Effect of Half and Whole Root Drying on Photosynthesis, Nitrate Concentration, and Nitrate Reductase Activity in Roots and Leaves of Micropropagated Apple Plants

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ABSTRACT. Half or whole root systems of micropropagated ‘Gala’ apple (*Malus ×domestica* Borkh.) plants were subjected to drought stress by regulating the osmotic potential of the nutrient solution using polyethylene glycol (20% w/v) to investigate the effect of root drying on NO₃⁻ content and metabolism in roots and leaves and on leaf photosynthesis. No significant difference in predawn leaf water potential was found between half root stress (HRS) and control (CK), while predawn leaf water potential from both was significantly higher than for the whole root stress (WRS) treatment. However, diurnal leaf water potential of HRS was lower than CK and higher than WRS during most of the daytime. Neither HRS nor WRS influenced foliar NO₃⁻ concentration, but both significantly reduced NO₃⁻ concentration in drought-stressed roots as early as 4 hours after stress treatment started. This reduced NO₃⁻ concentration was maintained in HRS and WRS roots to the end of the experiment. However, there were no significant differences in NO₃⁻ concentration between CK roots and unstressed roots of HRS. Similar to the effect on root NO₃⁻ concentration, both HRS and WRS reduced nitrate reductase activity in drought-stressed roots. Moreover, leaf net photosynthesis, stomatal conductance and transpiration rate of HRS plants were reduced significantly throughout the experiment when compared with CK plants, but the values were higher than those of WRS plants in the first 7 days of stress treatment though not at later times. Net photosynthesis, stomatal conductance and transpiration rate were correlated to root NO₃⁻ concentration. This correlation may simply reflect the fact that water stress affected both NO₃⁻ concentration in roots and leaf gas exchange in the same direction.

Localized irrigation, which includes drip irrigation and micro-sprinkling, is widely used for irrigation management in fruit production throughout the world. The responses of fruit trees to localized irrigation were largely studied in the field, specifically focusing on photosynthesis, vegetative growth, cropping and fruit quality (Bryla et al., 2003; Li et al., 1989; Punthakey, 1984; Zhu et al., 2004). Leaf photosynthesis of fruit trees, such as ‘Redchief Delicious’ apple (Xu, 1999), was generally higher under localized irrigation than under flood irrigation. Stomatal conductance (g_s), transpiration rate (Tr), and vegetative growth decreased significantly under drip irrigation or micro-sprinkling when compared with flood irrigation (Bryla et al., 2003; Punthakey, 1984). However, fruit growth rate, size, quantity, and quality do not decrease under localized irrigation, and they may be improved (Davies et al., 2000; Loveys et al., 2000).

Nitrogen nutrition plays an important role in vegetative growth and fruit quality of fruit trees. Severe drought affects not only NO₃⁻ availability at the root surface, but also the physiological capacity for root NO₃⁻ uptake (Buljovic and Engels, 2001). Moreover, evidence from other species showed that the sensitivity of g_s to N nutrition varied under different water conditions (Shangguan et al., 2000). In several prairie grasses, a close correlation existed between foliar N concentration and photosynthesis in response to water stress and recovery once water was restored (Heckathorn

et al., 1997). Study of sunflower (*Helianthus annuus* L.) revealed that root NO₃⁻ concentration decreased during soil drying (Correia et al., 2005). Usually, nitrate reductase [NR (EC 1.6.6.1)] activity decreases when plants are subjected to drought stress (Azedo-Silva et al., 2004; Chandrasekar et al., 2000; Foyer et al., 1998). Though NO₃⁻ acts as substrate and signal molecule in the N metabolic pathway (Foyer and Noctor, 2002; Gawronska et al., 2003; Goupil et al., 1998; Parsons and Sunley, 2001; Scheible et al., 1997; Tischner, 2000), very little attention has been paid to the contribution of drought-induced NO₃⁻ changes to photosynthesis, especially in fruit trees under partial root zone drying.

