field conditions, the photosynthetic apparatus are exposed to variable intensities of light, temperature and humidity that may result in a typical midday depression of CO_2 assimilation or photoinhibition of photosynthesis (Hirasawa and Hsiao, 1999). The midday depression of photosynthesis is a common phenomenon for many C_3 plants, including Citrus (Hu *et al*., 2008), Grapevine (M. Popescu and G. C. Popescu, 2014) and Greengram (Alagupalamuthirsolai and Mudit, 2015). Several study shown that Stomatal closure is partially responsible for the reduction of photosynthetic rate (Kamakura *et al*., 2011) and meanwhile some study reported that the photoinhibition and increase of photorespiration also responsible for midday depression of photosynthesis in leaves (Guo *et al*., 1994 and Debabrata Panda, 2011).

The aim of this study was to elucidate how the midday depression in CO_2 assimilation (photosynthesis) is related to variable intensities of light, temperature and humidity. Earlier studies have indicated that temporal variation in photosynthesis observed in small cardamom may either be associated with increase in light intensities (Murugan *et al*., 2008).In the present study, we have attempted to analyze the relative contribution of light in midday depression using gas exchange techniques and portable fluorometer, which is capable of true *in situ* measurements under field conditions. The present study will provide new insights into finding diurnal patterns of photosynthesis, maximum efficiency PSII centre (F_v/F_m) and stomatal status in leaves of cardamom and also enrich our knowledge about their eco-adaptation. The knowledge of the physiological characteristics of understorey crop plants is essential to our understanding of the functioning and effective management of agroforestry systems.

Materials and methods

The experiments were conducted during a warm-weather period after the south-west monsoon in the month of March, at the field of the ICAR-Indian Institute of Spices Research, Regional station, Madikeri. The study was carried out on one year old plants of Small cardamom (Variety: Appangala 1) grown in field under natural conditions. All the experiments were carried out in a 10 day period to avoid seasonal changes. Newly expanded mature leaves i.e., the third leaves were measured in experiments. Madikeri is located in the tropical rain forest in Western Ghats region of India. Its annual average temperature is 20.5^{o}C, and the annual precipitation is about 2783 mm.

Leaf gas exchange: The net photosynthesis rate (Pn), stomatal conductance (gs), Photosynthetic photon flux density (PPFD) and intercellular CO_2 concentration (Ci) were measured in the field on sun exposed leaves with a portable photosynthetic system (LCpro-SD Advanced Photosynthesis Measurement System, England) at 2 h intervals from 8:00-18:00 h local time. The measurements were recorded when both Pn and gs were stable.

Chlorophyll fluorescence: *In vivo* chlorophyll fluorescence was measured using chlorophyll flurometer (Os-30p) in 10-15 minutes dark adapted leaves. The maximum PS II quantum yield (Fv/Fm) was determined in dark-adapted leaves at 2h intervals from 8:00-18:00 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 replicates.

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

Diurnal changes in environmental variables and leaf gas exchange parameters:

Diurnal changes in environmental variables on a typical sunny day are presented in Fig. 1A. Air temperature (Ta) was 29.0^{o}C at 8:00 h and then gradually increased, reaching a maximum of 33.8 ^{o}C at 14.00 h. Photosynthetic photon flux density (PPFD) also reached its maximum (1.52 mmol m^{-2} s^{-1}) at 12:00 h. Relative humidity in atmosphere was 62% at 8.00 h and gradually decreased with time and reaching a minimum 50% at 16.00 thereafter increased and reached 71% at 18.00 h.

Leaf temperature was 33.4^{o}C and increased to 41.6^{o}C at 14.00 h and thereafter decreased with dependent of the decrease in PPFD (Fig.

