Article

The Effects of 1-Methylcyclopropene (1-MCP) and Ethylene on Lignification of Postharvest Common Bean (*Phaseolus vulgaris L*)

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Abstract: Postharvest 1-MCP treatment can inhibit lignification of fruit and vegetables. It has been suggested that the mode of action of 1-MCP is through inhibiting ethylene production, but the effect of 1-MCP and ethylene on lignification of common bean remain unknown. This work compared the effect of 0.5 μL L^{-1} 1-MCP and 100 μL L^{-1} ethylene on lignification of common bean during storage. Postharvest 1-MCP significantly inhibited the increase of lignified cell group, sclerenchyma became thicker, vascular bundles thickened and lignified cells grew during storage, while ethylene is the opposite. 1-MCP inhibited the increase in respiration rate, sucrose phosphate synthase (SPS), sucrose synthase (SuSy), phenylalanine ammonialyase (PAL), cinnamyl-alcohol dehydrogenase (CAD), and peroxidase (POD) activities, whereas ethylene hastened all. Ethylene treatment can be stimulated and 1-MCP inhibited the decline of reducing sugar and cellulose content. Expression of genes, including *PvACO1*, *PvAOG1*, *PvSuSy*, *PvPAL3*, and *Pv4CL1*, *PvCOMT1* together with lignin content were significantly increased in common bean during storage. 1-MCP treatment markedly inhibited expression of *PvACO1*, *PvSuSy2*,

PvPAL3, *Pv4CL1* and *PvCOMT1* genes, while strengthened expression of *PvETR1* and *PvAOG1*, while ethylene was opposite. This work provides evidence that ethylene plays a key role in regulating the lignin biosynthesis of common bean, and also provides strategies for the maintenance of fruit quality during storage.

Key words: *Phaseolus vulgaris*; 1-MCP; ethylene; lignification

1. Introduction

Fresh common bean (*Phaseolus vulgaris* L.) is appreciated due to their bright colour and nutritional characteristics. However, water loss, respiratory metabolism, and lignification lead to the lengthening of pod tendon, loss of eating quality of fresh common bean during postharvest storage.[1-3] Lignification is mainly caused by chilling injury in loquat,[4-6] bamboo shoot,[7] and kiwifruit,[8] or senescence in Tsai Tai,[9] bamboo,[10-12] *Rosa sterilis* D. shi,[13] and citrus fruit juice sac.[14]

Lignification in loquat fruit involved the coordinated regulation of lignin biosynthesis and cellulose hydrolysis.[15-17] It is found that many enzymes play a key role in the lignification of fruits and vegetables. Among them, sucrose phosphate synthase (SPS) is regarded as a major player in sucrose synthesis in photosynthetic and nonphotosynthetic tissues, sucrose synthase (SuSy) catalyzing sucrose synthesis and degradation, while diverting energy substrate for cellulose synthesis or ATP-conserving respiration path,[18] phenylalanine ammonialyase (PAL) is involved more particularly in the cleavage of phenylalanine to cinnamic acid,[19] phenolics are precursors of lignin,[20] cinnamyl-alcohol dehydrogenase (CAD) is considered a key enzyme involved in the conversion of p-hydroxy-cinnamaldehydes to the corresponding alcohols, the last step of monolignol biosynthesis before oxidative polymerisation in cell wall,[9] 4-coumarate: coenzyme A ligase (4CL) is also an important gene in citrus fruit juice sac lignification,[14] COMT not only regulates the S units and G units, but also affects the total lignin content,[21] peroxidase (POD) catalyze the final step of lignin biosynthesis in cell walls and polymerize the

lignin monomers to lignin polymers.[22,23]

The literature suggests that ABA is considered to be very important for lignification in bamboo shoots,[24] ethylene is involved in lignin growth in Tsai Tai.[9] Ethylene production is moderate in fresh common beans,[25,26] and it cannot be considered crucial for ripening control due to insensitive to the accumulation of ethylene,[17,27] but the effect of ethylene on the lignification in fresh common beans is not clear. Postharvest 1-MCP treatment can inhibit the lignification by chilling injury in loquat[26] and kiwifruit,[8] and by senescence in bamboo shoot,[28] Tsai Tai,[9] pears,[29] plum,[30] and *Rosa sterilis* D. shi[13]. 1-MCP delays the senescence and reduces the chilling injury symptoms of beans during storage.[31,32] Our previous proteomics study also found that 1-MCP can be effectively down-regulated the key protein related to synthesis and response of lignin, cellulose, ethylene and abscisic acid.[4] This study is to reveal the effects of ethylene and 1-MCP on postharvest lignification in fresh common beans. The study is helpful for the understanding of the effect of ethylene on postharvest lignification of *P. vulgaris* L.

MATERIALS AND METHODS

Plant material

Fresh common bean (*Phaseolus vulgaris* L., *cv*. Qingbangdou) of commercial maturity were hand-harvested from a garden in Guanling County, Guizhou, in China. The beans without physical defects and with uniformity in color and length were selected.

Three different treatments were performed, namely, 100 μL L^{-1} of ethylene, and 0.5 μL L^{-1} of 1-MCP for 20 h at 25 ℃, and the control with air, all treatment use a mini fan to maintain the air circulation. Three replicates were used for each treatment. Then all of common beans was placed into commercial polyethylene bags for storage at 12±1 ℃ for three weeks with relative humidity of 85 %. The beans were frozen immediately in liquid nitrogen and stored at −80 ℃ until further analysis. The treatments were carried out on three biological replicates.

Evaluation of respiration rate, relative thickness of pods

The determination of respiration rate and relative thickness of pods were determined according to Xie et al.[4] The respiration rate was reported in mg kg^{-1} h^{-1} CO_2 and the relative thickness of pod was reported in %.

Determination of reducing sugar, and cellulose, total polyphenols, lignin content

The reducing sugar content was measured by 3,5-dichlorosalicylic acid (DNS) method and the results were reported as % (Fw).[33] The cellulose content was determined by acid detergent method and results were reported as % (Fw).[1] Total polyphenols were quantified by Folin - C reagent and results were reported in g kg^{-1} (Fw).[34] The lignin content was determined as Liu et al and results were recorded as A_{280} g^{-1}.[35]

Histochemistry for identifying cellulose and lignin morphology and distribution

The 10-μm-thick paraffin sections was prepared according to the method described by Chu et al and Li et al.[8,36] The microstructure of beans under an optical microscope were captured with a microscopy imaging system (OLYMPUS, Japan).

