Effects of Root Restriction on Anatomical Structure of Grape Berry during Berry Development Phases

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ABSTRACT

In this research, the main aim was to study the response of the anatomical structure of Vitis vinifera ‘Kyoho grape fruit to root restriction. It is demonstrated that root restriction can lead to the increase of sugar accumulation in grape berry and that the berry weight is also increased under root restriction and at the same time, changes of berry structure including skin and flesh was observed under root restriction. The thickness of skin cell wall was larger under root restriction than control treatment, and after veraison, root restriction kept the flesh cells intact which in turn led to the increase in sugar accumulation. Therefore, these changes are the responses of fruit growth to root restriction stress, just as the response of other organs, including shoot, root and leaf which are regulated by a number of stress factors caused by root restriction. Our results strongly suggested that the improvement of berry quality under root restriction benefits from the change of berry structure.

Key words: Root restriction, ultrastructure, anatomical structure, sugar accumulation.

INTRODUCTION

Grapevine (Vitis vinifera L.) is a woody perennial plant providing fruit species used as fresh fruit, dried raisins and for wine making and distillation of liquors (Coombe, 1989). The development of grape fruits can be divided into three different phases, including two major stages of growth separated by a lag phase (Coombe, 1992). During the development stages, the grape berry undergoes a complex series of physical and biochemical changes. Environmental factors, such as light, water, soil temperature and nutrients can influence the process of berry development.

Root restriction has been studied all over the world in fruit tree cultivation as an effective method for improving fruit quality and getting earlier yields. The principle of root restriction is based on the fact that vegetative growth and reproductive growth of fruit trees can be regulated (Carmi, 1986). Previous studies have shown that root restriction influences the growth and composition of a wide variety of fruits, including apples, cherries, pears and grapes. Grape berries generally are higher in sugars, anthocyanins and phenolics and lower in titratable acidity under root restriction (Wang et al., 1998; Bo et al., 2012). As the root of fruit tree under root restriction has limited growth zone and become too dense and competition for water and nutrient occurred among roots, some parts of the root may generate stress signals to regulate the growth of the tree. It has been demonstrated that restriction to root growth by mechanical barriers can increase the rate of ethylene evolution, by as much as six times that of unrestricted roots (Webster et al., 1997).

In previous reports, it has been demonstrated that plants subjected to root restriction display distinctive differences in growth habit as compared with those under normal cultural conditions (Goto et al., 2002; Wang et al., 2001). In comparison with the improvement of fruit quality under root restriction, less is known about how the fruit development responds to root restriction even though it is
important to reveal the mechanism that determines the improvement of fruit quality under root restriction and few experiment have attempted to correlate the changes of berry structure with root restriction. The purpose of this investigation was to determine the influence of root restriction during specific stages of berry development on fruit growth and anatomical structure.

MATERIALS AND METHODS

Plant material

Plant material was sampled from an experimental orchard of the Shandong Institute of Pomology, located at Tai’an city, Shandong, China. Four-year-old own rooted grapevines of V. vinifera L. Kyoho cultivar were used in the study. Two groups of 20 vines were selected for the experiment; vines in one group used for root restriction were planted in 20 plastic pots (volume 10 L) containing 1 soil: 1 sand: 1 peat medium (by volume). Vines in the other group for controlling treatment were planted in a raised bed (50 cm deep) in the same medium with root restriction. At the initiation of the experiment, the vines were pruned to two-bud spurs, and all vines were staked upright and the shoots vertically trained. When shoot length was about 40 cm, each shoot was thinned to one cluster bearing and all other clusters were removed. The basal cluster was thinned to about 50 berries. The vines were maintained in a ventilated plastic house under natural light at the experimental farm. During shoot growth season, the complete liquid fertilizer (Hydro Co. Ltd., Israel) was used and one litre of the fertilizer applied to one vine once a week; a drip irrigation system was used to irrigate the vine tree and the soil moisture was kept at 3.0 kPa before veraison and 5.0 kPa after veraison. The soil moisture was monitored by soil tensiometers (Vacuum Pressure Gauge, China) inserted into the root-zone soil at a 15 cm depth.

