Effects of elevated CO_2 applied to potato roots on the anatomy and ultrastructure of leaves

Z.-P. SUN[1,2], T.-L. LI[1,2]* and Y.-L. LIU[1,2]

Key Laboratory of Protected Horticulture, Ministry of Education[1] *and College of Horticulture, Shenyang Agricultural University*[2]*, Shenyang 110866, P.R. China*

Abstract

The root system of potato (*Solanum tuberosum* L. cv. Favorita) plants was treated with different O_2 and CO_2 concentrations for 35 d in aeroponic culture. Under 21 or 5 % O_2 in the root zones, the thickness of leaves and palisade parenchyma significantly increased at 3 600 μmol(CO_2) mol^{-1} in the root zone, compared with CO_2 concentration 380 μmol mol^{-1} or low CO_2 concentration (100 μmol mol^{-1}). In addition, smaller cells of palisade tissue, more intercellular air spaces and partially two layers of palisade cells were observed in the leaves with root-zone CO_2 enrichment. Furthermore, there was a significant increase in the size of chloroplasts and starch grains, and the number of starch grains per chloroplast due to elevated CO_2 only under 21 % O_2. In addition, a significant decline in the thickness of grana and the number of lamellas, but no significant differences in the number of grana per chloroplast were observed under elevated CO_2 concentration. The accumulation of starch grains in the chloroplast under elevated CO_2 concentration could change the arrangement of grana thylakoids and consequently inhibited the absorption of sun radiation and photosynthesis of potato plants.

Additional key words: aeroponics, chloroplast grana ultrastructure, palisade parenchyma, *Solanum tuberosum,* starch grains.

Introduction

At the present, the atmospheric CO_2 concentration is about 380 μmol mol^{-1} and by the end of this century might be doubled (Pearson and Palmer 2000, Li *et al*. 2009). It is rather surprising that elevated atmospheric CO_2 usually stimulates soil respiration (Janssens and Ceulemans 2000). Kimball *et al*. (2002) found in average 24 % stimulation in soil respiration for a variety of crops grown in elevated CO_2. Luo *et al*. (1996) observed a 42 % stimulation of soil respiration under elevated CO_2 in natural Mediterranean grasslands, Ball and Drake (1998) reported 15 % stimulation in natural marsh vegetation. In experiments using tree species, Körner and Arnone (1992) reported that soil respiration doubled in artificial tropical ecosystems developing in mesocosms in CO_2-enriched environments. Longer term exposures also stimulated soil respiration in *Pinus ponderosa* (Johnson *et al*. 1994, Vose *et al*. 1997), *Acer rubrum* and *Acer saccharum* (Edwards and Norby 1999), and *Pinus sylvestris* (Janssens *et al*. 1998). Using FACE technology on natural soils, King *et al*. (2004) showed increased soil respiration (39 %) for *Populus tremuloides* and *Betula papyrifera*. As a result, the rising soil respiration leads to an increased CO_2 concentration in the root zones (Ineson *et al.* 1998, Diao *et al*. 2002, Kimball *et al.* 2002, Gonzalez-Meler *et al*. 2004), which, in turn, would have impact on the plant growth.

On the other hand, CO_2 is the primary substrate for photosynthesis in the green plants and the photosynthetic capacity of C_3 plants is limited by the CO_2 concentration in the present atmosphere. Various researchers have shown that the root system of some plants such as potato, barley, onion, citrus and soybean can absorb CO_2 (Overstreet 1940, Graf 1955, Coker 1981, Cramer and Richards 1999, Hibberd and Quick 2002). Arteca and Poovaiah (1982) and McGuire *et al*. (2009) further confirmed that the leaf photosynthesis of potato plant

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Abbreviations: ACES - the aeroponic culture experiment system; TEM - transmission electron microscopy.

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* Corresponding author; fax: (+86) 24 88487166, e-mail: tianlaili@126.com

could use of the CO_2 absorbed by root system. In addition, Arteca and Poovaiah (1979) reported the effects of 12 h CO_2 enrichment (45 % CO_2+ 21 % O_2+ 34 % N_2) in root zone on the growth of potato plant and the result showed significantly increased dry mass and tuberization compared with control (ambient air treatment). Therefore, we studied the effects of different CO_2 concentrations in the root zone in combination with different O_2 concentrations on the anatomy and ultrastructure of potato leaves.