FTIR measurements

The FTIR spectra of beans were recorded with an IRAffinity-1 FTIR spectrometer, using the KBr disk standard technique (1 mg of beans powder with 100 mg KBr). Each spectrum was an average of 32 scans over the range 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1}. In order to eliminate the influence of beans powder difference and operation error on the experimental results, I_{1379}/I_{1508} and I_{1740}/I_{1508} were used to characterize the cellulose content and I_{1508}/I_{1379}, I_{1508}/I_{1425} and I_{1508}/I_{1740} were used to characterize the lignin content.[37]

Evaluation of SPS, SuSy, Cx, PAL, 4CL, CAD, and POD activity

Sucrose phosphate synthase (SPS) and SuSy activity were carried out according to Grof et al and

Cunha et al,[18,38] and results were expressed as mol h^{-1} kg^{-1}. Cellulase (Cx) activity was determined according to Cai et al,[15] and results were expressed as U g^{-1}. Phenylalanine ammonialyase (PAL) and CAD activity were carried out as Li et al,[8] and results were expressed as U g^{-1}. 4CL activity was determined according to Luo et al,[39] and results were expressed as U g^{-1}. POD activity was carried out as Xie et al,[13] and results were expressed as U g^{-1}.

Total RNA isolation, cDNA synthesis, and Real-time q-PCR assays

Based on preliminary proteomic results,[4] genes involved in lignification, ethylene and abscisic acid were identified and confirmed. The expression of genes related to ethylene synthesis and response (*PvACO1* and *PvETR1*), ABA synthesis and response (*PvAOG1* and *PvPYR1*), cellulose synthesis gene (*PvSuSy2*), and lignin synthesis genes (*PvPAL3*, *Pv4CL1*, *PvCOMT1*, *Pv*CAD6, and *Pv*POD1) (Supplementary Fig. S1) were examined in fresh common bean. The primers were designed by Primer Premier 5.0 software and listed in Supplementary Table S1. The Real-time q-PCR was carried out as Xie et al method.[4] Samples from day 0 (assigned an arbitrary quantity of "1") were used as a calibrator to calculate the relative quantity of the results. Three replicates were performed for each sample.

Statistical analysis

Statistical tests were performed using the SPSS Statistical Software 22.0 (IBM). The means and significant differences were carried out by Duncan's multiple range tests at the 0.05 probability ($P<0.05$). The results were reported as the mean ± standard error.

RESULTS

Respiration rate and relative thickness of pods

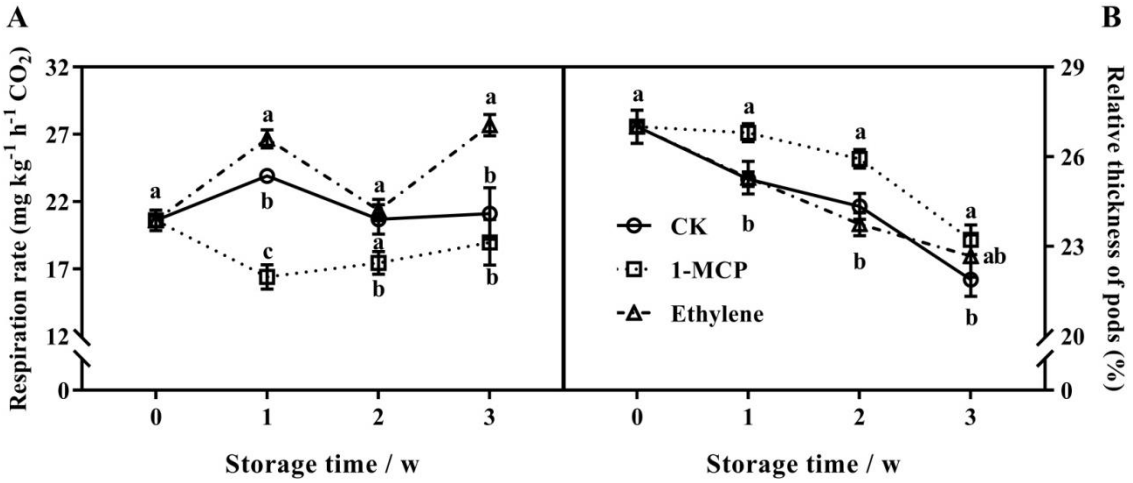

Fig.1 (A) Respiration rate and (B) relative thickness of pods in Control, Ethylene and 1-MCP treated beans during storage. Vertical bars represent standard error of three replicates (10 beans of each). Different letters within each parameter indicate statistically significant differences ($P< 0.05$)

The respiration rate of control and ethylene in fresh common beans increased in the first weeks and then decreased with further storage. The respiration rate was significantly suppressed by 1-MCP, but ethylene enhanced the respiration rate (Fig. 1A). The relative thickness of pods declined in beans during storage, 1-MCP treatment significantly inhibited the decrease of relative thickness of pods (Fig. 1B).

Reducing sugar, cellulose, total polyphenols, and lignin content

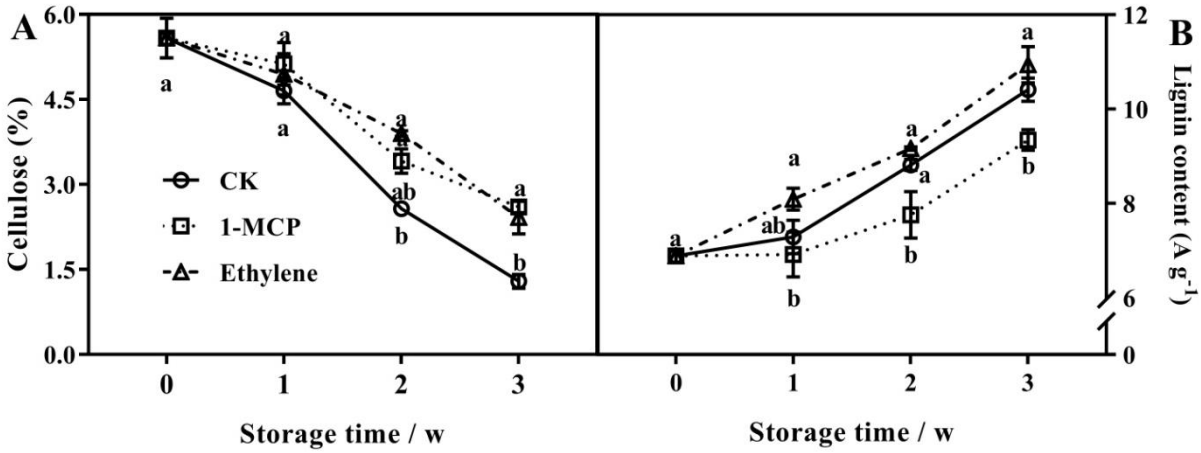

Fig. 2 (A) Cellulose and (B) lignin content of Control, Ethylene and 1-MCP treated beans during storage. Vertical bars represent standard error of three replicates (10 beans of each). Different letters within each parameter indicate statistically significant differences ($P < 0.05$)

