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EFFECTS OF ELEVATED CO_2 AND WATER STRESS ON ROOT STRUCTURE AND HYDRAULIC CONDUCTANCE OF *SOLANUM MELONGENA* L.

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Abstract

Pot experiment was conducted using eggplants (*Solanum melongena* L.) grown in a controlled growth chamber environment. Ambient (370 ppm) and elevated (700 ppm) CO_2 concentrations, were used. Hydraulic conductance in stressed eggplants was lower than that of control eggplants and this phenomenon was enhanced under elevated CO_2 environment and also due to plant age. Hydraulic conductance through the root system in free water was comparatively higher than that of soil medium. As in the pressure plate apparatus technique with moist soil, the eggplants that experienced repetitively shorter stress showed the lowest conductance (6.38×10^{-5} kg/m^2/s/MPa) under the interactive effects of high CO_2 and water stress while control and longer stressed plants had 9.11×10^{-5} and 7.19×10^{-5} kg/m^2/s/MPa, respectively. It appeared that eggplants grown in a high CO_2 environment might be efficient in water use under soil water shortage conditions.

Introduction

The movement of water in the Soil-Plant-Atmospheric-Continuum (SPAC) is controlled by the conductance of the components of water pathways. In general root conductance (inverse of root resistance) and stomatal conductance have been considered to be the main controlling factors of water flow in plants (Jones 1992). When plants were exposed to water stress, they showed some changes in water flow rate. Modifications of conductivity components of the transpiration pathway (root, shoot and stomata) contribute to determine such changes. It was reported that water stress affects root and shoot conductivity by inducing embolism in the root cells and xylem vessels by anatomical changes (Lo Gullo *et al.* 1998). Root hydraulic resistance increased in plants subjected to water stress (North and Nobel 1991).

It was reported that increasing CO_2 concentrations in the atmosphere stimulates root growth and enhanced soil exploration (Rogers *et al.* 1992, Wechsung *et al.* 1999, Obrist and Arnone 2003). Such beneficial effects of elevated CO_2 enhanced the proliferation of roots into resource-rich microsites (Arnone 1997) and increased water and nutrient use efficiency (Rogers *et al.* 1994, Rogers *et al.* 1999, Van Vuuren *et al.* 1996). In some cases, increasing levels of CO_2 concentration negated the effect of water stress (Rogers *et al.* 1994) due to improving the water status of the plant reflected by lowering Evapotranspiration (ET) and increasing water-use efficiency (Owensby *et al.* 1997, Conley *et al.* 2001). Elevated CO_2 concentration caused partial closure of stomata, thereby decreasing leaf conductance to CO_2 and H_2O vapor (Morison 1985) and reducing transpiration (Kimball and Idso 1983). It is expected that not only lower leaf conductance is responsible for lowering ET and higher water-use efficiency, but root conductance and internal anatomy might contribute to water movement in plants. There is some information on the root growth and water relationship for crop plants but little information is available on root hydraulic conductance and root anatomy under elevated CO_2 for eggplants. The present study was aimed to examine the response of hydraulic conductance of the eggplant root system for water

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translocation to global changes in the CO_2 in the air under different levels of water stress. The objectives of this work are (i) to study root anatomical structure under the interactive effects of elevated CO_2 concentration and water stress and also (ii) to quantify the hydraulic conductance of the eggplant root system.

Materials and Methods

Growth conditions, plant culture and treatment description: The experiment was conducted with eggplants (*Solanum melongena* L.) cv. Senryo No. 2). The growth chamber was controlled at 25^{o} C from 8.00 a.m. to 6.00 p.m. and at 20^{o} C from 8.00 p.m. to 6.00 a.m. at artificial lighting of 40 klux provided by lamps, with a photoperiod of 12 h. The relative humidity was 70% all day long and the wind speed fluctuated between 0.4 and 0.8 m/s. Ambient CO_2 concentration was 370 ppm while 700 ppm was used for elevated CO_2 treatment keeping other conditions fixed for both experiments. Volcanic ash soil in a pot at a bulk density of 0.61 to 0.63 g/cm^3 was used for this study**.** The test soil was previously well incorporated with mixed granular fertilizer.

