

Full Length Research Paper

Effect of root restriction on vegetative growth and leaf anatomy of 'Kyoho' grapevines cultivar

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The effects of root restriction on leaf and shoot growth and leaf anatomy of grape were investigated. Root restriction delayed leaf appearance, decreased leaf growth and reduced the rate of leaf expansion during all stage of berry development. Shoot growth of root-restricted vines was also reduced, with the length and thickness of shoots being lower than those of the control. Root restriction also induced adaptive changes in the leaf anatomy. Somata density of leaves from root-restricted vines was higher than that of controls, but the size of the stomata was smaller. The thickness of the leaf, cuticle, palisade layer, spongy mesophyll, and leaf structure tense ratio (CTR) were increased, however, the leaf tissue loose ratio (SR) and the size of guard cells were decreased. In conclusion, our results suggest that root restriction not only reduces growth of vegetative organ, but also alters their anatomy. These changes may be one of the reasons that change the physiological functions of the leaf.

Key words: Root restriction, shoot and leaf growth, leaf anatomy.

INTRODUCTION

Leaves are the main source of CO₂ assimilation in plants. Root restriction can control the size of the shoot and partitioning of assimilates between vegetative and reproductive organs (Carmi, 1986). Under root restriction, the plant displays distinctive differences in growth habit compared with that under normal field cultivation. Root restriction increased root mass, the amount of fibrous roots, reduce shoot growth, and improve both fruit set and fruit quality (Bar-Tal et al., 1995; Wang et al., 1998, 2001). Many investigators report that root restriction decrease vegetative growth, and alter the metabolism of vegetative organs (Wang et al., 2001; Zhu et al., 2006). Root restriction decreased leaf nitrogen content of *Euonymus* (Dubik et al., 1990), grapevine (Zhu et al., 2006), and peach (Mark and Marra, 1994; Boland et al., 2000), which may reduce the rate of leaf expansion (Radin, 1983; Gastal and Nelson, 1994).

Although, many studies have reported that root restriction affects leaf photosynthesis and nutrient content, information on its effects on leaf growth and anatomy is limited. The importance of leaf anatomy for the main physiological functions of photosynthesis and transpiration, and its response to the environment, has long been recognized (Pachepsky et al., 1999). The objective of this study was to examine the effects of root restriction on vegetative growth and leaf anatomy of 'Kyoho' (*Vitis vinifera* × *Vitis labrasca*) grape cultivar.

MATERIAL AND METHODS

Plant material

The experiment was carried out during the 2008 growing season at the experimental farm at Shanghai Jiao tong University, Shanghai,

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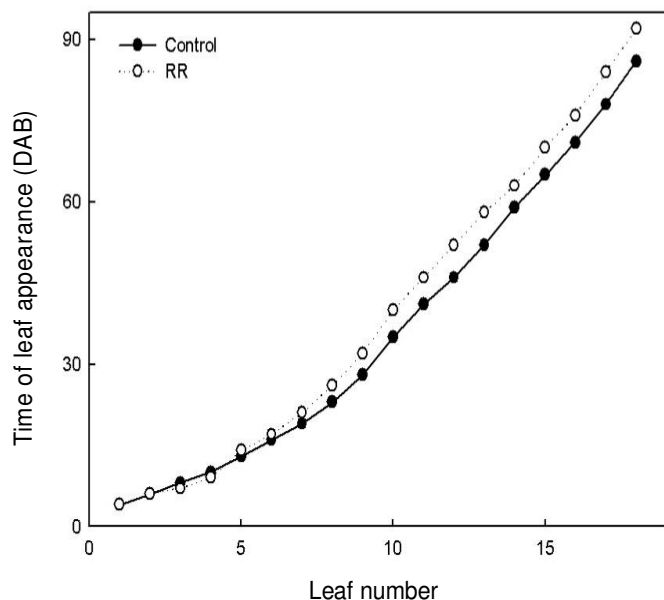


Figure 1. The time of leaf appearance in plants subjected to root restriction (RR) and control. Points represent the mean of 5 shoots.