The content of reducing sugars in fresh common beans reduced during storage. Treatments with 1-MCP and ethylene did not affect the decrement of content of reducing sugars during storage (Supplementary Fig. S2A). Cellulose of beans decreased during storage, but 1-MCP and ethylene delayed

the decrease of cellulose content before the third week, and no significantly differences in cellulose content were found among beans treated with 1-MCP and ethylene (Fig. 2A). The total polyphenols of control in fresh common beans increased in the second week of storage and then declined with further storage, with a remarkable highest value appearing in the second week. The increase in total polyphenols in fresh common beans was suppressed by 1-MCP and ethylene treatment (Supplementary Fig. S2B). The lignin content of fresh common beans increased during storage. However, ethylene promoted the accumulation of lignin in fresh common beans before the second week (Fig. 2B).

Anatomical structure analysis of fresh beans under ethylene and 1-MCP treatment

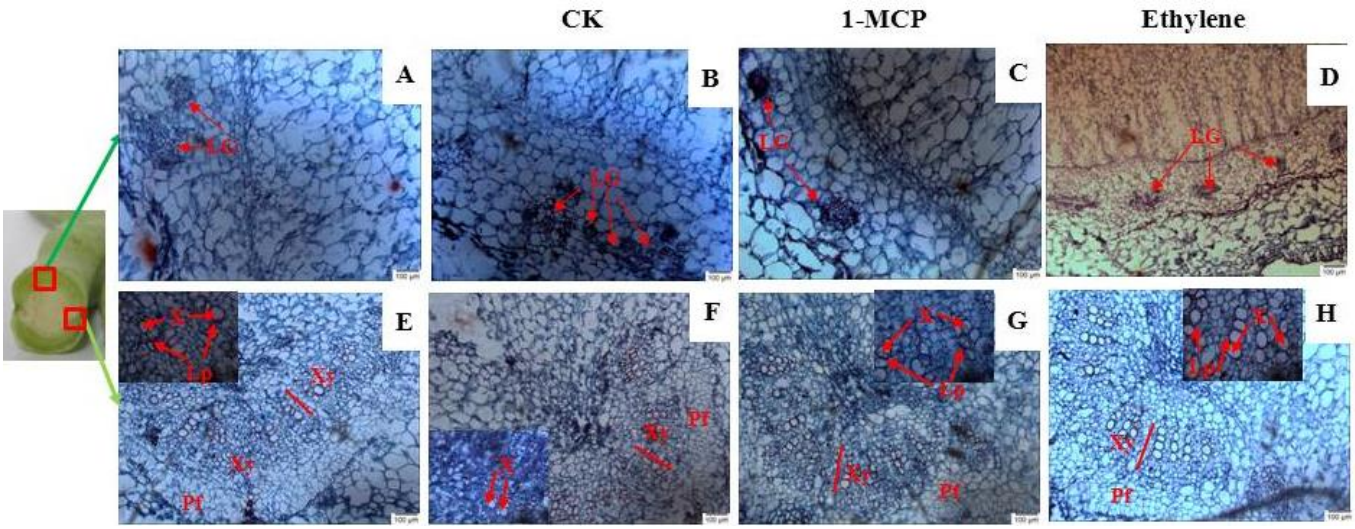

Fig.3 Effects of ethylene and 1-MCP treatment on the anatomical structure in beans. A-D represent the middle sections of fresh common beans, E-H represent the lower tendon sections of fresh common beans; (A and E) at harvest day; (B and F) Control group at 2 weeks; (C and G) 1-MCP treatment at 2 weeks; (D and H) ethylene treatment at 2 weeks. Abbreviations: LG, lignified cell group; LP, lignified cell; Pf, phloem fibers with primary cell wall; Xy, xylem; V: vascular bundles.

To look into the response to ethylene and 1-MCP treatment on the lignin level in beans, longitudinal sections from pods of common beans at harvest day and storage at 2 weeks was analyzed by Safranin-fast green staining for lignin identification (Fig. 3). The vascular bundles and collenchyma can be clearly observed, the xylem and phloem fibers with primary cell wall were visible, lignified xylem vessel appeared a clear purplish red (Fig. 3). Lignin was mainly located in the sclerenchyma and vascular bundles (Fig. 3). Lignified cells emerged around vascular bundles and were smaller than surrounding

parenchymal cells, occurring in clusters. The lignified cell group in fresh bean increased significantly during storage, 1-MCP could significantly inhibited the increase and enlargement of lignified cell group in fresh bean, while ethylene was the opposite (Fig. 3A-D). At 2 weeks of storage, sclerenchyma became thicker than those observed on the harvest day, vascular bundles thickened and lignified cells grew in beans during storage, 1-MCP significantly inhibited those changes, while ethylene is the opposite (Fig. 3E-H).

FTIR spectra

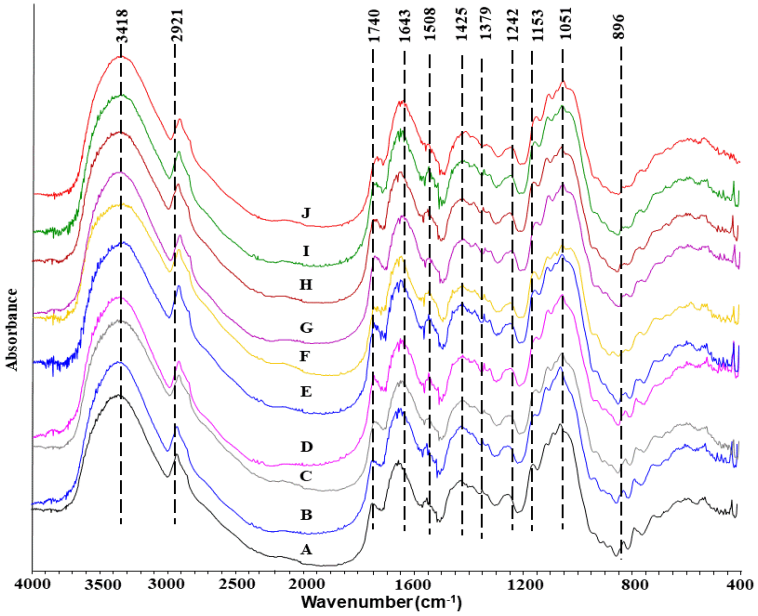

Fig. 4 FTIR spectra of control, ethylene and 1-MCP treated beans. (A) harvest; (B) control, (C) ethylene and (D) 1-MCP treatment at 1 weeks, respectively; (E) control, (F) ethylene and (G) 1-MCP treatment at 2 weeks, respectively; (H) control, (I) ethylene and (J) 1-MCP treatment at 3 weeks, respectively.

