



## Abstract

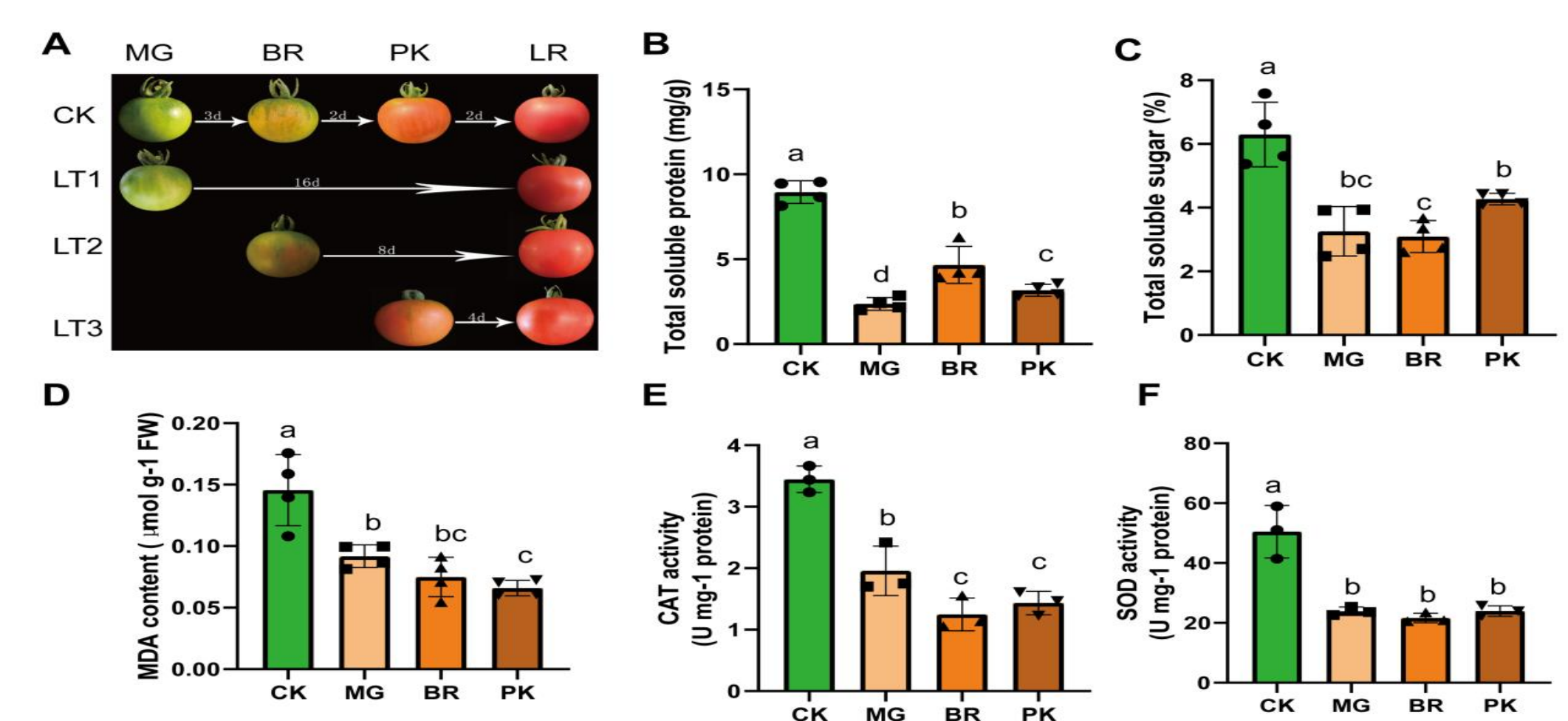
An integrative analysis of transcriptomics and metabolomics was used to elucidate the reprogrammed metabolic pathway related to fruit flavor in response to low temperature (LT) at different ripening stages. The results showed the metabolites related to Calvin cycle and sucrose metabolic pathways, together with volatile precursor substances (including valine and leucine) are reduced under LT treatments. In contrast, the LT treatment enhanced the accumulation of flavonoids related metabolites, such as quercetin and rutin, consistent with up-regulation of most flavonoid structural genes induced by LT treatment. The expression levels of volatile synthesis related gene (FLORAL4) and LT responsive genes, such as CBF1 and GS2 were dramatically induced, while CCH1 was significantly inhibited by all three LT treatments relative to control. To further elucidate the CBF1 roles for LT tolerance and fruit flavor, we performed EMSA and luciferase assays suggesting that CCH1 inhibits the expression of CBF1 by binding to its G-box at promoter region, and CBF1 stimulates the expression of both GS2 and FLORAL4 by binding to DRE binding motif. In addition, CBF1 stimulates the expression of FLS1 which explains well the upregulation of flavanol metabolic pathways. This study comprehensively illustrated the molecular mechanism of cross-talk for LT responsive pathway and fruits flavor, which expands our understanding of the flavor changes and the complex regulatory mechanisms in response to LT in tomato fruits at ripening stages.

