Growth dynamics and spatial variation of the eggplant root system under elevated CO₂ and soil moisture stress

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Abstract: A complementary root growth under the interactive effect of elevated CO_2 and water stress has been hypothesized and increased root biomass and surface area for economical use of water and mineral, resulted in a better drought survival morphological characteristic. Eggplants were grown under two atmospheric CO₂ concentrations, ambient 365~370 ppm for control and 700 ppm for elevated CO_2 concentration, using environment control growth chambers. In addition to the two levels of atmospheric CO_2 , two levels of conditioned water treatments; frequently irrigated control and 21-d lag of irrigation for water stress was maintained for a 63-d period which consisted of three irrigation-drying cycles. The objectives of this study were to quantify the interactive effects of rising CO_2 and water stress on root morphological growth in special reference to root biomass and surface area. The temporal trend in root growth and spatial distribution at different growth profiles were investigated at three different growth stages. Eggplants showed positive regulation with an increased root growth under elevated CO2 at any developmental stages. Eggplants subjected to periodically subsequent water stress had a complementary root growth (root biomass and root surface area) under elevated CO₂ environment. Roots of water stressed eggplants grown with 700 ppm CO_2 had a greater dry biomass and surface area than roots of well-watered eggplants grown with ambient CO₂ environment. The relative increase in percentage (RE) due to elevated CO₂ was transient and varied during any plant developmental stage. A maximum of 82% RE in root dry biomass (RE_{RDW}) in well-watered eggplants and also additional 130% RE_{RDW} in the eggplants at 21-d lag water supply were due only to elevated CO_2 over ambient CO_2 . The strongest stimulation in root growth occurred in water stressed eggplants under elevated CO₂. Elevated CO₂ markedly stimulated the root biomass and exposed more root surface area at all depths of soil profiles but the greatest stimulation occurred at the top soil layer (0-20 cm), during all growth stages. Eggplants subjected to periodically subsequent water stress had greater stimulation at deeper bottom soil profiles during the reproductive stage. The results suggested that the elevated CO_2 (700 ppm) can compensate for restriction in root growth by water stress in improving morphological feature, and can increase net input organic C in soil.

Key words: Acclimation, eggplant, elevated CO₂, root growth, root surface area, water stress.

Introduction

Atmospheric CO₂ concentration is steadily increasing and this concentration is expected to continue to increase, double from the current level by the end of 21st century (Keeling et al. 1976; Trabalka et al. 1986). Increased CO₂ concentration along with other greenhouse gases have resulted in warmer temperature globally, a trend that is expected to continue. Changes in air temperature are likely to change precipitation pattern, worldwide, creating dry environment in many regions (Houghton et al. 2001). This, together with higher evapotranspiration resulting from warming conditions is expected to subject the agricultural crops to greater risk of more severe and prolonged water deficiency (Sengupta and Sharma, 1993; Samarakoon and Gifford, 1995; Ellsworth, 1999). 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). Some plant species are not sensitive to short-term stress and can acclimatize themselves with less damage. In nature, drought may last for a long period, its intensity may differ and sometimes may happen repeatedly.

Critical to understanding the consequences of global climate change for terrestrial ecosystems is the role of root system and belowground processes in regulating plant responses to rising CO_2 . It is reported that root growth pattern is more flexible than above-ground parts, a less genetically limited potential for taking up additional carbon in roots compared with the above-ground biomass (Thomas and Strain, 1991). A larger root system under

elevated CO_2 is supposed to achieve resulting in increased net input of organic carbon to soil. The root growth in terms of root biomass and root length density is increased in both time and space reported by several studies (Wechsung *et al.* 1999; Rogers *et al.* 1994). Elevated CO_2 may induce a greater root system with improved root physiological and morphological features that may counteract by facilitating nutrient acquisition when water stress inhibit the flux of soil water to the plant (Bassirirad *et al.* 1997; Van Vuuren *et al.* 1996).

