Pak. J. Bot., 46(2): 483-488, 2014.

CHANGES IN WATER TRANSLOCATION IN THE VASCULAR TISSUE OF GRAPE DURING FRUIT DEVELOPMENT

XIE ZHAOSEN[1*], CHARLES F. FORNEY[2], CAO HONGMEI[1] AND BO LI[3]

[1]*Hebei Normal University of Technology and Science Qinhuangdao 066600, China*
[2]*Atlantic Food and Horticulture Research Centre, Agriculture and Agri-Food Canada*
[3]*Breeding Laboratory, Shandong Institute of Pomology, Taian 271000, Shandong, China*
**Corresponding author's e-mail: xiezhaosen@sina.com*

Abstract

The relationship between vascular water translocation in grapes and berry growth was investigated. Berry growth, firmness and turgor were measured, and the structure and function of the vascular bundles for water translocation was observed. During phase I fruit development, the dorsal and central vascular bundles rapidly translocated introduced dye in the pedicle. The speed of dye translocation was highest in the dorsal vascular bundles of phase I fruit with a speed of 0.97cm/h. After phase II, both the distribution of dye and the speed of dye translocation in the fruit vascular tissue decreased, with speeds in the dorsal and central vascular bundles being 0.08 cm/h and 0.72 cm/h, respectively. During phase III, the distribution of dye was still lower than phase I. After phase II, the walls of some xylem vessels were indistinct and broken. After phase III, even though the water translocation efficiency of the xylem decreased, sugar accumulation in the berry as well as osmoregulation increased.

Introduction

Fruit quality of grapes affects their market value and the income of grape growers. Water is the most important factor that affects the growth and development of grapes (Delrot *et al.*, 2001). Water accumulation in grapes promotes volumetric growth and plays a key role in fruit quality formation, by serving as the medium by which solutes are transported into and throughout the fruit pericarp (Keller *et al.,* 2006). Water and solute transport is facilitated by the xylem and phloem vessels of the vascular network (Coombe & Bishop, 1980). The vascular system of the grape consists of central bundles and a peripheral network. Current evidence indicates that xylem is functional in grapes before veraison, but after verasion function of xylem is reduced or eliminated (Bondada *et al.,* 2005).

Berry growth, including the increase in weight, volume, and diameter, follows a double-sigmoid curve, which results from two consecutive rapid growth stages separated by a slow stage of growth. Grapes require a significant amount of water for the two rapid growth stages, and water typically contributes almost 80 percent of berry fresh weight during the second rapid growth stage (Lang & Thorpe, 1989). An understanding of water translocation in grapes is the first step in understanding the relationship between water and berry development. Although it is known that mineral elements are transported by the xylem and phloem vascular systems, and that water flow changes from mainly xylemic to being mainly phloemic during development (Chatelet *et al.,* 2008), there is no direct evidence to show the changes of water translocation in berry vascular bundles during different stages of fruit development. The objective of this study was to investigate the changes of water translocation in grape vascular bundles during fruit development.

Materials and Methods

Plant materials: All experiments were conducted on uniform 3-year-old grapevines (*Vitis Vinifera*×*Vitis Labrasca* cv. Kyoho), that were grown in a ventilated green house under natural light at the experimental farm located at Shanghai Jiaotong University, Shanghai, China (3111N, 12129W). Dormant grapevines were pruned to retain 3 to 5 buds. Vines were staked upright and shoots were trained vertically. One good strong shoot on each vine was chosen and the other shoots were removed. Only the basal fruit cluster, which consisted of about 50 berries, was retained.

Berry growth measurements: A total of 30 berries were chosen from each of three grapevines for berry growth measurements. Berry diameters were measured with a pair of calipers (± 0.02 mm precision) every 3 days starting at the twentieth day after anthesis (DAA).

