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College of Agriculture Vellayani, Thiruvananthapuram

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PREFACE

Scientific Agriculture played an important role in the process of enrichment of developed countries. Any country seeking to develop its economy has to give significant priority to Agriculture, particularly Scientific Agriculture, and scientific agriculture is intimately associated with climate and weather.

The impact of drought and flood has underlined the urgency of understanding the interrelationships between crops and weather. We have unusual opportunities today to develop crop saving techniques in areas affected by adverse weather conditions and suitable compensatory production programmes in favourable areas. Thus scientific management of monsoon behaviour is becoming feasible. However, to do this in a meaningful manner it is important to understand the effects of weather on the different components of the biosphere. With this in view, the Kerala Agricultural University, College of Agriculture, Vellayani has taken the lead to organize a Workshop on Climate and Development under the sponsorship of the Kerala State Planning Board, Government of Kerala.

I am very much thankful to Dr. P. Rajasekharan, Chief, Kerala State Planning Board for encouraging me to organise a Workshop of this kind so as to make awareness among the scientific community, planners and ultimately the farming community about the importance of weather, the latest methods of weather forecasting and its importance in the current scenario of global agriculture importance. Thanks are also due to our beloved Vice-Chancellor, Sri. K. R. Viswambharan IAS, Dean, Faculty of Agriculture,Dr.K.Harikrishnan Nair and Associate Director of Research (Meteorology)Dr. G.S.L.H.V. Prasada Rao for their keen interest and flagging support. I gratefully acknowledge the benevolence and immeasurable help rendered from Dr. T. N. Balasubramonian, Advisor to National Insurance, Chennai and former Head of Department, Agricultural Meteorology, Tamil Nadu Agricultural University, Coimbatore. I also acknowledge with thanks all the authors who have contributed papers to this Workshop. I thank all the invited speakers, Chairman, Co-chairman and Rapporteur of different technical sessions for taking part in the conduct of technical sessions and actively deliberating on the occasion. Last but not least the hard work and sincere efforts of my collegues at College of Agriculture, Vellayani is also acknowledged.

I am confident that the Workshop will lead to a greater understanding of methods of weather forecasting, its importance and methods of weather management in relation to crop production. The Proceedings of the workshop represents an up-to-date reference of ideas and discussions of the Workshop concerning Climate and Development. On behalf of the organizing committee, I have great pleasure in presenting this Proceedings of the Workshop to the delegates of the National Workshop on Climate and development conducted jointly by the Kerala Agricultural University and the Kerala State Planning Board, Government of Kerala.

The financial assistance received from the Kerala state Planning Board is gratefully acknowledged.

Thiruvananthapuram | L.Girija devi

22.06.2009 | Organising Secretary

Elevated carbon dioxide and temperature reduce stomatal density in coconut (*Cocos nucifera* L.)

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Abstract

Climate change is looming large with immense impact on plants. As per IPCC reports, atmospheric concentration of CO_2 will further rise leading to increased temperatures. Thus it is important to study the adaptation capacity of plants so as to choose suitable crops or cultivars. A study was conducted with coconut seedlings of three cultivars viz. WCT, LCT, COD, and two hybrids viz., WCT x COD, and COD x WCT, which were grown in Open Top Chamber facility, where CO_2 levels were maintained at 550 and 700ppm and in a separate OTC, temperature was elevated at 2°C above ambient. Apart from these, seedlings were also grown in control chamber and in shade net conditions. After exposure to above treatments for about 2 years, the leaf stomatal density was counted. Results indicated that the elevated CO_2 and temperature caused significant reduction in leaf stomatal density. The reduction was more in LCT and COD x WCT. Interestingly, the reduction in stomatal density did not influence the net photosynthetic rates, indicating significant adaptation strategy by coconut to changing climate.

Introduction

Global climate change is a cause of great concern worldwide. The CO_2, a major green house gas, levels will rise further and will lead to elevated temperatures as par the fourth assessment reports brought out by IPCC (2007). These climatic changes have significant influence on plants. Stomata, through which plants exchange gases with atmosphere, are found to be sensitive to changing climate. Earlier observations on herbarium records covering about 200-years period indicated that the stomatal density of eight species of forest trees showed a 40% reduction in relation to a rise in atmospheric CO_2 of 60 mmol/mol over that period (Woodward 1987). Similar observations were made on stomatal density in 14 species of herbarium material collected 240 years ago (Penuelas and Matamala 1990), in fossil sample and present samples of Ginkgo leaves in relation with increasing CO_2 (Li-quan chen *et al.* 2001). Stomatal response of four native North American conifer species (*Tsuga heterophylla*, *Picea glauca*, *Picea mariana*, and *Larix laricina*) to a range of historical CO_2 mixing ratios (290 to 370 ppmV) indicated that stomatal frequency, based on the number of stomata per millimeter of needle length, decreased significantly with increasing CO_2 (Lenny *et al.* 2003).