The eggplant growth is markedly decreased due to a lack of sufficient moisture during growth and developmental stages (Sarker et al. 2005). Water stress may affect plants in very different ways when subjected to elevated CO₂. The responses of plant roots and shoots to environmental change determine the growth, survival and abundance of individual plants and communities. It is important how below-ground plant part acclimate under elevated CO₂ when plant subjected to sequential and periodic water stress. Measurement of root growth response to elevated CO₂ is critical to a understanding plant responses and organic C sequestration. Particularly, there is limited quantitative understanding of this effect of interaction between elevated CO₂ and water stress on some vegetable crops. In this study, repetitive soil moisture stress of varying degrees was simulated in eggplants, an economically important vegetable. It is hypothesized that eggplants under elevated CO₂ may ameliorate the water stress effects by improving morphological features that is important for soil carbon sequestration and taking up soil water under soil water shortage condition. The objectives of this study were (i) to quantify the root dynamics of eggplants in terms of root weight and surface area at different times of growth to elevated CO₂ interacting with subsequently imposed periodic soil water stress, and (ii) to

investigate the temporal and spatial growth pattern under ambient and elevated CO_2 concentration (700 ppm) in interaction with water stress. Such information would help to determine to what extent root morphology and net C input in soil are altered by changing of two important environmental factors, elevated atmospheric CO_2 and soil moisture stress, in agricultural crop production.

Materials and Methods

Plant and soil culture: Pot experiments were conducted in the environment controlled growth chamber at the Laboratory of Agro-Environmental Sciences in the Iwate University, Morioka (North-eastern Japan) using ambient CO_2 environment (365~370 ppm) and using elevated CO_2 environment (700 ppm). The eggplant (Solanum melongena L. cv. Senryo No. 2) was used as plant material in the study. Each of selected seedlings was 18 to 20 cm in height and was transplanted one in each pot on May 12 in 2004; documented as 0 days after transplant (DAT). The pot used for this study was 90 cm in height and 15 cm in inner diameter in which volcanic ash soil was used up to 0.80 m height for the eggplants growing purposes. The details of pot design for collecting layer based root growth was presented in Fig. 1. The soil was previously well incorporated with mixed granular fertilizer of 1:1:1 for N, P, and K @ 50 g/20 L soil as maintenance dose along with 10 g lime/20 L soil. The experiments lasted for 63 days after transplanting (DAT) in the pot.

Growth chamber environment: The experiment was conducted in the environment controlled growth chamber for crop culture. Environment controlled growth chambers were used separately for the ambient and elevated CO_2 condition. The chamber was controlled at 25 $^{\circ}C$ from 8.00 AM to 18.00 PM and at 20 $^{\circ}C$ from 20.00 PM to 6.00 AM, and artificial lighting of 40 klux was provided by lamps with a photoperiod of 12 h. The relative humidity was controlled at 70% all day long and the wind speed fluctuated between 0.4 to 0.8 m s⁻¹. Ambient CO₂ concentration containing air was supplied in one growth chamber while 700 ppm CO₂ concentration was maintained in another chamber during study period by supplying CO₂ gas with a regulator from cylinder.

Experimental design and moisture stress treatment: A completely randomized design with three replications was followed for the study purposes. The experiment was started on May 13, 2004 as the day 1st of experiment and was continued for 63 days period. Thirty six pots (18 for ambient and 18 for elevated CO₂ condition) with test plants were used for plant parameter (like root weight, surface area, length and volume) measurement. Three replicated plants from each treatment were harvested at 21 DAT and 42 DAT and 63 DAT, respectively. Note is that four pots (two from ambient and two from elevated CO_2 condition) of the last twelve were equipped with soil moisture measuring sensors and used for soil parameter measurements. In the present study, the irrigation schedule was sequenced as T1 (control, well-watered)- irrigating the eggplant pots once at every two to three days interval and T2 (water stress)- irrigating the eggplant pots once every 21 day interval. The Amb_T1 and Ele_T1 were used for well-water condition under ambient and elevated CO₂

concentrations, while Amb_T2 and Ele_T2 were used for water stress condition under ambient and elevated CO₂ concentrations. Note that the pot was irrigated to raise its soil moisture status up to pot capacity level by just replenishing the total amount of water lost by ET after each wetting and drying cycles. The soil moisture content at pot capacity level was tested in this study on the basis of watering the pot at saturation level and then the moisture was allowed for depletion to the pot soil up to its water holding capacity level against gravitational force. All the holes of the pot were completely sealed with silicon grease before starting the experiment. Growth stage of eggplants for this study was assigned by 0-21 DAT as vegetative stage:1st irrigation-drving cycle, 21-42 DAT as early reproductive stage; 2nd irrigation-drying cycle and 42-63 DAT as peak reproductive stage; 3rd irrigation-drying cycle, respectively.

