Combining acetic acid and ethanol as an anti-browning treatment for lettuce butt discoloration through repression of the activity and expression of phenylalanine ammonia lyase

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Abstract

The enzymatic browning of lettuce (*Lactuca sativa* L.) is a main cause of postharvest quality loss, and is controlled by the enzyme phenylalanine ammonia lyase (PAL). However, effective browning inhibitors that prevent lettuce butt discoloration have not been commercially developed, so the effects of such inhibitors on PAL are largely unknown. Here, we not only developed an anti-browning treatment, but also explored the mechanisms of the PAL-associated browning of Iceberg lettuce by profiling all homologs of *PAL* genes at transcript level. The anti-browning treatment used a combination of 0.25 M acetic acid and 200 mL L^{-1} ethanol and was able to repress enzymatic browning and microbial growth for two weeks. Notably, the lettuce butt discoloration in stem disks was repressed by 0.5 M acetic acid by inhibiting PAL activity, and this inhibition of PAL activity was also observed *in vitro* using a crude PAL enzyme extract from lettuce stems. To investigate the anti-browning mechanism at the transcriptional level, we identified and cloned six predicted *LsPAL* genes in the Lettuce Genome Resource, and further found that four of these (*LsPAL1* to *LsPAL4*) were wound-inducible in the lettuce stem. Among these four wound-inducible *LsPALs*, *LsPAL4* showed the highest wound-induced fold-change, suggesting that *LsPAL4* has a key role in lettuce browning. Interestingly, wound-induction of *LsPAL* genes was dramatically downregulated by application of acetic acid. Taken together, acetic acid treatment of lettuce stems repressed butt discoloration by repressing PAL both enzymatically and transcriptionally, and ethanol provided complementary antimicrobial activity. A combination treatment with acetic acid and ethanol therefore has commercial potential in lettuce head processing.