Response of stomata to elevated levels of CO_2 was also studied in controlled conditions, where the crops are being exposed to enriched levels of CO_2. In growth experiments with elevated CO_2 levels, the number of leaf stomata decreased in 40% of the species studied (Woodward and Bazzaz 1988; Woodward and Kelly 1995). Reports indicate that elevated CO_2 caused a significant reduction in stomatal density on both surfaces in couch grass leaves (*Elymus repens*) (Engloner *et al.* 2003), in *Opuntia ficus-indica* (Morse *et al.* 1993) and in *Salix cinerea* (McElwain *et al.* 1995)

Though many citations conclude enrichment of CO_2 reduces the stomatal density still there are enough literature indicating stomata numbers unchanged due to elevated CO_2 levels. The Stomatal density is reported to be unchanged under elevated CO_2 in selected

17 plant species (Bettarini *et al.* 1998). No effect of elevated CO_2 on stomatal distribution was observed in longleaf pine (*Pinus palustris* Mill.) needles. (Pritchard *et al.* 1998) and in *Vicia faba* L seedlings (Radoglou *et al.* 1993).

High temperature also results in slight reduction of stomatal density in *Quercus robur*, which indicates stomatal density is negatively correlated with the mean temperature under which the leaves were formed. (Beerling and Chaloner 1993). In contrast to this, elevated temperature resulted in higher stomatal density, irrespective of seasons, in perennial Rye grass, *Lolium perenne* (Ferris *et al.* 1996).

Elevated CO_2 caused improvement in growth of coconut seedlings grown in open. top chambers (Naresh Kumar, 2007) and the simulation modeling studies using InfoCrop-COCONUT (Naresh Kumar *et al.* 2008) projected increase in coconut yields in west coast of India, part of Karnataka and Tamil Nadu due to climate change (Naresh Kumar, 2008). Since coconut (*Cocos nucifera* L.) is a C3, source limited (Naresh Kumar *et al.* 2002) and hypostomatous (Naresh Kumar *et al.* 2000) plant, it is important to understand the influence of elevated CO_2 and temperature on several aspects of growth and development. Thus, a study was conducted to delineate stomatal response of seedlings to elevated CO_2 and temperature.

Materials and Methods

Experiment was carried out in Central Plantation Crops Research Institute, Kasaragod (12□18□ N, 75□ E, ~7m AMSL), which receives an average of ~3400 mm of annual rainfall. Seedlings of three cultivars of coconut viz: WCT, LCT, COD and two hybrids WCTxCOD and CODxWCT were grown for two years in Open Top Chamber (OTC) elevated CO_2 and Temperature facility. This is an SCADA based automated system consisting of six OTCs of $4m^3$ open top chambers. Seedlings are raised in poly bags with adequate irrigation and uniform nutrition in the form of vermicompost. Seedlings are exposed to two different concentrations of CO_2 viz., 550 and 700 ppm. The CO_2 levels are being maintained with constant supply of commercial CO_2 and are monitored with computerized (SCADA) system. In a separate OTC, elevation of temperature by 2□C above ambient, using hot air blower was maintained. For comparison, a set of seedlings are grown in OTC which served as chamber control. Apart from these, a set of seedlings are also grown under shade net.

For taking stomatal prints, clear transparent nail varnish was applied on the abaxial (dorsal) surface of the middle portion of middle leaflets of the third frond from top. Dried peel was removed using forceps and was mounted on glass slide. For each cultivar prints were taken from six seedlings. At least 10 images from each slide were captured using Leitz Diaplan (Germany) microscope connected with camera, which in turn is connected to a computer. Images were captured at 10x resolution and were analyzed using Leica Q win software (Leica, Germany) and stomata are counted for fixed area.

The net photosynthetic rates were recorded on the middle leaflets of the third frond from top, using infrared gas analyzer (LiCor-6400; LiCor Inc. USA). The recordings were done in a bright sunny day between 9 and 11 am. At least 30 readings in each seedling were taken and in each cultivar observations were made on six seedlings in each treatment. All the data were statistically analyzed and standard error of mean was calculated.

Results and discussion

Since seedlings were grown for over two years in respective treatment conditions (shade net, chamber control, elevated CO_2 -550 and 700 ppm and elevated temperature (+2°C)), the leaves from which samples were drawn were formed from their initiation to full growth in respective treatment conditions. Leaf stomatal density of coconut cultivars grown in OTC was significantly less as compared to that in shade net grown seedlings. This may be attributed to chamber effect, as the micro-climate of chamber is different from that of shade net. Similar chamber effects were drawn with *Andropogon gerardii*, a C4 grass (Knapp *et al.* 1994). Stomatal density decreased further in seedlings exposed to elevated levels of CO_2 (Table 1; Fig 1), indicate role of CO_2 on stomata formation. In this regard the crop behaved in such a manner that stomatal density is reduced with elevated CO_2 as observed in several other crops (Woodward and Bazzaz, 1988; Morse *et al.* 1993; Woodward and Kelly, 1995; Enlonger *et al.* 2003)

Not-withstanding slight difference observed among cultivars, generally the decrease in stomatal density was more (14%) in seedlings exposed to 700 ppm CO_2 than those exposed to 550 ppm of CO_2 (10%). A slight reduction in stomatal density (5%) is also observed in coconut seedlings exposed to higher temperature (Table 1; Fig.1). Similar result was reported in *Quercus robur,* where stomatal density was negatively correlated with the mean temperature under which the leaves were formed (Beerling and Chaloner, 1993). The results clearly indicate that elevated CO_2 and temperature reduce the stomatal density in leaves.