1B). Leaf photosynthetic rate (Pn) showed a unimodal diurnal pattern (Fig. 1C). Leaf net photosynthetic rate (Pn) was low in the early morning, increasing with time and reaching a maximum (10.34 µmol CO_2 m^{-2} s^{-1}) at 10:00 h. Thereafter, Pn decreased independent of the increase in incident PPFD. Stomatal conductance (gs) was 0.098 mol H_2O m^{-2} s^{-1} at 8:00 h and increased to 0.225 mol H_2O m^{-2} s^{-1} at 10:00 h thereafter decreased and reaching 0.046 mol H_2O m^{-2} s^{-1} at 18:00 h (Fig. 1D).

Ci was 300.8 (ppm) at 8.00 h and decreased to lowest 269.5 (ppm) at 12.00 h and thereafter increased to highest 316.4 (ppm) at 18.00 h. Ci did not change significantly between 10:00 and 14:00 h (Fig. 1D).

Diurnal changes in chlorophyll a fluorescence parameters:

The maximum PSII efficiency (Fv/Fm) was 0.804 at morning (08.00 h), afterwards it decreased with the increase of PPFD (Fig. 2A). Although Fv/Fm fell to 0.689 at midday (12.00 h), it recovered to a value 0.803 in the evening (18.00 h). The minimal fluorescence (Fo), on the contrary, was low in the early morning, then increased with PPFD and reached its highest value 105.5 at 12:00 h and gradually decreased and reached lowest 86.2 at 18.00 h (Fig. 2B). Over the course of the sunny day the initial increase and subsequent decrease in the PPFD incident on the cardamom leaves were reflected by initial decrease and subsequent increases in PSII efficiency.

Discussion

Previous studies have shown that midday depression of photosynthesis is a common phenomenon in C_3 plants. This phenomenon is thought to be mainly caused by stomatal closure, elevated respiration and photoinhibition resulting from strong light, high temperatures and VPD in summer (Medina *et al*., 2002; Pons & Welschen, 2003; Oso´ rio *et al*., 2006).

The present study showed that midday depression of Pn and gs in cardamom occurred in summer with high PPFD, Ta under atmospheric CO_2 (Fig. 1A and 1D), which is consistent with previous observations on cardamom (Murugan *et al*., 2008) and other C_3 plant species (Pons & Welschen, 2003). Our results also showed that Pn and gs of leaves declined from 10:00 to 18:00 h (Fig. 1D). The high correlations of Pn to gs (P < 0.01) in leaves indicate that stomatal closure may play an important role in midday depression of photosynthesis in cardamom leaves rather than leaf temperature (T_l) (Table 1). Similar results have been obtained in citrus (Hu *et al*., 2008).

Although PPFD was highest at 12:00 h, a drop of photosynthesis rate was observed (Fig. 1A and 1C). Apparently, light absorbed by the plants exceeded the photo-utilization capacity in chloroplast. Mid day depression of photosynthesis, in turn, increases excessive excitation energy which may have led to the inactivation of PSII. Our results showed that the mid-depression of P_n leaves were accompanied by the significant decrease in PSII activity (Fv/Fm) (Fig. 2A).

Plants have developed several strategies to minimize the harmful effects of excess energy. Mechanisms such as xanthophylls cycle-dependent energy dissipation as heat from antenna in PSII, the D1 repair cycle, photorespiration, the operation of water–water cycle and cyclic electron flow around PSI, are recognized as important players in photoprotection (Ort and Baker, 2002).

Photorespiration has been shown to play a role in preventing the plant from photoinhibition under strong light at low CO_2 concentration since it can dissipate excessive light energy (Osmond, 1981). The maximum PSII efficiency Fv/Fm is widely used as an indicator of photoinhibition (Ort and Baker, 2002). In this study, reversible change in Fv/Fm was found during the day, suggesting that photoprotection rather than photodamage occurred. The decrease in Fv/Fm is likely to be due to the reversible inactivation or downregulation of PSII, rather than the photodamage to PSII or loss of D1 protein (Critchley and Russell, 1994; Demmig-Adams and Adams, 1996; Huang *et al*., 2006). The sharp increase in F_o at 12:00 h was likely to be caused by PSII inactivation (Demmig-Adams and Adams, 1992).

