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Vine growth and nitrogen metabolism of 'Fujiminori' grapevines in response to root restriction☆

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Abstract

Two-year-old 'Fujiminori' grapevines (*Vitis Venifera* × *V. Labrasca*) planted in plastic pots (10 L) were used to evaluate vine growth and nitrogen metabolism in response to root restriction. Results show that root restriction reduced shoot growth and photosynthetic rate, but promoted root growth in vines. NO_3^--N concentration in all plant parts, and total N concentrations in brown roots and new leaves were decreased by root restriction, and chlorophyll and carotenoid concentrations in mature leaves were also reduced. Nitrate and nitrite reductase activities in brown roots and mature leaves were significantly reduced in root-restricted vines. The results suggest that the reduction of nitrate and nitrite reductase activities caused the inhibition of nitrogen assimilation, and this might be an important reason for root restriction inhibiting shoot growth.

Keywords: Root restriction; Vine growth; Nitrogen metabolism

1. Introduction

Normal field grape cultivation usually causes extensive root growth, making it difficult to control the absorption of soil moisture and nutrients. Sometimes the effects of fertilizer may continue until maturation, and consequently delay berry colouration and maturation, resulting in poor berry quality (Wang et al., 2001). In recent years, root restriction has been developed to manipulate tree vigor and control the environment of root systems in grape (Wang et al., 2001), apple (Bar-Yosef et al., 1988; Myers, 1992), mandarin (Yakushiji et al., 1996), peach (Costa et al., 1992; Mark and Marra, 1994; Boland et al., 2000), cherry (Webster et al., 1997), persimmon (Ogawa et al., 1997) and fig (Matsuura et al., 1992).

Under root restriction, the plant displays distinctive differences in growth habit compared with that under normal field cultivation. Root restriction can increase root mass and the amount of fibrous roots, reduce shoot growth, and improve fruit set and fruit quality (Bar-Tal et al., 1995; Wang et al., 1998, 2001). Moreover, some reports have revealed that root restriction decrease the concentration of nitrogen in shoots, flower clusters, trunks and canes of grapevines (Wang et al., 1998), and of leaves of *Euonymus* (Dubik et al., 1990) and peach (Mark and Marra, 1994; Boland et al., 2000). Reduction of nitrogen concentrations in root-restricted plants is believed to be one of the important reasons why root restriction reduced shoot growth in 'Kyoho' grapevines (Wang et al., 1998). Nitrate reductase (NR) and nitrite reductase (NiR) are the key metabolic enzymes in plants regulating reduction of nitrogen (Datta and Sharma, 1999). In previous reports, it has been demonstrated that water stress, salinity and heavy metal stress usually results in a reduction of vegetative growth and nitrogen reductase activities in wheat (El-Komy et al., 2003), *Nicotiana tabacum* (Sweby et al., 1994), maize (Bardzik et al., 1971), mustard (Singh et al., 2002), and tomato (Chiraz et al., 2003). Similarly, root restriction might be regarded as one type of physical stress for roots of fruit trees. Certainly, in comparison unrestricted root growth, development of fruit trees under root restriction should show many differences in

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metabolism, including nitrogen metabolism. However, there is little information available on nitrogen metabolism under root restriction. Therefore, in the following study, 2-year-old 'Fujiminori' grapevines were used to study the effects of root restriction on nitrogen metabolism, by principally determining NR and NiR activities.

2. Materials and methods

2.1. Plant material and growth conditions

In the spring of 2003, two groups of 20 vines of 2-year-old uniform 'Fujiminori' (*Vitis Venifera* × *V. Labrasca*) grapevines were selected. Vines in one group planted in 20 plastic pots (volume 10 L) were used for root restriction. The medium was a mixture of sand, loam and perlite (1:1:1). Vines in the other group planted in a raised bed (50 cm deep) in the same medium served as the controls. One shoot was trained vertically on each vine and spikes were removed. The space between each vine was 60 cm. From February to September, vines were maintained in a ventilated plastic house under natural light at the experimental farm, Shanghai Jiaotong University, Shanghai, China. After budburst, 1 L of complete liquid fertilizer (Hydro Co. Ltd., Israel) containing 120 mg/L N were applied to each vine once a week. The composition of the liquid fertilizer was: NO_3^--N 32.7 mg/L, NH_4-N 22.0 mg/L, UREA-N 58.7 mg/L, P_2O_5 120 mg/L, K_2O 120 mg/L, MgO 20.0 mg/L, B 0.167 mg/L, Cu 0.067 mg/L (as EDTA-Cu 0.047 mg/L), EDTA-Fe 0.467 mg/L, Mn 2.667 mg/L (as EDTA-Mn 0.187 mg/L), Mo 0.027 mg/L, Zn 0.167 mg/L (as EDTA-Zn 0.120 mg/L). Tensiometers were placed at 15 cm depth in rooting-zone to monitor soil moisture. Vines were watered by drip irrigation system to maintain the soil moisture ≥ -3.0 kPa from replanting to veraison, and ≥ -5.0 kPa from veraison to harvest, respectively.