The effect of drought-induced NO₃⁻ change and its relationship to photosynthesis in fruit trees under localized irrigation has been largely ignored due to the large plant size of fruit trees and the heterogeneity of water conditions in the field and in pots. Since the osmotic potential of aqueous solutions of polyethylene glycol (PEG) was related to PEG concentration and was mainly changed with temperature variation (Michel and Kaufmann, 1973), the use of PEG solutions to simulate water deficits on micropropagated plants can create uniform drought stress conditions to roots. The entire root zone is not uniformly wetted when water is provided under localized irrigation; rather, only a part of root system of the tree gets water while the other roots are still subjected to drought stress throughout the growing season in arid and semi-arid regions. This soil water condition can be simulated by a split root technique (Loveys et al., 2000). The objective of the present study was to use the split root techniques to define the effect of drought stress applied to half of the root system to simulate the condition of trees under localized irrigation versus drought stress applied to the whole root system of micropropagated apple plants

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on NO_3^- and NH_4^+ concentrations in the roots and leaves and on photosynthesis. In addition, the relationship between drought-induced NO_3^- change to photosynthesis was investigated.

Materials and Methods

PLANT MATERIALS AND EXPERIMENTAL CONDITIONS. Micropropagated 'Gala' apple plants were grown in plastic pots containing culture media of 1 vermiculite : 3 peat : 6 field soil (by volume) in a greenhouse for 1 year. Two well-developed buds were kept on each trunk at winter pruning, and one new shoot was kept after bud break in early spring. When the new shoots reached a length of ≈ 20 cm, a uniform set of 16-month-old plants were repotted into black glass containers (15 $\times$ 20 $\times$ 80 cm, with two independent compartments of equal volume) containing modified Hoagland nutrient solution. The solution contained 0.5 mM KNO_3 , 0.3 mM $\text{Mg}(\text{NO}_3)_2$, 0.11 mM $\text{Ca}(\text{NO}_3)_2$, 0.5 mM CaCl_2 , 0.5 mM MgSO_4 , 0.5 mM KH_2PO_4 , 3.9 μM ZnSO_4 , 2.6 μM CuSO_4 , 4.6 μM MnCl_2 , 0.44 μM $\text{EDTA}\cdot\text{Fe}\cdot\text{Na}$, 0.25 μM H_3BO_3 , and 0.33 nM $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$. Roots of each plant were washed before putting in the container and were divided equally into the two compartments. The glass containers were covered with wooden boards to maintain a dark environment for the roots, and the nutrient solutions were aerated continuously. The plants were grown under ambient conditions at 20–32 °C and a 17-h photoperiod of natural light.

TREATMENTS. When plants were about 25 cm in height with 10 mature leaves, after 20 d in the nutrient solution, treatments were initiated. Half root stress or whole root stress treatments were imposed starting at 0600 HR on the morning on 4 May 2004, and were compared with a control. HRS and WRS were applied by regulating the osmotic potential of the solution using 20% w/v PEG (PEG-6000; Wako Pure Chemical Industries, Osaka, Japan), which corresponds osmotic potential of -0.511 MPa at 25 °C based on the calculation formula of Michel and Kaufmann (1973). For HRS, one compartment of the glass container was filled with modified Hoagland solution containing 20% PEG-6000 and the other one received regular solution. For WRS, both compartments were filled with Hoagland solution containing 20% PEG-6000. For CK, both compartments were filled with regular Hoagland solution. The nutrition solution was replaced at 0600 HR on day 4 and day 7 after starting the treatments. There were four replications of each treatment with 60 plants per replication, 30 of which were used for NO_3^- , NH_4^+ , and other biochemical analyses, and 30 were used for leaf water potential and photosynthesis measurements. Plants were arranged in a completely randomized design in the greenhouse.

MEASUREMENTS OF LEAF WATER POTENTIAL. Predawn and diurnal variation in leaf water potential was determined from two fully expanded mature leaves per plant from the middle of the shoot (Scholander et al., 1964). Measurements were made on one plant from each replication on each sampling date using a pressure chamber (ZLZ-4; Lanzhou Univ., Lanzhou, China). Diurnal variation of leaf water potential was measured on the fifth day after initiating treatments.

LEAF AND ROOT SAMPLING FOR BIOCHEMICAL ANALYSIS. Well-developed leaves and fine roots were harvested from five plants per replication of each treatment on the day prior to starting the experiment, and around 1000 HR at 1 (i.e., 4 h), 2, 4, 7, and 11 d after starting drought stress treatments. All the sample tissues were rinsed thoroughly in tap water first and then again in deionized water to remove attached PEG-6000 or unabsorbed nutrition salts. The clean samples were blotted dry with paper towels and

immediately frozen in liquid nitrogen. Then, they were pooled and ground to fine powder in liquid nitrogen and stored at -80 °C until used for biochemical analyses.

