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Effects of severe water stress on partitioning of ^{14}C-assimilates in tomato plants

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Summary

Tomato plants (*Lycopersicon esculentum* Mill. cv Nikita) were grown hydroponically and subjected to severe water stress induced by addition of PEG-6000 to the nutrient solution. The PEG-treatment clearly impaired growth. Leaf photosynthesis decreased during the experiment. Moreover, the decrease in photosynthesis was associated with a decrease in dry weight of the shoot compared to the root. Also leaf area expansion, stomatal conductance and transpiration decreased. Water stress enhanced the transport of ^{14}C-assimilates from the source leaf to the lower parts of the plant where the assimilates were incorporated in the lower stem, the leaves below the source leaf and the roots. It was observed that ^{14}C was much more concentrated in the roots compared to the other plant parts.

Introduction

Water stress is one of the most important environmental constraints that limit plant productivity (BRADFORD and HSIAO, 1982). Plant productivity is determined by a complex series of events leading from CO_2 fixation in the chloroplasts, formation of phloem-mobile and storage metabolites, and delivery of these metabolites to sink tissue (MADORES and LUCAS, 1995). This partioning of carbon determines plant health and vigor during times of stress and subsequently leads to good or bad harvests (HOUPIS et al., 1999).

The relationship between carbon translocation and water stress is interesting to study as water is a major constituent of tissue, a reagent in chemical reactions, and a solvent for and mode of translocation for metabolites and minerals. With the occurrence of water stress many of the physiological processes associated with growth are affected. Photosynthetic rate reductions due to increased water stress have been observed in a wide variety of plant species. Stomatal effects are usually considered to be the first and the major limitation to CO_2 fixation (CORNIC and MASSACCI, 1996; ZGALLAÏ et al., 2005). However, leaf photosynthesis may also be affected by accumulation of photosynthates due to changes in translocation or utilization of the assimilates (SHINOHARA et al., 1995). Inhibition of assimilate translocation by water stress has been observed in a number of plant species (GEIGER and FONDY, 1991). WARDLAW (1990) has indicated that the major effect of water stress on translocation is the delay and the reduction of transfer rate of assimilates from the assimilating tissues to the sink tissues. MUNNS (1988) reported that the reductions in translocation observed under water stress are probably the result from the reduction in growth and the slowing down in carbohydrate utilization prior to a reduction in photosynthesis. Disagreement exists as to the relative sensitivity of photosynthesis and translocation to water stress (SUNG and KRIEG, 1979).

Determination of translocation or the carbon balance of the whole plant requires that the carbon balance of the source leaf is determined by the [14]CO_2 steady-state feeding method (SHISHIDO et al., 1999). This method allows calculation of the proportion of total carbon accumulated in the sink organ that was supplied by the source leaf, as well as the growth contribution of the source leaf to the main plant sinks. These data are vital to determine the productivity of the source leaf under severe water stress.

The modified sink-source relationship caused by water stress has been largely neglected in the past, although the assessment is of high importance to gain fundamental insights into the carbon metabolism during stress conditions (SHISHIDO et al., 1999). In this paper, we examined the effects of severe water stress induced by polyethylene glycol-6000 (PEG-6000) on the carbon metabolism in tomato plants (*Lycopersicon esculentum* Mill. cv. Nikita). Both photosynthesis and carbon translocation were measured in this study to elucidate the effects of severe water stress on the carbon metabolism. To investigate the relationship between translocation and water stress, the ^{14}C incorporation of a leaf exposed to [14]CO_2, and the subsequent distribution of the C-assimilates in different parts of the plants were analysed.

Materials and methods

Plant material and treatment

Tomato (*Lycopersicon esculentum* Mill. cv. Nikita) seeds were germinated in pots containing sand and peat. Seedlings were grown in a greenhouse at 20/32 °C, a relative humidity of 60 %/70 %, and under normal day-light conditions. After 14 d, plants were transplanted to vermiculite and irrigated with a nutrient solution. The nutrient solution was aerated using an air compressor. The pH of the nutrient solution was monitored daily and kept at 6.0 with addition of HCl (0.1 mol/l) or NaOH (0.1 mol/l). At this time, the plant material was subdivided into two sets: one control and one treatment group. Each group consisted of 16 plants, with the plants being randomly arranged over two nutrient film drains. The osmotic potential of the nutrient solution (Ψ_s) of the treatment group was changed gradually at a rate of -0.04 MPa/d by addition of polyethylene glycol-6000 (PEG-6000). The addition of PEG was suspended when Ψ_s reached -1.22 MPa (after 32 d; progressive severe stress) and a series of comparative investigations on treated and non-treated plants were performed. The concentration of PEG-6000 (in g/g water) needed for this level of water stress was determined using the equation given in MICHEL and KAUFMANN (1973).