Eggplants were grown in plastic pots (15.5 cm inner diameter, 20 cm tall). Almost uniform sized seedlings of five to seven leaves were transplanted singly in pots. Three water treatments were sequenced as (i) watering the pot at two to three days interval at pot capacity level (Control-WW), (ii) withholding water for five days (shorter stress-SS) and (iii) withholding water for ten days (longer stress-LS). A completely randomized design with three replications ($3 \times 3 \times 2 = 18$ pots, nine pots for ambient CO_2 and others for elevated CO_2 treatment) was used. Out of 3 plants two were used for water flux volume and other plant parameter measurements for each treatment while a single plant was used for anatomical study. All the plants were watered from October 5, 2003 at two to three days interval for two weeks to establish them well in the pot. Then conditioned water treatment was applied to nine pots for the 1st experiment with ambient CO_2 concentration (370 ppm) up to November 15, 2003 and test plants were harvested separately as conditioned and used for water flux volume measurement. Simultaneously, nine other pots were maintained under sufficient moisture conditions up to November 20, 2003 for the high CO_2 experiment. Higher CO_2 concentration (700 ppm) plus conditioned water treatment was applied from November 20 to December 10, 2003, respectively. The soil moisture content at pot capacity level was tested in this study on the basis of watering the pot to saturation level and then the moisture was allowed to deplete its water holding capacity level against gravitational force.

Leaf water potential and root parameters: For leaf water potential measurement, the uppermost 3rd/4th leaf was sampled and quickly measured for different plants with a pressure chamber. Roots were collected through careful washing of the soil by spraying water keeping on a sieve to minimize root loss and damage. The fresh root was gently blotted for root surface area measurement. The roots were spread on a contrast background and photographs were taken with digital camera. Root surface area (RSA) was calculated by image analysis with LIA32 software using the formula

$$R_{SA} = \frac{I_{TR} \times A_{Ri}}{I_{AR}} \times \pi \qquad (m^2) \qquad [1]$$

where I_{TR}, A_{Ri} and I_{AR} are the image area of total root measured by software, actual reference index area on background, and average image of reference index area measured by software, respectively while π is used for 3-D factor.

Root anatomy: Ten root samples of secondary root were blotted well and preserved in a preservative of 50% ethanol. For anatomical observation, free hand cross-sections were prepared with a sharp razor blade and stained with Sudan Red 7B (Brundrett *et al.*1991). Random cross sections were made at 0.07 to 0.12 m distance from root apex. Stained suberin lamella in the root cell was observed under a light microscope (Model: LEICA DMIRB, Germany) and photographs were taken by a digital camera.

Measurement of hydraulic conductance by pressure plate apparatus: The designed eggplant pot was maintained at full saturation by putting the pot in a bigger pot containing water 2 h prior to root excise on the day of experiment (Fig. 1a). The root was excised at 4 - 5 cm above the soil surface by a sharp knife. The pot was immediately placed vertically inside the pressure plate apparatus (Model: DIK-3480, Daiki Rika Kogyo Co. Ltd., Japan). The pressure (positive pressure) in the chamber was applied in steps of 0.02, 0.04, 0.06 and 0.10 MPa. Flux volume was measured by collecting water at 1 minute interval in a calibrated beaker and data stored in a computer. This technique was also used for measuring flux volume coming out through root (carefully washed) immersed in free water (Fig. 1b). Hydraulic conductance ($C_{R[+]}$) was calculated by the following equation:

$$C_{R[+](SS\ or\ FW)} = \frac{F_{A[+]}}{P_P \times R_{SA}} \qquad (kg/m^2/s/MPa) \qquad [2]$$

where, $C_{R[+]}$, $F_{A[+]}$, P_P and R_{SA} represent hydraulic conductance, total amount of volume flux per plant, applied pressure and root surface area, respectively, used for pressure plate apparatus. The subscripts SS and FW indicate saturated soil and free water.