China (3111N, 12129E). Two groups of 20 uniform 3-years-old 'Kyoho' grapevines (*V. vinifera* × *V. labrasca* cv.) were selected. The first group was subjected to root restriction by planting in 10 L plastic pots, however, the second one, served as the control, was planted in a raised bed 50 cm in deep in a medium composed of a mixture of sand, loam, and perlite (1:1:1) by volume and served as the control. In late winter of 2007, each vine was pruned to 3 to 5 buds. In spring of 2008, one vigorous shoot on each vine was left and the other shoots were removed. Only the basal cluster was retained on the remaining shoot after flowering. The number of berries in those clusters was thinned to about 50. The space between each vine was 60 cm; vines were maintained in a ventilated greenhouse under natural light at the experimental farm from February to September. The greenhouse was covered with a plastic sheet with a transmission of about 87%. Natural ventilation was used to maintain the air temperature inside the greenhouse near from outside air temperature. After budburst, each vine was fertilized with 1 L of complete liquid fertilizer (Hydro Co. Ltd., Israel), containing 32.7 mg NO₃⁻, 22.0 mg NH₄⁺, 120 mg P₂O₅, 120 mg K₂O, 20.0 mg MgO, 0.167 mg B, 0.067 mg Cu, 0.467 mg EDTA-Fe, 2.667 mg Mn, 0.027 mg Mo, and 0.167 mg Zn, once a week. Tensiometers were placed at a 15 cm depth in the rooting-zone to monitor soil moisture. Drip irrigation system was used to maintain the soil moisture ≥ -3.0 kPa from replanting to veraison, and ≥ -5.0 kPa from veraison to harvest.

Leaf and shoot growth measurements

Five grapevines in each treatment were marked and all major features of development were monitored on the marked shoots, including the time of new leaf appearance. Leaf area (cm²) of the second leaf from the shoot apex was measured during different stages of berry development. The leaf area for each leaf was calculated from the non-destructive measurements of leaf length using the following equation, $Y = 0.6933X^2 + 5.5069X - 15.1966$ ($R^2 = 0.9419$) where the X is the length of leaf midvein. The length and thickness of tagged shoots were measured every 3 days after 20 days of anthesis.

Observation of the stomata density and anatomic structure of leaf

Clear nail polish was applied to the adaxial and abaxial leaf surfaces on opposite sides of the leaf to measure the stomata density. The transparent of the leaf surface impression was removed and attached to a glass slide (Abraham et al., 2009).

To measure the anatomical features of the leaves, the lower surface of one fully-expanded leaf from six different individuals for each treatment was assessed. 3 × 3 mm segments were cut from the central area of the cleared leaf blade tissue, then were fixed by FAA, and cut into 10 μm thickness by paraffin sectioning technology (Nii et al., 1995).

Statistical analysis

The comparison of pairs of values was analyzed by t-test and levels of significance are represented by *P < 0.05, **P < 0.01 (Xie et al., 2012).

RESULTS

New leaf appearance

The time of leaf appearance was recorded after bud break. The second leaf appeared 1 to 2 days later than the first leaf, the third and the fourth leaves appeared at interval of 2 to 3 days (Figure 1). The difference in time of leaf appearance was not significant from the first to the fourth leaf between root restriction and control treatments. After the fifth leaf, the rate of leaf appearance was at an interval of 4 to 5 days. The time of leaf appearance in plants subjected to root restriction was delayed 2 to 3 days when compared with the control after the fifth leaf (Figure 1).

Leaf growth

The expansion of new leaves was observed during different stages of berry growth, including inflorescence, the first rapid growth stage, the lag stage and the second rapid growth stage (Figure 2). During the first rapid growth stage (15 to 50 days after anthesis), the new leaf had the maximum rate of expansion compared with the other stages (Figure 2B). During all stages of berry growth, the leaf expansion was reduced in plants subjected to root restriction than in control plants (Figure 2).