DETERMINATION OF NO_3^- , NH_4^+ , FREE AMINO ACID, AND PROLINE CONCENTRATIONS. To determine NO_3^- and NH_4^+ concentrations, 1.0 g of the leaf or root powder was homogenized with 10 mL double distilled water together with polyvinylpyrrolidone in a mortar precooled by liquid nitrogen (Jiang et al., 2003). The homogenates were extracted under ultrasonic conditions at 4 °C for 30 min and then centrifuged for 10 min at 4 °C and 18,000 g_n . Then, the supernatants were passed through a Supelclean ENVI-Carb SPE column (Sigma-Aldrich Corp., St. Louis). Thereafter, NO_3^- in the effluent was determined by ion exchange chromatography (DX-600; Dionex Corp., Sunnyvale, Calif.) using an IonPacAS11 column (Dionex Corp.) with a solution containing 50 mM NaOH and detected with a conductivity detector module (ED50; Dionex Corp.). The same supernatant was used for NH_4^+ determination by the same ion exchange chromatography using an IonPacCS12A column (Dionex Corp.) with a solution containing 20 mM methanesulfonic acid and detected by the same conductivity detector module.

Total free amino acids were extracted and quantified as follows: 0.3 g of frozen sample was boiled in 10 mL deionized water for 30 min, centrifuged for 20 min at 20,000 g_n , and the supernatant was collected. The residue was washed and centrifuged twice with 10 mL deionized water. The supernatants were combined and made up to 50 mL with distilled water. Total free amino acids were determined spectrophotometrically using the ninhydrin method described by Tang (1999).

Determination of proline content was according to methods described by Bates et al. (1973). Frozen leaf sample of 1.0 g was homogenized with 10 mL of 3% sulfosalicylic acid (w/v) and boiled for 10 min. Then, the homogenates were centrifuged at 3000 g_n for 20 min and the supernatants were collected for proline content determination. The reaction mixture contained 1.2 mL of the supernatant, 2 mL of glacial acetic acid, and 3 mL of 2.5% acid ninhydrin, and was boiled for 40 min. After termination of the reaction in an ice bath, the reaction mixture was extracted by 5 mL toluene and the absorbance was determined at 520 nm.

NR ACTIVITY. To prepare the enzyme extracts, 1.0 g of frozen root sample was extracted with 25 mM phosphate buffer (pH 7.5) containing 5 mM L-cysteine and 5 mM EDTA- Na_2 in a mortar precooled with liquid nitrogen. After rapid homogenization while ice-cold, the homogenates were centrifuged for 10 min at 4 °C and 12,000 g_n , and then dialyzed at 4 °C by stirring against 0.067 M phosphate buffer (pH 7.5) for 48 h (Grasmanis and Nicholas, 1967). Thereafter, NR activity was measured immediately in the supernatant according to methods described previously (Tang, 1999). The reaction mixture contained 1.2 mL of 0.1 M KNO_3 , 0.4 mL of 2.5 mM NADH_2 in phosphate buffer (pH 7.5) and 0.4 mL of extract. The reactions proceeded at 30 °C for 30 min, and were then stopped by adding 1 mL of 1% sulfanilamide in 3 M HCl. Thereafter, 0.2% 1-naphthylamine was added and the mixture was centrifuged 20 min at 1000 g_n for 5 min. The absorbance was determined at 540 nm. NR activity was defined as amount (micrograms) of NO_2^- produced per milligram protein. The content of soluble protein was determined by the Bradford protein assay (Bradford, 1976).

MEASUREMENTS OF NET PHOTOSYNTHESIS (Pn) AND RELATED PARAMETERS. Net photosynthesis and related photosynthetic parameters of fully expanded leaves were measured between 0830 to 1100 HR on all sunny days throughout the experiment.

Measurements were performed on three randomly selected plants in each replication using a portable photosynthesis system (LCA-4; ADC, Hoddesdon, U.K.).

STATISTICAL ANALYSIS. All statistical analyses was performed using the SPSS statistical package (version 11.0; SPSS Inc., Chicago). Moreover, correlations between NO_3^- concentration in leaves and roots and photosynthesis or the other photosynthetic parameters were determined. Only the data obtained from CK, WRS, and stressed roots of HRS were used for the regression analyses concerning NO_3^- concentration in roots.