Berry firmness and turgor: About 40 berries were chosen with uniform shape and color from different grape vines to measure berry firmness and turgor. Berry and flesh firmness (g/cm^2) were measured using a berry firmness meter. Berry turgor was measured using the method of Huang & Huang (2001). The berry skin was pierced 2 mm deep by the needle of a micro-syringe and the quantity (μL) of juice that effused into the micro-syringe after 1 minute was measured.

Berry dye uptake: Water transport in the berry vascular tissue was evaluated using the dye uptake method of Bondada *et al.,* (2005). The berries were excised from the cluster at different days of after anthesis(DAA), and the pedicel of an intact berry was trimmed and immediately dipped in a small reservoir of apoplastic dye solution, basic fuchsin 0.1% aqueous (Talboy, 1955). Infusion through the cut pedicel was allowed to occur for 1 to 6 h under laboratory conditions, the infusion was performed in a humidified chamber maintained at 100% RH. Berries were then either cross-sectioned at the pedicel or the blossom end, or were sectioned longitudinally through the centre, berry skin was removedm, and the pattern and extent of vascular staining was observed and photographed, and the length and quantity of staining was measured.

Dorsal vascular vessel anatomy, berry skin and flesh: To measure the anatomical features of the vascular tissue of the skin and flesh of grapes, the berries were sampled before and after veraison(50 DAA). All experimental material were fixed in FAA (Formaldehyde acetic acid and alcohol), embedded in paraffin and cut into 10 µm thick sections following the method of Osman *et al*., (2012).

Results

Changes in berry diameter, firmness and turgor: After fruit set berry growth followed a double sigmoidal curve, which was divided into three phases, Phase I, Phase II and Phase III (Fig. 1). During Phase I the berry underwent rapid growth and the berry was hard, green and sour; during Phase II, the berry showed slower growth and initiated softening; during Phase III, the berry entered the second rapid growth stage, color changed, and size and sugar increased. Phase III was the key period for the berry to accumulate sugar.

Berry firmness was one of the main factors that determined berry quality, and berry development played an important role in determining berry firmness. Changes in berry firmness result from physiological processes in the berry. During phase I, berry firmness did not change significantly, firmness values remained at about 200 g/cm^2 (Fig. 2A). During the lag growth stage (phase II), berry firmness decreased sharply which continued until harvest. Flesh firmness decreased during the whole berry development process reaching a minimum value during phase III (Fig. 2A). These results suggest that the structure and firmness of berry skin was the major factor determining berry firmness. The juice effused from the berry was collected to measure turgor. During phase I, turgor reached the highest value, after phase II, it decreased with the berry softening, but during the second rapid growth stage (phase III), the turgor value show a slight increase (Fig. 2B).

Water translocation during berry development: During phase I of berry development, rapid uptake of dye was observed. The dye was clearly visible in all vascular bundles, including dorsal and central vascular bundles. (Fig. 3A, B, G, H, M and N); However during phase II, the number and length of dyed dorsal vascular bundles significantly decreased and in some dorsal vascular bundles no dye was visiable. (Fig. 3C, D, I and G). Dye movement into the berry appeared to stop at the end of pedicel and little dye was visible in the central and ovular vascular bundles of longitudinal-sections (Fig. 3O, P). During phase III, the extent of dyed vascular bundles in the berry continued to remain at a low level, but the extent of vascular dying was greater than that in phase II (Fig. 3E, F, K and L).

Dye movement into berries was observed for 6 hours and the transport speeds were recorded. The results showed that during phase I dye uptake was fastest with a speed of 0.97 cm/h. During phase II the speed dropped significantly, and fell to a minimum rate. During phase III the speed increased slightly, but was lower than in phase I (Table 1).

Structure differences between pre–veraison and post-veraison berries: The differences in the structure of skin flesh and dorsal vascular bundles between pre and post-veraison berries were observed. As seen in Fig. 4, the walls of xylem vessels were visible and intact in pre-veraison berries (Fig. 4A, C). However, after phase II, the wall of the outer xylem vessel broke down and became invisible, while the central xylem vessel was intact (Fig. 4B, D).