The water potential of the nutrient solution (Ψ_s) was measured using filter paper discs (0.25 cm^2). Measurements were made with a thermocouple psychrometer (sample chambers type C52; Wescor Inc, Logan, Utah, USA) following the method described by SPANNER (1951).

Measurement of photosynthesis, transpiration and stomatal conductance

Photosynthesis (P_n), transpiration (E) and stomatal conductance (g_s) of four fully expanded mature leaves of different control (Ψ_s = -0.16 MPa) and treated plants (Ψ_s = -1.22 MPa) were determined using an infrared gas analysis system for CO_2 and H_2O respectively (Walz compact CO_2/H_2O porometer; Heinz Walz, Efeltrich, Germany). The analyzer was connected to a leaf chamber in which the leaf received a photosynthetic photon flux density of 900 µmol m^{-2} s^{-1}. The flow of the air through the chamber was 600 ml min^{-1}. After the measurement the leaf areas were measured using an automatic leaf area meter (Li-Cor, Walz, Germany) calibrated to ± 0.1 cm^2.

Spatial distribution of photosynthetic assimilates

Translocation was measured using the $^{14}CO_2$ steady-state feeding method (SHISHIDO et al., 1999). The radioactive pulse was given to the fourth leaf counted from the base of the shoot of tomato plants (Fig. 1). The treated leaf consisted of only the three terminal leaflets (the other leaflets were removed just before treatment). The remaining leaflets were confined hermetically in a transparent cylindrical cuvette (CRS087, PP systems, UK). 15 μl $Na_2^{14}CO_3$ (specific activity 53 mCi mmol^{-1}) (Amersham, UK) was put in a reservoir inside the cuvette and 100 μl HCl (10^{-5} M) was added to the carbonate to form $^{14}CO_2$. The $^{14}CO_2$ pulse was given during 15 min under natural light conditions. At the end of the translocation period (23 h), the plants were quickly divided into different parts (Fig.1).

For quantitative analysis of the amount of radioactivity in the different plant parts the sample was placed in a scintillation counter. The sample was combusted in oxygen (dry combustion) by a sample oxidizer (Packard Instrument Co. Model 306 Tri-carb Sample Oxidizer) by which the carbon is evolved as $^{14}CO_2$. Extracts were counted in a liquid scintillation counter (Nuclear Chicago Isocap. 300) using a toluene-base scintillation liquid containing 0.015 % POPOP (W/V), 0.6 PPO (W/V) and Triton X (2 parts of toluene/ 1 part Triton X).

The radioactivity in the various parts of the plants was expressed in two ways as described by MOR and HALEVY (1979): (a) as the percentage of radioactivity recovered in the plant, excluding the source leaf, and (b) as a relative specific activity (RSA), which is the ratio of the specific activity in a given part (soluble radioactivity/g dry weight) to specific activity of the whole plant.

Fresh and dry weight of different parts of the plant were determined with a digital balance (Sartorious B310S, Göttingen, Germany), calibrated to ± 0.001 g.

Results

Effect of severe water stress on photosynthesis, transpiration and stomatal conductance

The effect of severe water stress on the assimilation rate (P_n), transpiration rate (E) and stomatal conductance (g_s) are shown in Tab. 1. After the severe water stress, the assimilation rate and the stomatal conductance decreased to about 71 % of the control, but the intercellular partial pressure of CO_2 was identical (data not shown). The results indicate that the stomata of the leaves of the PEG-treated plants were nearly completely closed, causing stomatal limitation of photosynthesis. The decrease in photosynthesis and stomatal conductance was associated with a reduction in transpiration rate (Tab. 1).

Tab. 1: Effects of severe water stress on the net photosynthesis rate (P_n), the stomatal conductance (g_s), the transpiration rate (E) and the shoot to root ratio of tomato plant grown in a nutrient solution. Values indicate the mean (n = 4). Different letters within the column show significantly differences using Duncan's test.