Results

LEAF WATER POTENTIAL. Predawn leaf water potential (Ψ_{pd}) of CK was nearly constant at a high level (-0.36 to -0.22 MPa) throughout the experiment, and no significant difference in Ψ_{pd} was found between HRS and CK (Fig. 1A). However, Ψ_{pd} of WRS was significantly lower than CK and HRS starting on day 2 after plants were subjected to drought stress. The Ψ_{pd} of WRS plants decreased gradually to -1.2 MPa until 8 d after starting treatments, and stayed at about -1.0 MPa to the end of the experiment (Fig. 1A).

The diurnal variation in leaf water potential (Ψ_d) is shown in Fig. 1B. It declined rapidly in the morning in all treatments, reached the lowest value at about 1000 HR, and slowly recovered in the afternoon. There were significant differences between treatments, especially from 1000 to 1900 HR. The Ψ_d from HRS plants was significantly lower than CK, but higher than WRS during most of daytime. While Ψ_d of CK and HRS had recovered fully by 2300 HR, Ψ_d of WRS was still significantly lower than CK and HRS.

Fig. 1. Response of predawn leaf water potential (Ψ_{pd}) throughout the experiment (A) and diurnal leaf water potential (Ψ_d) on the fifth day after initiating treatments (B) of micropropagated apple plants to half root drought stress (HRS) and whole root drought stress (WRS) when compared with control (CK). Each point is mean $\pm$ SE ($n = 4$).

NO_3^- CONCENTRATION AND NR ACTIVITY. Similar tendency of change in foliar NO_3^- concentration of HRS and WRS to CK throughout the experiment was obtained in this study (Fig. 2A). The lowest and highest foliar NO_3^- concentration were observed on both stressed treatments and CK on day 2 and 7, respectively. However, foliar NO_3^- concentration was very low, 4.3 to 23.6 $\mu\text{g}\cdot\text{g}^{-1}$, for all treatments when compared with roots and there were no significant differences in foliar NO_3^- concentration among CK, HRS, and WRS throughout the experiment, indicating that drought stress had no effect on foliar NO_3^- concentration.

In contrast, both HRS and WRS had marked effects on root NO_3^- concentration (Fig. 2B). Nitrate concentration in the stressed

Fig. 2. Nitrate concentration in leaves (A) and roots (B), and nitrate reductase (NR) activity in roots (C) of micropropagated apple plants under half root drought stress (HRS) and whole root drought stress (WRS) when compared with control (CK). Nitrate concentration is expressed on a fresh weight basis and NR activity is defined as amount (μg) of NO_2^- produced per milligram protein. Each point is mean $\pm$ SE ($n = 4$).

roots of WRS and HRS decreased to about 50% of CK, significantly lower as early as the day 1, within 4 h after treatments were initiated. This decreased NO_3^- concentration was maintained in the stressed roots of WRS and HRS to the end of the experiment. However, there was no significant difference in NO_3^- concentration between CK roots and unstressed roots of HRS.

Similar to the effect on root NO_3^- concentration, both HRS and WRS reduced root NR activity (Fig. 2C). Root NR activity declined to 32%–46% and 36%–60% of CK in stressed roots of HRS and WRS, respectively. However, NR activity in unstressed roots of HRS remained at a level similar to CK. Moreover, a significant quadratic correlation was found between NR activity and NO_3^- concentration (Fig. 3, $r^2 = 0.638$, $P < 0.001$).

NH_4^+ CONCENTRATION. Drought stress had no effect on foliar NH_4^+ concentration when compared with CK (Fig. 4A), while NH_4^+ concentration in stressed roots of HRS and WRS treatments increased markedly 7 d after plants were subjected to drought stress, and rose to 2.7-fold and 2.2-fold of CK values by the end of experiment, respectively (Fig. 4B). However, NH_4^+ concentration was not altered in unstressed roots of HRS, and there were no significant difference in NH_4^+ concentration between CK and unstressed roots of HRS throughout the experiment.

Pn, g_s , and Tr. Drought stress applied to both half and whole root systems influenced Pn, g_s , and Tr of micropropagated apple plants (Fig. 5A–C). HRS resulted in between 30% to 74% lower Pn, 25% to 54% lower g_s , and 48% to 62% lower Tr compared to CK plants. However, Pn, g_s , and Tr were significantly higher in HRS than those in WRS during days 1–7, though there was no significant difference between HRS and WRS thereafter.

