Using RNA-Seq and integrative analysis to study transcriptomic changes in Chinese bayberry ripening fruit

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1. RNA-Seq

To obtain a general overview of the Chinese bayberry transcriptome, four libraries, MR1-4 were designed for RNA-Seq. MR1 was a mixture of equal amounts of RNA from stems and leaves (Fig.1A), buds (Fig.1B), flowers (Fig.1C), and young fruit (Fig.1D, E), while MR2-4 were fruit at three different ripening stages, respectively (Fig.1F, G, H) .

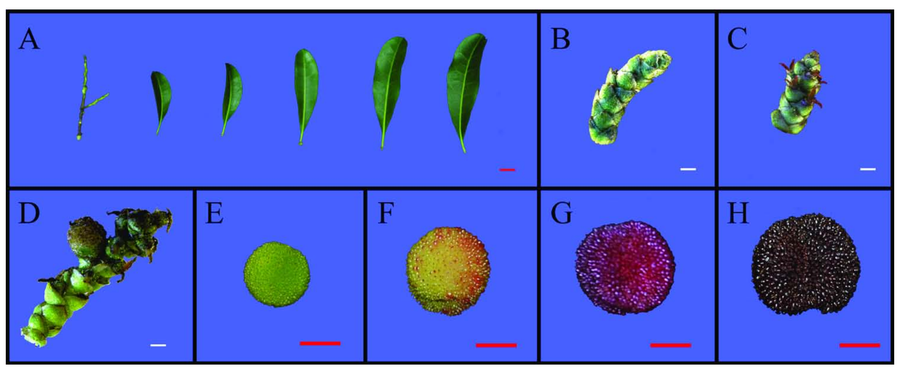

Figure 1 - Tissues of Chinese bayberry used in RNA-Seq.

(A) Stem and leaf, (B) Bud, (C) Flower, (D) young fruit at 15 DAF, (E) young fruit at 45 DAF, (F) breaker stage fruit at 75 DAF, (G) red ripe stage fruit at 80 DAF, (H) dark red ripe stage fruit at 85DAF. Red bar = 1 cm, white bar = 1 mm.

2. Gene expression profiles of ripening bayberry fruit

41,239 UniGenes were *de novo* assembled from bayberry RNA-Seq data, 3,644 of which were differentially expressed during fruit ripening (Fig.1A), with 826 up-regulated and 1,407 down-regulated (Fig.1B). GO comparisons between the UniGenes of these two types found that energy-related metabolism and catalytic activity was increased (Fig.1C).

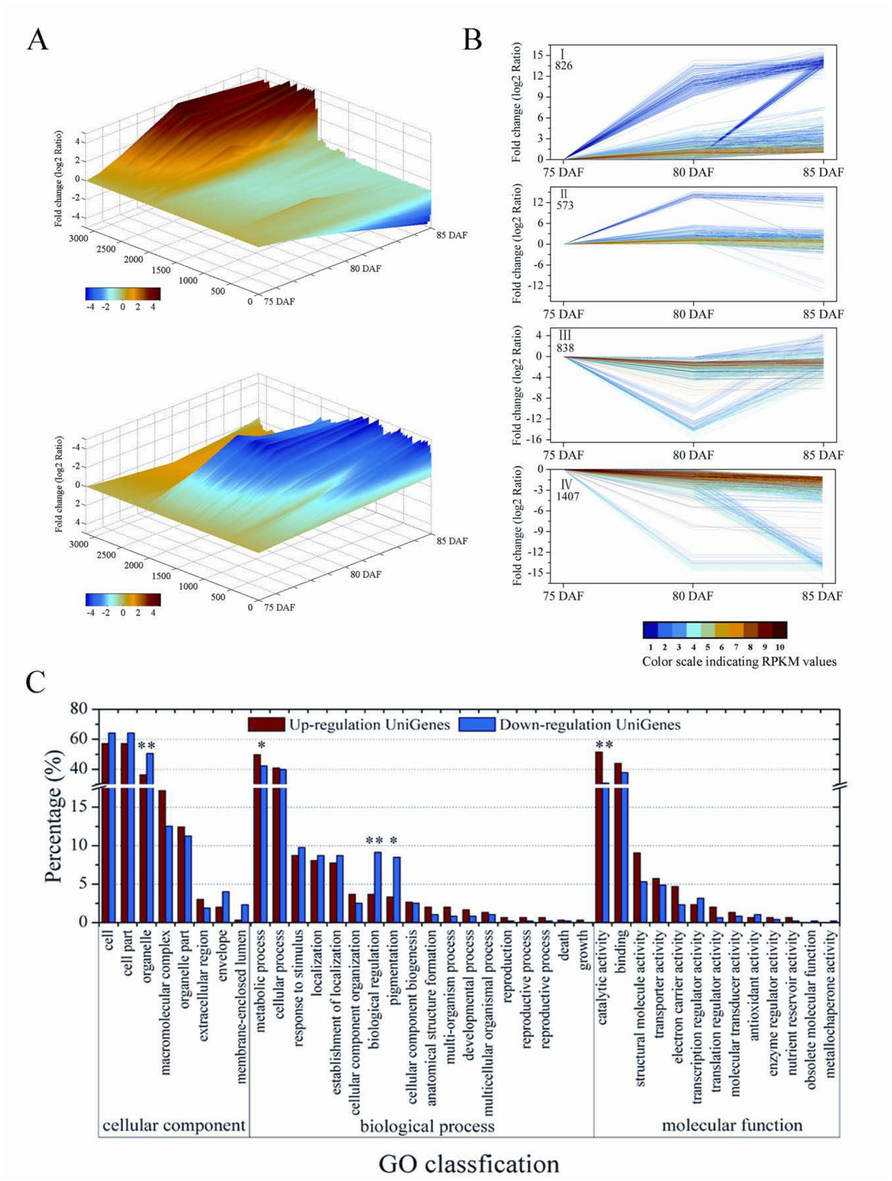

Figure 2 – UniGene expression profiles during bayberry fruit ripening.

