

**EFFECTS OF ELEVATED CO₂ AND WATER STRESS ON THE ADAPTATION
OF STOMATA AND GAS EXCHANGE IN LEAVES OF EGGPLANTS
(*SOLANUM MELONGENA* L.)**

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Abstract

Effects and possible interaction of elevated CO₂ and water stress on the stomatal morphology and the pattern of gas exchange in leaves of eggplants (*Solanum melongena* L. cv. Senryo No. 2) were investigated. Seedlings were grown to maturity in controlled growth chamber using ambient CO₂ (365~370 ppm) and elevated CO₂ (700 ppm) and at frequent watering as control and watering after every 21 days for simulating periodic water stress and recovery from stress. A complementary acclimatisation of photosynthesis in water stressed eggplants grown under elevated CO₂ was evident. Despite decreased stomatal conductance and transpiration rate under elevated CO₂ conditions, increased net leaf photosynthesis rate was observed. Stomatal length and width decreased under water stress and increased CO₂.

Introduction

As atmospheric CO₂ concentration and global temperature rise, potential shifts in regional scale precipitation patterns are expected in many areas of the world. In addition, shifts in regional precipitation will probably result in decreased soil water availability or drought conditions in some regions of the world (Keeling *et al.* 1976, Allen 1994). The relationship between elevated CO₂ and water in the plant is complex because water and CO₂ interact on an instantaneous and microscopic scale in the mass flux of molecules in the stomatal pore during gas exchange; on a longer, organ scale of the determination of plant photosynthetic capacity by plant water status; and on the whole-plant scale because of the influence of the history of CO₂ fixation on leaf area, plant stature and developmental stage, and root mass and distribution in the soil (Morison 1993).

Stomatal density is an important ecophysiological parameter that affects gaseous exchange. Instantaneous water use efficiency is improved as a result of declining stomatal numbers (Woodward 1987). Negative correlation with stomatal properties under water stress and increasing CO₂ concentration is observed. It has been extensively reported that water stress influences plant performance through effects on stomatal aperture, mesophyll photosynthesis and growth. Transpiration rate, stomatal conductance and photosynthetic rate are directly related to water stress. Increasing CO₂ concentration in the air changes in gas exchange and biochemical processes (Hsiao 1973, Tyree and Jarvis 1982). However, important is that how rising CO₂ and periodic soil moisture stress interact to leaf characteristics, i.e., leaf structure and pattern of gas exchange is a vital question in the consideration of continuous changes of global environment. Elevated CO₂ concentration with limited supply of water increase photosynthetic rate and the decrease the water use because of reduction in stomatal aperture become important.

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Drought stress restricts plant growth and economic yield not only in areas classified as arid and semi-arid, but also in areas where transpirational demand exceeds the water supply capability of soil. The effect of moisture stress on plant responses depends not only on lack of sufficient soil water, but also on the microclimatic environments in which the plant grows and develops, and the duration and severity of soil moisture depletion (Dale 1988). In nature, drought may last for a long period, its intensity may differ and sometimes it may appear repeatedly. However, the degree of adaptation to stress appears to vary considerably within the genera and also within the species (Torrecillas *et al.* 1996). The overall objective of this study is to investigate how elevated CO₂ conditions interact to leaf level micro-morphology and physiological process during periodic soil moisture stress and after stress recovery.

Materials and Methods

Eggplant (*Solanum melongena* L. cv. Senryo No. 2) seedling was grown one in each pot in controlled growth chamber. Seedlings were 18 to 20 cm in height and at 5-6 leaves stage. Eighteen seedlings were grown under increased CO₂ concentration in a chamber. Seedling were transplanted on May 12 in 2004 and studied up to 63 days after transplanting (DAT). Ambient (365~370 ppm) and elevated (700 ppm) CO₂ conditions were maintained in separate growth chambers. The chamber was maintained at 25°C during 12 h photoperiod (at 40 K lux) and at 20°C during dark period. The relative humidity was controlled at 70% all day long and the wind speed fluctuated between 0.4 and 0.8 m/s. This is maintained by setting CO₂ sensor equipped in the growth chamber and checked regularly from the chamber environment control monitor. The Amb_T1 and Ele_T1 were for well-water condition under ambient and elevated CO₂ concentrations, while Amb_T2 and Ele_T2 were used for water stress condition (watering every 21 days interval) under ambient and elevated CO₂ concentrations. The pot was irrigated to raise its soil moisture status up to the pot capacity level by just replenishing the total amount of water lost by ET after each wetting and drying cycles.