Volumetric water content: The four pots with test plants applying four different treatments were used for soil volumetric water content (VWC) measurement with Time Domain Reflectrometry (TDR) sensor (Model: Campbell Scientific Inc., USA). The measurement was monitored in each pot during whole growing period at every 30 minutes interval at three different layers (upper 0-30 cm, mid 20-50 cm and bottom 50-80 cm pot soil depth (Fig. 1) to monitor VWC during growth time. The data was collected instantly at 7-d interval for this study through data logger CR10X (Model: Campbell Scientific Inc., USA).



Fig.1. Schematic diagram of experimental pot equipped with data logging system; thermo domain reflectometry (TDR) and data logger CR10X connected with computer and position of three soil cores (100 mL).

Root growth parameters: Roots in each pot were collected by carefully washing soil from the root mass on a sieve, and were finally washed by using an ultrasonic cleaner to remove fine particles adsorbed onto the root mass. Layer-based root data was collected by removing the gum tape, and the soil was cut by using a sharp knife and was washed carefully with spraying water. The fresh root biomass (g) was recorded just after gently blotting. To calculate the root surface area (RSA) (cm²), roots were spread on a contrast background and photographs were taken by using a digital camera (Olympus Optical Company, Model No. C 2000 Zoom, Japan). The RSA was calculated by using image analysis with the software LIA32 using the following formula:

$$RSA = \frac{ITR \times ARi}{IAR} \times \pi$$
[1]

where, ITR, ARi and IRA are the image area of total root measured by the software, the actual reference index area on a background, and the average image of reference index area measured by the software, respectively, and π is used as a 3-D factor. The collected roots were dried at 65 0 C in an oven for 96 hours until a constant dry biomass (g).

Relative enhancement (RE) of root dry biomass and root surface area due to elevated CO_2 over ambient CO_2 was calculated as:

$$RE_{RDW} = 100 \times [(RDW_{Ele} - RDW_{Amb})/RDW_{Amb}]$$
[2]

$$RE_{RSA} = 100 \times [(RSA_{Ele} - RSA_{Amb})/RSA_{Amb}]$$
[3]

where, RE_{RDW} and RE_{RSA} are the relative enhancement of root dry weight and root surface area due to elevated CO₂. RDW and RSA indicate root dry biomass and root surface area and subscript Ele and Amb are for elevated and ambient CO₂ condition.

Statistical analysis: A statistical analysis following completely randomized design (CRD) with three replications was performed. The three factorial (Time, Water and CO₂) analysis of variance (ANOVA) test was done separately for the total root dry biomass and surface area. 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 root dry biomass and root surface area collected at different growth stages in the study. When (Time×CO₂×Water) interaction and (CO₂×Water) interaction were insignificant, the individual effect of elevated CO₂ and soil water was analyzed and used for interpretation.

Results

Volumetric water content (VWC) of the pot soil at different depth of layers during entire experimental period has been depicted in Fig. 2 which indicated the dynamic changing pattern with time under different soil moisture and elevated CO_2 treatments. As the time progress, significantly variation in soil moisture content was observed at different pots due to different irrigation frequency and evapotranspiration. The initial water

content was about 0.55 to 0.60 m³ m⁻³ at upper 0-30 cm, 0.65-0.70 m³ m⁻³ at mid 20-50 cm and 0.65-0.80 m³ m⁻³ at bottom 50-80 cm soil layer, respectively, for all the TDR equipped pots soil. The Amb_T1 and Ele_T1 eggplants showed a small decrease at upper 0-30 cm soil layer and returned to its original level or little higher after replenishment. Rate of depletion in water content was increased with the increased growth rate of plant. At the end of 3rd irrigation-drying cycle, VWC reduced to as low as 0.20 to 0.24 m³ m⁻³ at the mid and bottom pot soil layers. It was visualized that leaf showed wilting while the VWC of soil reached below 0.30 m³ m⁻³. Thus, this VWC was considered the relative critical point to wilting for this experiment. For Amb_T1 and Ele_T1 eggplants, VWC reached below wilting point recorded from the end of 2nd irrigation-drying cycle due to higher transpirational demand. The VWC in Amb_T2 and Ele_T2 eggplants pots continued to reduce and it approached to about 0.20 m³ m⁻ ³ at the end of the 3rd irrigation-drying cycle, which was below the wilting point under this experiment.