In order to identify the changes of lignin and cellulose in control, ethylene, and 1-MCP treated beans during storage, a profile of beans based on specific spectra was detected by FTIR. The peaks of FTIR spectra in beans were ascribed as follows: 1740 cm^{-1} for unconjugated carbonyl (C=O) stretching vibration in hemicellulose, 1643 cm^{-1} for conjugated carbonyl (C=O) stretching vibration in absorbed water, 1508 cm^{-1} for the telescopic carbon skeleton (C=C-OH, benzene) vibration in lignin, 1425 cm^{-1} for the combination the benzene (C=C-OH) ring skeleton and hydrocarbon (C-H) stretching vibration in lignin, 1379 cm^{-1} for the hydrocarbon (C-H) bending vibration in cellulose and hemicellulose, 1242 cm^{-1}

for the benzene epoxy bond (CO-OR) stretching vibration in lignin, 1153 cm^{-1} for the ether ethanol and tertiary asymmetric scale (C-O-H) stretch in cellulose and hemicellulose, and 1051 cm^{-1} for the C-O-C stretch in cellulose, hemicellulose, and lignin (Fig. 4). The large peak at 3418 cm^{-1} corresponded to hydroxyl (O-H) stretching vibration, whereas 2921 cm^{-1} and 896 cm^{-1} corresponded to C–H stretching vibration in cellulose. The ratio of FTIR characteristic peak in cellulose decreased during storage, ethylene and 1-MCP suppressed the decrease in I_{1740}/I_{1508}, which was consistent with changes in lignin content in beans (Fig. 2A), but enhanced the decrease in I_{1379}/I_{1508}. The ratio of FTIR characteristic peak in lignin increased during storage, 1-MCP inhibited the increase in I_{1508}/I_{1379}, I_{1508}/I_{1425} and I_{1508}/I_{1740}, while ethylene enhanced it (Supplementary Fig. S3), which was consistent with changes in lignin content of common beans (Fig. 2B).

SPS, SuSy, Cx, PAL, 4CL, CAD, and POD activity

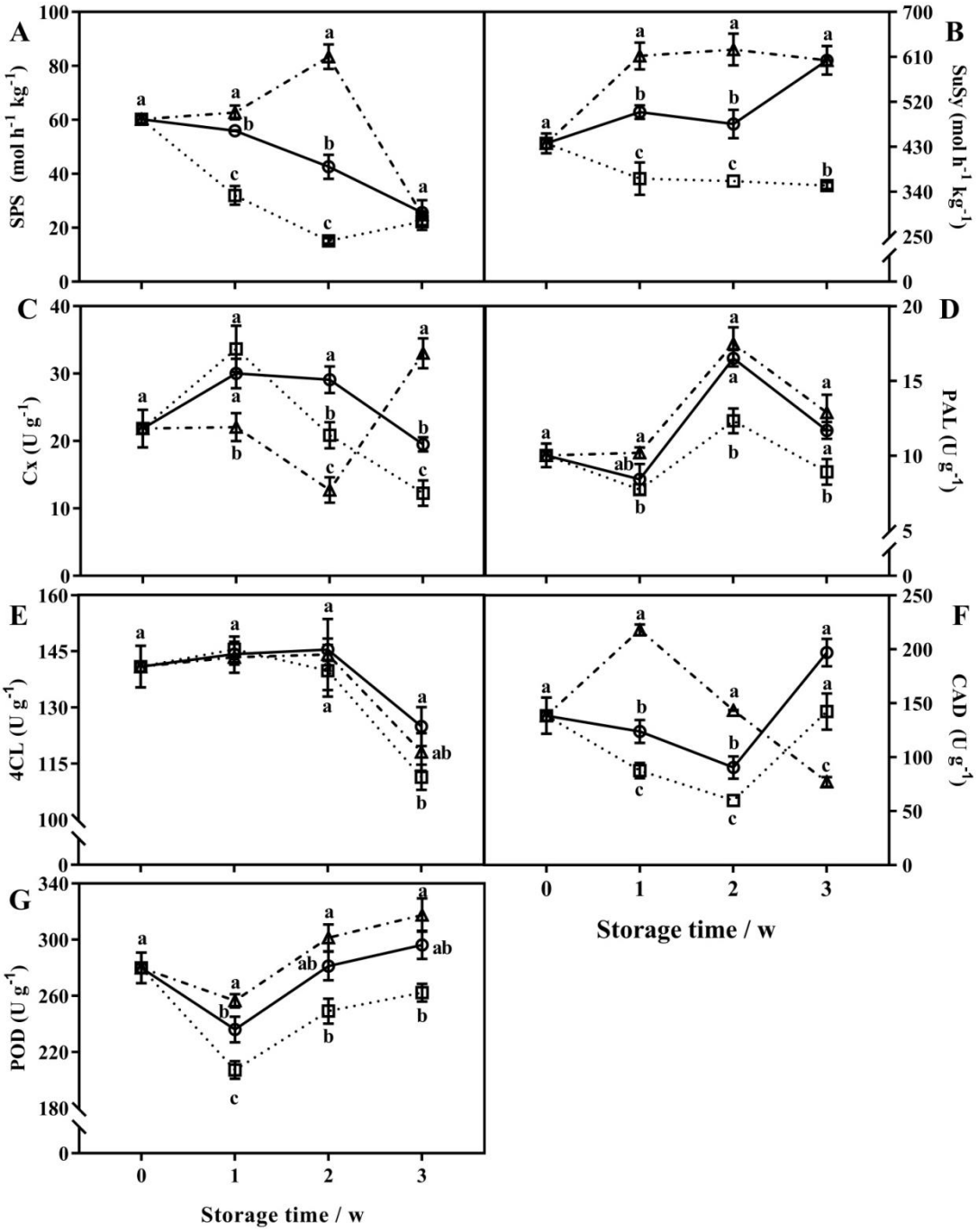

Fig. 5 (A) SPS, (B) SuSy, (C) Cx, (D) PAL, (E) 4CL, (F) CAD, (G) POD activity and of Control, Ethylene and 1-MCP treated beans during storage. Vertical bars represent standard error of three replicates. Different letters within each parameter indicate statistically significant differences ($P < 0.05$).