2.2. Growth measurements

Five vines in each treatment were marked and their shoot growth measured weekly over the whole growing season. Shoot growth of vines with root restriction was inhibited significantly 127 days after planting compared with control vines, as determined by the shoot length. Then three vines per treatment were taken to measure the length and fresh weight of primary and secondary roots. For fresh weight measurements, roots in each treatment were separated into fibrous roots (<2 mm), medium roots (2–5 mm) and large roots (>5 mm) according to the root diameter 3 cm from the root apex.

2.3. Photosynthetic and pigment measurements

Since there were significant differences in shoot growth between treatments (127 days after planting), from 06:00 to 18:00 h, diurnal variation of the photosynthetic rate was measured with a portable photosynthesis system (CIRAS-2, PP Systems, UK), in the seventh leaf from the shoot base on a sunny day.

Concentrations of chlorophyll and carotenoids in fresh leaves were determined by the spectrophotometric method of Wellburn (1994).

2.4. Determination of NH_4^+, NO_3^- and total N

Three vines from each treatment were removed and each was separated into four parts: (1) new leaves (the fully expanded new leaves on the upper shoot), (2) mature leaves (the fully expanded leaves from middle shoot), (3) white roots (the white-coloured roots near the root apex), (4) brown roots (the brown-coloured roots near the white roots). The samples were thoroughly rinsed with distilled water, dried with filter paper, and then freeze-dried (ModulyoD-115, Thermo Savant, USA), and used for further quantitative analysis of NH_4^+, NO_3^- and total N.

NH_4^+ was extracted by grinding 0.5 g samples in 5 ml 10% acetic acid (v/v) and the volume was made up to 100 ml with deionised water (EC <1 μs/cm). Then the solution was filtered with filter membrane (0.45 μm) and the concentration of NH_4^+ was measured by ion chromatography (ICS-90, Dionex, USA) using an IonPac CS12A separation column (Dionex, USA), with a solution of 20 mM methanesulfonic acid as eluent, at a flow rate of 1 ml/min. The concentration of NO_3^- was determined according to the procedure described by Silvia et al. (2003). NO_3^- was extracted by grinding 0.5 g samples in 10 ml of 10 mM HCl. The extract was filtered through a membrane (0.45 μm) and the concentration of NO_3^- was determined by ion chromatography (ICS-90, Dionex, USA) using an IonPac AS9-HC separation column (Dionex, USA), with a solution of 8.0 mM Na_2CO_3 and 1.0 mM $NaHCO_3$ as eluent, at a flow rate of 1 ml/min. NH_4^+ and NO_3^- quantification was obtained using a calibration curve. Total N concentration was determined using a 2300 Kjeltec Analyzer Unit (Foss Tecator, Sweden) by the Kjeldahl digestion method.

2.5. NR and NiR analysis

Leaves and roots were sampled to measure the diurnal variation of NR and NiR activity upon the appearance of significant differences in shoot growth between treatments.

NR activity in new leaf, mature leaf, white root and brown root samples was determined according to Hageman and Hucklesby (1971). 0.4 g of leaf discs or root slices were placed in vials containing 5 ml ice-cold incubation medium, consisting of 0.1 M potassium phosphate buffer (pH 7.5), 50 mM KNO_3^- and 1% (v/v) iso-propanol. The fresh tissues were infiltrated by vacuum for 5 min, at −67 kPa, then incubated in the dark for 30 min at 30 °C. After incubation, the vials were placed in a boiling water bath for 5 min to stop the enzyme activity and to extract all nitrite formed. The nitrite released to the medium was determined by adding

1 ml of sulfanilamide (1% (w/v) in 3 M HCl and 1 ml 0.05% (w/v) *N*-naphtyl-ethylenediamine solution), and the absorbance was read at 540 nm after 30 min.