Temporal trend and spatial distribution of RDW: No significant $(Time \times CO_2 \times Water)$ interaction and (CO₂×Water) interaction were observed on total RDW at three growth stages but (Time \times CO₂) and (Time \times Water) interaction. ANOVA study indicates that the elevated CO₂ and water effects were predominant, and total RDW was stimulated due to the increasing levels of CO_2 concentration in the air environment of the growth chamber irrespective of their pot soil moisture status, for most of the developmental stages. Elevated CO₂ concentrations markedly stimulated root growth (Table 1). The stimulatory effect of elevated CO₂ on RDW was consistent throughout the experiment time. The strongest stimulation of RDW occurred during reproductive stage, i.e., 21 to 63 DAT of the experiment and the slowest stimulation during vegetative stage, i.e., 0 to 21 DAT. A marked decrease in eggplant RDW was observed due to water stress in both levels of CO₂ environments during the early reproductive stage and peak reproductive stages. The Amb_T2 eggplants had 73%, 91% and 73% RDW of the Amb_T1 eggplants during the vegetative, early and peak reproductive stages, i.e., 21, 42 and 63 DAT and the Ele_T2 eggplants had 92%, 82% and 76% RDW of the Ele T1 eggplants.

Table 2 shows the relative increase in RDW due only to elevated CO_2 over ambient in pot culture. Both fresh and dry matter in root markedly increased due to elevated CO_2 . The RE due to elevated CO_2 was comparatively greater in water stressed eggplants than their corresponding wellwatered plants. The greater RE in eggplants for both water conditions under elevated CO_2 was at the vegetative stage. Furthermore, the Ele_T1 eggplants increased 82%, 58% and 42% more RDW than Amb_T1 eggplants and more importantly, Ele_T2 eggplants produced 67%, 30% and 8% more RDW than Amb_T1 eggplants during the vegetative, early and peak reproductive stages.

The differences in spatial and temporal distribution in eggplant RDW at different depth of layers are presented in Fig. 2. In general, the highest RDW was always observed at the top 0-20 cm soil layer. The highest RDW was at the

top layer and a gradual decrease in RDW at the subsequent bottom layers was the feature during the vegetative stage.



Fig.2. Time course volumetric water content (VWC) along the vertical 0-80 cm pot soil profile for eggplants grown under ambient and elevated CO_2 environments at different times of experiments. (A) and (B) represent the VWC at 7-d interval for ambient and elevated CO_2 environments. Vertical bar indicates the day of irrigation for Amb_T2 and Ele_T2 pots at 21-d lag of period.

However, RDW at bottom 65-80 cm layer gradually increased during the early reproductive and peak reproductive stage. Periodically and subsequently developed water stress reduced root growth at almost every depth of layers with few exceptions. During the peak reproductive stage, the bottom 65-80 cm soil layer had a greater RDW than 35-50 cm and 50-65 cm depth of layers, in all the eggplants. Greater enhancement in eggplants under stress at bottom 65-80 cm soil layer under elevated CO₂ than ambient CO₂ was the characteristic of the present study.

Relative increase of RDW at different growth profiles (Layer based) due only to elevated CO₂ over ambient CO₂ is presented in Table 3 which indicates the greater enhancement during the vegetative stage. The deepest bottom 65-80 cm soil layer had the highest enhancement during the vegetative stage, irrespective of their soil water status. At the early reproductive stage and peak reproductive stage, RE gradually slowed down. The Ele_T1 eggplants had some negative values indicating reduced root growth at 65-80 cm soil layer during peak reproductive stage than Amb_T1 eggplants and the Ele_T2 had greater RE than the Ele_T1 eggplants during the peak reproductive stage.

Temporal trend and spatial distribution of RSA: Water stress had marked influence on root surface area (RSA)



Fig. 3. Distribution of root dry weight along the vertical 0-80 cm pot soil profile for eggplants grown under ambient and elevated CO_2 environments at different times of experiment. (A), (B) and (C) represent the layer based root weight at 21, 42 and 63 DAT.