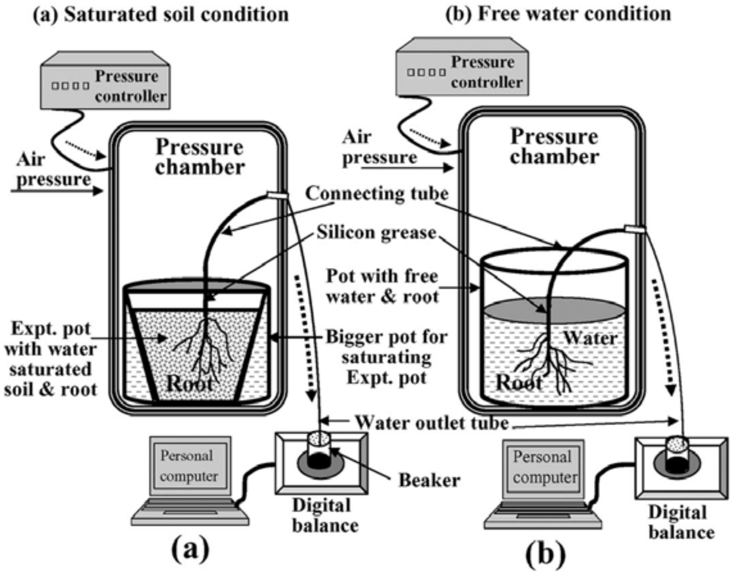

Fig. 1. Schematic diagram of experimental set-up for measuring volume flux density through root system of eggplants with a pressure plate apparatus. Fig. 1a represents the set-up for measuring volume flux density of non-disturbed root system of eggplants at water saturated soil condition and Fig. 1b represents the set-up for measuring volume flux density of washed root system at free water condition.

Measurement of hydraulic conductance by vacuum aspirator: After measuring flux volume at full saturation by pressure plate apparatus technique, an aspirator was used for measuring root water flow separately at full saturation (Fig. 2a) and also after washing the root immersed in free water (Fig. 2b). The suction pressure from the aspirator was applied in steps of 0.026, 0.053, 0.079 and 0.10 MPa, respectively. The $C_{R[-]}$ was calculated using the following formula:

$$C_{R[-](SS\ or\ FW)} = \frac{F_{A[-]}}{(\Psi_{Plant} - \Psi_{Out}) \times R_{SA}} \qquad (kg/m^2/s/MPa) \qquad [3]$$

where $C_{R[-]}$, $F_{A[-]}$, Ψ and R_{SA} represent hydraulic conductance, total amount of volume flux per plant, pressure potential and root surface area, respectively.

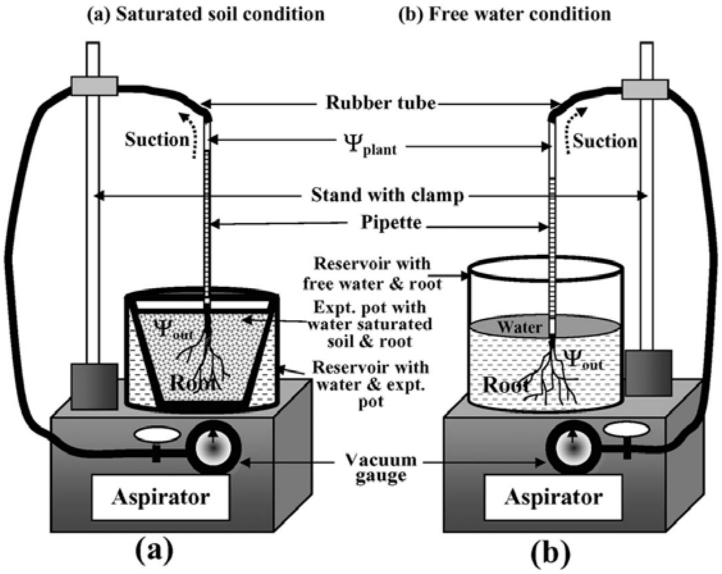

Fig. 2. Schematic diagram of experimental set-up for measuring volume flux density through root system of eggplants with a vacuum aspirator. Fig. 2a represents the set-up for measuring volume flux density of non-disturbed root system of eggplants at water saturated soil condition and Fig. 2b represents the set-up for measuring volume flux density of washed root system at free water condition.