The nitrite reductase assay was performed according to Datta and Sharma (1999). One gram fresh leaf tissue was homogenized in 2 ml of 50 mM potassium phosphate (pH 8.8) buffer consisting of 1 mM EDTA, 25 mM cysteine and 3% (w/v) BSA. The assay mixture consisted of 1.4 ml of 100 mM potassium phosphate buffer (pH 7.5), 100 μl of 5 mM KNO_2^-, 100 μl of enzyme extract and 100 μl of methyl viologen (2 mg/ml). The volume was made up to 1.8 ml with distilled water. To start the assay, 200 μl of sodium dithionite (25 mg/ml in 190 mM $NaHCO_3$ solution) was added and incubated for 30 min at 30 °C. At the end of the incubation period, 100 μl of the assay mixture was added to 1.9 ml of water and vortexed immediately to oxidize the dithionite. The amount of nitrite used up by nitrite reductase was estimated by adding 1 ml of sulfanilamide (1% (w/v) in 3 M HCl) and 1 ml of 0.05% (w/v) *N*-naphtyl-ethylenediamine solution. The solution was incubated at 30 °C for 30 min and the absorbance was measured at 540 nm. The amount of nitrite used up by nitrite reductase was estimated from a standard curve of nitrite.

2.6. Statistical analysis

The experiments were repeated at least three times. Where appropriate, standard deviations are indicated in tables and figures. Significant differences were determined by a standard t-test and levels of significance are represented by $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ and NS, not significant.

3. Results

3.1. Growth of shoot and roots

Up until 92 days after planting, no differences in shoot growth were observed between treatments ($P < 0.05$). Subsequently, the shoot growth of vines with root restriction was weaker than that of control vines. When root-restricted vines showed significant differences in shoot growth compared with that of control vines 127 days after planting

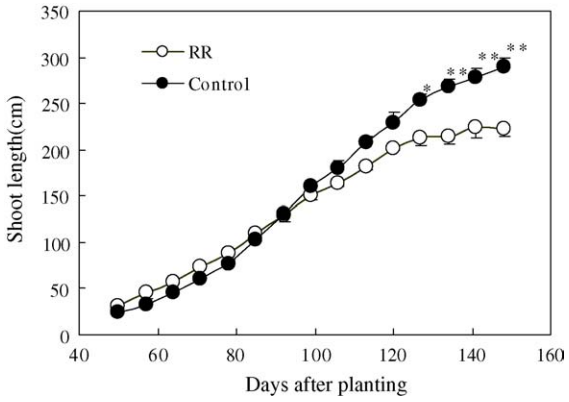

Fig. 1. Effect of root restriction on shoot length in 'Fujiminori' grapevine. Vertical bars indicate S.E. of the values. RR indicates root restriction treatment.

($P < 0.05, 0.01$) (Fig. 1), fresh weight of secondary roots in vines with root restriction was 5.7-fold more than that in control vines (Table 1). However, there was no difference in the length of primary and secondary roots between treatments. Root-restricted vines produced more fibrous roots (about 4.7-fold compared with control) and medium roots than unrestricted ones (Table 1).

3.2. Photosynthetic rate

Diurnal variation of the photosynthetic rate in the seventh leaf from the shoot base is shown in Fig. 2. The curves of photosynthetic rate showed similar changes. The maximum photosynthetic rate was reached at about 10:00 h and subsequently declined rapidly, but there are large differences between two treatments. The photosynthetic rate in root-restricted vines is 16.9%, 29.1% and 61.1% of the control at 8:00, 10:00 and 12:00 h, respectively, while after 12:00 h, the photosynthetic rate remained at a low level and there was no difference between the treatments.

3.3. Concentration of NH_4^+, NO_3^-, total N, chlorophyll and carotenoids

Nitrate concentrations in roots and leaves of root-restricted vines ranged from 1% to 29% of those in the control vines (Table 2). In addition, the concentrations of

Table 1
Effect of root restriction on length and weight of root system

Treatment	Length (cm)		Fresh weight (g)				
	Primary roots	Secondary roots	Primary roots	Secondary roots	Fibrous roots (<2 mm)[a]	Medium roots (2–5 mm)[a]	Large roots (>5 mm)[a]
Control	44.8	20.8	41.3	32.1	35.3	24.7	6.1
RR	47.9	24.3	61.3	184.2	164.5	43.9	8.6
Significance	NS	NS	NS	***	***	*	NS

$^{*}P < 0.05$, $^{***}P < 0.001$ and NS, non-significant.
[a] Diameter measured at 3 cm from the root apex. RR indicates root restriction.