FREE AMINO ACID AND PROLINE CONCENTRATION. The effect of HRS on foliar free amino acid concentration varied throughout experiment. Significantly lower foliar free amino acid concentration was observed from HRS plants than CK on days 4 and 7 after initiating water stress. However, there were no significant difference between HRS and CK for the other days (Fig. 6A). As regards WRS, significantly increased foliar free amino acid concentration was observed only on day 11 when compared with CK.

A dramatic increase in foliar proline concentration was found in plants receiving WRS from day 2 after starting the treatment (Fig. 6B). It increased by 1.4-fold on day 2 and by 2.6-fold on day 11 when compared with CK. Foliar proline concentration of HRS plants did not differ from CK plants throughout the experiment (Fig. 6B).

CORRELATIONS BETWEEN NO_3^- CONCENTRATION AND PHOTOSYNTHESIS. A significant quadratic correlation was found between Pn and NO_3^- concentration in roots (Fig. 7A). Net photosynthesis increased sharply with NO_3^- concentration in roots until NO_3^- concentration in roots was above $1200 \mu\text{g}\cdot\text{g}^{-1}$. Moreover, g_s and Tr were linearly correlated with NO_3^- concentration in roots (Fig. 7B and C). However, no significant correlation was found between foliar NO_3^- concentration and Pn, g_s , and Tr, respectively (data not shown).

Discussion

The foliar NO_3^- concentration was extraordinarily low when compared with that in roots of both drought-stressed and control apple plants in this study (Fig. 2). This differs from herbaceous plants such as maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), and lettuce (*Lactuca sativa* L.), which have shown higher foliar NO_3^- concentrations (Correia et al., 2005; Ferrario-Méry et al.,

Fig. 3. Correlation between root nitrate reductase (NR) activity and NO_3^- concentration of micropropagated apple plants under control, whole root drought stress and half root drought stress. Nitrate concentration is expressed on a fresh weight basis and NR activity is defined as amount (μg) of NO_2^- produced per milligram protein. Each point is mean $\pm$ SE ($n = 4$). The asterisks *** indicate significance at 0.001.

Fig. 4. Ammonium concentration in leaves (A) and roots (B) of micropropagated apple plants under half root drought stress (HRS) and whole root drought stress (WRS) when compared with control (CK). Ammonium concentration is expressed on a fresh weight basis. Each point is mean $\pm$ SE ($n = 4$).

Fig. 5. Leaf net photosynthesis (Pn) (A), stomatal conductance (g_s) (B), and transpiration rate (Tr) (C) of micropropagated apple plants under half root drought stress (HRS) and whole root drought stress (WRS) when compared with control (CK). Each point is mean $\pm$ SE (n = 4).

1998; Foyer et al., 1998). In these species, NO_3^- can accumulate to a high concentration in the vacuoles of leaf cells as a result of the shortage of carbon skeletons for synthesis of nitrogen-containing organic compounds (Demšar et al., 2004). Prior studies also showed that NO_3^- was very low in parts of the apple plant other than roots (Lee and Titus, 1992). It seems that absorbed NO_3^- is mainly reduced in the roots of apple trees.

Drought stress from both HRS and WRS treatments influenced NO_3^- concentration in the roots. Root NO_3^- concentration only decreased in drought-stressed roots (Fig. 2B). Low NO_3^- concentration was accompanied by low NR activity in the roots under drought stress (Fig. 2C). Regression analyses showed that there was a significant correlation between NR activity and NO_3^- concentration in roots (Fig. 3). In maize leaves, decreased NO_3^- may lead to a decrease in quantity of NR mRNA (Foyer et al., 1998). The decrease of root NR activity observed following the onset of drought stress in this study could have been caused by the decrease in root NO_3^- . The latter would directly result from a decrease in NO_3^- absorption ability of the stressed roots (Ferrario-Méry et al., 1998).