## Materials and Methods

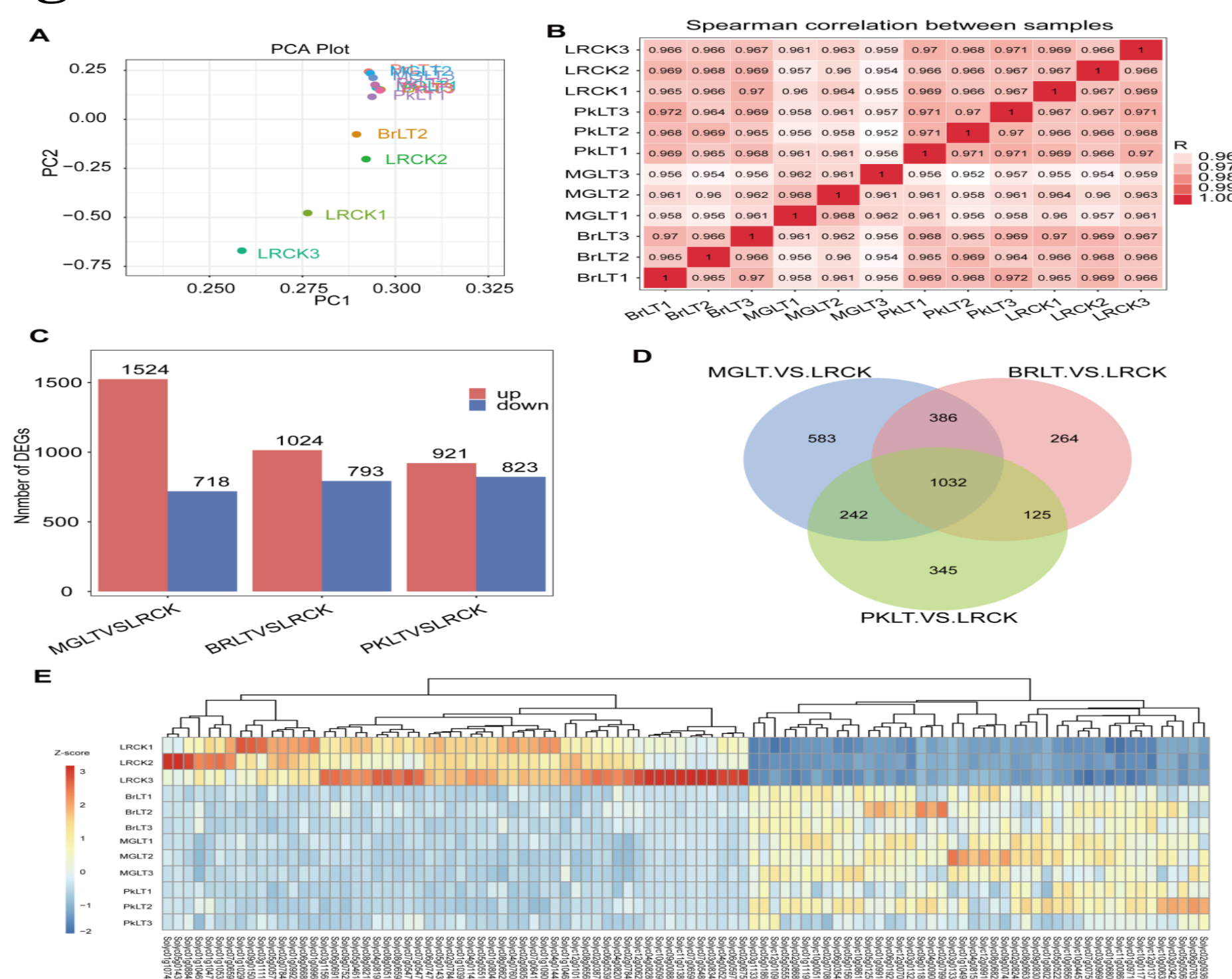
- 1.LC-MS/MS based untargeted metabolism and identification
- 2.Solid- phase micro extraction (SPME) and analyzed