Shoot growth

Shoot growth was monitored following 20 days after anthesis (Figure 3). The shoot growth of grape vines, including the length and thickness, was reduced in plants subjected to root restriction compared to controls (Figure 3). The increase of shoot length followed a single sigmoid curve. The shoots of root restriction vines were significantly shorter than control following 50 days after

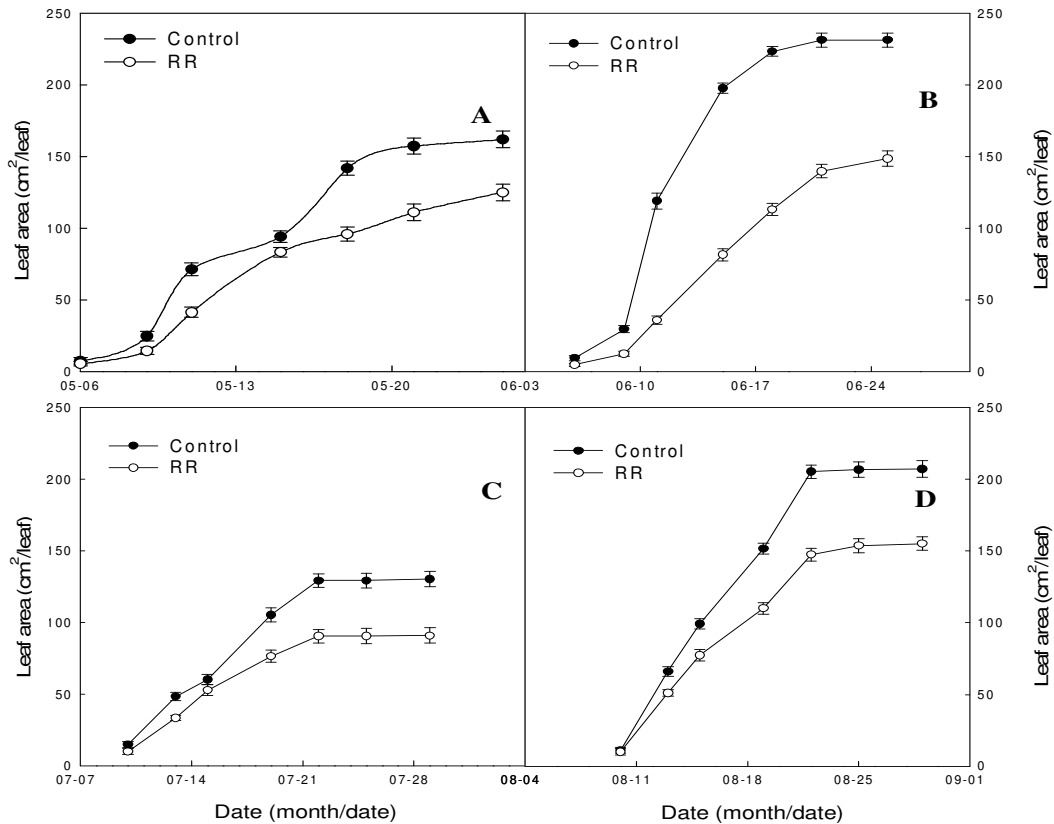


Figure 2. The changes in leaf area of the second leaf from the shoot apex in plants subjected to root restriction (RR) and control during anthesis (A), the first rapid stage of berry growth (B), the lag stage of berry growth (C), and the second rapid stage of berry growth (D).