The negative relationship between C_i and photosynthesis in leaves (Fig. 1C) suggests that high C_i may have contributed to mid-depression of photosynthesis. Since the decrease in Pn at midday was accompanied by an increase in Ci, the midday depression in Pn could be attributed to the decreased photosynthetic activity of mesophyll cells. This is in agreement with an early study by Guo *et al*. (1994) who found that C_i is responsible for the decreased CO_2 assimilation in cotton during the noon.

Conclusion

Leaf net photosynthetic rate in small cardamom canopies showed a unimodal diurnal pattern. The midday depression is mainly due to decline of leaf stomatal conductance (gs) and reversible inactivation of photosystem II reaction centers under high light intensities in summer is an important factor contributing to midday depression of photosynthesis in small cardamom. Photoinhibition in the midday occurred as a photoprotective mechanism, which induced a reversible inactivation of PSII centers and increased thermal energy dissipation in the antennae. The enhancement of photorespiration consumed excess light energy, thereby avoiding the photodamage to photosynthetic apparatus.

Acknowledgments

This work was supported by the ICAR-Indian Institute of Spices Research as an institute project.

Table.1. Correlation coefficients between physiological parameters of small cardamom and the main environmental factors

	Ta	RH	PPFD	T_l	C_i	gs	Pn
Ta	-	-0.747**	0.434**	0.652**	-0.358**	0.371**	0.408**
RH	-0.747**	-	-0.393**	-0.480**	0.436**	-0.365**	-0.422**
PPFD	0.434**	-0.393**	-	0.858**	-0.814**	0.089	0.402**
T_l	0.652**	-0.480**	0.858**	-	-0.676**	0.035	0.285*
C_i	-0.358**	0.436**	-0.814**	-0.676**	-	-0.124	-0.514**
gs	0.371**	-0.365**	0.089	0.035	-0.124	-	0.895**
Pn	0.408**	-0.422**	0.402**	0.285*	-0.514**	0.895**	-

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

T_a-Air temperature (°C), RH-Relative humidity in atmosphere (%), PPFD-Photosynthetic photon flux density (mmol m^{-2} s^{-1}), T_l-Leaf temperature (^oC), C_i- intercellular CO_2 concentration (ppm), gs- Stomatal conductance (mol H_2O m^{-2} s^{-1}), Pn-Net photosynthetic rate (µmol CO_2 m^{-2} s^{-1}).

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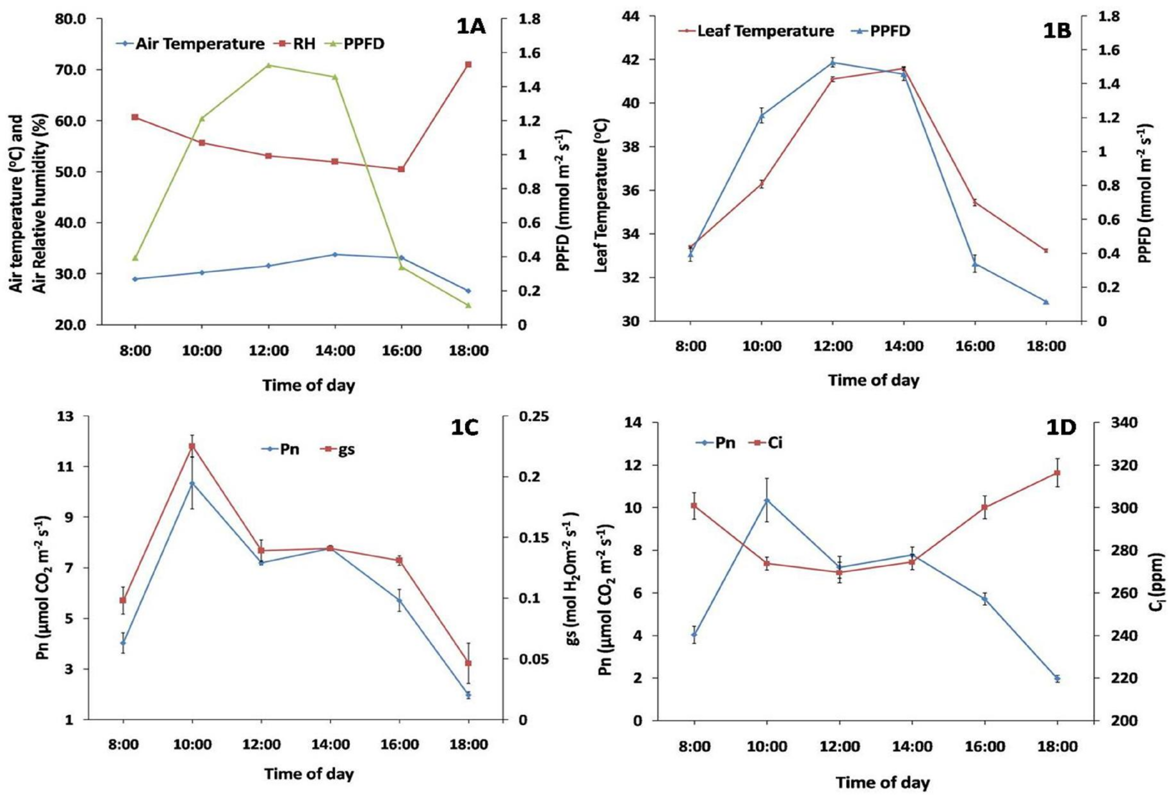