The cells in the berry skin were tightly arranged before veraison, but after veraison the cells became loosely associated (Fig. 4E, F). The flesh cells were tightly arranged and intact before veraison, but after veraison parts of the flesh cell showed a damaged cell wall (Fig. 4G, H).

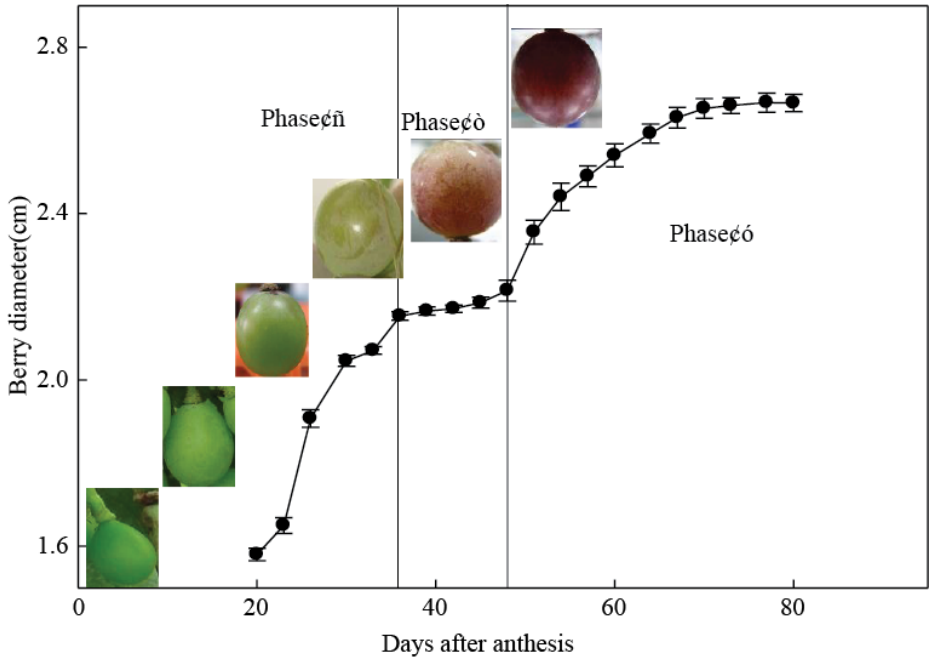

Fig.1 Changes in the diameter of grape berry during development.