The net photosynthetic rate (Pn) is measured to know whether decrease in stomatal density affect CO_2 assimilation. Pn rate was significantly higher in seedlings grown in enriched CO_2 concentrations and behaved similarly as most of the crops did under enriched CO_2 levels (Ryle *et al.* 1992, Woodward *et al.* 1991). Even at elevated temperature, no significant change in Pn was observed. Pn is found to be unaffected by decrease in stomatal density (Fig 2) at least in the range of observed decrease in stomata density. The Z-plot clearly indicates that in spite of change (reduction / increase) in stomatal density by 25% did not influence the Pn in a particular manner. Similar results were observed in *Ginkgo biloba* (Beerling *et al.* 1998). It will be interesting to see the gas exchange response of leaves in situations when the reduction in stomatal density is beyond 30%.

Hence, we conclude that the elevated CO_2 plays a key role in stomata formation and its density is inversely related to the higher CO_2 concentrations and temperatures under which the leaves have formed. It indicates that plants adapt to atmospheric CO_2 levels by modifying the stomatal density as well apart from other adaptation mechanisms.

Acknowledgment

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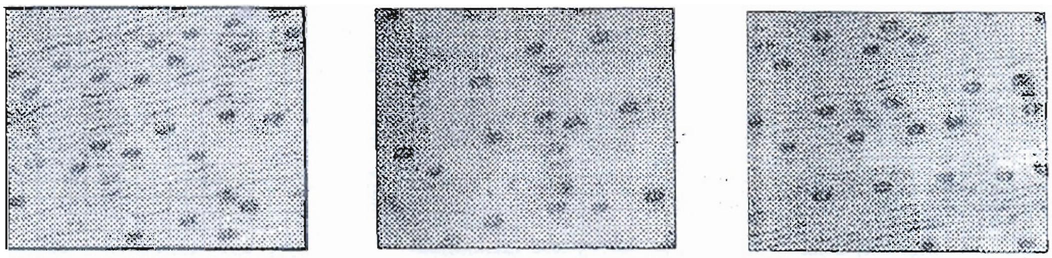
Table Stomatal density (SD) (number/mm^2 leaf area) in coconut cultivars and hybrids grown under different treatments viz., shade net (SN), control chamber (CC), elevated temperature (ET), elevated CO_2 levels at 550 ppm ($[CO_2]$ 550) and elevated CO_2 at 700 ppm ($[CO_2]$ 700). SEM-standard error of mean

Treatments	Stomatal density (number / mm^2 leaf area)					Mean	SEM
	LCT	WCT	COD	WCT x COD	COD x WCT		
SN	343.9	352.0	328.3	346.3	389.5	352.0	10.2
CC	290.9	276.6	331.3	268.9	377.8	309.1	20.3
ET	258.8	263.3	342.9	260.1	329.6	290.9	18.6
EC550	270.3	275.3	292.8	270.3	272.1	276.1	4.3
EC 700	228.6	251.4	290.4	279.8	278.5	265.7	11.3

Fig 1. Stomatal print from leaf dorsal surface of seedlings grown in (a) control chamber, (b) elevated CO_2 and (C) elevated temperature



a b c

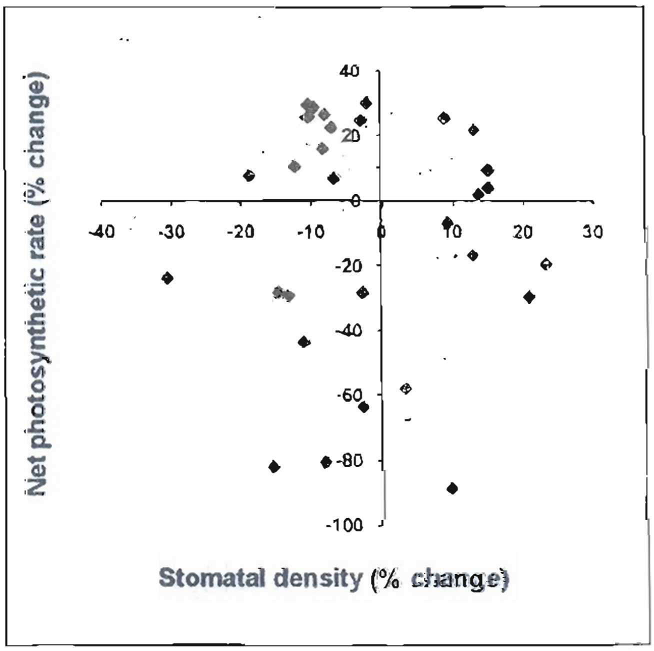


Fig 2. Z-plot between change in stomatal density and photosynthetic rate.

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