Treatment	P_n (μmol m^{-2} s^{-1})	g_s (mmol m^{-2} s^{-1})	E (mmol m^{-2} s^{-1})	Shoot/Root
Control	13.02±0.47[a]	131.72±12.65[a]	30.00±1.45[b]	1.70±0.01[a]
Stressed	3.87±0.02[b]	38.20±3.01[b]	17.19±4.14[a]	1.38±0.01[b]

The dry weight of the shoot, the root, the ratio shoot/root and the leaf area were significantly ($P \leq 0.05$) reduced by the imposed water stress (Fig. 2, Tab. 1).
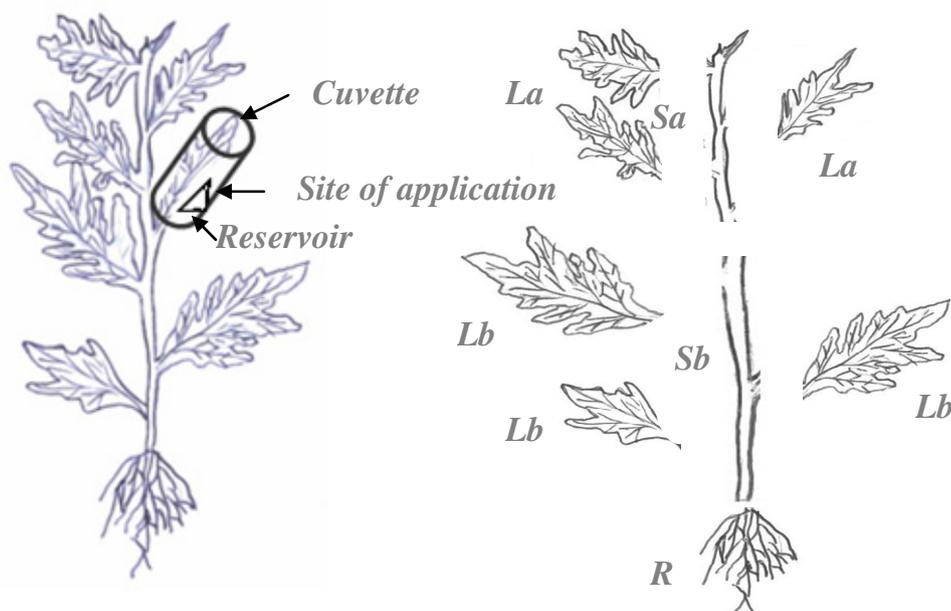

Fig. 1: Diagram showing the site of application of the solution containing the radiolabelled compound and the cut plant parts of tomato plant before counting. Symbols used: R = roots, Sb = stem below source leaf, Lb = leaves below source leaf, Sa = stem above source leaf, La = leaves above source leaf.
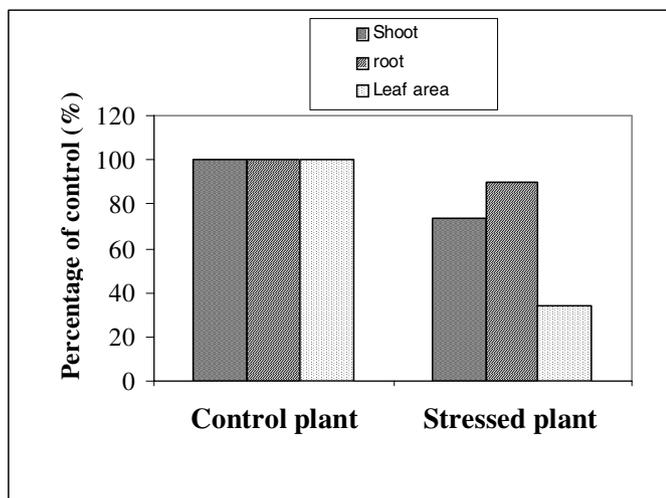
Fig. 2: Changes in shoot and roots dry weight and leaf area expressed as a percentage of the same parameters in the control plants.