reduction either in eggplants grown under ambient or elevated CO₂ environments. Likewise RDW, both eggplants grown either in well-watered or water stress condition increased RSA under elevated CO_2 concentration with compare to their corresponding counterparts, grown under ambient CO₂ (Table 1). A comparative statement on relative increase in RSA along with RDW due to elevated CO_2 is presented in Table 2. Statistical analysis showed no significant (Time \times CO₂ \times Water) and (CO₂ \times Water) interactions were observed on total RSA at three growth stages. But the independent effect of time, water and elevated CO_2 on RSA was existed for this study. The Ele_T1 eggplants increased additionally 43%, 25% and 36% RSA during vegetative, early and peak reproductive stages due to elevated CO₂, than those of Amb_T1 eggplants. Similarly, RE in Ele_T2 eggplants was 75%, 57% and 109% RSA during vegetative, early and peak reproductive stages due to elevated CO₂, than those of Amb_T2 eggplants. Further, eggplants under interactive effect of elevated CO₂ and water stress had more stimulatory effect on RSA than eggplants grown only under elevated CO₂ environment. Spatial distribution of RSA in different eggplants at different times of experiment grown under both CO₂

levels is presented in Fig 3. The growth pattern in regard to RSA at different depth of layers was almost as similar as RDW in all the eggplants under both CO₂ levels. Table 3B shows that a wide variation in RE_{RSA} was observed at different soil profiles. The Ele_T1 eggplants had lower RSA at 65-80 cm layer than Amb_T1 but periodically water stressed Ele_T2 eggplants always stimulated their RSA at the bottom layers. At peak reproductive stage, the RE_{RSA} in Ele_T1 eggplants had 139%, 88%, 40% 8% and -26% while the RE_{RSA} in Ele_T2 eggplants had 77%, 109%, 206%, 95% and 184%, respectively, at 0-20 cm, 20-35 cm, 35-50 cm, 50-65 cm and 65-80 cm depth of layers. The Ele T1 plants had negative RE_{RSA} for the bottom 65-80 cm indicating less root growth than Amb T1 eggplants but more importantly, the Ele T2 eggplants that experienced periodically water stress induced a growth pattern with increased RSA and RE_{RSA} at every depth of layers (Table 3; Fig. 4).



Fig. 4. Distribution of root surface area along the vertical 0-80 cm pot soil profile for eggplants grown under ambient and elevated CO_2 environments at different times of experiment. (A), (B) and (C) represent the layer based root surface area at 21, 42 and 63 DAT

Discussion

The soil moisture deficit in different levels experienced by eggplants from the seedling to harvesting stage showed a distinctive change in root growth in terms of root dry weight and root surface area. A marked variation in VWC at different soil layers was due to different root water extraction and evapotranspiration.

Relating the response of eggplant roots to soil moisture stress under both CO₂ conditions in the growth environment provided information both on stress sensitivity aspects of root dynamics and how root growth of stressed eggplants was acclimatized under elevated CO₂ environment. Temporal behavior of eggplant root dynamics showed a definitive pattern, with a peak RDW during peak reproductive stage (Table 1). Water stress reduced the root growth under both CO₂ concentrations at any growth stage but a significant reduction was observed during the peak reproductive stage. Statistical analysis indicated that there was no significant effect on the interaction (Time×CO₂×Water) among growth time, soil water and elevated CO₂, and also soil water and elevated CO_2 but there exists independently significant effect on root dry weight.

The eggplants at the end of 1st irrigation-drying cycle with 21-d lag of watering during vegetative stage, reduced soil moisture but that have above wilting range showed an increased root growth, about two-third of well-watered eggplants grown under ambient CO_2 . Similarly, eggplants at early reproductive and peak reproductive stage had negative root growth under subsequently developed water stress. Interest is that root biomass in eggplants at well-watered conditions under ambient CO_2 was similar and sometimes lower than those of eggplants grown at water stressed conditions under elevated CO_2 (700 ppm) environment. In this study, it is evident that eggplant root acclimatized well showing a complementary root growth under the environment of elevated CO_2 interacting with water stress condition.

The RE_{RDW} due to elevated CO₂ is transiently variable during different growth stages of eggplants either in wellwatered or water stressed conditions. A remarkable increase in RE_{RDW} was always observed in well-watered eggplants under elevated CO₂ over ambient CO₂ environment. The well-watered eggplants had a greater increase in RE_{RDW} at vegetative stage (82%) followed by gradual decrease at early reproductive stage (58%) and then peak reproductive stage (42%). Eggplants grown under the interaction of elevated CO₂ and water stress had also greater increase during vegetative stage (130%). During early and peak reproductive stage, 44% and 48% RE_{RDW} occurred under periodical soil moisture stress. For eggplants those grown at both soil water conditions, a maximal effect reached at vegetative stage, i.e. 21 DAT (Table 3B). In general, results agreed well with those obtained from many other environmental studies but different crops; wheat (Wechsung et al. 1999) and cotton (Roggers et al. 1994).