Results and Discussion

The leaf water potential of eggplants was high at well water condition but it decreased when exposed to stress and again it regained its potential after re-watering the pots (Fig. 3). Under ambient condition, SS and LS eggplants had leaf water potential of 1.0 and 1.8 (-MPa) at the end of 4th and 2nd irrigation-drying cycles which were three and six times lower leaf water potential than the control. Similarly, under elevated CO_2, SS and LS plants showed decreased leaf water potential during the drying period and again increased their potential after relieving their stress. The lowest leaf water potential was recorded 4.0 (-MPa) for LS at the end of the 2nd irrigation-drying cycles while SS showed as low as 1.25 (-MPa) at the end of the 4th irrigation-drying cycle followed by 0.01 (-MPa) recorded for WW under well watered condition (Fig. 3). The stress history either in shorter or longer period, plant water status represented by leaf water potential showed a remarkable variation in leaf water status. Leaf water potential also dropped as the eggplants were exposed to water stress repeatedly.

Shorter and longer stress did not significantly affect root growth characteristics although control eggplants showed higher growth than those of shorter and longer stress. Under elevated CO_2, root growth of all plants irrespective of their stress was higher than that of ambient CO_2 (Table 1).

Conditioned treatments SS and LS under ambient CO_2 showed little changes in the root structure of eggplant, producing layered endodermis accumulating a visible amount of suberin lamellae (Fig. 4). The deposition of suberin lamellae in exodermis and especially in endodermis observed in roots of stressed plants might be responsible for resisting radial conduction of water from soils to xylem vessels. Under high CO_2 condition, a considerable amount of suberin lamellae at the exodermis was observed for all the root structure irrespective of their stress. Deposition of secondary suberin was also laid down on the inner walls of endo-exodermal cells. Plant age and water stress had the principal cause for this suberin deposition in the root cells. It is assumed that high CO_2 environment may induce the suberin deposition at the roots. In the present study, this evidently shows that eggplant roots acclimatize under high CO_2 in air by accumulating suberin lamellae in both endodermis and exodermal cells which might be able to compensate for water uptake from soil and transpiration loss to the global atmosphere.

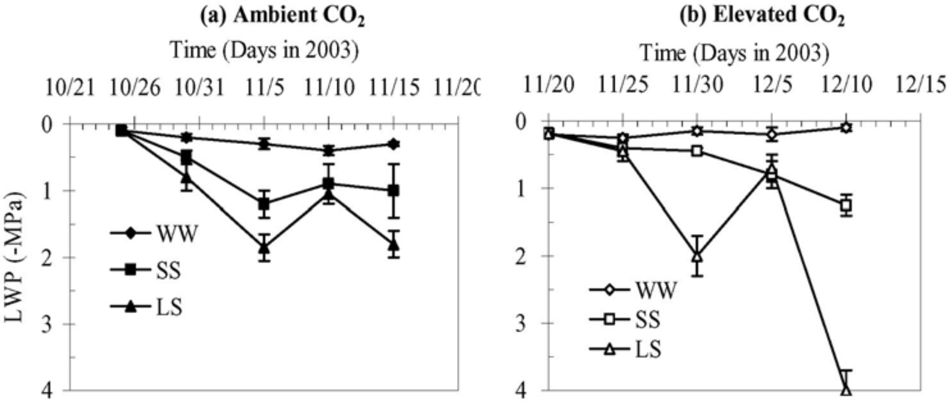

Fig. 3. Leaf water potential (LWP) of eggplants under different conditioned water levels at different growing periods. 3a. Ambient, 3b. Elevated CO_2 conditions.

Table 1. Root growth parameters of eggplants grown under ambient and short-term elevated CO_2 interacting with water stress.

Root parameter	Ambient CO_2			Elevated CO_2		
	WW	SS	LS	WW	SS	LS
Root surface area (m^2)	0.163 ± 0.018	0.170 ± 0.014	0.153 ± 0.018	0.315 ± 0.014	0.320 ± 0.028	0.255 ± 0.035

± = Standard deviation.