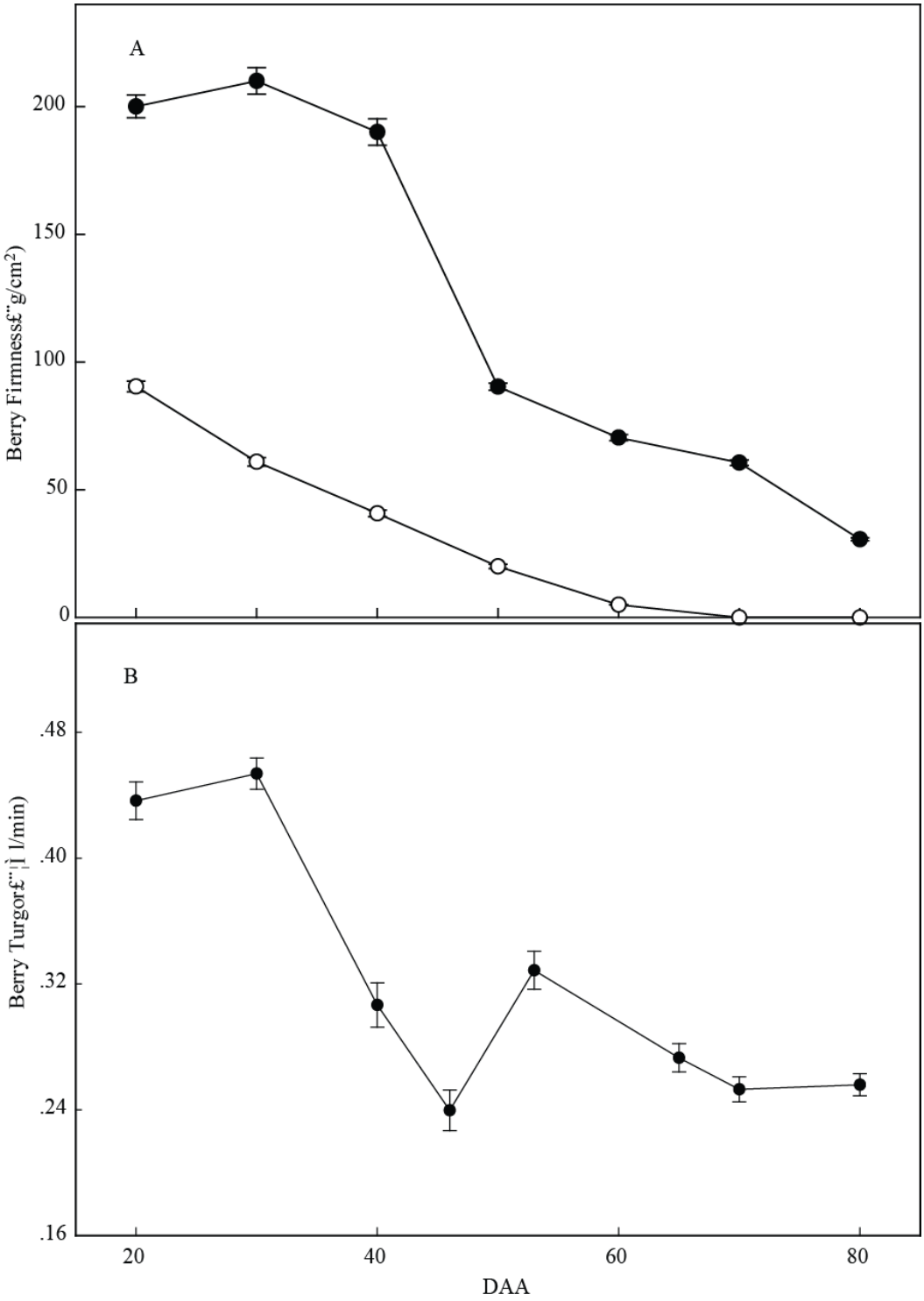

Fig. 2 Changes in berry firmness, flesh firmness and turgor during development.
Note: Days after anthesis was abbreviated to DAA

Table 1. The speed of dye transport in vascular bundles of grape.

Phase of berry development	The average speed in dorsal vascular bundle (cm/h)	The average speed in central vascular bundle (cm/h)
Phase I(30 DAA)	0.97	1.51
Phase II(60 DAA)	0.08	0.72
Phase III(80 DAA)	0.12	0.74