The third or fourth leaf from the apex of an individual plant were collected and about 2 × 2-mm leaf parts fixed in a 2% glutaldehyde immediately. These were then passed in an ethanol series. The processed samples were then dried (using HCP-1, Hitachi Critical Point Dryer, Hitachi) and then set up on the aluminium plate for coating with gold metal. The samples were studied by scanning electron microscope (Model: S-2250N, Hitachi, Japan) and microphotographed and measured using Quartz PCI V.5 software. Stomata counts were made on the middle portion of the leaf excluding any lateral rib and midrib.

The stomatal index was calculated according to Salisbury (1927) as follows:

$$\text{Stomatal index} = [\text{No. of stomata}/(\text{No. stomata} + \text{No. of epidermal cells})] \times 100$$

Photosynthetic rate, transpiration rates and stomatal conductance were monitored for a single leaf. A portable porometer with leaf cuvette (Model: CIRAS-1, PP System, Koito, Japan) with controlled environment was used to measure the gas exchange variables of a single leaf in the growth chamber. Measurements were made by using leaf cuvette on an upper fully expanded leaf of an individual eggplant pot representing each treatment, typically between 12.15 p.m. to 13.30 p.m. hrs local time. Upper most third or fourth from the top belonging fully expanded young to mid-aged leaves were selected. For each measurement, the leaf was allowed to acclimate to the leaf cuvette for 5 minutes, and then the average photosynthesis rates, transpiration rates and stomatal conductance over next 5 minutes period were collected for the purposes. Data from the porometer were stored instantly in the internal data logging system and finally transferred to the computer and used for analysis and interpretation. The measurement was taken initially at seven days interval and then after two to one day.

ANOVA using CRD with ten replications was performed to investigate the treatment difference on stomatal density. The three factorial (time, water and CO₂) ANOVA was followed separately for the stomatal density on adaxial and abaxial surfaces of eggplants. This analysis was carried out using statistical software MSTAT-C computer program to compare the treatments effect and differences between two CO₂ levels and water treatments for stomatal density in the study.

Results and Discussion

The typical features of stomatal density on adaxial and abaxial surfaces of eggplant leaves have been presented in Table 1, Fig. 1A-E. Eggplants grown under elevated CO₂ environment had reduced stomatal density. The phenomenon was common in both adaxial and abaxial surfaces. Water stress also reduced the stomatal density of all leaves collected from ambient and elevated CO₂. At 63 DAT, Amb_T1 and Amb_T2 eggplants had 21% and 20% on adaxial layer, and 30% and 31% on abaxial surface. Similarly, Ele_T1 and Ele_T2 eggplants had 17% and 18% on adaxial surface and 22% and 25% on abaxial surface. Statistically significant differences were observed in stomatal index between adaxial and abaxial surfaces of leaves under ambient and elevated CO₂. The stomatal index was always bigger on abaxial surface than on adaxial one.

Table 1. Epidermal micromorphological characteristic of eggplant leaves grown under ambient and elevated CO₂ environments.

Times	1st day of transplanting		63rd day after transplanting							
	Watered		Watered		Water stressed		Watered with elevated CO ₂		Water stressed with elevated CO ₂	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
Stomatal density/mm ²	413 (± 43)	630 (± 80)	340 (± 46)	678 (± 110)	375 (± 35)	770 (± 149)	258 (± 39)	645 (± 147)	363 (± 46)	720 (± 111)
Epidermal cells/mm ²	1260 (±214)	1493 (± 247)	1320 (± 130)	1595 (± 260)	1513 (± 159)	1520 (± 127)	1265 (± 60)	2345 (± 478)	1655 (± 157)	2369 (± 540)
Stomatal index (%)	25 (± 3)	30 (± 3)	21 (± 3)	30 (± 3)	20 (± 0)	33 (± 3)	17 (± 2)	22 (± 1)	18 (± 3)	25 (± 3)
Stomatal length (µm)	12.8 (± 0.4)	11.7 (± 5.4)	9.4 (± 2.4)	11.1 (± 1.1)	6.4 (± 0.8)	8.2 (± 1.5)	8.6 (± 1.0)	10.9 (± 2.7)	7.0 (± 1.5)	9.5 (± 2.4)
Stomatal width (µm)	4.3 (± 0.6)	3.8 (± 1.2)	3.6 (± 0.6)	4.3 (± 0.5)	2.0 (± 0.6)	2.1 (± 0.7)	2.9 (± 1.2)	5.0 (± 1.1)	1.4 (± 1.3)	1.9 (± 1.3)