Berry quality analysis

Berry quality was evaluated through measuring of average berry size, TSS (“brix”), pH value, and titratable acidity. Fresh weight of both berries was taken (no pedicel, but receptacle intact), using a bench top AX205 Delta Range (Mettler Toledo, Switzerland), and then averaged. TSS value was observed in the field at harvest. Fifty berry samples were randomly selected from each treatment. TSS was determined using a refractometer (PAL-1, Atago Co., LTD., Tokyo, Japan) and pH determined using a pH meter (PHR-146; Lazar Research Laboratories, Inc.). Titratable acidity was measured indirectly by how much 0.1 N NaOH was titrated into 5 ml of juice until a pH of 8.2 was reached.

Forty (40) berries in one group were randomly sampled, placed in an ice box and taken back to the laboratory within half an hour, and then were immediately frozen in liquid nitrogen and stored at -80°C until compositional analysis. For each sample, before analysis, berries were pressed. The resulting juice mix was first heated at 40°C for 30 min in a Accublock™ Digital Dry Bath (Labnet International, Inc., Woodbridge, NJ), then centrifuged (5415D; Eppendorf, Hamburg, Germany) for 2 min at 10,000 rpm. The supernatant was again centrifuged at the same rate using a centrifuge tube with a 0.45 μm filter before HPLC analysis.

Acid and sugar levels were determined using High Pressure Liquid Chromatography (HPLC) (Agilent 1100 Liquid Chromatograph, Hewlett-Packard, Waldbronn, Germany). The method consisted of injecting 0.5 μl of juice sample into a 0.004M H2SO4 solvent at a flow rate of 0.700 ml/min through a 50 × 7.7 mm Hi-Plex H, guard column and 300 × 7.7 mm, Hi-Plex H, ligand exchange column (Agilent Technologies, Waldbronn, Germany), capable of both sugar and organic acid analysis. Samples injected into the HPLC were diluted 5-fold to prevent over saturation of the column. Column temperature was 72.5°C and Refractive Index Detector (RID) temperature was 55°C. The Diode Array Detector (DAD) was set to measure at 210 nm.

The RID was used in determining glucose, fructose, tartaric acid and citric acid. The DAD was also used for measuring malic acid and the unidentified peak. The reasons for two detectors being used were based on selectivity and baseline integrity. Glucose and fructose cannot be measured through DAD. The DAD baseline was too irregular for accurate readings during the retention times for tartaric and citric acids. Malic acid co-eluted with glucose and fructose in a way (between or within the 2 peaks) that made analysis difficult. The peak area given by the HPLC through HP ChemStation software (version 10.02) was converted to g/L using a calibration curve made from known standards, according to Dolenc-Sturm et al. (1999).

Anatomic observation of berry skin and flesh

We sampled the berries during different growth phases of grape berry. All experimental material including berry skin and flesh separately were fixed in FAA (Formaldehyde acetic acid and alcohol), embedded in paraffin and cut into 10 nm thick sections following the method of Osman et al. (2012).

Observation of berry skin and flesh ultrastructure

During different development stages of grape berry, a sampling of 10 berries from 3 clusters for each treatment were carried into the laboratory and the grape berry flesh and skin cut into small cubes (approximately 1 to 2 mm) immediately in the laboratory and fixed immediately with 3% glutaraldehyde in 0.1 M potassium phosphate buffer at
RESULTS

The changes of fruit weight throughout the development of grape berry

Figure 1 shows the changes in fresh and dry weight of berry and seed during different development stages of fruit. During the first rapid growth of grape berry, there was no evident difference for the berry growth between control and root restriction, but during the second rapid growth phase, root restriction resulted in a more rapid increase of berry growth compared to control treatment expressed from berry fresh weight and dry weight. These results showed that root restriction promoted the growth of berries grown under during the second rapid growth stage. The rapid increase of seed weight happened mainly in the first rapid growth of grape berry and after veraison, the increase of seed weight slowed down. The seed growth under both treatments showed the same trend, but both the seed fresh weight and dry weight were significantly higher when under root restriction as compared to control treatment.