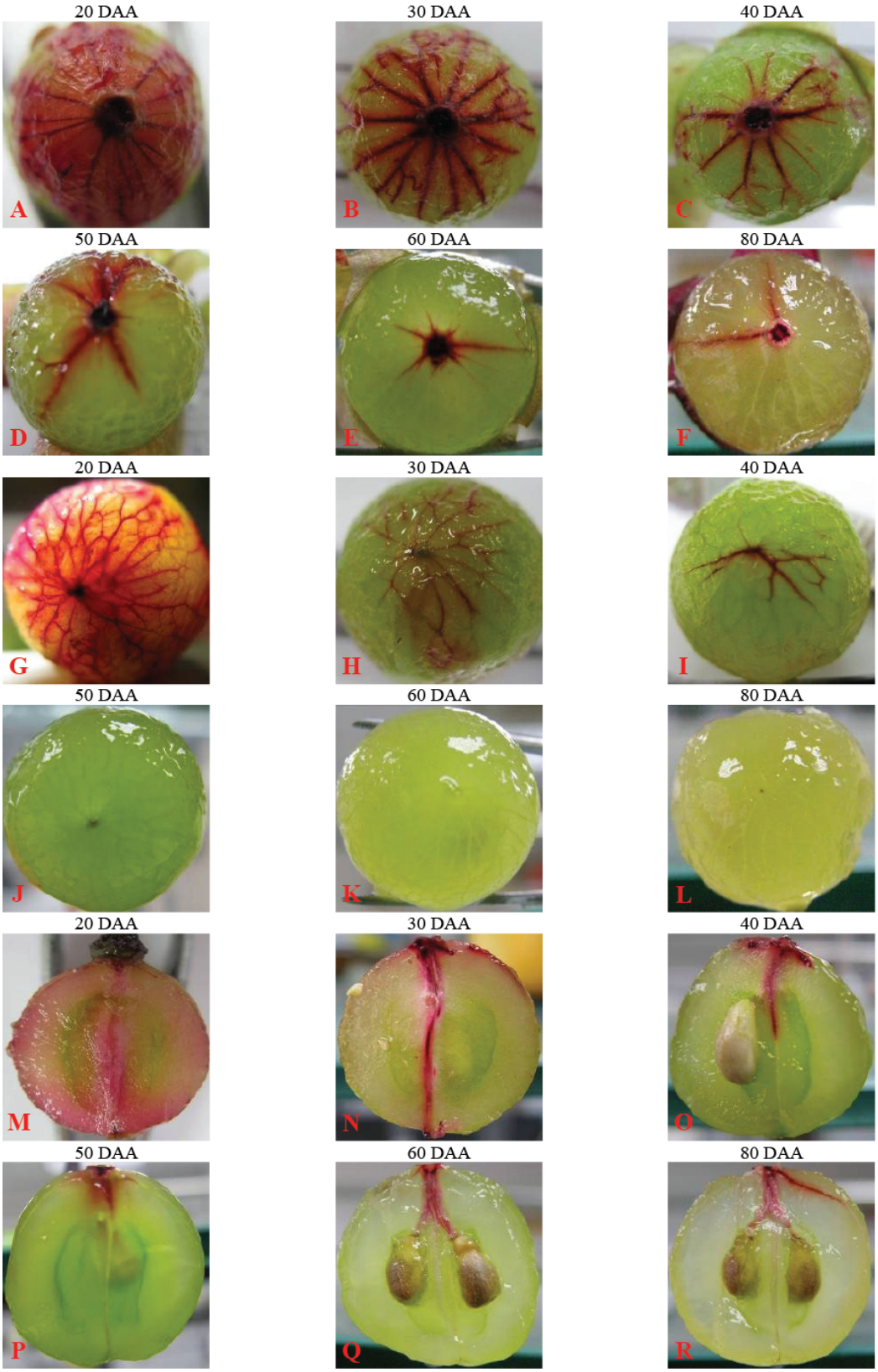

Fig.3 The transport of dye transport in grape berries after the berries were exposed to the dye for 6hours.
Fig.3A-F, Distribution of dye in dorsal vascular bundles viewed from the stem end, Fig.3G-L, Distribution of dye in dorsal vascular bundles viewed from the blossom end, Fig.3M-R, Distribution of dye in central vascular bundles viewed from a cross section.