SPS activity of control and 1-MCP treated beans decreased during storage, but ethylene enhanced SPS before the second week and then decreased with further storage (Fig. 5A). SuSy activity in beans increased during storage, the SuSy activity was significantly decreased by 1-MCP treatment, while ethylene treatment enhanced the SuSy activity of beans to higher values than controls (Fig. 5B). Cx activity in beans increased in the first week and then decreased with storage time. Ethylene treatment

significantly inhibited the increase of Cx activity, but 1-MCP treatment enhanced Cx activity (Fig. 5C). PAL activity in fresh common beans increased in the second week, and then decreased with storage time. The increase of PAL activity in fresh common beans was significantly suppressed by1-MCP treatment (Fig. 5D). 4CL activity in fresh common beans remained basically unchanged until the second week of storage and then decreased with further storage. The decline in 4CL activity in beans was improved by 1-MCP and ethylene treatment (Fig. 5E). CAD activity of control and 1-MCP treated beans decreased in the second week, and then increased with further storage. However, CAD activity of ethylene in fresh common beans increased in the first week, and then decreased with further storage (Fig. 5F). POD activity of fresh common beans decreased in the first week, and then increased with further storage. The POD activity in fresh common beans was significantly suppressed by 1-MCP ($P<0.05$). However, the ethylene treatment enhanced POD activity in fresh common beans during storage (Fig. 5G).

Expression of *Pv*ACO1, *Pv*ETR1, *Pv*PYR1, and *Pv*AOG1 genes during postharvest storage

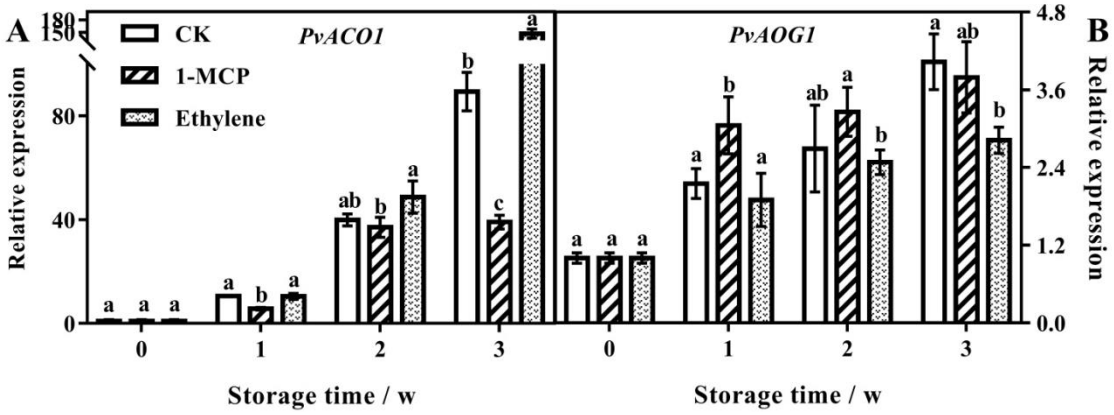

Fig. 6 Expressions of (A) *PvACO1* and (B) *PvAOG1* genes of Control, Ethylene and 1-MCP treated beans during storage. Vertical bars represent standard error of three replicates (10 beans of each). Different letters within each parameter indicate statistically significant differences ($P < 0.05$)

The *PvACO1* genes showed differential expression between control and treated fresh common beans during storage (Fig. 6A). *PvACO1* in fresh common beans showed an increasing trend during storage. *PvACO1* gene was significantly enhanced by ethylene, but significantly suppressed by 1-MCP during storage. Expression of *PvETR1* was stimulated by ethylene and 1-MCP (Supplementary Fig. S4A). Expression of *PvETR1* was significantly increased by 1-MCP before the second week of storage.

However, expression of *PvETR1* was significantly inhibited by 1-MCP and ethylene treatment at the third week of storage, while there was no significant difference between 1-MCP and ethylene treatment. *PvPYR1* in control and treated fresh common beans showed a downward trend during storage. The effect of 1-MCP and ethylene was a maintained expression pattern of *PvPYR1* at second week of storage, but there was no significant difference between 1-MCP and ethylene treated fresh common beans (Supplementary Fig. S4B). Expression of *PvAOG1* showed an upward tendency during storage. Increased expression of *PvAOG1* at the second weeks of storage was noted in 1-MCP treatment, though no significant changes between control and 1-MCP treated fresh common beans were observed, while expression of *PvAOG1* was inhibited by ethylene at the third week of storage (Fig. 6B).