The stomatal size was always greater on the adaxial surface than on abaxial one at 63 DAT (Table 1, Fig. 1E-H). The adaptation of stomatal properties to elevated CO₂ concentration in eggplants occurred on both adaxial and abaxial surfaces. Stomatal density on adaxial surface was 24% lower and abaxial surface was 5% lower in elevated CO₂ than in ambient CO₂ concentration. Water stress induced this effect negatively on by decreasing stomatal density on both surfaces and reduced 3 and 6% on adaxial and abaxial surfaces. Elevated CO₂ had negative correlation on stomatal density. The stomatal index also showed a consistency as the stomatal density did (Table 1). Eggplant leaves grown under elevated CO₂ had lower stomatal index than the leaves under ambient CO₂. Well-watered eggplants under elevated CO₂ had 7 and 8% less stomatal index on adaxial and abaxial surfaces while water stressed eggplants had 7 and 5% less stomatal index on adaxial and abaxial surfaces than their corresponding counterparts grown under ambient CO₂.

Some herbs and tree species showed the similar relationship between CO₂ concentration and stomatal density (Oberbauer *et al.* 1985, Woodward and Bazzaz 1988). However, in the present study, inverse regulation or negative adaptation of stomatal properties of eggplants under elevated CO₂ occurred to interacting with water stress, i.e., less stomatal density under high CO₂ conditions. Consequently, stomatal size on both surfaces also adapted inversely with the increase in elevated CO₂ concentration and water stress.

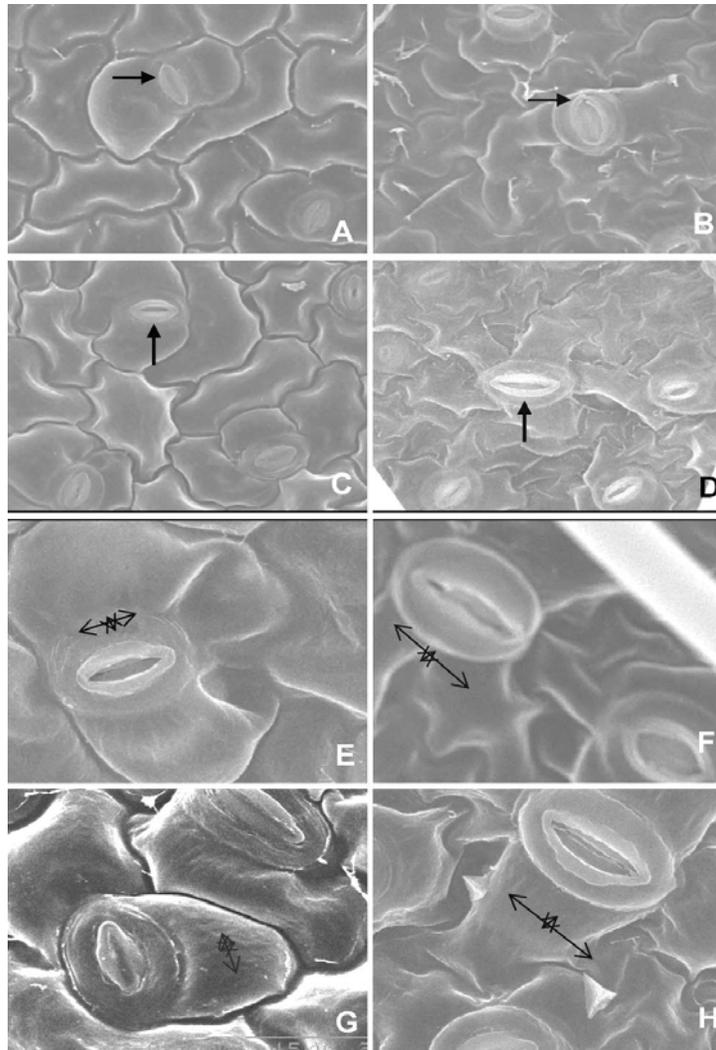


Fig. 1. A-H. Scanning electron micrographs of eggplant grown under ambient and elevated CO₂ environments for 63 days. A and B are adaxial (upper) and abaxial (bottom) surfaces, respectively, of well-watered eggplants. C and D are adaxial and abaxial surfaces, respectively, of water stressed eggplants. Bars = 20.0 μm with $\times 2000$ magnifications. E and F are for stomatal length on adaxial and abaxial surfaces of well-watered eggplants (ET_1). G and H are for stomatal length on adaxial and abaxial surfaces, respectively, of water stressed eggplants (Ele_T2). Arrow inside the circle indicates stoma length and width. All figs $\times 550$.

