

Genetic and molecular approaches reveal fruit-specific mutation inside Anthocyanidin Synthase exhibiting pathways controlling pigmentation in raspberries (*Rubus Idaeus L.*).

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Abstract

Raspberries (*Rubus idaeus L.*) are rich sources of variety of poly(phenols) especially ellagitannins and anthocyanins those account for broad range of biological functions in plants (1,2,3). These compounds have been described to have potential health beneficial effects in humans against coronary heart diseases, blindness, cancer, aging, inflammation, cardiovascular and neuro-degenerative diseases (4,5,6). Anthocyanins and carotenoids are known to be the main pigments, while colour of red fruits is mainly considered due to varying anthocyanin contents (4). Despite the interest in raspberry anthocyanins, little is known about the genetic control and their regulation process during fruit development. Here, we describe the a detailed study conducted to identify the key structural genes involved in genetic block or control involved in pigmentation of yellow raspberry fruits.

An LC-MS based method allowed to determine the polyphenol composition focusing on (poly)phenolic pattern in red and yellow raspberries to better understand the origin of yellow pigmentation. Selected yellow and red varieties revealed some differences in terms of polyphenol composition, but the data output didn't gave clear clue for major metabolite differences between red and yellow genotypes under study besides the presence or absence of anthocyanins (3) but indicate the possible deviation of secondary metabolism into other directions (3,7). Expression analysis of flavonoid pathway genes of two raspberry varieties "Tulameen (red)" and "Anne, Yellow" was carried in out to get insight into the possible pathway mutants. Interestingly, a clear decrease in transcripts of anthocyanidin synthase (ANS) step was observed on turning stage of fruit colour. Molecular cloning of ANS gene revealed a 5 bp mutation/insertion leading to a truncated protein, which lacks conserved region for substrate, cosubstrate and iron binding sites in "Anne".

Furthermore, complementation of ANS gene via *Arabidopsis* line (tds4-2) mutant for ANS and functional characterization of ANS gene through recombinant protein expression elaborates the role of this gene in the flavonoid biosynthesis pathway in raspberry. Based on these results and further proceedings a pathway map for pigmentation in raspberry will be predicted and utilized for biotechnological production of specific aroma compounds having beneficial effects on health.

Keywords: pigmentation, pathway block, yellow raspberry, mutation, anthocyanidin synthase, complementation

References

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