The measured flux volume (F_A) coming out through the root systems at a specific applied hydrostatic pressure varied considerably in response to the stress intensity and elevated CO_2 concentration (Fig. 5). The $F_{A[+]}$ and $F_{A[-]}$ of SS eggplant roots at different applied pressures were always lower than those of WW and LS eggplant roots. This is also evident in the case of both ambient and elevated CO_2. Under ambient CO_2, SS eggplants that experienced repetitive shorter stress showed lower $F_{A[+]}$ than those under longer stress while the control eggplants always had the highest. Under elevated CO_2, all the plants showed a lower quantity of measured $F_{A[+]}$ than ambient CO_2 measured at saturated soil. The $F_{A[+]}$ increases linearly with the increase in pressure applied and time (Fig. 5). A similar phenomenon occurs in the roots of many species like rice, maize and olive, where applied hydrostratic driving force changes the contribution of water flow (Lo Gullo *et al.* 1998, Ranatunge *et al.* 2003). In the present study it is evident that the $F_{A[+]}$ of roots of elevated CO_2 was always smaller than that of ambient CO_2. Under high CO_2, the $F_{A[+]}$ of roots was always smaller than that of ambient concentration. This might be due to some anatomical changes which resist the water permeability of roots under stress and high CO_2.

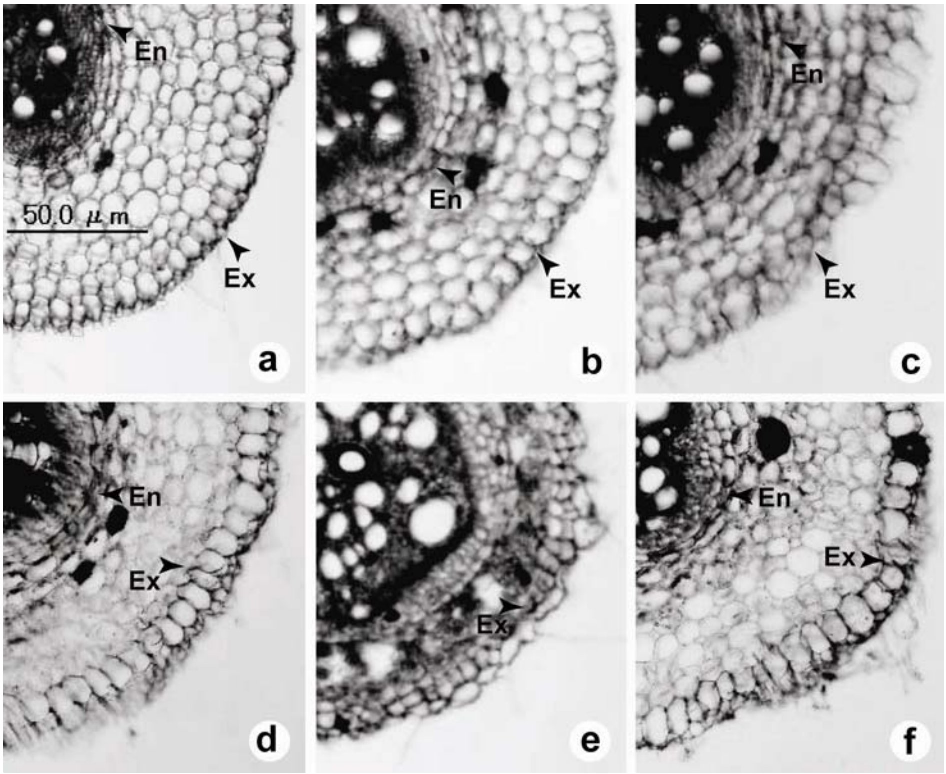

Fig. 4. Free hand cross-sections of a part of the eggplant roots for showing locations of the suberin lipids at the root cells grown under ambient and elevated CO_2, interacting with different water stresses. 4a, b and c representing the endodermis (En) and exodermis (Ex) cells in the root sections of eggplant grown at well-water (WW), shorter-stress (SS) and longer-stress (LS) under ambient CO_2. 4d, e and f representing the location of suberin lipids at endodermis and exodermis layers of root sections of eggplant grown at well-water (WW), shorter-stress (SS) and longer-stress (LS) under elevated CO_2. The arrow-heads indicate the locations of suberin lipids at endodermis and exodermis layers.