Materials and methods

Potato (*Solanum tuberosum* L. cv. Favorita) virus-free plantlets grown in the test tubes were firstly planted in peat:*Vermiculite* mixture (1:1/ v:v). After 21 d, twelve uniform single plantlets with height approximately 15 cm were transplanted on the culture barrel heads at equal spacing around the circumference of circle (30 cm diameter) in the aeroponic culture experiment system (ACES). The roots of each plantlet were inserted through holes (2 cm diameter). The stems were wrapped with asbestos and the lacuna of holes was filled with asbestos in order to prevent CO_2 leakage. The root zones of potato plants were continuously aerated with a gas stream at the flow rate of 3 - 10 dm^3 min^{-1}.

The ACES system, developed by the Protected Horticulture Lab, Shenyang Agricultural University, included a plastic barrel for cultivation, a spray nozzle, a pump, an electronic timer, an electromagnetic valve, a control valve, a manometer, a solution strainer, a PVC pipe and reservoir (150 dm^3). The nutrient solution, which contained [mg dm^{-3}] 527 NO_3^-, 16.2 NH_4^+, 240 $H_2PO_4^-$, 220 K^+, 80 Ca^{2+}, 28 Mg^{2+}, 93 SO_4^{2-}, 2 Fe^{2+}, 1 Mn^{2+}, 0.4 Zn^{2+}, 2.1 $H_2BO_4^-$, 0.03 Cu^{2+}, and 0.1 MoO_4^{2-}, was supplied for half min every 5 min by pump to the root zone of potato plants at a flow rate of 1 dm^3 min^{-1} and a pressure of 0.2 MPa. The solution in the reservoir (pH 6.0) was replaced biweekly and solution temperature was kept at 17 - 20 °C.

Four CO_2 treatments of root zone were used: 380 μmol(CO_2) mol^{-1} + 21 % O_2, 3 600 μmol(CO_2) mol^{-1} + 21 % O_2, 100 μmol(CO_2) mol^{-1} + 5 % O_2 and 3 600 μmol(CO_2) mol^{-1} + 5 % O_2). The first treatment corresponded to the ambient atmosphere level and the air was supplied by a compressor outside the greenhouse. The combination 3600 μmol mol^{-1} (CO_2) + 21 % O_2 was a mixture of pure CO_2 and ambient air; 100 μmol mol^{-1} (CO_2) + 5 % O_2 was made from a mixture of pure N_2 and current atmosphere; 3600 μmol mol^{-1}(CO_2) + 5 % O_2 was a mixture of pure CO_2, pure N_2 and ambient air. CO_2 concentration of every treatment was monitored by a portable photosynthesis system *Li-Cor 6400* (Lincoln, NE, USA) twice a week.

In the greenhouse, the CO_2 concentrations varied from 380 μmol mol^{-1} (day) to 920 μmol mol^{-1} (night). A photosynthetic photon flux was 600 - 870 μmol m^{-2} s^{-1} at noon of a sunny day, at the same time there was ventilation from 9:30 to 14:30. Photoperiod was 10 - 12 h and day/night temperature 20 - 27/13 - 16 °C.

After 35-d treatment, samples from the tip leaflet of the fourth fully expanded leaf from the top were collected, immediately fixed in 3 % glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2), thoroughly washed with 0.1 M cacodylate buffer and postfixed in 2 % osmium tetroxide in the same buffer, dehydrated in a graded series of ethanol, and embedded in *Epon 812*. For light microscopy, thin sections (2 - 4 μm) were made with a *AO-820* (*Scientific instruments*, USA) rotary microtome and then observed and photographed using an *Olympus BH-2* (Tokyo, Japan) microscope. Quantitative data were based on 9 - 21 measurements per item. For electron microscopy, ultrathin sections collected on *Formvar*-coated copper grids were stained with uranyl acetate and lead citrate. Observations and photographs were taken using a *JEM-100CX/II* (*JEOL*, Japan) transmission electron microscope (TEM). Quantitative data were based on 50 - 180 measurements per item.

Experiments were conducted twice from October 2004 to June 2005. *ANOVA* was used to analyze the results and the means were compared by Duncan's multiple range test.

Results

The root-zone CO_2 enrichment had a significant influence on the leaf structure of potato plants (Table 1). Under a 21 % (normal) O_2 concentration in the root zone, plants treated by 3 600 μmol(CO_2) mol^{-1} showed 10.6 % increase in the thickness of leaves and 5.7 % increase in the thickness of palisade parenchyma compared with the leaves of plants treated with ambient CO_2 concentration (380 μmol mol^{-1}). Under a 5 % O_2, the thickness of leaves and palisade parenchyma increased by 21.4 and 11.4 %, respectively, under elevated CO_2 concentration compared with the 100 μmol(CO_2) mol^{-1}.