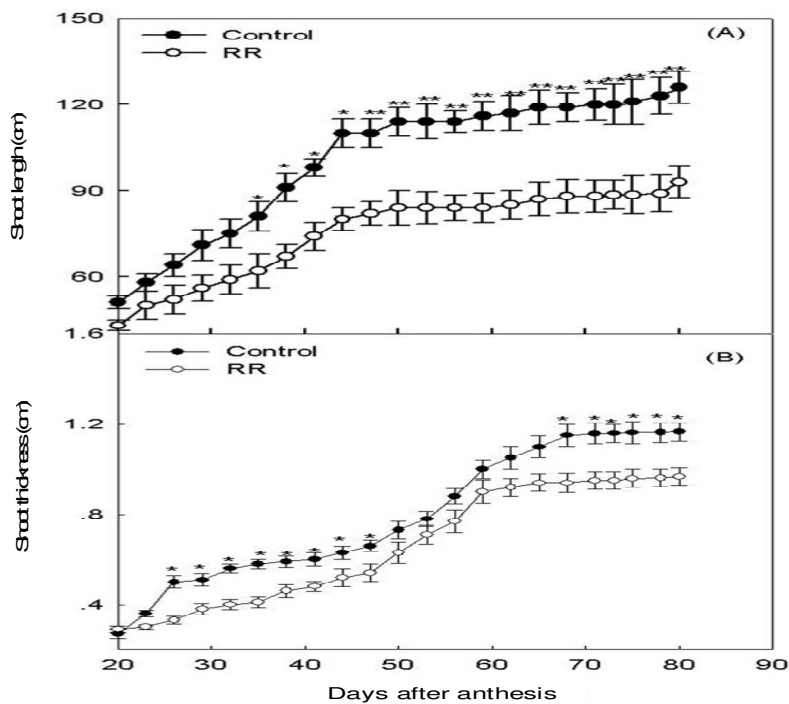


Figure 3. The length (A) and thickness (B) of shoots in plants subjected to root restriction (RR) and control during fruit development.

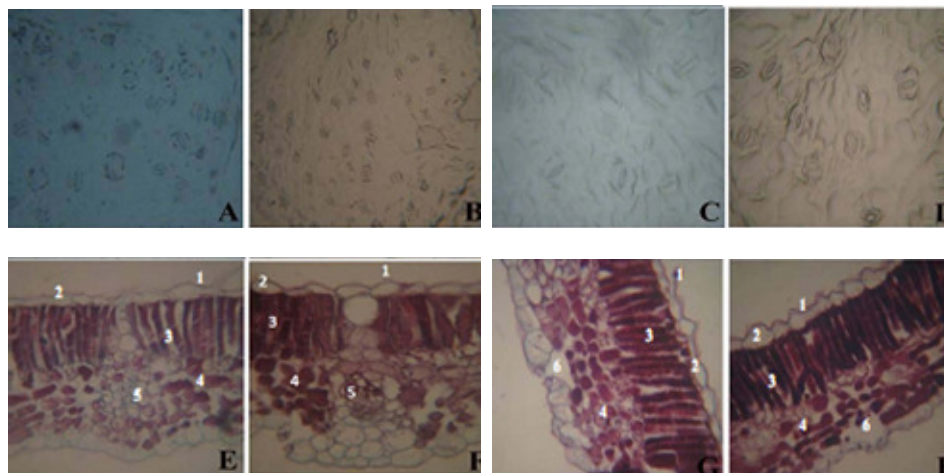


Figure 4. The stomata (A-D) and comparative anatomical structure (E-H) of grape leaves from plants subjected to root restriction (B, D, F, H) and control (A, C, E, G). 1: Cuticle, 2: epidermis, 3: palisade layer, 4: spongy tissue, 5: vascular tissue; 6: stomata.

Table 1. Indices of anatomical structure of grape leaves from plants subjected to root restriction (RR) and control.

Treatment	Control	RR	Significance
Leaf thickness(μm)	319.4	456.8	**
Cuticle thickness(μm)	1.5	2.1	**
Thickness of palisade layer (μm)	176.84	236.13	**
Thickness of spongy mesophyll (μm)	78.35	85.14	*
Thickness of upper epidermis cell (μm)	41.12	52.25	*
Thickness of lower epidermis cell (μm)	16.84	22.7	*
CTR ^z (%)	42.84	51.69	**
SR ^y (%)	24.53	18.64	**
Stomata density (number/ mm^2)	72	101	**
Stomata longitudinal diameter (μm)	26.6	21.3	*
Stomata horizontal diameter (μm)	4.9	3.2	*

Values represent the means of six replicates. *, Significant at $p < 0.05$; **, significant at $p < 0.01$ based on a paired t-test. ^zCTR (leaf tissue structure tense ratio): the thickness of (palisade + lower tight tissue) / mesophyll. ^ySR (leaf tissue structure loose ratio): the thickness of spongy/mesophyll.

anthesis (Figure 3A). The change of shoot diameter followed a double sigmoid curve; the maximum significant difference between the two treatments was achieved 67 days after anthesis (Figure 3B).