Effect of severe water stress on the distribution of photosynthetic assimilates

The radioactive pulse was given to the fourth leaf counted from the base of the shoot (Fig. 1). The relative distribution of total ^{14}C radioactivity 23 h after $^{14}CO_2$ exposure recovered in each plant part is shown in Fig. 3. The pattern of ^{14}C distribution exhibited some marked differences between control and PEG-treated plants. For control plants, ^{14}C was found mainly in the upper and lower stem and in the roots. The leaves contained less than 4 % of the recovered radioactivity (Fig. 3) which was equally distributed between the leaves below and above the source leaf. The main difference in the radioactivity pattern found after application of severe water stress concerned the distribution of the ^{14}C in the lower parts of the plants. The largest increase was observed in the roots (an increase of

10.2 % compared to control plant), but also in the stem and the leaves below the source leaf an increase was detected (6.2 and 2.7 % respectively).

Fig. 4 also shows the distribution of the radioactivity expressed in terms of relative specific activity (RSA) in the different parts of the tomato plants 23 h after application of the pulse to the source leaf. The RSA indicates that the stem and the roots are labelled most (the source leaf excluded). Compared to the control plant, the distribution of radioactivity in the whole plant was significantly different for tomato plants exposed to severe water stress. Except for the stem part above the source leaf, the RSA was higher in all distinguished parts of the PEG-treated plants compared to the control plants.

Discussion

In this study we investigated the response of hydroponically grown tomato plants to long-term severe water stress. Addition of PEG-6000 to the nutrient solution was used to impose gradual water stress on the plants by exposing their root system to the solution. No toxic effects of PEG were observed and, hence, this osmoticum was considered as suitable to induce water stress (KOCHEVA et al., 2005; ZGALLAÏ et al., 2005). The water-stressed plants were clearly retarded in growth. Both photosynthesis and transpiration decreased significantly (Tab. 1) due to the rapid response of stomata to decreasing water availability (BUCKLEY, 2005). The closure of the stomata was observed by the sharp decrease in g_s during severe water stress. This was also demonstrated by BÜSSIS et al. (1998) and ZGALLAÏ et al. (2005). Hence, photosynthesis and transpiration are very sensitive to severe water stress.

Another well-observed adaptation response in relation with water stress is a reduction in shoot growth relative to root growth (TURNER and BEGG, 1981; BOTA et al., 2004). Also our study indicates a decrease in shoot/root ratio for the water-stressed tomato plants (Tab. 1). MEYER and BOYER (1981) and SHINOHARA et al. (1995),
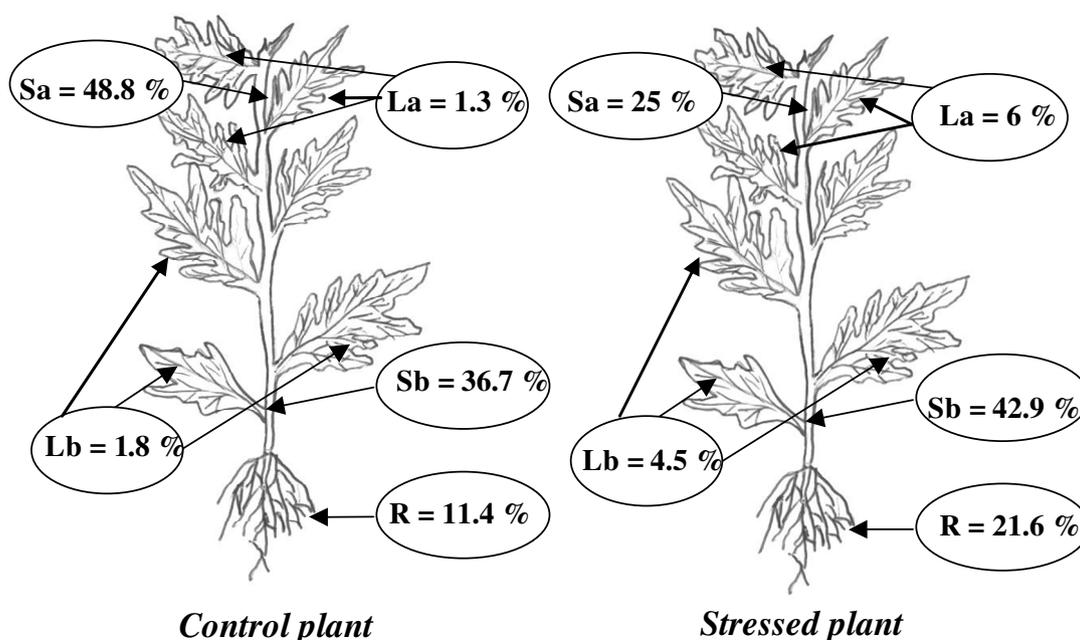