The calculated hydraulic conductance ($C_{R[+]}$) following equation [2] for different conditioned plants. There were significant variations in $C_{R[+]}$ among different conditioned treatments. Repetitive shorter stress showed the lowest conductance than those of control followed by longer stress. $C_{R[+]}$ of test plants under elevated CO_2 was lower than those of the plants grown under ambient CO_2. The mean $C_{R[+](SS)}$ of WW, SS and LS plants grown under ambient CO_2 was 2.44×10^{-4}, 1.57×10^{-4} and 2.01×10^{-4} kg/m^2/s/MPa, respectively, while corresponding $C_{R[-](SS)}$ under elevated CO_2 were 0.911×10^{-4}, 0.638×10^{-4} and 0.719×10^{-4} kg/m^2/s/MPa, respectively. The percentage of conductance with respect to control under the effect of water stress and high CO_2 and their interaction (at 0.10 MPa) is presented in detail in Table 2.

Table 2. Water stress, elevated CO_2 and their interactive effects on root hydraulic conductance in percentage (0.10 MPa pressure) at saturated soil and free water conditions.*

a: Conductance at saturated soil condition of the undisturbed root system in pot soil.

Effect	CO_2 level	$C_{R[+]}$			$C_{R[-]}$		
	(ppm)	WW	SS	LS	WW	SS	LS
Stress	370	100	66	84	100	58	60
	700	100	72	68	100	48	58
CO_2	370	100	100	100	100	100	100
	700	38	42	31	38	32	37
Stress × CO_2	370	100	64	82	100	46	76
	700	37	36	46	38	18	22

b: Conductance at free water condition after collecting roots from the pot soil

Stress	370	100	50	96	100	64	64
	700	100	26	34	100	36	37
CO_2	370	100	100	100	100	100	100
	700	95	50	31	62	35	36
Stress × CO_2	370	100	26	62	100	64	64
	700	95	25	32	62	22	23

*Shorter stress (SS) and longer stress (LS) were compared with control plants (WW) as 100% while the plants grown under elevated CO_2 was compared with the plants grown under ambient CO_2 as 100%, respectively.

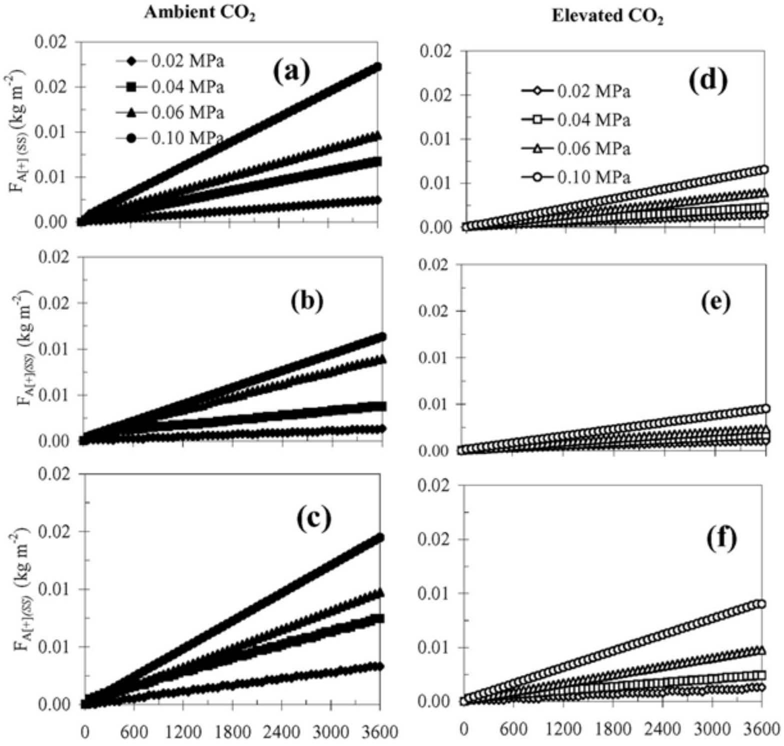

Fig. 5. Flux volume density ($F_{A[+]}$) of roots for different conditioned water treatments measured using undisturbed eggplant roots in water saturated soil. 5a, b and c. Ambient CO_2 condition. 5d, e and f. Elevated CO_2 condition.