Leaf anatomy

Grape leaves are comprised of a cuticle, epidermis, mesophyll, and vascular tissue (Figure 4E and F). The thickness of the leaf and cuticle in plants subjected to root restriction was greater than that of control (Table 1). Root restriction increased the leaf tissue structure tense ratio (CTR) and decreased the leaf structure loose ratio (SR) compared with those of control (Table 1, Figure 4G

and H). The leaves of plants subjected to root restriction had better uniformity epidermis cells than that of control. The thickness of the upper epidermis of leaves subjected to root restriction and control were 52.25 and 23.12 μm , respectively, however, the thickness of lower epidermis of leaves were 22.7 and 16.84 μm , respectively (Table 1, Figure 4G and H). The palisade layer and spongy mesophyll of leaves subjected to root restriction were thicker than those of controls. Leaves subjected to root restriction had thicker palisade layer, thicker spongy mesophyll, and thicker layers of smaller and more closely-arranged diachyma cells than control (Table 1, Figure 4G and H). Stomata and guard cells of leaves from root restriction plants were significantly smaller than those of control; however, the density of stomata was

higher (Table 1, Figure 4A-D).

DISCUSSION

Root restriction, as one type of physical stress, can induce changes in the development of grapevines and other plants (Wang et al., 2001; Xie et al., 2009). Reduced vegetative growth as a result of root restriction is a well recognized phenomenon (Peterson et al., 1991a). In agreement with previous reports, our results showed that leaf and shoot growth was reduced by root restriction. This decline in vegetative growth in plants subjected to root restriction was often interpreted as a feedback inhibition caused by the stress on the roots (Shi et al., 2008).

Leaf structure in plants is determined by both genetic and environmental factors. The total thickness of leaves from root restricted plants was higher than in control (Table 1). Furthermore, an increase in the density of palisade and spongy mesophyll cells and a decrease of intercellular spaces were also observed (Table 1, Figure 4G and H). The arrangement of spongy mesophyll cells in root-restricted leaves may result in a reduction of the CO₂ conductance. These results proved that root restriction, environmental stress, can induce changes in leaf structure.

Wang et al. (2001) reported that water stress occurred almost every day in plants subjected to root restriction, because the amount of available water was reduced. Several reports have suggested that the reduction in shoot growth due to root restriction is partly attributed to disturbance of the internal water relations (Tschaplinski and Blake, 1985; Arp, 1991). Garg et al. (2001) reported that increasing water stress progressively decreased plant growth potential, leaf area, net photosynthetic rate and nitrate reductase activity. It has been reported that root restriction reduced the number of leaves, total leaf area, and shoot initiation in peach (Richards and Rowe, 1977). Leaf growth was the most sensitive process to water stress (Dry and Loveys, 1998).

The growth of leaf and shoot can be mediated by chemical signals synthesized in the roots. The effect of root-sourced signals on leaf growth has been reported for apple, tomato and grape plants. Ternes et al. (1994) indicated that ABA in xylem of sunflower plants is subjected to 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). Root restriction also affected the capability of the root to absorb nutrients. Some reports have revealed that root restriction decreased the concentration of nitrogen in shoots, flower clusters, trunks and canes of grapevines (Wang et al., 1998; Zhu et al., 2006), 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).

In conclusion, this study has shown that the dynamic process of leaf and shoot growth was affected by root restriction. The decrease in leaf growth also affected leaf anatomy, which may affect its function.

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