Growth profiles of root dry biomass over depth (Fig. 2) showed an exponential distribution, and top layer (0-20 cm) had the highest root biomass in all the eggplants irrespective of their water and CO_2 levels and a similar

	(A) Root biomass (dry weight) and acclimation				(B) Root surface area and acclimation			
Time	Treatment	Growth	Root weight	Regulation	Remarks	Root surface area	Regulation	Remarks
DAT		Condition	(g)	(%)		(cm ²)	(%)	
0	Control	Water	0.79	-	-	397	-	-
		$CO_2 \times Water$	0.83	-	-	391	-	-
21	Amb_T1	Water	3.75	100	Control	3861	100	Control
egetative	Amb_T2	Stress	2.73	73	Down regulation	2523	65	Down regulation
	Ele_T1	CO ₂ ×Water	6.84	182	Up regulation	5529	143	Up regulation
	Ele_T2	CO ₂ ×Stress	6.28	167	Up regulation	4409	114	Up regulation but complementary
-	Amb_T2	Stress	2.73	100	Control	2523	100	Control
	Ele_T2	CO ₂ ×Stress	6.28	230	Up regulation	4409	175	Up regulation
42	Amb_T1	Water	13.02	100	Control	10156	100	Control
ė	Amb_T2	Stress	11.82	91	Down regulation	4844	48	Down regulation
/ reproductiv	Ele_T1	CO ₂ ×Water	20.63	158	Up regulation	12711	125	Up regulation
	Ele_T2	CO ₂ ×Stress	16.97	130	Up regulation	7613	75	Down regulation but complementary
Sarl	Amb_T2	Stress	11.82	100	Control	4844	100	Control
Щ	Ele_T2	CO ₂ ×Stress	16.97	144	Up regulation	7613	157	Up regulation
reproductive 89	Amb_T1	Water	23.20	100	Control	12532	100	Control
	Amb_T2	Stress	16.92	73	Down regulation	4675	37	Down regulation
	Ele_T1	$CO_2 \times Water$	33.02	142	Up regulation Complementary	16996	136	Up regulation
	Ele_T2	CO ₂ ×Stress	25.02	108	regulation	9785	78	Down regulation but
eak	Amb T2	Stress	16.92	100	Control	4675	100	Control
<u>н</u>	Ele_T2	$CO_2 \times Stress$	25.02	148	Up regulation	9785	209	Up regulation

Table 1. Root biomass and surface area of	eggplants and their	acclimation during	the experiment un	der elevated CO ₂
interacting with soil water stress				

Table 2. Relative enhancement (RE) of eggplant root biomass and root surface area due

to elevated CO_2 over ambient CO_2 in the growth environment.								
Time	(A) Root fresh biomass		(B) Root dry biomass		(C) Root surface area			
DAT	Ele_T1	Ele_T2	Ele_T1	Ele_T2	Ele_T1	Ele_T2		
	(%)	(%)	(%)	(%)	(%)	(%)		
21	50	92	82	130	43	75		
42	31	37	58	44	25	57		
63	58	93	42	48	36	109		

Table 3. Relative enhancement of eggplant roots at different depth of layers under ambient and elevated CO₂ environments at different times of experiment

Time	Depth	Root dry biomass		Root surface area	
DAT	of layers	Ele_T1	Ele_T2	Ele_T1	Ele_T2
		(%)	(%)	(%)	(%)
21	0-20	59	117	17	56
	20-35	97	152	46	82
	35-50	160	169	72	118
	50-65	186	152	80	76
	65-80	917	354	663	264
42	0-20	91	53	86	53
	20-35	55	23	59	49
	35-50	1	39	0	42
	50-65	38	36	-14	51
	65-80	21	37	-21	97
63	0-20	98	41	139	77
	20-35	30	35	88	109
	35-50	-13	54	40	206
	50-65	9	47	8	95
	65-80	-28	94	-26	184