Fruit sugar and organic acid contents

The grape berry quality was largely decided by the sugar contents at harvest, but the sweetness of the berry depended on the ratio of sugar to acid. At harvest time, the levels of fructose and glucose under root restriction were significantly higher than those of the control, indicating that root restriction promoted the accumulation of sugar in grape berry. The level of malic acid in root restriction treatment berries was considerably lower than in control berries; in consequence, the sugar/acid ratio in root

Figure 1. The fresh weight and dry weight of grape berry and seed under control and root restriction.
Table 1. Fruit sugar and organic acid contents.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TSS(Brix)</th>
<th>TA (%)</th>
<th>Glucose (g/100 g)</th>
<th>Fructose (g/100 g)</th>
<th>Tartaric acid (g/100 g)</th>
<th>Citric acid (g/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>17.5a</td>
<td>0.63a</td>
<td>0.675a</td>
<td>0.463a</td>
<td>0.532a</td>
<td>0.215a</td>
</tr>
<tr>
<td>Root restriction</td>
<td>19.8b</td>
<td>0.48b</td>
<td>0.959b</td>
<td>0.768b</td>
<td>0.424b</td>
<td>0.301b</td>
</tr>
</tbody>
</table>

Figure 2. Anatomical structure of pericarp and flesh of grape berry under control and root restriction. 1, 2, 3) anatomical structure of pericarp under control treatment during phases I, II and III; 4, 5, 6) anatomical structure of pericarp under root restriction treatment during phases I, II and III; 7, 8, 9) anatomical structure of flesh under control treatment during phases I, II and III and 10, 11, 12) anatomical structure of flesh under root restriction treatment during phases I, II and III.

Restriction treatment was higher than those in the control treatment and the sweetness of grape berry was increased under root restriction (Table 1).

Structural changes in fruit skin during different development stages

The structure of grape skin during different development stages under root restriction and control treatments are shown in Figures 2 and 3. The grape skin consisted of cuticle, intermediate epidermis and hypodermis. The cuticle was covered by waxes and the berry skin under root restriction had thicker waxes and cuticle than those under control treatment after veraison (Figures 2: 1, 4 and 3: 1, 2); this would benefit the decrease of water loss from berry. During the first rapid growth stage, the outermost three layers of skin were compact, the cells have narrow cellular cavities and thick protoplasm and the root restriction treatment decreased the cell size of outermost layers, but increased the thickness of the cell wall (Figure 3: 1, 2).

The inner layers of berry skin under two treatments have much larger and highly vacuoles, and with the development of grape berry, the size of skin cells expanded and the radial
Figure 3. Ultrastructure of grape berry skin under control and root restriction. 1, 2) ultrastructure of berry skin under control and root restriction treatment during phase I; 3, 4) ultrastructure of berry skin under control and root restriction treatment during phase I; 5, 7) ultrastructure of berry skin under control treatment during Phase I; 6, 8) ultrastructure of berry skin under root restriction treatment during phase I.

widths of inner layer cells increased, but compared to the flesh cells, the increase of size in the epidermis and subepidermis three layers was less (Figures 2: 2, 5 and 3, 4). The control treatment showed a larger increase in cell size of epidermis and hypodermis than that under root restriction (Figure 2: 3, 6). During the second rapid growth phase, the thickness of cell walls of the outermost three layers increased under both root restriction and control treatment, while the cell wall thickness of inner layers decreased (Figure 3: 5, 6, 7 and 8). The reasons for such changes were mainly the fruit plasticity increasing and the cell wall loosening. During the second rapid growth phase, more breakdown of the middle lamella was observed in some inner cells under control treatment (Figure 3: 7, 8).

Structural changes in fruit flesh during different development stages of fruit

Compared to skin cells, the flesh cells of grape berry were bigger with thinner cell walls and contained less cytoplasm (Figure 2: 1, 4, 7 and 10). During the first rapid growth, the flesh cells showed a rapid expansion, the size of outer mesocarp cells were bigger than those of the inner mesocarp and the shape of the outer mesocarp cells was more oval than those of the inner mesocarp (Figure 2: 7 and 10). During this period, a large central vacuole was observed in the mesocarp cells, and the mesocarp cell wall was compact, numerous highly developed mitochondria and evenly stained material were also observed in the wall.
In the present study, the anatomical and ultrastructure changes were examined. In this study, we examined the anatomy and ultrastructure changes in berry under root restriction treatment. As described in the previous reports, root restriction could alter a range of plant processes at anatomical and physiological levels, depending upon the plant varieties, extent of root restriction stress and time of root restriction stress. Under root restriction, the increase of root density induces the competition for water and nutrients among roots, and some signals interrelated with these stresses were transferred to above-ground parts of plant, yet the supply of water and nutrients can satisfy the demand of plant growth, therefore, in a certain sense, these signals are false and leads to adaptive response.