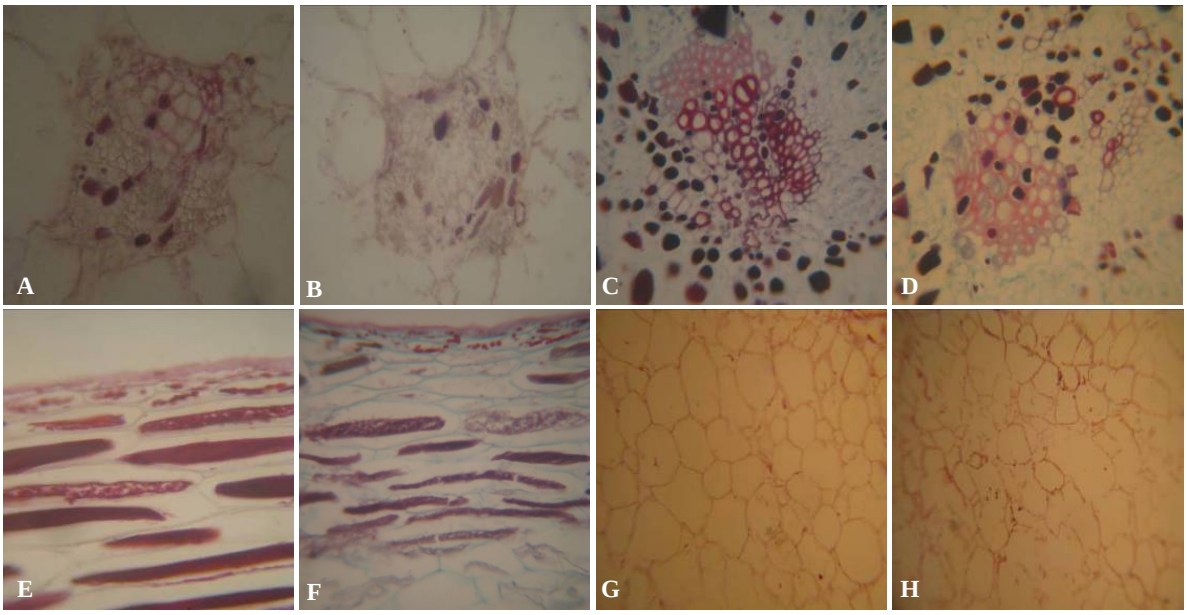

Fig. 4. Anatomical structure of the dorsal vascular bundle in berry skin and flesh during phase I and phase III development.
A and C: Anatomical structure of dorsal vascular bundle during phase I;B and D: Anatomical structure of dorsal vascular bundle during phase III:, E and F: Anatomical structure of grape skin during phase I and III; G and H: Anatomical structure of berry flesh during phase I and III.

Discussion

Kunmazawa (1961) used dye uptake methods to study the ability of water to be tranlocated in maize vascular bundles. Findlay *et al*., (1987) used dye infusion in grape to study water movement in berry xylem. In this study, we used the methods of dye uptake and anatomical structure observation to study changes in water translocation in berry vascular tissue during fruit development. These results suggested that there was a substantial reduction in water movement through the vascular bundles after phase I.

Water transport into fruit can occur through both phloem and xylem. Xylem and phloem are interconnected and can readily exchange water and solutes (Zwieniecki *et al.*, 2004). Many studies formed the hypothesis that xylem of post-verasion berries are non-functional as a direct or indirect consequence of post-veraison berry growth (Coombe & McCarthy, 2000). Bondada *et al*., (2005) reported that although xylem conduits in the berry were intact and apparently functional after veraison, the proportion of water was transported to the berry through the xylem was substantially reduced compared to the phloem.

After veraison, although the berry xylem function broke down, the water uptake power remained adequate to support berry growth. Nearly all sugar accumulation in grapes occured during phase III, and fruit quality was determined in this phase (Coombe, 1992; Zhang *et al*., 2006). Huang *et al*., (2005) monitored the diurnal contraction and expansion of grape berries and noticed that during the post-veraison phase a net increase in diameter was achieved during the day-time when fruit shrinkage normally should occur. Zhang *et al*., (2006) reported that there was a shift in the phloem unloading pathway during the onset of ripening of grapes, from the symplasmic to apoplasmic pathway. A change of water transport paths from the xylem to the phloem would be helpful for sugar accumulation in the fruit during phase III. Sugar accumulation in the fruit would decrease its water potential and increase the water potential gradient between the inside and outside of the berry; the drop of water potential in the berry may be the major driver of berry growth.

In conclusion, during phase III fruit development, the decrease of turgor pressure and berry firmness and the lose of xylem function resulted in the decrease of water transport, but sugar accumulation in the berry dropped its water potential, creating the driving force for water uptake, and resulting in the second rapid phase of berry growth.