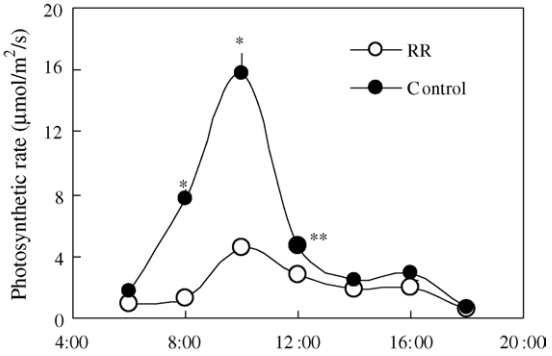

Fig. 2. Effect of root restriction on diurnal variation of photosynthetic rate in the seventh leaf from shoot base.

total N in new leaves and brown roots and the concentrations of NH_4^+ in new leaves were also significant lower in root-restricted vines. Chlorophyll and carotenoid concentrations were also reduced in mature leaves as a result of root restriction (Table 3).

3.4. Activity of NR and NiR

NR and NiR activities from 06:00 to 18:00 h in roots and leaves as in Figs. 3 and 4. NR activity of mature leaves was reduced by root restriction over the whole day and that of brown roots from 10:00 to 18:00 h. On the contrary, there was no difference in NR activity of new leaves between treatments (Fig. 3A). NR activity in white roots was reduced by root restriction after 12:00 h. The highest activity of NR in both treatments occurred in mature leaves and brown roots at the 10:00 h sampling. NiR activities of leaves and roots in root-restricted vines were lower than those in control vines at 10:00 h, in new and mature leaves, and in white and brown roots NiR activity of root-restricted was 84%, 54%, 90% and 85% of that in the control vines respectively at 10:00 h (Fig. 4). Subsequently, the NiR activity of mature leaves, brown roots in root-restriction vines declined and were also showed significantly different between treatments at 14:00 h, and a further difference appeared in new leaves at 18:00 h.

Table 2
Effect of root restriction on concentration of NH_4^+, NO_3^- and total N in vine organ (%DW)

Treatment	White roots	Brown roots	New leaves	Mature leaves
NH_4^+				
Control	0.17	0.02	0.10	0.02
RR	0.15	0.03	0.03	0.02
Significance	NS	NS	*	NS
NO_3^-				
Control	2.48	0.51	0.13	0.06
RR	0.24	0.15	0.03	0.02
Significance	**	**	***	***
Total N				
Control	1.95	1.27	2.66	2.08
RR	1.87	1.23	2.27	2.03
Significance	NS	*	**	NS

$^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ and NS, non-significant.

Table 3
Effect of root restriction on concentration of chlorophyll and carotenoid in leaves

Treatment	Chlorophyll (mg/g fw)		Carotenoid (mg/g fw)	
	New leaves	Mature leaves	New leaves	Mature leaves
Control	0.97	1.78	0.40	0.59
RR	1.02	1.37	0.41	0.44
Significance	NS	**	NS	**

$^{**}P < 0.01$ and NS, non-significant.

4. Discussion

The experiment showed that root restriction inhibited shoot growth (Fig. 1), and significantly increased the fresh weight of secondary, fibrous and middle roots in grapevines (Table 1). The findings agree with results of several previous studies (Bar-Yosef et al., 1988; Mark and Marra, 1994;