Stomata closure, decline in Pn, accumulation of carbohydrates, and changes in activities of related carbon metabolic enzymes

Fig. 6. Foliar free amino acid (A) and proline (B) concentration of micropropagated apple plants under half root drought stress (HRS) and whole root drought stress (WRS) when compared with control (CK). The concentration is expressed on a fresh weight basis. Each point is mean $\pm$ SE (n = 4).

usually result from drought stress applied to whole roots in apple (Li and Li, 2005; Rufty et al., 1988), grape (*Vitis vinifera* L.) (Rodrigues et al., 1993), and peach [*Prunus persica* (L.) Batsch] (Chai et al., 2001; Escobar-Gutiérrez et al., 1998). The results obtained in this study show that drying half the root system influenced Pn in micropropagated apple plants (Fig. 5A), although Ψ_{pd} of HRS plants did not differ from that of CK plants with significant differences between them during the daytime (Fig. 1A–B). Leaf Pn, g_s , and Tr decreased significantly after half or the whole root system of apple plants were subjected to drought stress (Fig. 5A–C). Usually the presence of NO_3^- not only triggers changes in the NO_3^- assimilation pathway but it also reprograms several pathways of carbon metabolism (Krapp et al., 2002). In the presence of NO_3^- , carbohydrate synthesis decreases and more carbon is converted via glycolysis to phosphoenolpyruvate and enters organic acid metabolism (Krapp et al., 2002). In ‘Gala’ apple, Pn and g_s decreased with decreasing leaf N, and the activities of Rubisco and other photosynthesis-related enzymes were limited by N limitation (Chen and Cheng, 2004). Also, a close relationship was found between foliar N content and CO_2 assimilation in ‘Fuji’ apple (Cheng and Fuchigami, 2000a, 2000b). However, there were no significant differences in foliar NO_3^- (Fig. 2A) and NH_4^+ concentration (Fig. 4A) between either drought stress treatment and CK, and no significant correlation between foliar NO_3^- concentration and photosynthesis was found in the present study. It is possible that the response of leaf photosynthesis and related photosynthetic parameters would not be influenced directly

Fig. 7. Correlations between root NO₃⁻ concentration and leaf net photosynthesis (Pn) (A), stomatal conductance (g_s) (B), and transpiration rate (Tr) (C) of apple plants subjected to drought stress, respectively. As regards NO₃⁻ concentration of half root drought stress, only the data obtained in the stressed roots were used in regression analysis. Each point is mean ± SE for root NO₃⁻ concentration (horizontal, n = 4) and the dependent variables (vertical, n = 4). The asterisks *, **, and *** indicate significance at 0.05, 0.01, and 0.001, respectively.

by changes in foliar NO₃⁻ and NH₄⁺ concentration under drought stress. However, both HRS and WRS induced significantly lower root NO₃⁻ concentration in stressed roots as early as the day 1, 4 h after drought stress treatments were initiated (Fig. 2B), as well as higher NH₄⁺ concentration in stressed roots from day 7 to 11 after plants were subjected to drought stress (Fig. 4B). Furthermore, Pn, g_s, and Tr were each positively correlated only with root NO₃⁻ concentration (Fig. 7A–C). This correlation may simply reflect the fact that water stress affected both NO₃⁻ concentration in roots and leaf gas exchange in the same direction.

Though HRS affected root NO₃⁻ metabolism, its negative impact on photosynthesis was not as large as that of WRS. The negative influence on photosynthesis from NO₃⁻ metabolism in the stressed root may have been compensated by normal NO₃⁻ metabolism in the unstressed roots. This may be why no apparent nitrogen-related metabolic changes were observed in leaves of micropropagated apple plants under HRS, such as free amino acid (Fig. 6A) and proline accumulation (Fig. 6B). Though no correlation between foliar NO₃⁻ concentration and photosynthesis was found, it would be useful to know if specific localization of NO₃⁻ in certain cells or different compartments of a given cell may be taking part in regulating photosynthesis and related processes.

In conclusion, drought stress applied to half root system can induce a similar effect on leaf Pn and nitrogen nutrition to that resulted from whole root drying in apple plants in this study although there were considerable differences in water status between the plants subjected to half and whole root drying. Both HRS and WRS decreased significantly leaf Pn, g_s, and Tr, as well as the NR and NO₃⁻ concentration in stressed root. Quick response of HRS plants to g_s and Tr confirms the advantage of economizing water use of trees by using localized irrigation technique. Moreover, limitation in use of assimilates and nitrogen nutrition, represented by low Pn and NO₃⁻ concentration in stressed roots, of the HRS plants in this study may explain in part why the fruit trees in the field under localized irrigation have decreased vegetative growth when compared with those under flood irrigation.

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