Under elevated CO_2 concentration (3 600 μmol mol^{-1}) together with 21 or 5 % O_2, smaller cells of palisade parenchyma, more intercellular air spaces and two partial layers of palisade cells were observed in the leaves (Fig. 1*C*,*D*). At 100 or 380 μmol(CO_2) mol^{-1}, the palisade

parenchyma consisted of one layers of long, uniform and tightly arranged cells (Fig. 1*A*,*B*).

Further, the CO_2 enrichment caused a significant decrease in the number of chloroplasts per palisade parenchyma cell, significant increase in the length of chloroplasts and no significant difference in the width. Under a 5 % O_2 concentration, the CO_2 enrichment led to increase in the length of chloroplasts, but no significant difference in the width and number of chloroplasts. In addition, under 3 600 μmol(CO_2) mol^{-1} and 21 % O_2, a highly significant decrease in the number of starch grains per palisade cell, but an increase in the number, length and width of starch grains per chloroplast were observed. Under the same CO_2 enrichment and 5 % O_2, there was a significant increase in the number, length and width of starch grains per palisade tissue cell but no significant difference in the number of starch grains per chloroplast. The root-zone CO_2 enrichment caused a significant decline in the number of lamella per granum, and the thickness of grana per chloroplast, but there was no significant difference in the number of grana per chloroplast whether under 21 or 5 % O_2. Electron micrographs of chloroplast from 3 600 μmol(CO_2) mol^{-1} treated plants (Fig. 2*D-F*) showed increase in the size of starch grains per chloroplast, resulting in a distorted and swelled chloroplasts. In addition, the lamellar structure grana and the disk-structure grana were coexistent in a transverse section of chloroplast.

Table 1. Effects of 35-d CO_2 treatments in root zones on the leaf anatomy and chloroplast ultrastructure of potato plants. Means of three plants (50 -180 measurements per item). * - significantly different at $P \leq 0.05$ according to Duncan's multiple range test.

Leaf and chloroplast feature	21 % (O_2)		5 % (O_2)	
	380 μmol mol^{-1}(CO_2)	3600 μmol mol^{-1}(CO_2)	100 μmol mol^{-1}(CO_2)	3600 μmol mol^{-1}(CO_2)
Leaf thickness [μm]	243.40	269.20*	241.00	292.60*
Palisade layer thickness [μm]	110.60	116.40*	108.80	121.20*
Palisade/leaf thickness ratio	0.45	0.43	0.45	0.41
Number of chloroplasts [$cell^{-1}$]	26.80	23.00*	21.33	23.00
Chloroplast length [μm]	3.23	3.40*	2.67	3.60*
Chloroplast width [μm]	1.67	1.84	1.50	1.57
Number of starch grains [$cell^{-1}$]	44.20	40.40*	36.67	39.33*
Number of starch grains [$chlp.^{-1}$]	1.65	1.76*	1.72	1.71
Starch grain length [μm]	1.30	1.51*	1.01	1.30*
Starch grain width [μm]	0.87	1.02*	0.60	0.84*
Number of grana [$chlp.^{-1}$]	20.00	18.33	14.00	16.33
Thickness per granum [μm]	0.11	0.09*	0.09	0.07*
Number of lamellas [$granum^{-1}$]	18.00	11.33*	11.00	8.96*