agreement was with other findings (Wechsung et al. 1999; Gerwitz and Page, 1974). All the eggplants under elevated CO₂ markedly increased root biomass at top 0-20 cm layer than those of eggplants grown under ambient CO_2 . Elevated CO₂ also induced to increase root biomass at all other depth of layers with plant growth. Amb_T1 eggplants had higher root biomass at bottom layer (65-80 cm) than Ele_T1 eggplants, at peak reproductive stage. The fact is that eggplants grown under the interactive effect of elevated CO₂ and water stress also had higher root biomass at the deepest bottom layer (65-80 cm) showing a positive and complementary root growth in eggplants, at the early and peak reproductive stage. This would be explained as more branching of lateral roots under elevated CO₂ may occur due subsequently to intensify water stress during the reproductive stage. Such phenomenon in soil might enhance the greater root proliferation and C allocation at deeper bottom soil layers. An increased root at a deeper soil layer play an important role for using water and nutrient capture from deeper soil zone during soil water deficit period and sequestrate more organic C in soil. In general, deep-rooted plants survive under drought better than those with shallow roots, and extensive root development is an important factor for drought tolerance suggested by several studies (Smucker et al. 1991; Carrow, 1996; Qian et al. 1997). In the present study, biomass allocation in roots in water stressed eggplants at deeper bottom (65-80 cm) layer under elevated CO₂ environment might be important for survival during drought or water shortage period. The RE_{RDW} increase at different depth of soil layers due only to elevated CO₂ was also greater in water stressed eggplants than well water stressed eggplants (Table 2). The greater enhancement was at vegetative stage observed for the eggplants irrespective of their soil water status. Water stressed eggplants had higher RE_{RDW} at top (0-20 cm) and 2nd most upper layer from the top (20-35 cm) than wellwatered eggplants and lower RE_{RDW} had at deeper bottom layers (35-50 cm, 50-65 cm and 65-80 cm), during the vegetative stage. However, this trend of spatial distribution over depth altered during the early and peak reproductive stages; characterized by lower RE_{RDW} at upper soil layer and greater RE_{RDW} at deeper bottom layers, due to the effect of elevated CO₂, respectively. An increased root biomass at different soil layers under both soil water conditions and elevated CO2 concentration resulted in increased net organic C sequestration in different soil layers and might increased more soil moisture and nutrient ions acquisition.

A complementary root growth under elevated CO_2 was observed for the plants those experienced with periodically subsequent soil water stress at different levels. Eggplants increased RSA in eggplants under elevated CO_2 as root biomass did. Soil water and elevated CO_2 condition had independent effect on RSA at all growth stages as per ANOVA result. No significant interaction was observed but had independent effect of water and elevated CO_2 . Greater and positive acclimation occurred during vegetative stage then declined the acclimation percentage. Well-watered eggplants increased 43% at vegetative stage, 25% at early reproductive stage and 36% at peak reproductive stage. The highest increase in RE_{RSA} in water stressed eggplants under elevated CO_2 was during peak reproductive stage and RE_{RSA} declined during early reproductive stage which likely similar to well-watered eggplants. The RSA growth profiles at different depth of soils for all eggplants under study had exponential distribution at vegetative stage. The RSA growth continued to increase with the increase in plant growth. Likewise RDW, RSA of water stressed eggplants under elevated CO_2 had greater than those of eggplants grown under ambient CO_2 , at all depth of layers.

A comparative study indicated that RE_{RSA} due to elevated CO_2 among different soil layers was greater at top layer (0-20 cm) during vegetative stage. During early and peak reproduction stage, water stressed plants had greater RE_{RSA} at 2nd most upper layer from the top to following deeper bottom layers (20-35 cm, 35-50 cm, 50-65 cm and 65-80 cm), indicating stronger stimulation on RSA under the interactive effect of water stress and elevated CO_2 than well-watered plants. Fact is that, root growth for well-watered eggplants under elevated CO_2 had negative enhancement at deeper bottom layers during reproductive stages indicating lower root growth than ambient CO_2 but striking RE_{RSA} at the top layer is the feature of well-watered condition.

In conclusion, elevated CO_2 environment increased root growth in relation to biomass and surface area expansion in soil leading to acclimation, with increased root growth at top soil layer; water stress increased root biomass and surface area at deeper bottom soil layers. Indeed, the root dry biomass is as larger in water stressed eggplants under elevated CO_2 (700 ppm) as in well-watered eggplants in ambient CO_2 environment. Stimulated RDW and RSA at both soil water conditions under elevated CO_2 concentration resulted in increased net organic C sequestration in soil and may enhance more soil water and nutrient acquisition. A complementary root growth in water stressed eggplants under elevated CO_2 indicated better survival ability and might be beneficial during future global environment change.

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