Fig. 1. Diurnal variations of photosynthetic photon flux desity (PPFD), air temperature (Ta) and relative humidity (RH) in a clear day (Fig. 1A) and Leaf temperature (T_l), Photosynthetic Photon Flux Desity (PPFD) (Fig. 1B); Net photosynthetic rates (Pn), Stomatal conductance (gs) (Fig. 1C); Net photosynthetic rates (Pn), intercellular CO_2 concentration (C_i) (Fig. 1D) in small cardamom leaves. Data are the means of five replicates with standard errors shown by vertical bars.

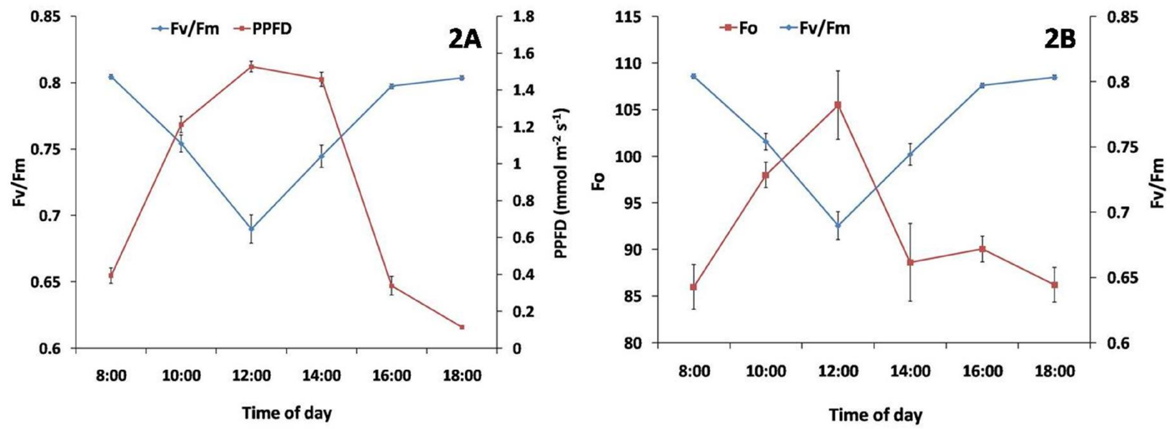

Fig. 2. Diurnal variations of maximum efficiency of PSII (Fv/Fm) (Fig. 2A) and minimal fluorescence (F0) (Fig. 2B) in small cardamom leaves. Data are the means of five replicates with standard errors shown by vertical bars.