

Metabolite profiling and proteome analysis of genetically modified lettuce plants (*Lactuca sativa* L.) that produce astaxanthin and its esterified derivatives

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Abstract

Plant genetic engineering research has been used to confer metabolic pathways to fortify nutritional traits of crops. In these metabolically engineered crops, endogenous metabolic status should be inevitably influenced by the newly designed and introduced enzymatic steps with the aid of metabolic genes from other organisms. Here, we report metabolomic and proteomic comparisons between genetically modified (GM) high astaxanthin-producing lettuce (*Lactuca sativa* L.) that was generated with chloroplast transformation and its parental cultivar, together with commercial lettuce varieties as controls. It was demonstrated that the higher adenine levels in the GM lettuce samples were the only difference consistently observed between the GM and control lettuce samples. In addition, no significant differences were observed in the levels of nitrate-nitrogen and the allergenic protein Lac s 1. These results indicate that the endogenous metabolic status of lettuce is not noticeably modified by genetic engineering of the high astaxanthin-producing trait.

Keywords : astaxanthin, carotenoid, lettuce (*Lactuca sativa* L.), metabolite profiling, proteome analysis

I Introduction

Since the introduction of biotechnology to agriculture, conventional cultivars have been engineered metabolically to produce nutritionally valuable metabolites, such as vitamins and fatty acids, at higher levels^{1, 2)}. Generally, this metabolic engineering is executed by introducing foreign genes to add new routes branching from endogenous biosynthetic pathways, which should affect the original pathway activities. To clarify and diagnose those genetic engineering events, it is critical to know how such expression of foreign metabolic genes may impact endogenous metabolic properties. It is thus expected that omics technologies should enable holistic comparison of metabolic activities between such genetically modified crops and their parental conventional varieties^{3, 4)}.

Astaxanthin, a red-colored natural product, is classified as a ketocarotenoid that contains 13 conjugated double bonds, which are responsible for its antioxidant and radical

scavenging activities. In addition to its use as a food colorant⁵⁻¹⁰⁾, astaxanthin is expected to exert supportive effects in a variety of clinical areas including inflammation, tumor, cardiovascular disease, immune disorder and eye health¹¹⁻¹³⁾. To meet the increasing demands, the industrial production of astaxanthin now relies on both the chemical synthesis^{14, 15)} and extraction from biological sources¹⁶⁾. Genetic modification has also been used to produce astaxanthin in microorganisms and plants^{17, 18)}.

In nature, only a limited species of microalgae, bacteria, fungi and plants are known to produce astaxanthin. In microalgae, astaxanthin accumulates in cytosolic lipid bodies especially under environmental stress conditions, such as high light, high salinity and nutrient deprivation¹⁹⁻²¹⁾. Astaxanthin production in the green microalga *Haematococcus pluvialis*, one of the most promising astaxanthin producers in nature, reached 4% of dry weight under stress conditions¹⁶⁾. In plants, however, astaxanthin is uncommon with the exception of a