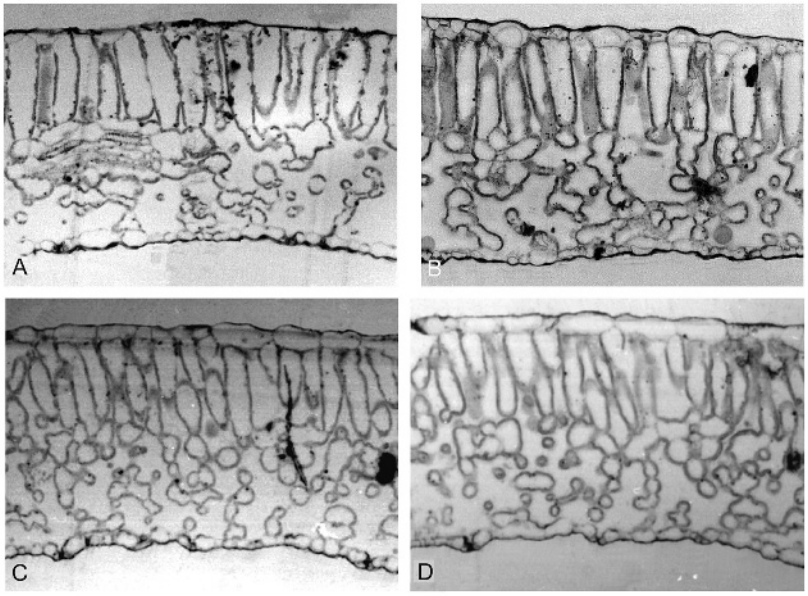

Fig. 1 Light micrographs of transverse sections of leaf tissues from 35-d potato plants grown under 380 μmol(CO_2) mol^{-1} + 21 % O_2 (*A*), 100 μmol(CO_2) mol^{-1} + 5 % O_2 (*B*), 3 600 μmol(CO_2) mol^{-1} + 21 % O_2 (*C*) and 3 600 μmol(CO_2) mol^{-1} CO_2 + 5 % O_2 (*D*) in the root zone.

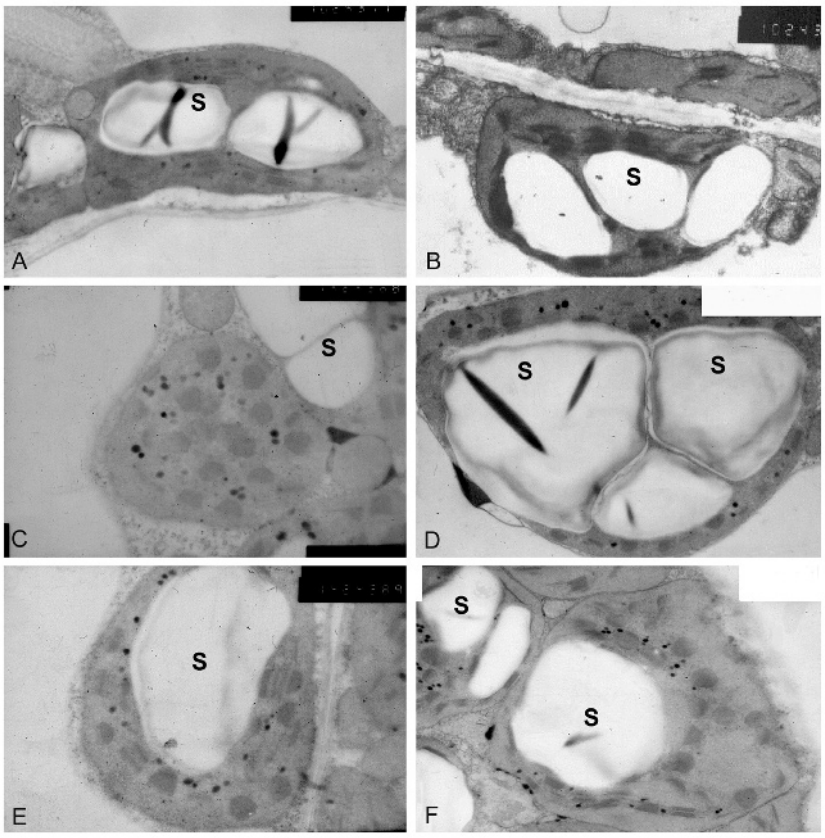

Fig. 2. Electron micrographs (×10 000) of transverse sections of chloroplast from 35-d potato plants grown under 380 μmol(CO_2) mol^{-1} + 21 % O_2 (*A,C*), 3 600 μmol(CO_2) mol^{-1} + 21 % O_2 (*D,E*), 100 μmol(CO_2) mol^{-1} + 5 % O_2 (*B*) and 3 600 μmol(CO_2) mol^{-1} CO_2 + 5 % O_2 (*F*) in the root zone (S - starch grain; chloroplasts located in the side of palisade parenchyma cell (*A*,*B*,*D*,*E*), chloroplast located in the bottom of palisade layer (*C*,*F*).

