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Changes in Lignin Content and Activity of Related Enzymes in the Endocarp During the Walnut Shell Development Period

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Abstract

Lignification was observed using the phloroglucinol–HCl method and the content of lignin, cellulose, and polyphenol and the activity of related enzymes were measured during development with the materials of walnut of 'Zanmei' and 'Zhenzhuxiang'. Results showed that lignification occurred at the beginning of June, initially took shape in mid-June and finished in late July. From shell lignification to harvest, the concentrations of lignin, cellulose, and phenolic content increased before harvest, but phenolic content decreased slightly. The activities of both POD and PAL decreased and PPO varied somewhat. Correlation analysis showed lignin content to be significantly positively correlated with the cellulose content. Phenolic content had a significant positive correlation with that of lignin. Phenolic content was positively correlated with cellulose. The activity of POD had a significant negative correlation with lignin, cellulose, and phenolic content, but it was positively correlated with the activity of PAL.

Keywords: walnut; shell; lignin; cellulose; PAL; POD; PPO

1. Introduction

In recent years, with the transition from seedling to grafted varieties in China, the area of thin-shell walnut varieties has also increased rapidly. However, the problems of naked kernels, dissilient nuts and stained nuts have become serious in some areas with extensive cultivation and poor management. Varieties of *Juglans hopeinsis* Hu have been cultivated in different provinces in China. These nuts have white tips and colored exteriors, both of which affect their value. These problems are due to abnormal development of the nut shell.

Correlations between structure indices and kernel qualities were analyzed. Results showed that there are significant correlations among the structural indices, which were also significantly correlated with kernel quality (Zhao et al., 2007). The tighter seal grade and the thicker the shell, the lower the strain rate, rate of dissilient nuts, and rate of wormy nuts, MDA content of oils in stored walnut kernel and the stronger the antioxidation capacity of walnut. The results suggested that shell structures should be taken into account to evaluate the quality of the walnuts. The indices of shell structure, such as seal grade and thickness, should receive sufficient attention. More recent studies have shown shell thickness have a significantly positive correlation with nut fruit weight, which itself is significantly negatively correlated with the kernel rate (Arzani et al., 2008). Walnut nut shell structure is closely related to species, time of harvesting (Zhao et al., 2011), light (Cui and Guo, 2008) and other factors. Components of walnut shells were analyzed, and results showed lignin and cellulose to be the major components (Zheng et al., 2006). The lignin content of shells was closely related to seal grade, mechanical strength, and the thickness of the shell (Li et al., 2012).

However, the study of walnut shells has focused primarily on pericarp structure (Xiao et al., 1998; Wu et al., 2005), chemical composition of the shell, and constituents of lignin (Zheng et al., 2007). Few studies have evaluated the process of shell development. Systematic study of changes in lignin, cellulose, polyphenol, and the activity of related enzymes during the walnut

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shell development period can provide a theoretical foundation suitable for revealing the mechanism underlying walnut shell differentiation and may facilitate measures to decrease the number of dissilient nuts.

2. Materials and methods

2.1. Plant material

Plant material was grown in the walnut germplasm nursery in Zanhuang County in Hebei Province. Two walnut cultivars (*Juglans regia* L.) were sampled: 'Zanmei' (Zhao et al., 2013a), a cultivar with a thick nutshell (1.23 mm), and 'Zhenzhuxiang' (Wang et al., 2013b), a cultivar with a thin nutshell (0.93 mm). All samples were from 12-year-old walnut trees grown naturally and fruiting normally. Five trees from each cultivar, which showed similar growth and no plant pests or diseases, were selected. Samples were collected once every 14 days from May 19 to August 19 in 2012, but once a week in the hardcore period. Fifteen nuts from each cultivar were randomly selected from peripheral tree-crowns. The lignin, cellulose, and phenolic contents and the activity of related enzymes were measured in the endocarp with three replications.