(A) Overall expression profiles for the UniGenes expressed in fruit libraries of three different maturity stages, (B) Four expression profiles are shown, with I and IV indicating UniGenes with up-regulated and down-regulated expression, respectively, and II and III indicating those with irregular expression, (C) GO classification for up-regulated and down-regulated UniGenes, with * and ** indicating significant difference at 5% and 1%, respectively.

3. Metabolic pathway analysis during fruit ripening

Interactive Pathways analysis (ipath) was used to provide a global view of metabolism during fruit ripening. Pentose phosphate metabolism (Fig. 3A), which generates NADPH for increasing the metabolic rate, showed enhancement. Fruit colour changes can be explained by the transcriptional profiling of anthocyanin biosynthesis pathway (Fig.3B) and carotenoid biosynthesis pathway (Fig.3C). Changes in flavour are likely to be associated with sucrose phosphate synthase and glutamate decarboxylase (Fig. 3D, E).

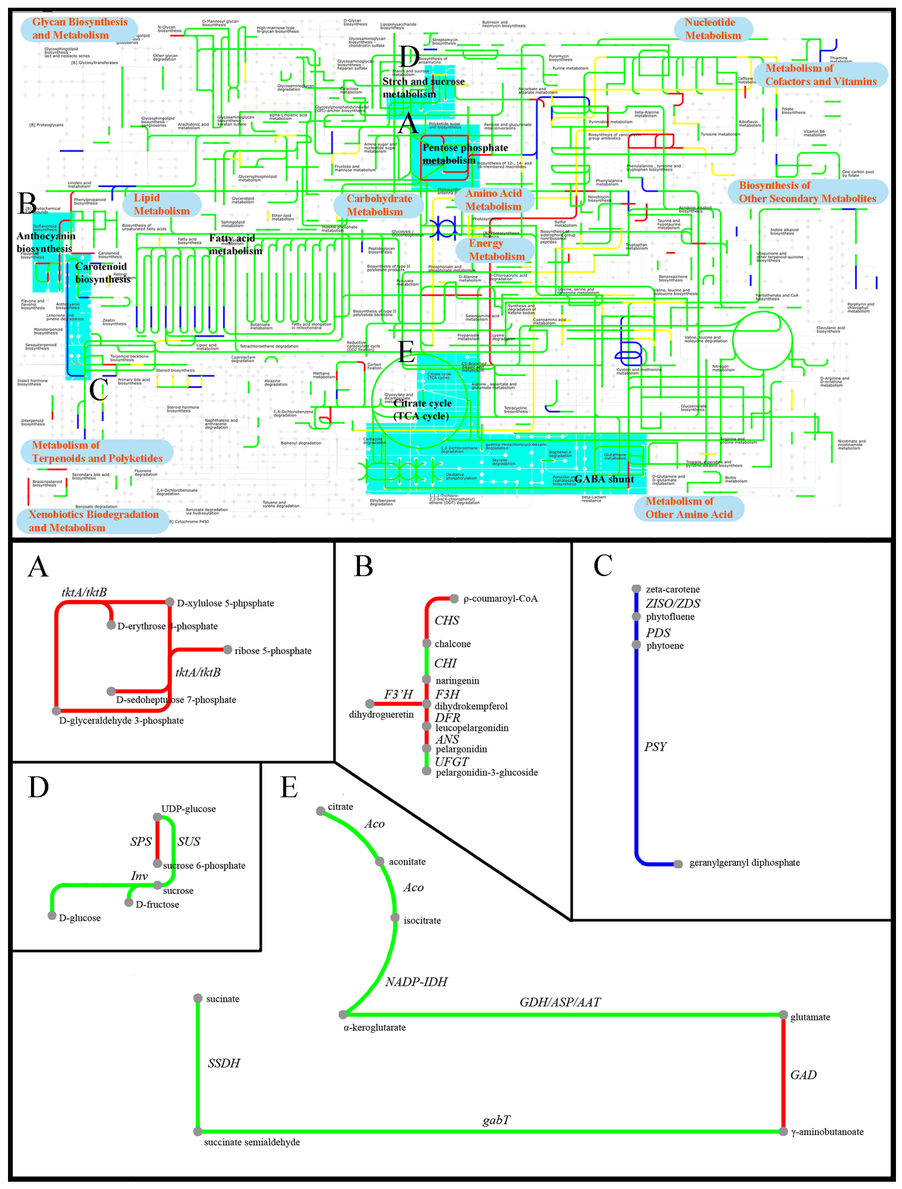

Figure 3 - Interactive pathways analysis during bayberry fruit ripening.

The green, the red, the blue and the yellow lines indicate genes with non-significant expression change, up-regulated, down-regulated, and irregularly regulated, respectively. The areas with sky blue background indicate the metabolic pathways related to fruit color, sugar and organic acids. (A) Pentose phosphate metabolism, (B) Anthocyanin biosynthesis, (C) The upstream part of carotenoid biosynthesis, (D) Sucrose biosynthesis, (E) GABA shunt.

Conclusions:

Mass sequence data of Chinese bayberry was obtained and the expression profiles were examined during fruit ripening. The UniGenes were annotated, providing a platform for functional genomic research with this species. Using pathway mapping and expression profiles, the molecular mechanisms for changes in fruit color and taste during ripening were examined. This provides a reference for the study of complicated metabolism in non-model perennial species.

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International Symposium on Integrative Bioinformatics
The 8th annual meeting
2nd-4th April, 2012, Hangzhou, China