Expression of lignin synthesis genes during postharvest storage

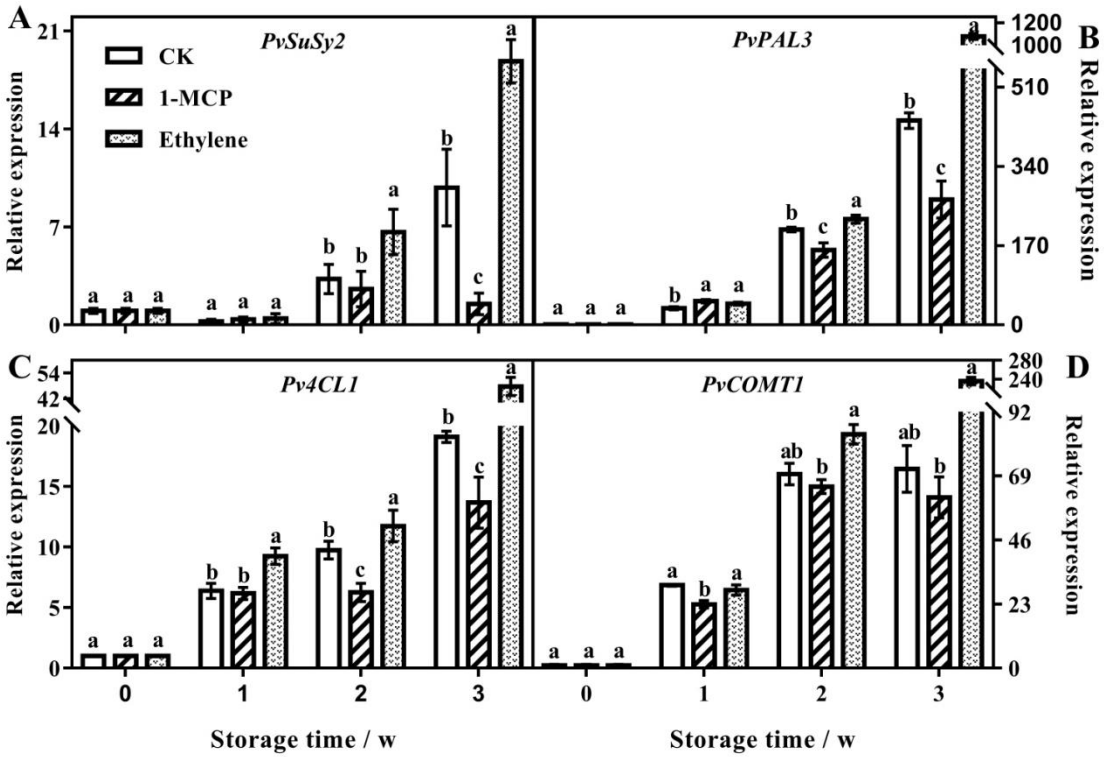

Fig. 7 Expressions of (A) *PvSuSy2*, (B) *PvPAL3*, (C) *Pv4CL1*, and (D) *PvCOMT1* genes of Control, Ethylene and 1-MCP treated beans during storage. Vertical bars represent standard error of three replicates (10 beans of each). Different letters within each parameter indicate statistically significant differences ($P < 0.05$)

The expression of six lignin synthesis related genes were selected and explored during postharvest storage (Fig. 7 and Supplementary Fig. S5). Expression of *PvSuSy2*, *PvPAL3*, *Pv4CL1*, and *PvCOMT1* in

control and 1-MCP treated-beans increased, these genes were significantly stimulated by ethylene, while 1-MCP treatment significantly suppressed their expression (Fig. 7A, B, C, and D). Expression of *PvCAD6* in fresh common beans decreased during storage, and the decrease of expression was accelerated by 1-MCP and ethylene treatment by the first week of storage, and then the decline of expression was inhibited by 1-MCP treatment (Supplementary Fig. S5A). Ethylene and 1-MCP significantly increased expression of *PvPOD1* before the second weeks of storage. However, expression of *PvPOD1* was significantly inhibited by treatment compared with the control, but there was no significant difference between the ethylene and 1-MCP at 3 weeks of storage (Supplementary Fig. S5B).

DISCUSSION

In the senescence process in lignification of fruits and vegetables, cellulose is synthesized and forms fibre bundles. Lignin is synthesized and deposited in the fiber bundle grid, as the cell lengthens and the secondary wall thickens, the tissue became rough and fibrous, which leads to lignification.[1] Tissue lignification accumulated the increase of firmness in loquat fruit and accompanied by lignin biosynthesis and cellulose hydrolysis.[15,40] In this work, postharvest 1-MCP treatment suppressed the respiration rate and retarded the decrease in relative thickness of pods (Fig. 1A). Others have reported that 1-MCP treatment remarkably suppressed respiration rate of fresh common beans during storage.[31,32] Our results showed the relative thickness of the pods decreased because of the consumption carbohydrates by self-respiration during storage (Fig. 1A and Fig. 2B). Previously reported that phenolics are precursors of lignin, and coumaric acid, caffeic acid and ferulic acid, are the major phenolics in asparagus stalks.[16,20] Our results showed that total polyphenols increased first and then decreased with storage time. The increase in total polyphenols was significantly suppressed by ethylene and 1-MCP treatment (Supplementary Fig. S2A). Our result showed that fresh common beans treated with 1-MCP effectively inhibited the decrease in cellulose and increase in lignin during storage, and these were enhanced by

ethylene treatment (Fig. 2A and Fig. 2B). Cai et al. and Zeng et al. also found that lignification in loquat fruit involved the coordinated regulation of lignin biosynthesis and cellulose hydrolysis,[15,40] while its transformation mechanism is not clear. Our results showed the lignified cell group in fresh bean increased, sclerenchyma became thicker, vascular bundles thickened and lignified cells grew during storage (Fig. 3), which was consistent with report of Li et al. noted that lignification was appeared in core of 'Xuxiang' kiwifruit. 1-MCP inhibited significantly those changes in common bean (Fig. 3).[8] Moreover, the ratio of FTIR characteristic peak in cellulose decreased during storage, ethylene and 1-MCP suppressed the decrease in I_{1740}/I_{1508}, which was consistent with changes in lignin content in beans (Fig. 2A), but enhanced the decrease in I_{1379}/I_{1508}. The ratio of FTIR characteristic peak in lignin increased during storage, 1-MCP inhibited the increase in I_{1508}/I_{1379}, I_{1508}/I_{1425} and I_{1508}/I_{1740}, while ethylene enhanced it (Supplementary Fig. S3), which was consistent with changes in lignin content of common beans (Fig. 2B).

Our results showed that postharvest treatment with 1-MCP treatment inhibited lignin accumulation in fresh common beans and this was mainly due to restraint of the SPS, SuSy, PAL, CAD and POD activities, while ethylene treatment enhanced (Fig. 5A,B,D,F, and G), consistent with research on loquat fruit,[26] water bamboo shoot,[28,41,42] *Rosa sterilis* D. shi,[13] and common beans.[4]

The literature suggests that lignification was involved in ethylene in Tsai Tai,[9] while ABA/GA_3 in bamboo shoots,[24] the phytohormones that affect the lignification in fresh common beans are not clear. The presented results show a significant increased the expression of *PvACO1* by ethylene treatment during storage. 1-MCP treatment produced effects opposite to those of ethylene (Fig. 6A). However, treatment with ethylene and 1-MCP heightened the *PvETR* in beans in the second week, and then inhibited it during further storage (Supplementary Fig. S4A). Treatment with 1-MCP enhanced expression of *PvAOG1* in beans and inhibited the decline of *PvPYR1* during storage (Fig. 6B and

Supplementary Fig. S4B). The significant increase in expression of *PvAOG1* during storage as the result of the 1-MCP treatment was pronounced. Treatment with 1-MCP produced effects opposite to those of ethylene, which provides added evidence for the role of these genes concerning lignin content.[24]

The expression levels of genes related to lignin biosynthesis, including *PAL*, *4CL*, *CAD*, and *COMT*, are up-regulated in the process of lignification.[5-9,12,14,21] Our results showed that 1-MCP treatment dramatically suppressed expression of genes, including *PvSuSy2*, *PvPAL3*, *Pv4CL1*, and *PvCOMT1* in beans, while ethylene treatment increased (Fig. 7A, B, C, and D). Expression of *PvCAD6* was suppressed by 1-MCP and ethylene treatment for the first week of storage, but then it inhibited the decline of expression (Supplementary Fig. S5A), are in contrast to results in Tsai Tai treated with ethylene and 1-MCP,[9] but are consistent with results in kiwifruit treated with 1-MCP.[8] Expression of *PvPOD1* gene was enhanced in beans by ethylene and 1-MCP treatment compared with control before the second week of storage (Supplementary Fig. S5B). These results agree with previous findings that 1-MCP markedly increases the expression levels of *AcPOD1* and *AcPOD1* in kiwifruit core tissue, but decreased in pulp tissue,[8] those results showed that the expression of the same gene is different in different tissues.