Fig. 3: Effect of severe water stress on the distribution pattern of (^{14}C) carbon accumulated in each sink from a common source leaf. Distribution values of the sinks are expressed as percentages of the total ^{14}C accumulated in the source leaf. Same symbols are used as in Fig. 1.
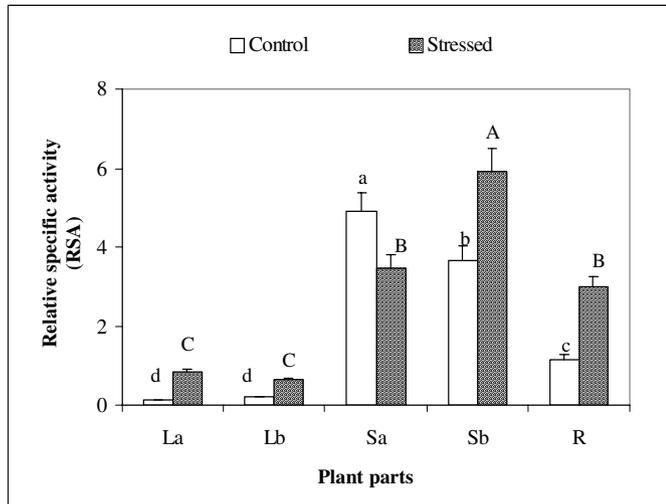
Fig. 4: Effect of severe water stress on the distribution of radioactivity (expressed in terms of relative specific activity) in tomato plants 23 h after exposure to $^{14}CO_2$. The radioactive pulse was given to the fourth leaf counted from the base of the plant. Averages of three replications are presented. The different letters within the histogram indicate significantly differences at a 5 % level. Same symbols are used as in Fig. 1.

reported that the decrease in shoot/root ratio mostly resulted from relative reductions in leaf growth. This significant shift in the shoot/root ratio during water stress supports the idea of changes in biomass allocation (SHARP and DAVIES, 1985; SHINOHARA et al., 1995; BOTA et al., 2004).

Changes in translocation were studied using the $^{14}CO_2$ steady-state feeding method (SHISHIDO et al., 1999). The total $^{14}CO_2$ fixation in control and treated source leaves was set equal to 100%. The proportion of carbon supplied by the source leaf to sink organs could be analysed. It was observed that water stress clearly affected the export of photosynthetic assimilates from the source leaf in the plant. After 23 h of translocation, the portion of ^{14}C moved out of the source leaf in the control plant was equally distributed between the upper (50.1 %) and lower parts (49.9 %) (Fig. 3). In contrast, in severe stressed plants, the percentages of radioactive distribution were 31 % and 69 % for the upper and lower plant parts, respectively. Hence, the largest portion of ^{14}C fixed by the source leaf was transferred to the lower parts of the plant (Fig. 3) and there was much less movement of ^{14}C towards the upper parts. This was confirmed by the relative specific activity measurements (Fig. 4). Hence, severe water stress had a significant effect on the movement of C-assimilates, whereby the roots received more photosynthetic assimilates compared to the control plants. These results suggest that tomato plants under severe water stress translocate more C-assimilates towards the roots in order to maintain root growth, which means survival (or uptake of water), even during conditions of severe water stress. These findings were supported by SHINOHARA et al. (1995) and OHASHI et al. (2000) who also observed changes in the distribution of assimilates, together with a decrease in shoot/root ratio and an increase of assimilates in the roots during water stress.

In this study we used ^{14}C to estimate the relative distribution of radiocarbon in tomato plants under severe water stress. The parameters we used were the percentage of radioactivity recovered in the plant and the relative specific activity (RSA), which emphasized the ability of various organs to draw metabolites (MOR et al., 1981). This method enabled us to analyse the carbon balance of each

individual sink, thereby obtaining information necessary to evaluate the productivity of plants under water stress condition. The higher translocation of photosynthetic assimilates towards the roots indicated that water-stressed tomato plants tried to maintain root growth and, hence, tried to survive even during the unfavourable growing conditions imposed.

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