with gas chromatography-mass spectroscopy (GC-MS)
- 3.Transcriptome analysis
- 4.EMSA experiments
- 5.Luciferase assays

## Results

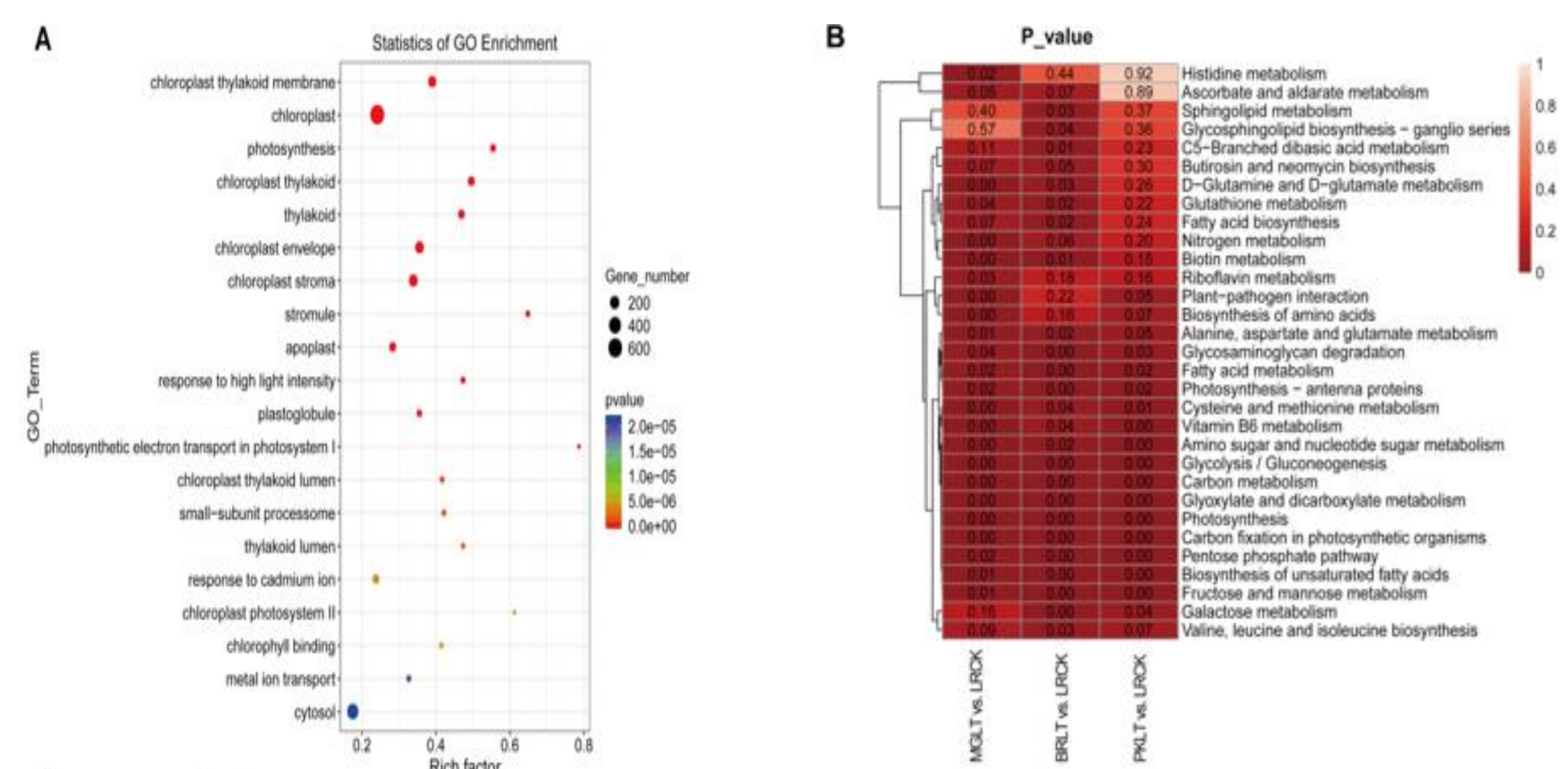