It is especially challenging to identify the extent of root restriction stress that is positive or harmful. Actually, the plants under root restriction normally showed similar features to that under water stress, such as the allocation of photosynthetic product to fruit increase (Boland et al., 2000a, 2000b). It has been reported that during the second rapid growth phase of grape berry, water stress occurred almost every day due to the smaller amount of available water under root restriction and high environmental temperature (Wang et al., 2001); at same time, moderate water stress retards secondary shoot growth without notably affecting photosynthetic activity (Carbonneau and Deloire, 2001), thus, favoring the redistribution of sugar into the berries and affecting the growth of grape berry. It is reported that water stress can lead to the anatomical and ultrastructural modifications of fruit, but there is no report about anatomical and ultrastructural changes of grape berry under root restriction.

During the process of berry growth and development, the berries showed big changes in size, composition, color, texture and flavor. According to the changes of berry growth speed, berry growth phases were divided into 3 phases and exhibited a double sigmoid growth pattern (Coome, 1992). The first rapid growth phase occurred after flowering, and lasted for approximately 60 days. After the first rapid growth, berry went through a lag growth phase. The second growth phase is a lag growth phase which followed the first rapid growth phase, at the start of this phase, berries have reached at least half of their final size and seeds especially reached their final size. After this lag growth phase, berries moved to the second rapid growth phase, in which many traits related to the grape berry’s quality changed, the berries became larger, softer, sweeter, less acidic and strongly flavoured and coloured than the first rapid growth phase (Deloire et al., 2004, 2005b). An increase in the thickness of the epidermis and hypodermis cell walls of berry skin and flesh was observed under root restriction; this result showed that root restriction stress has a positive impact on fruit firmness and sugar accumulation in fruit during the second rapid growth phase. After veraison, the flesh cells under root restriction extended to more rounded shape than control treatment, and the intercellular space among cells in berry of root restriction became larger than the control treatment.

Many studies showed that the thickness of cell wall had close relation with fruit firmness, and with the development of the berry, the size and amount of intercellular space increased; at the same time, cellulose depolymerisation and arabinose loss occurred in the cell wall which led to fruit softening.

Many studies suggested that water status in soil affect grape fruit growth, depending on the extent of water stress and the stage of berry development. Ojeda et al. (2001) reported that cell volume of grape berry decreased as a result of an early water stress from flowering to veraison. In this study, we did not find that the size of berry cell decreased under root restriction; a possible cause for the decrease in cell size under root restriction is a shortage in supply of water and nutrients during the second rapid growth phase of grape berry. Some studies suggested that in some cases root restriction did not implement effects on plant growth through water stress and some researchers also reported that root-restricted tomato plants (Ruff et al., 1987) and peach seedlings (Richards, 1977) developed more densely branched root systems than root-unrestricted plants. The resultant change in root morphology may lead to changes of other organs of the plant. Some other
Figure 4. Ultrastructure of flesh of grape berry under control and root restriction. 1, 3, 5) ultrastructure of flesh under control treatment during phase I; 2, 4, 6) ultrastructure of flesh under root restriction treatment during phase I; 7, 8) ultrastructure of flesh under control and root restriction treatment during phase I; 9, 11) ultrastructure of flesh under control treatment during phase I; 10, 12) ultrastructure of flesh under root restriction treatment during phase I.
researchers reported that the reduction in plant growth under conditions of root restriction or pruning is probably related to hormone synthesis and metabolism in the root system (Boland et al., 1994; Girona et al., 2003). These results partly explain the reason for the difference of berry structure between root restriction and control treatments during the first rapid growth phase of fruit.

Conclusion

In conclusion, results demonstrated that root restriction can lead to changes of the berry structure, and these changes are the responses of fruit growth to root restriction stress, just as the response of other organs including shoot, root, leaf regulated by a number of stress factors caused by root restriction. Our results strongly suggested that the improvement of berry quality under root restriction benefit from the change of berry structure.

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REFERENCES


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