Acknowledgement

This study was supported by the National Natural Science Foundation of China (31101508), Natural Science Foundation of Hebei and province of china(C2013407107, ZR2012CQ036, YQ2013010) respectively.

References

Bondada, B.R., M.A. Matthews and K.A. Shackel. 2005. Functional xylem in the post-veraison grape berry. *J. Exp. Bot.,* 56: 2949-2957.

Chatelet, D.S., T.L. Rost, M.A. Matthews and K.A. Shackel. 2008. The peripheral xylem of grapevine (*Vitis vinifera*) berries. Anatomy and development. *J. Exp. Bot.,* 59: 1997-2007.

Coombe, B.G. 1992. Research on development and ripening of the grape berry. *Am. J. Enol. Vitic.*, 43(1): 101-110.

Coombe, B.G. and G.R. Bishop. 1980. Development of the grape berry. II. Changes in diameter and deformability during veraison. *Aust. J. Grape. Wine. Res.,* 31: 499-509.

Coombe, B.G. and M.G. McCarthy. 2000. Dynamics of grape berry growth and physiology of ripening. *Aust. J. Grape Wine Res.,* 6: 131-135.

Delrot, S., S. Picaud and J.P. Gaudillère. 2001. Water transport and aquaporins in grapevine. In: *Molecular Biology and Biotechnology of the Grapevine,* (Ed.): K.A. Roubelakis-Angelakis, Kluwer Academic Publishers, Dordrecht, the Netherlands, pp. 241-262.

Findlay, N., K.J. Oliver, N. Nii and B.G. Coombe. 1987. Solute accumulation by grape pericarp cells. IV. Perfusion of pericarp apoplast via the pedicel and evidence for xylem malfunction in ripening berries. *J. Exp. Bot.,* 38: 668-679.

Huang, X.M. and H.B. Huang. 2001. Early post-veraison growth in grapes: Evidence for a two-step mode of berry enlargement. *Aust. J. Grape. Wine. Res.*, 7: 132-136.

Huang, X.M., H.B. Huang and H.C. Wang. 2005. Cell walls of loosening skin in post-veraison grape berries lose structural polysaccharides and calcium while accumulate structural proteins. *Sci. Hortic.,* 104: 249-263.

Keller, M., J.P. Smith and B.R. Bondada. 2006. Ripening grape berries remain hydraulically connected to the shoot. *J. Exp. Bot.,* 57: 2577-2587.

Kumazawa, M. 1961. Studies on the vascular course in the maize plant. *Phytomorphology,* 11:128-139.

Lang, A. and M.R. Thorpe. 1989. Xylem, phloem and transpiration flows in a grape: application of a technique for measuring the volume of attached fruits to high resolution using Archimedes' principle. *J. Exp. Bot.,* 40: 1069-1078.

Osman, A.K., M.A. Zaki, S.T. Hamed and N.R.A. Hussein. 2012. Comparative anatomical study on fruits of some tribes of family Gramineae from Egypt. *Pak. J. Bot.,* 44(2): 599-618.

Talboy, P.W. 1955. Detection of vascular tissues available for water transport in the hop by colorless derivatives of basic dyes. *Nature,* 175: 510.

Zhang, X.Y., X.L. Wang, X.F. Wang, G.H. Xia, Q.H. Pan, R.C. Fan, F.Q. Wu, X.C. Yu and D.P. Zhang. 2006. A shift of phloem unloading from symplasmic to apoplasmic pathway is involved in developmental onset of ripening in grape berry. *Plant Physiol*., 142: 220-232.

Zwieniecki, M.A., P.J. Melcher, T.S. Field and N.M. Holbrook. 2004. A potential role for xylem-phloem interactions in the hydraulic architecture of trees: Effects of phloem girdling on xylem hyfraulic conductance. *Tree Physio.,* 24: 911-917.

(Received for publication 18 November 2012)