### 1.Performance of tomato fruits exposed to LT treatments during ripening



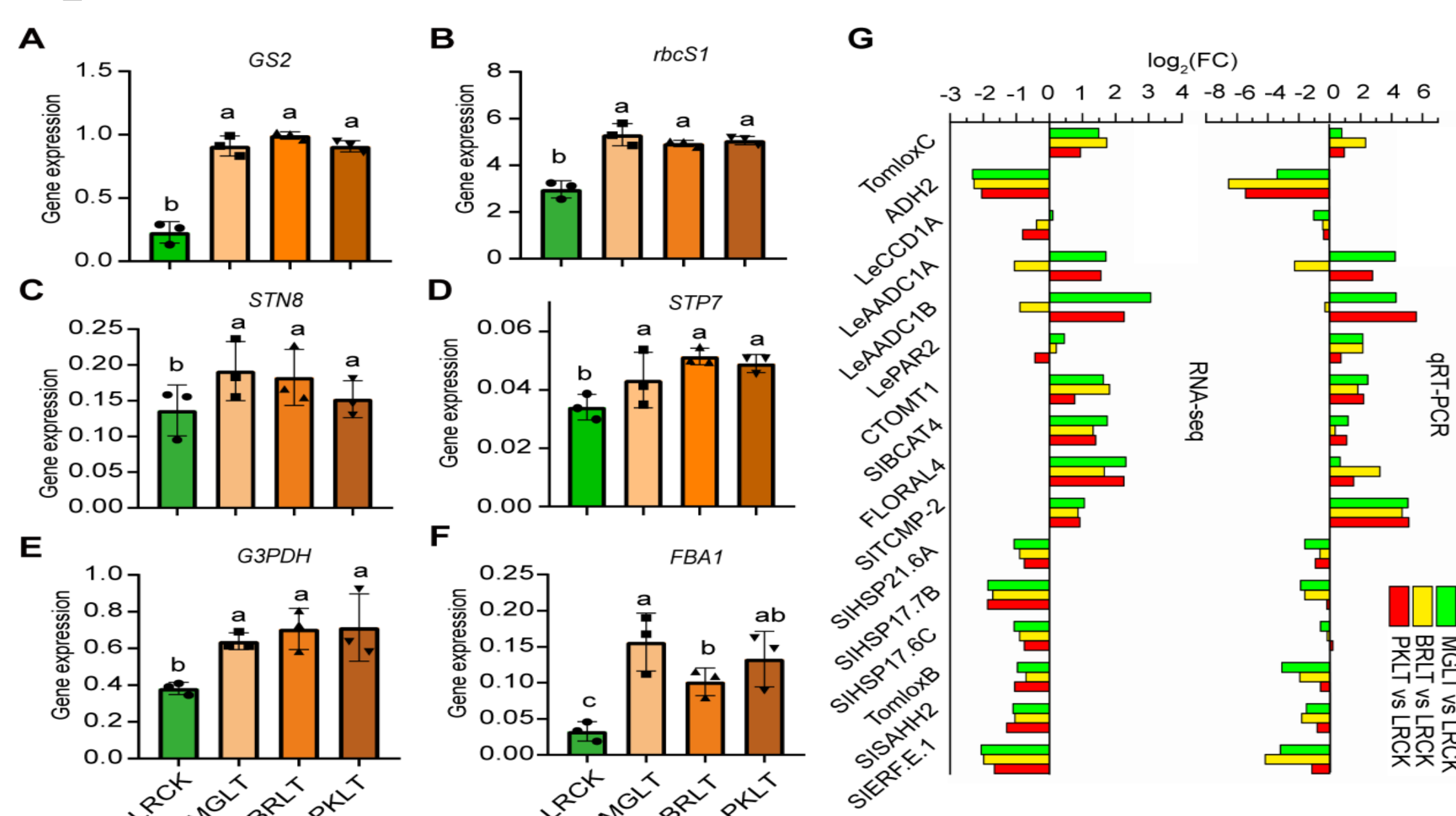
### 2.Transcriptomes analysis on different treatments of low temperature (LT) together with control condition in tomato



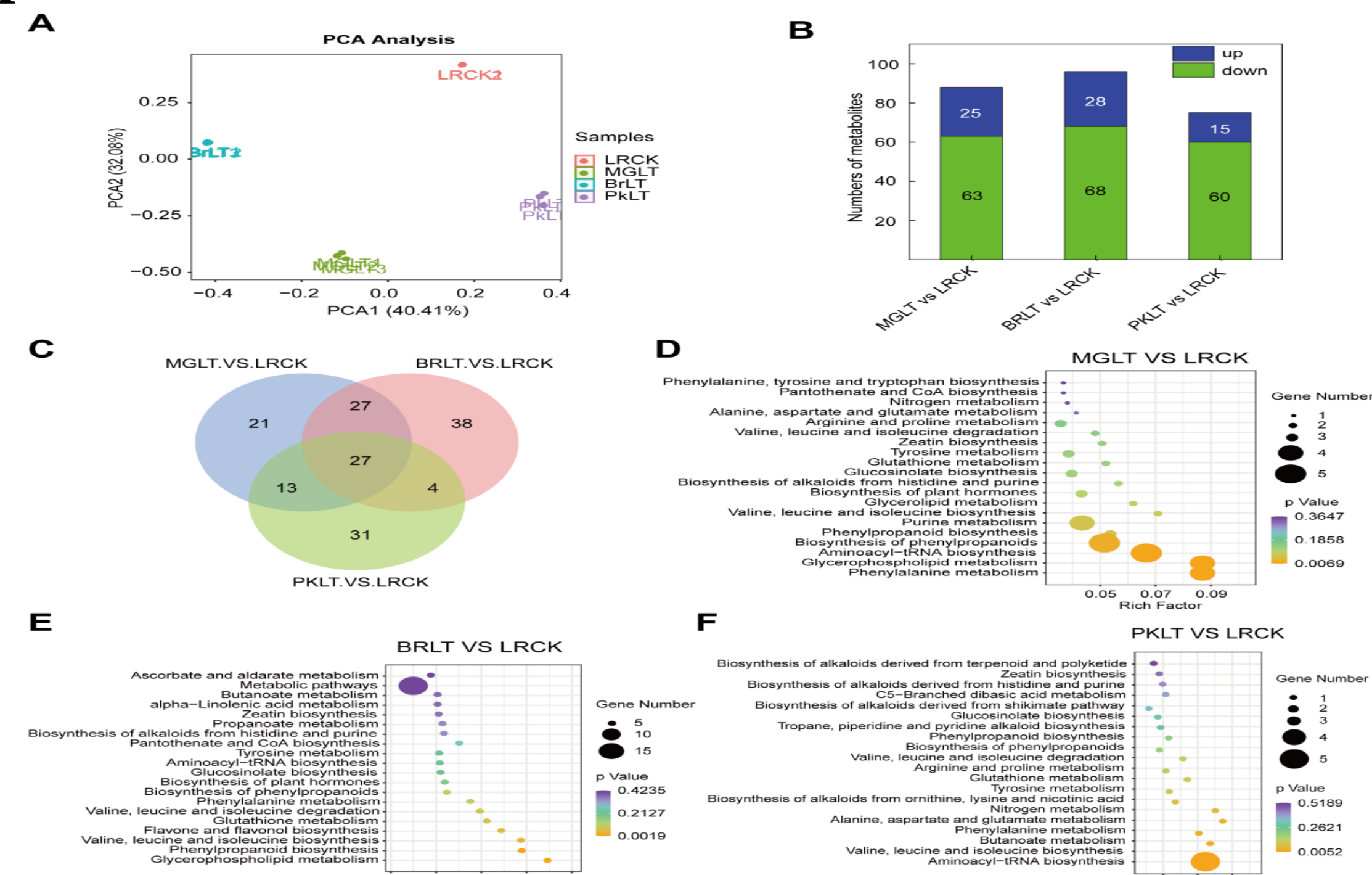
### 3.GO and KEGG analysis across different LT treatments relative to control for tomato