The $C_{R[-]}$ of eggplant root systems under saturated soil and free water conditions was calculated using equation [3]. In this technique, the eggplants grown under elevated CO_2, demonstrated comparatively lower root water conductance. Under ambient condition, the highest hydraulic conductance was in saturated soil ($C_{R[-](SS)}$) by the control eggplants at 0.10 MPa with an average value of 2.67×10^{-4}. The mean hydraulic conductance in saturated soil ($C_{R[-](SS)}$) of WW, SS and LS eggplants root grown under ambient CO_2 was 0.708×10^{-4}, 0.391×10^{-4}, and 0.667×10^{-4} kg/m^2/s/MPa, respectively. Under elevated CO_2, WW and LS eggplants showed no significant variation in $C_{R[-](SS)}$ but were higher than that of SS eggplants. Root conductance in free water ($C_{R[-](FW)}$) either under ambient or elevated CO_2 was higher that those of conductance measured in saturated soil ($C_{R[-](SS)}$). The highest calculated $C_{R[-](FW)}$) in free water under ambient was for WW followed by LS and SS, respectively. In case of elevated CO_2, the mean $C_{R[-](FW)}$) of WW, SS and LS was 2.69×10^{-4}, 0.573×10^{-4} and 1.00×10^{-4} kg/m^2/s/MPa, respectively, of the corresponding $C_{R[-](FW)}$ measured in ambient CO_2.

The absolute values of hydraulic conductance of eggplants appeared differently due to differences in stress level and CO_2 interactions. Shorter stressed eggplants experienced several times repetitive stress. Higher CO_2 in the air decreased the root water conductance from soil or water medium to root xylem bundles. Stress level and frequency along with a high CO_2 level had a pronounced effect on root hydraulic conductance reduction. In addition, plant age also had an important factor to decrease C_R which is consistent with report of Fiscus and Markhart (1979), that conductance first increased, then decreased over time, probably because of changes in the proportion of root system in various stages of maturation. In comparison between the two media used for $F_{A[+]}$ measurement, conductance from soil was lower than free water. The resistance of moist soil might contribute to such lower conductance while there was no external barrier apparent to water movement from free water to root. Furthermore, the decline of $C_{R[\pm]}$ for SS eggplants seemed to be due to acclimation of root structure. The anatomical features revealed by free hand cross-sections, showed that both stress and high CO_2 altered the structure of endo- and exodermis layers. The deposition of suberine lamellae might have a positive role in reducing the absolute C_R of eggplants. Steudle (2000) revealed that changes in the suberization of roots are most relevant to water permeability. Suberization of roots increases with plant age and stress intensity. In the present study, $C_{R[\pm]}$ under elevated CO_2 had a much lower level than that of ambient CO_2 irrespective of the stress. This is also explained, as above, that high CO_2 in the air accelerates the changes of root structure, synthesizing more suberin in the endo- and exodermis cells that may contribute to limiting conductance.

In conclusion, eggplant roots showed low hydraulic conductance at repetitive stress, even experiencing a shorter or longer stress. Plant age and water stress contributed to synthesis and accumulation of suberin in the root cells resulted in lower hydraulic conductance. Elevated CO_2 may alter the root structure by synthesizing suberin lipids in endo- and exodermis layers regardless of their soil water availability or stress intensity, although, quantitative analysis of suberin at different parts of roots and at different growth stages may provide actual information which deserves further study. Hydraulic conductance through the roots was higher than that of soil medium. Lowering conductance is highly desirable for agricultural plants when water supply is scarce and uneven in the growth and development stage. Therefore, it indicates that the increasing CO_2 concentration in the global atmosphere might be beneficial for utilizing soil water by minimizing transpirational loss. The low values of hydraulic conductance under higher CO_2 are of wider significance in irrigated agriculture.

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