2.2. Lignin deposition

Lignin was stained with Wiesner reagent. Fruit that was cut from different sections was treated for 5 min with 2% phloroglucinol in 95% ethanol and mounted in 6 mol · L^{-1} HCl to indicate the presence of cinnamaldehyde groups in lignin (Alba et al., 2000).

2.3. Lignin, cellulose, and phenolic contents

Analysis of lignin content was carried out according to the method described by Li et al. (2006). Cellulose content was determined using the methods described by Xiong et al. (2005). Phenolic content was determined using the Folin–Ciocalteu method (Wang et al., 2008).

2.4. PAL, POD, and PPO activities

PAL and POD activities were determined as described by Tao et al. (2004). PPO activity was assessed using a modified version of the method described by Wu and Chen (2003): with the same the extraction, catechol was the reaction substrate (0.1 mol · L^{-1}) and the absorbance at $A_{420\ nm}$ was measured. This was performed three times.

2.5. Statistics

The experiment was arranged with a completely randomized design. Each point in time sampled for each variety comprised three independent replicates. Standard deviation and correlation analysis were calculated to compare significant treatment effects at the 5% level and regression analysis was conducted using SPSS and Excel software.

3. Results

3.1. Changes in lignin deposition in the endocarp during the walnut development period

On May 19, the exocarp, mesocarp, and endocarp of 'Zanmei' and 'Zhenzhuxiang' were not colored, which meant that tissue cells in the endocarp had not deposited lignin (Fig. 1). On June 2, the diaphragm and endocarp near the top of the fruit appeared light pink dyed by phloroglucinol. Additionally, the color of 'Zanmei' was deeper than that of 'Zhenzhuxiang', which demonstrated that lignification began in the endocarp and diaphragm near the upper part of the shell, and the shell of 'Zanmei' forms earlier than that of 'Zhenzhuxiang', after which lignin gradually accumulated. On June 16, the whole endocarp in 'Zanmei' and 'Zhenzhuxiang' appeared pink, but the color was light and thin, which indicated that the shells of nuts began to form during this period, and the shapes of the nuts ceased to change. On June 30, the kernels took shape and the shells became thicker with deeper color. This suggested that shell had become thick and lignin accumulated rapidly. The endocarps stopped thickening in both varieties, which showed shell layer had finished differentiating by July 20. At this time, the shells of both varieties had become stained deep pink by phloroglucinol which was the result of the deposition of large amounts of lignin.

3.2. Changes in lignin content in the endocarp during the walnut development period

With differentiation of the walnut shell, the lignin content of the endocarp increased gradually (Fig. 2), which indicated that lignin precipitated in the differentiation of the walnut shells. The lignin content of the endocarp increased rapidly from June 2 to 16, and then the fruit entered a stage of endocarp hardening. Thereafter, the lignin content rose steadily.

The thickness of the walnut shells became stable and ceased to increase after July 20, but the lignin content did continue to increase, which demonstrated the rapid deposition of lignin within the secondary cell wall of the shell.

It is important to note that the moisture content in the endocarp remained relatively stable during the early stage of development, but the water content decreased sharply after August 19, resulting in a rapid increase in the relative amount of lignin (Fig. 2).

3.3. Changes in cellulose content in the endocarp during the walnut development period

The trends in cellulose changes were similar to that of lignin (Fig. 3). With the differentiation of shell cells, the cellulose content increased rapidly when the endocarp thickened gradually. The cellulose content in the endocarp of 'Zanmei' climbed steadily from 1.17% to 5.45% from June 2 to August 4. The changes in cellulose content in the endocarp of 'Zhenzhuxiang' reached 5.88% on June 23, after which it was similar to that of 'Zanmei'. Shell thickness plateaued after July 20 and cellulose accumulated in the cell, which improved the strength of cells considerably. A sudden increase in cellulose content in the endocarp was observed on August 19, probably the result of the sharp decline in moisture content in the endocarp during this period.