CONCLUSIONS

The effects of ethylene and 1-MCP on postharvest lignification of fresh common beans were evaluated. Where lignification was reinforced by ethylene treatment, or retarded by 1-MCP, the inhibition of lignin biosynthesis and the enzyme were consistently enhanced or retarded. Expression correlated well with the lignification and in response to the treatments by ethylene and 1-MCP. This work provides further information on the role of gene expression in lignin biosynthesis in fresh common beans during storage and the influence of ethylene or 1-MCP and may be helpful in understanding ethylene involvement in lignification in fresh common beans. Overall, the results indicate that ethylene or ABA plays an important role in lignification in fresh common beans, while the interaction of ethylene

and ABA on the lignification of fresh bean still needs further study.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests in the research.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

References:

1. Miao Y, Tian WN, Hao CM, Rao L, Cao JK and Jiang WB, Study on pods fibrosis delaying of postharvest common bean by chitosan treatment. *J China Agr Univ* **17**: 132-137 (2012).
2. Wang LB, Jiang L, Shi Y, Luo HB, Yu ZF, Physiobiochemical Characteristics of *Phaseolus vulgaris* L. during Controlled Atmosphere Storage. *Food Sci* **34**(8): 289-293(2013).
3. Xie GF, Yang C, Fei Y and Ma LZ, Physiological and proteomic analyses of 1-MCP treatment on lignification in fresh common bean (*Phaseolus vulgaris* L) during storage. *Postharvest Biol Tec* https://doi.org/10.1016/j. postharvbio.2019.111041(2020).
4. Yang SL, Sun CD, Wang P, Shan LL, Cai C, Zhang B, Zhang WS, Li X, Ferguson I, Chen KS, Expression of expansin genes during postharvest lignification and softening of 'luoyangqing' and 'baisha' loquat fruit under different storage conditions. *Postharvest Biol Tec* **49**: 46-53(2008).
5. Ge H, Zhang J, Zhang YJ, Li X, Yin XR, Grierson D and Chen KS, EjNAC3 transcriptionally regulates chilling-induced lignification of loquat fruit via physical interaction with an atypical CAD-like gene. *J Exp Bot* **68**:5129-5136(2017).
6. Xu M, Zhang MX, Shi YN, Liu XF, Li X, Grierson D and Chen KS, EjHAT1 participates in heat alleviation of loquat fruit lignification by suppressing the promoter activity of key lignin monomer synthesis gene *EjCAD5*. *J Agr Food Chem* **67**: 5204-5211 (2019).
7. Luo ZS, Xu XL and Yan BF. Use of 1-methylcyclopropene for alleviating chilling injury and lignification of bamboo shoot (*Phyllostachys praecox, f. prevernalis*) during cold storage. *J Sci Food Agr* **88**(1): 151-157 (2008).
8. Li H, Suo JT, Han Y, Liang CQ, Jin MJ, Zhang ZK and Rao JP, The effect of 1-methylcyclopropene, methyl jasmonate and methyl salicylate on lignin accumulation and gene expression in postharvest 'xuxiang' kiwifruit during cold storage. *Postharvest Biol Tec* **124**: 107-118 (2017).
9. Zhang LB, Wang GA, Chang JM, Liu JS, Cai JH and Rao XW, Effects of 1-MCP and ethylene on expression of three CAD genes and lignification in stems of harvested Tsai Tai (*Brassica chinensis*). *Food Chem* **123**:

32-40(2010).

10. Luo ZS, Feng SM, Pang J, Mao LC, Shou HL and Xie JW, Effect of heat treatment on lignification of postharvest bamboo shoots (*Phyllostachys praecox f. prevernalis.*). *Food Chem* **135**: 2182-2187(2012).
11. Liu ZL, Li L, Luo ZS, Zeng FF, Jiang L and Tang KC, Effect of brassinolide on energy status and proline metabolism in postharvest bamboo shoot during chilling stress. *Postharvest Biol Tec* **111**: 240-246(2016).
12. Jiang HG, Wang SG, Liu Y, Shen YH, Li XN and Cao Y, Transcriptome-wide identification of lignin gene families of bambusa emeiensis shoots and their differential expression. *Bull Bot Res* **38**(3): 415-421 (2018).
13. Xie GF, Wang LR, Fan KX, Liu N, Liu YL and Zhao ZB, Postharvest 1-methylcyclopropene treatments maintain the quality of *Rosa sterilis* D. shi during storage. *Food Sci Tec* http://dx.doi.org/10.1590/fst. 32818(2019).
14. Jia N, Liu JQ, Tan PH, Sun YF, Lv YM, Liu JM, Sun J, Huang YT, Lu J, Jin N, Li MM, Imam KMSU, Xin FJ and Fan B, Citrus sinensis MYB transcription factor CsMYB85 induce fruit juice sac lignification through interaction with other CsMYB transcription factors. *Frontiers Plant Sci* **10**:213(2019).
15. Cai C, Xu CJ, Li X, Ferguson I and Chen KS, Accumulation of lignin in relation to change in activities of lignification enzymes in loquat fruit flesh after harvest. *Postharvest Biol Tec* **40**(2): 163-169(2006).
16. Chiaiese P, Ruotolo G, Matteo AD, Virzo ADS, Marco AD and Filippone E, Cloning and expression analysis of kenaf (*Hibiscus cannabinus* L.) major lignin and cellulose biosynthesis gene sequences and polymer quantification during plant development. *Ind Crop Prod* **34**: 1072-1078(2011).
17. Zong RJ, Huang B, Lu YX and Li W, Studies of optimizing temperature, humidity and atomoshpere for prolonging cold storage of green bean, cucumber and sweet pepper. *J Refrig* (3):22-28(1984).
18. Cunha, C. P., Roberto, G. G., Vicentini, R., Lembke, C. G., Souza, G. M., Ribeiro, R. V., Machado, E. C., Lagôa, Ana MMA and Menossi M, Ethylene-induced transcriptional and hormonal responses at the onset of sugarcane ripening. *Sci Rep-UK* **7**: 43364 (2017).
19. Liu J, Huang QH, Kang PZ, Liang L and Chen JJ, Lignin Accumulation in Three Pumelo Cultivars in Association with Sucrose and Energy Depletion. *Biomolecules* **9**: 701(2019).
20. Liu ZY and Jiang WB, Lignin deposition and effect of postharvest treatment on lignification of green asparagus (*Asparagus officinalis L.*). *Plant Growth Regul* **48**(2):187-193(2006).
21. Robbins ML, Roy A, Wang PH, Gaffoor I, Sekhon RS, Buanafina MMO, Rohila JS and Chopra S, Comparative proteomics analysis by DIGE and iTRAQ provides insight into the regulation of phenylpropanoids in maize. *J Proteomics* **93**(19): 254-275(2013).
22. Li SM, Su XP, Jin Q, Li GH, Sun YM, Abdullah M, Cai YP and Lin, Y, iTRAQ-based identification of proteins related to lignin synthesis in the pear pollinated with pollen from different varieties. *Molecules* **23**:548(2018).
23. Tobimatsu Y and Schuetz M, Lignin polymerization: how do plants manage the chemistry so well? *Curr Opin Biotech* **56**: 75–81(2019).
24. Luo ZS, The Relationship Between Lignification and endogenous hormone content of excised bamboo shoots. *Sci Agricultura Sinica* **39**:792-797(2006).
25. Wills RBH and Kim GH, Effect of ethylene on postharvest quality of green beans. *Aust J Exp Agr* **36**(3): 335-337(1996).
26. Cao SF, Zheng YH, Wang KT, Rui HJ and Tang SS, Effect of 1-methylcyclopropene treatment on chilling