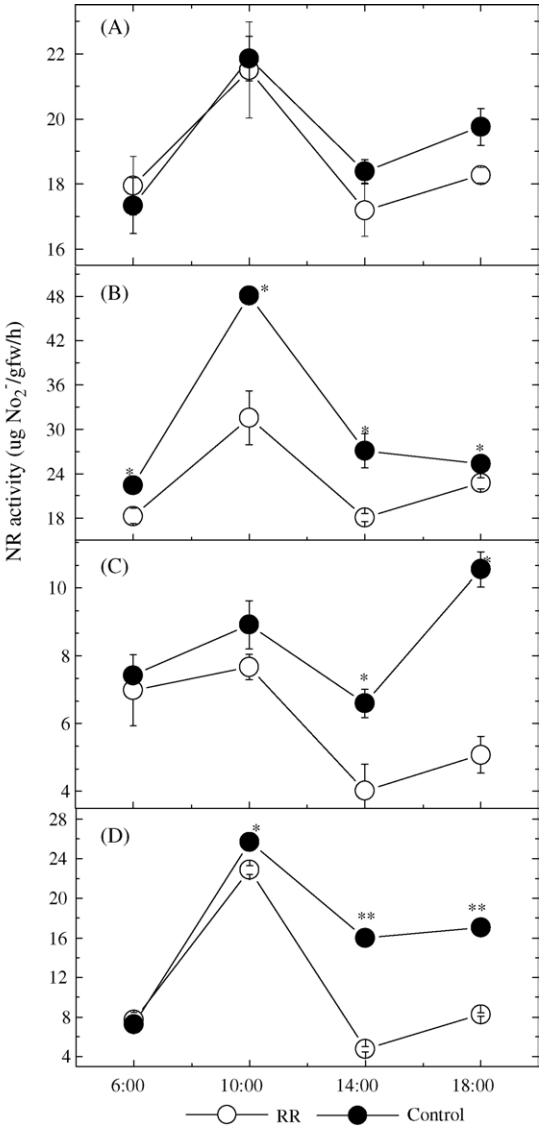

Fig. 3. Effect of root restriction on diurnal variation of NR activity in new leaves (A), mature leaves (B), white roots (C) and brown roots (D).

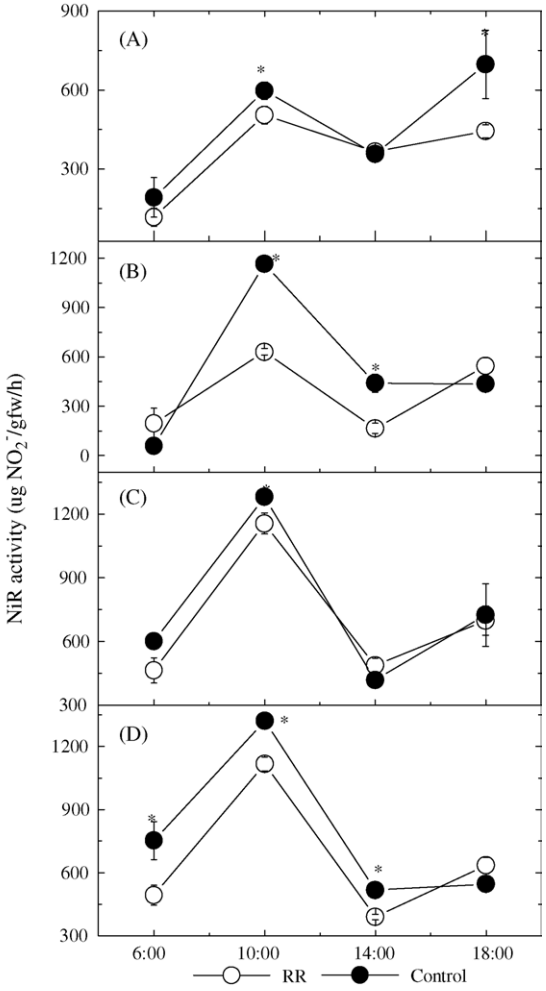

Fig. 4. Effect of root restriction on diurnal variation of NiR activity in new leaves (A), mature leaves (B), white roots (C) and brown roots (D).

Boland et al., 1994, 2000; Wang et al., 2001), which showed that vines' growth could be substantially inhibited by root restriction.

Nitrogen metabolism has been implicated in the inhibitory effects of shoot growth in relation to reduced root volume and function. Mark and Marra (1994) and Boland et al. (2000) reported that leaf N in peach decreased as root volume declined. Root restriction in Euonymus reduced the concentration of some mineral nutrients in leaves such as N, P, Ca, Mg, Al, Fe and Cu (Dubik et al., 1990), it have also suggested that root restriction decreased the nitrogen levels in root-restricted plants. Our unpublished work showed that the amount of ^{15}N (NO_3^-) accumulation of whole vine under root restricted was only about 50% of the control. Our findings on the reduction of total N in 'Fujiminori' grapevines in root-restricted vines support the above results.