Fig. 2A shows that photosynthetic rate of eggplant leaves was affected by soil moisture stress. Net photosynthetic rate of leaves exposed to elevated CO₂ was always greater than to ambient CO₂, irrespective of their soil moisture status. Withholding water reduced photosynthetic rate of leaves at both CO₂ concentrations but fall at ambient CO₂ concentration was proportionally greater

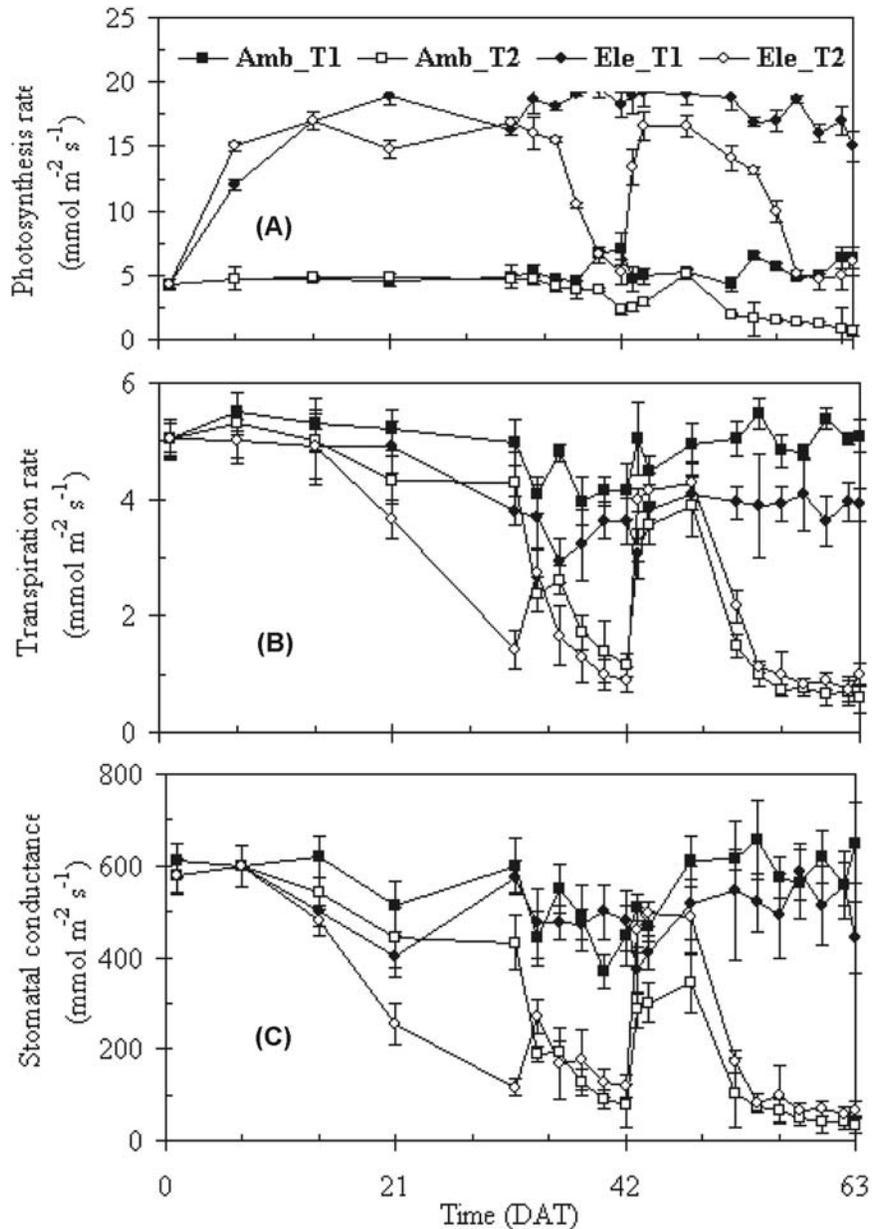


Fig. 2. Photosynthesis rate (A), transpiration rate (B) and stomatal conductance (C) of eggplant leaves. Lines with ■ and □ represent well-watered and water stressed at ambient CO₂, respectively. Lines with ◆ and ◇ represent well-watered and water stressed at elevated CO₂ conditions, respectively.

than elevated CO₂ such that the ratio of elevated to ambient net photosynthesis increased from 2 - 4 : 1 in well-watered eggplants to approximately 3-8 : 1 during the subsequent soil drying and after recovery of stress by re-watering the eggplants. The highest photosynthetic rate was 7.0 and 5.0 (μmol CO₂/m²/s) in Amb_T1 and Amb_T2 plants, respectively, at 42nd and 48th day of experiment. The Ele_T1 and Ele_T2 eggplants had the highest average, 19.0 and 17.0 (μmol CO₂/m²/s) at 44th and 32nd day of experiment. The photosynthetic rate in Amb_T2 and Ele_T2 leaves gradually decreased during subsequent moisture stress but the rate increased after recovery of stress by re-watering the test pots.

