Pigmentation “clue” in Yellow Raspberries - An Insertion/Mutation in Anthocyanidin Synthase Gene

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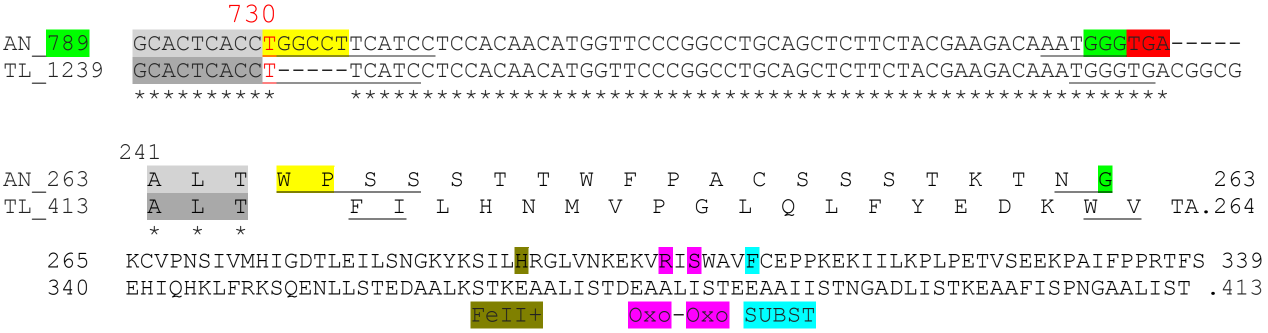
Genetic control of fruit pigmentation in raspberries is a complex phenomenon. Pigmentation itself is one of the most important traits where a range of colour patterns from deep purple to yellow exists. Anthocyanins and carotenoids are known to be the main pigments in raspberries; however colour of fruits is mainly considered due to varying anthocyanin contents (1). These compounds have been described to have potential health beneficial effects against cancer, aging, inflammation and neuro-degenerative diseases (2). Aside from the health benefits, anthocyanins play basic role as an indicator of fruit quality and consumer acceptance. The role of anthocyanins *in planta* is not known exactly, however protective role against biotic and abiotic stresses has been discussed. Apparently it seems that carotenoids are responsible for colour in yellow raspberries, due to the loss of masking effect of anthocyanins (3). Thus, in yellow raspberries there might be a block of anthocyanin biosynthesis and that can be the possible reason behind the yellow pigmentation.

To better understand the origin of colour differences observed in raspberry varieties, “Anne” (yellow) and “Tulameen” (red), fruits were analyzed to identify the genetic block or control and regulation processes during fruit development. Sequence and transcriptional analysis of the gene encoding anthocyanidin synthase (*ANS*) was performed from genomic DNA and RNA of independent samples of “Anne” and “Tulameen”, respectively. A five base pairs insertion/mutation at position 731 bp of the coding region of “Anne” *ANS* was found in the gene that leads to a truncated protein of 28.9 kDa (789 bp) in comparison to “Tulameen” *ANS* (45.4 kDa; 1239 bp) which lacks conserved region for substrate, cosubstrate and iron binding sites in “Anne” (Fig. 1). Most likely the truncated protein is inactive and is apparently the reason for the lack of the red colour at-least in this yellow variety. The expression analysis of key steps of the flavonoid pathway on “Anne” and “Tulameen” also indicates the down-regulation of gene coding for *ANS* enzyme in later fruit development stages. Furthermore, functional characterization of *ANS* gene through recombinant protein expression and transformation to *ANS* mutant line of *Arabidopsis thaliana* will elaborate the role of this gene in the flavonoid biosynthesis pathway in raspberry.



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                  730
AN_789     GCACTCACCTGGCCTTCATCCTCCACAACATGGTTCCCGGCCTGCAGCTCTTCTACGAAGACAAATGGGTGA-----
TL_1239    GCACTCACCT-----TCATCCTCCACAACATGGTTCCCGGCCTGCAGCTCTTCTACGAAGACAAATGGGTGACGGCG
           **********     *************************************************************

          241
AN_263      A  L  T  W  P  S  S  S  T  T  W  F  P  A  C  S  S  S  T  K  T  N  G       263
TL_413      A  L  T        F  I  L  H  N  M  V  P  G  L  Q  L  F  Y  E  D  K  W  V  TA.264
            *  *  *

   265    KCVPNSIVMHIGDTLEILSNGKYKSILHRGLVNKEKVRISWAVFCEPPKEKIILKPLPETVSEEKPAIFPPRTFS 339
   340    EHIQHKLFRKSQENLLSTEDAALKSTKEAALISTDEAALISTEEAAIISTNGADLISTKEAAFISPNGAALIST .413
                                     FeII+       Oxo-Oxo SUBST
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Fig. 1: Sequencing analysis indicating 5bp insertion to cause frameshift and truncated protein in yellow cultivar “Anne” (AN) in comparison to red cultivar “Tulameen” (TL) having conserved regions for substrate binding.

References
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