If nitrogen levels are so affected, then we might expect this to be reflected in levels and activities of N metabolites and enzymes. Wang et al. (1998) reported that in root-restricted vines the total amino acid concentration in xylem sap was lower than that in control vines, which indicated that the nitrogen assimilation is likely to be inhibited in root-restricted vines. Our results show that root restriction reduced activities of the two key nitrogen reductase activities (NR and NiR). A consequence of this, may be reduced N assimilation in the vines and affect on the synthesis of amino acids, protein and therefore shoot growth, and it is probably responsible for the inhibition of plant growth.

We also found further physiological consequences of root restriction. In root-restricted vines, the NO_3^- concentration in all parts of the vines and total N concentrations in new leaves and brown roots were lower than those in the control vines (Table 2). Concentrations of chlorophyll and carotenoids in mature leaves, and photosynthetic rate in the seventh leaves from the shoot base in root-restricted vines were also lower (Table 3 and Fig. 2). Wang et al. (1998) reported that root restriction increased sugar levels in both roots and shoots of 'Kyoho' grapevines. Nitrate is an important factor regulating NR and NiR activity (Datta and Sharma, 1999), and NO_3^- uptake by plants is followed by an increase in the NO_3^- present in the leaves (Eilrich and Hageman, 1973; Aslam, 1981) and, in controlled conditions, by an increase in the activity of nitrate reductase (Przemeck and Kucke, 1986). Vouillot et al. (1996) suggested in N-deficient plants, the nitrate reductase activity was lower than in well-fertilized plants. Carbon and nitrogen metabolism share organic carbon and energy supplied from photosynthesis and respiration and this is supported by recent work with transgenic plants of *N. plumbaginifolid* with low levels of NR expression, which had lower levels of chlorophyll, protein and amino acids and higher contents of carbohydrate (starch and sucrose) than high NR expressors (Foyer et al., 1994). Majerowicz et al. (2000) suggested that restriction of absorption and assimilation of nitrogen into organic molecules could be associated with the high levels of sugars in the metabolic pool of the nitrate-fed *C. fimbriatum* plants. Therefore, the concentration of NO_3^- and leaf pigments in tissues, photosynthesis and carbon metabolism can all be affected by the activity of NR and NiR in root-restricted vines.

In the present study, water potential in the root zone could decrease to the critical value before the next noon (replanting-veraison) or intraday noon (veraison-harvest) on a sunny day; although it was irrigated well in the morning, while the water potential remained stable in control (data not shown), which was in accordance with our previous study (Wang et al., 2001). That is to say water stress occurred almost every day because of the smaller amount of available water under root-zone restriction. Gray et al. (2001) reported that increasing water stress progressively decreased plant growth potential, leaf area, net photosynthetic rate and nitrate reductase activity. Vyas et al. (1996) and El-Komy et al. (2003) also found water stress decreased nitrogen reductase activity of leaves and roots in moth bean and wheat. Therefore, the decline of net photosynthetic, NR

activity and NiR activity was tightly related to frequent water stress under root restriction. In addition, it has been proposed that Cd or Pb and salinity could inhibit nitrate reductase activity and decrease organic nitrogen in leaves of mustard seedling (Singh et al., 2002). Furthermore, salinity has also been shown to inhibit nitrogen assimilation, which was found to be unaffected even by exogenous supply of either NO_3^- in *N. tabacum* (Sweby et al., 1994) or NH_4^+ in peanut and cotton (Leidi et al., 1992). Therefore, the reduction of NR and NiR activities in plants is likely one of the characteristics under stress conditions, such as water and salinity stress.

Some earlier studies have also suggested that root restriction may affect shoot growth through additional metabolism processes (Krizek et al., 1985; Ismail and Noor, 1996). For example, root restriction might alter plant water balance and consequently affect leaf growth (Hameed et al., 1987; Peterson et al., 1991). It has also been proposed that the reduction of plant growth under root restriction may be caused by a decrease in the synthesis and translocation of growth substances from the roots (Richards and Rowe, 1977; Carmi and Heuer, 1981; Ismail and Davies, 1998). Ternesi et al. (1994) indicated that ABA in xylem of sunflower plants under root restriction increased seven-fold, which may be associated with a reduction of leaf growth. Similar evidence was found in watermelon (Liu and Latimer, 1995). Our unpublished data also showed that ABA concentration in xylem sap in root-restricted vines was much higher than that in control vines.

Although these results suggest that there may be a number of physiological consequences of root restriction, all contributing to reduction in shoot growth, our results strongly suggest that the reduction of NR and NiR activity is one of the crucial factors responsible for changes in nitrogen metabolism and consequent weak vigor of vines.

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