A consequence of elevated CO₂ enabling photosynthesis to continue at high levels under soil moisture stress conditions (Fig. 2A). Similar findings have been reported for wheat (Nie *et al.* 1995), rice (Baker *et al.* 1997), maize (Maroco *et al.* 1999) and tomato (Bestford *et al.* 1990). In the present study, a complementary acclimation in leaf level photosynthesis occurred indicating that the eggplants have the potential to water stress condition under elevated CO₂ environments.

There is general agreement in literature from a wide range of species that elevated CO₂ leads to a reduced transpiration rate with the decrease in stomatal conductance (Tyree and Alexander 1993, Clark *et al.* 1999, Kang *et al.* 2002). Fig. 2B shows the instantaneous transpiration rate of eggplant leaves grown under ambient and elevated CO₂ environments. Leaf transpiration rate decreased when eggplant grown under elevated CO₂ concentration. Water stress also markedly reduced the transpiration rate per unit leaf surface area. Transpiration rate increased after recovery of stress by re-watering the eggplant pots and again it declined gradually during subsequent soil moisture stress. Under elevated CO₂ environment, eggplants had lower stomatal conductance than ambient CO₂ environment, and subsequently developed soil moisture stress gradually regulated to down and reduced stomatal conductance but the conductance increased again just after recovery of stress by re-watering. Consistent is that transpiration rate also reduced with the increase in water stress intensity as stomatal conductance did. Reduced stomatal conductance as well as reduced transpiration rate is expected to be a feature of plants exposed to increasing CO₂. Stomata do not appear to limit photosynthesis with elevation of CO₂ any more than do at normal ambient CO₂, even though stomatal conductance is usually decreased. Furthermore, reduced transpiration rate under elevated CO₂ by lowering conductance saves the soil water in some extent and such phenomena increased the availability of soil water (Field *et al.* 1995).

There was a remarkable reduction in stomatal conductance when the eggplants subjected to periodic water stress at both CO₂ concentrations (Fig. 2C). Elevated CO₂ had an influence to reduce stomatal conductance at well-watered plants. Fact is that the stomatal conductance of stressed plants, i.e., Ele_T2 was always little greater in comparison to Amb_T2 eggplants during stress period and this was pronounced after recovery of stress by re-watering the pots. The stomatal conductance of well-watered eggplants under both CO₂ concentrations fluctuated in between 369 and 660 mmol/m²/s but stressed eggplants showed gradual decline during subsequent stress and had as low as 34 mmol/m²/s in Amb_T2 and 57 mmol/m²/s in Ele_T2 eggplants at 63 DAT, i.e., during peak reproductive stage.

Transpiration rate and stomatal conductance of plant leaves dynamically respond to soil moisture deficit. In this study, the transpiration rate of eggplants was highly sensitive to soil moisture stress, lower in stressed eggplants than in eggplants with no stress. The effect of water deficit and CO₂ enrichment on transpiration rate has been described for sunflower (Tezara *et al.* 2002) and for sorghum (Conley *et al.* 2001). As the soil moisture stress increased, the volumetric water content continued to decrease, which then reduced the transpiration rate of eggplant leaves. The transpiration rate and stomatal conductance increased after rewatering, but sometimes leaves showed no higher conductance just after rewatering. Plant leaves cannot transpire at their full potential once their stomata begin to close because of stress that readily occurs when the

transpiration rate exceeds the rate of water supply to the leaves (Kramer 1950, Sarker *et al.* 2004); this may have happened in both CO₂ concentrations in this study. In some cases when prior stress was prolonged, the photosynthesis did not return to previous levels within a day. In the present study, the CO₂ history for the eggplants had great influence on lower stomatal conductance since the stomatal density decreased under elevated CO₂ condition. Many pot experiments have indicated that changes in stomatal conductance along with reduced transpiration rate are the main cause of the great decrease of photosynthesis with declining soil water content.

It is revealed that soil moisture stress greatly influences on stoma and physiology of leaves. In this study, eggplants under elevated CO₂ increased photosynthesis, but led to acclimatization, with decreased stomatal density, stomatal index and stomatal conductance; water stress increased photosynthesis compared to ambient CO₂. However, increased photosynthesis alone or combined with reduced transpiration rate fundamentally beneficial under elevated CO₂. Reduction in both stomatal density and conductance affect reduction in transpiration rate which might be important for improved water status. Photosynthesis remained less affected and maintained physiological activity during dry period.

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