injury, fatty acid and cell wall polysaccharide composition in loquat fruit. *J Agr Food Chem* **57**: 8439(2009).

27. Han YZ, Xue YJ and Song SY. Research progress on postharvest physiology and storage technology of snap bean. *Food Sci* **34**(13):345- 349(2013).
28. Song LL, Gao HY, Chen WX, Chen HJ, Mao JL, Zhou YJ, Duan XW and Joyce DC, The role of 1-methylcyclopropene in lignification and expansin gene expression in peeled water bamboo shoot (*Zizania caduciflora L.*). *J Sci Food Agr* **91**: 2679-2683(2011).
29. Chen YH, Sun JZ, Lin HT, Hung YC, Zhang S, Lin YF and Lin T, Paper-based 1-MCP treatment suppresses cell wall metabolism and delays softening of huanghua pears during storage. *J Sci Food Agr* **97**:2547-2552(2016).
30. Lin YH, Lin YY, Lin HT, Lin MS, Li H, Yuan F, Chen YH and Xiao JB, Effects of paper containing 1-MCP postharvest treatment on the disassembly of cell wall polysaccharides and softening in younai plum fruit during storage. *Food Chem* **264**: 1-8(2018).
31. Cao DA, Liu DZ, Li XH, Shen LM and Wang M, Effect of controlled atmosphere storage combined 1-methylcyclopropene on snap bean for alleviating chilling injury. *Adv Mater Res* **798-799**: 1041-1044(2013).
32. Cho MA, Hurr BM, Jeong J, Lim C and Huber DJ, Postharvest senescence and deterioration of 'Thoroughbred' and 'Carlo' green beans (*Phaseolus vulgaris L.*) in response to 1-Methylcyclopropene. *Hortscience* **43**:427-430(2008).
33. Teixeira RSS, Silva ASA, Ferreira-Leitão VS and Bon EPS, Amino acids interference on the quantification of reducing sugars by the 3,5-dinitrosalicylic acid assay mislead carbohydrase activity measurements. *Carbohyd Res* **363**: 33-37(2012).
34. Nuncio-Jáuregui N, Munera-Picazo S, Calín-Sánchez Á, Wojdyło A, Hernández F, Ángel A, Carbonell-Barrachina. Bioactive compound composition of pomegranate fruits removed during thinning. *J Food Compos Anal* **37**: 11-19(2015).
35. Liu JX, Feng K, Wang GL, Xu ZS, Wang F and Xiong AS, Elevated CO_2 induces alteration in lignin accumulation in celery (*Apium graveolens* L.). *Plant Physiol Bioch* **127**: 310-319(2018).
36. Chu J, Ma L and Zhang JH. The Chemical Composition of Bamboo after Heat Pretreatment with Fourier Infrared Spectrum Analysis. *Spectrosc Spect Anal* **36**:3557-3562(2016).
37. Liu CW, Su ML, Zhou XW, Zhao RJ, Lu JX and Wang YR, Analysis of content and distribution of lignin in cell wall of transgenic poplar with fourier infrared spectrun (FTIR) and confocal laser scanning microscopy (CLSM). *Spectrosc Spect Anal* **37**:3404-3408(2017).
38. Grof CPL, Albertson PL, Bursle J, Perroux JM, Bonnett GD and Manners JM, Sucrose-phosphate synthase, a biochemical marker of high sucrose accumulation in sugarcane. *Crop Sci* **47**: 1530(2007).
39. Luo HB, Jiang J, Zhang L, Jiang L, Fu LR, Dai YY and Yu ZF, Effect of gibberellic acid and 6-benzylaminopurine on lignification of fresh-cut *Zizania latifolia* during refrigerated (1 ℃) storage. J Food Process Pres **37**: 864-869 (2013).
40. Zeng FF, Luo ZS, Xie JW and Feng SM, Gamma radiation control quality and lignification of bamboo shoots (*Phyllostachys praecox f. prevemalis*.) stored at low temperature. *Postharvest Biol Tec* **102**: 17-24(2015).
41. Luo ZS, Xu XL and Yan BF, Accumulation of lignin and involvement of enzymes in bamboo shoot during storage. Eur Food Res Technol **226**: 635-640(2008).

42. Luo ZS, Xu XL, Cai ZZ and Yan BF, Effects of ethylene and 1-methylcyclopropene (1-MCP) on lignification of postharvest bamboo shoot. *Food Chem* **105**: 521-527(2007).