Discussion

Potato plants exhibited a significant increase in the total leaf thickness following the root-zone CO_2 enrichment. This is consistent with studies with various crops such as common bean, soybean, and cucumber grown at the CO_2 enrichment in root atmosphere. However, the causes of increased leaf thickness are not all consistent. Radoglou and Jarvis (1992) found that the increased thickness of the *Phaseolus vulgaris* leaf was particularly due to increase in the spongy parenchyma thickness. However there were no significant differences in the ratio of palisade to spongy parenchyma, the number of layers of palisade cells and the stomatal density. Wei *et al*. (2002) observed simultaneous increase in the thickness of leaf, epidermis and palisade tissue with longer and more tightly arranged palisade cells, and a slight increase in the ratio of palisade tissue to leaf thickness in the CO_2-enriched plants. Also, CO_2 enrichment around soybean roots (Vu *et al.* 1989, Lin and Hu 1996) caused an increase in leaf thickness, due to an increased number of palisade cells. In this study with the root-zone CO_2 enrichment, the thickness of potato leaves largely resulted from a simultaneous increase of palisade and spongy tissue, but the rate of spongy tissue thickening was greater than that of palisade tissue. There were also smaller and loosely arranged cells of palisade tissue and more intercellular air spaces in the leaves. Furthermore, two partial layers of palisade parenchyma cells were also observed under CO_2 enrichment (Fig. 1*D*). These results suggest that different plant species respond differently to the CO_2 enrichment.

Although there are some differences in the acclimation of the leaf chloroplast ultrastructure of various plants to CO_2 enrichment either around the aboveground plant parts or the underground plant parts, starch grain accumulation in the leaf chloroplast was always found. In this study, the CO_2 enrichment in rote zone significantly increased the size of chloroplast and number of starch grains per palisade parenchyma cell, and the number of starch grains per chloroplast. In addition, there was a significant decline in the thickness of grana, and the number of lamellas per granum. This was consistent with Zuo *et al*. (1996), who found that

under doubled root zone CO_2 concentration both the size and number of chloroplast in *Medicago sativa* increased, the grana became smaller and were infrequently dispersed among starch grains within the chloroplast. An accumulation of starch grains in the chloroplast of tomato leaves was also observed by Yelle *et al*. (1989). Many studies in potato also showed an increased leaf starch content following long-term exposure of roots to elevated CO_2 (Miglietta *et al*. 1998, Finnan *et al*. 2005). However, Wei *et al*. (2002) found in cucumber not only increased number and size of chloroplasts and starch grains in the palisade Parenchyma cells, but also the number of grana per chloroplast and lamella per granum under CO_2 enrichment.

According Gunning and Steer (1975), the grana of a mature chloroplast in higher plants consist of the flatted and disk-shaped thylakoids, which are typically stacked one on the top of the other in columns. Often these grana are erectly arranged along the long axis of chloroplast and interconnected by the stromal thylakoids. Such arrangement could significantly enlarge the effective area capturing sun radiation (Bunchanan *et al*. 2000, Perter and Johnson 2002). When observing the micrographs of transverse sections of chloroplast from TEM, the lamellar structure can be observed at the lateral view of the grana and the disk-shaped structure of grana at the top or bottom view of the grana. The lamellar structure of grana in the ultrathin sections of chloroplast was easily observed, but the disk-shaped-structure of grana was only seldom observed. In fact, under the ambient atmosphere of the root zone, the grana within the chloroplast in the palisade parenchyma cells had lamellar structure (Fig. 2*A*), and the disk-shaped-structure grana were occasionally observed only within the chloroplast located in the bottom of palisade layer (Fig. 2*C*). Under the CO_2 enrichment, coexistence of the lamellar-structure and the disk-shaped-structure grana was often observed. There were few in the chloroplast of the spongy parenchyma cells and in the bottom of palisade parenchyma layer (Fig. 2*D-F*). These results suggest that the accumulation and swelling of starch grains within the chloroplasts could influence the system of thylakoid membranes, change the grana position, and so reduce the capability of the thylakoid membranes for absorption and transformation of sun radiation.

The effect of starch accumulation on the depression of photosynthesis at CO_2 enrichment is not yet totally understood. Madsen (1975) demonstrated that tomato plants grown under high CO_2 concentrations in the root zone accumulated starch and had deformed thylakoids. Vu *et al.* (1989) and Zuo *et al*. (1996) also reported that the accumulated starch within the chloroplast damaged the thylakoids and grana, which consequently caused a long-term decline of photosynthesis at CO_2 enrichment. Yelle *et al*. (1989) also found the distorted thylakoids in the chloroplast of tomato plants grown at high CO_2 concentrations but they concluded the decrease of activated Rubisco was the main cause of the loss of photosynthetic efficiency and starch accumulation appeared to be a symptom. Based on the current study, we propose that the accumulation of starch grains in the chloroplast of potato leaves under long-term CO_2 enrichment in the root zone could alter the arrangement of grana thylakoids, which maybe result in an inhibition of sun radiation absorption and a decline of plant photosynthesis. However, the direct molecular, biochemical and physiological changes between starch accumulation and the depression of photosynthesis under long-term CO_2 enrichment still need a further research.

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