during different ripening stages



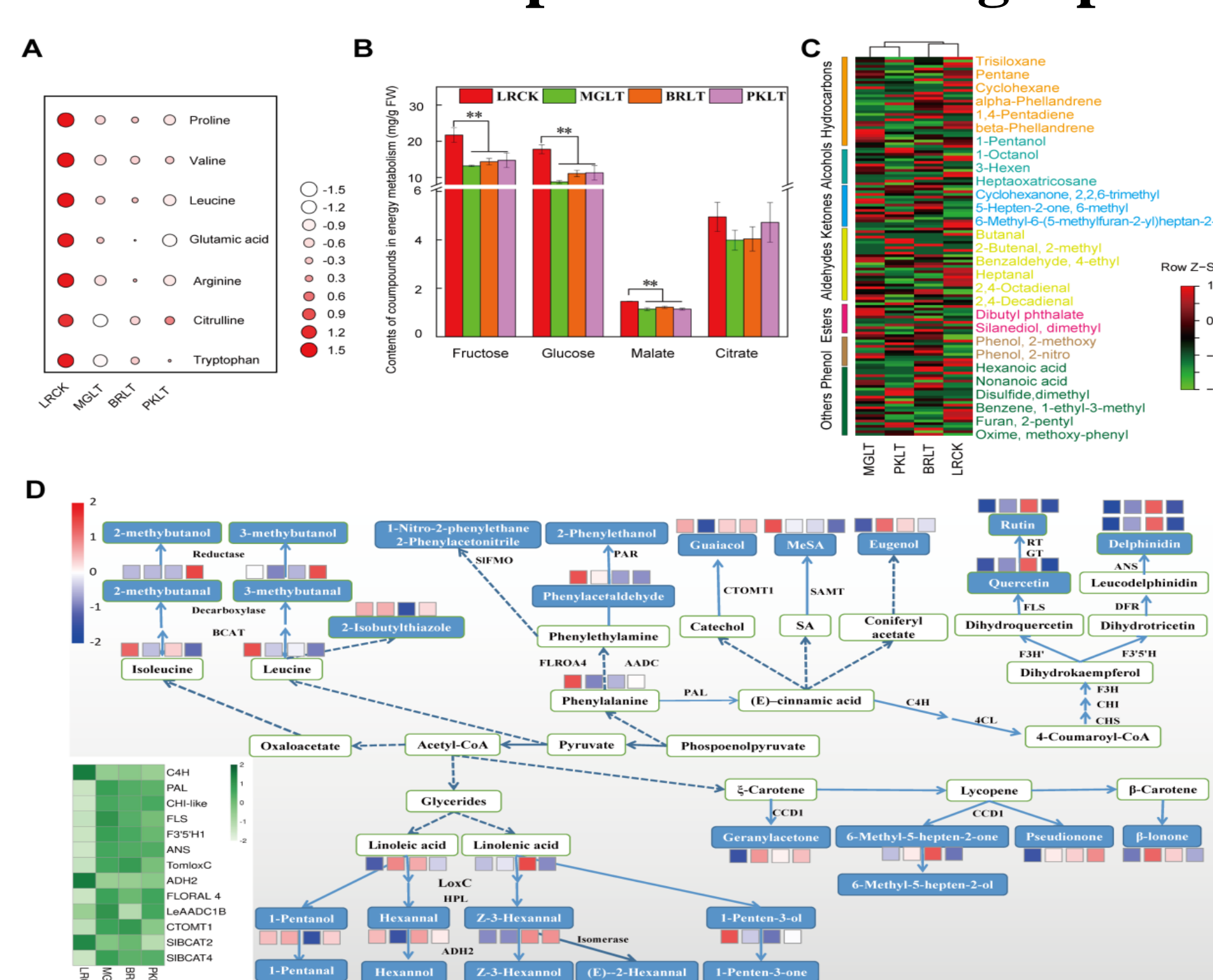
### 4.Differentially expressed genes in response to low temperature in tomato (AC) from different FRS



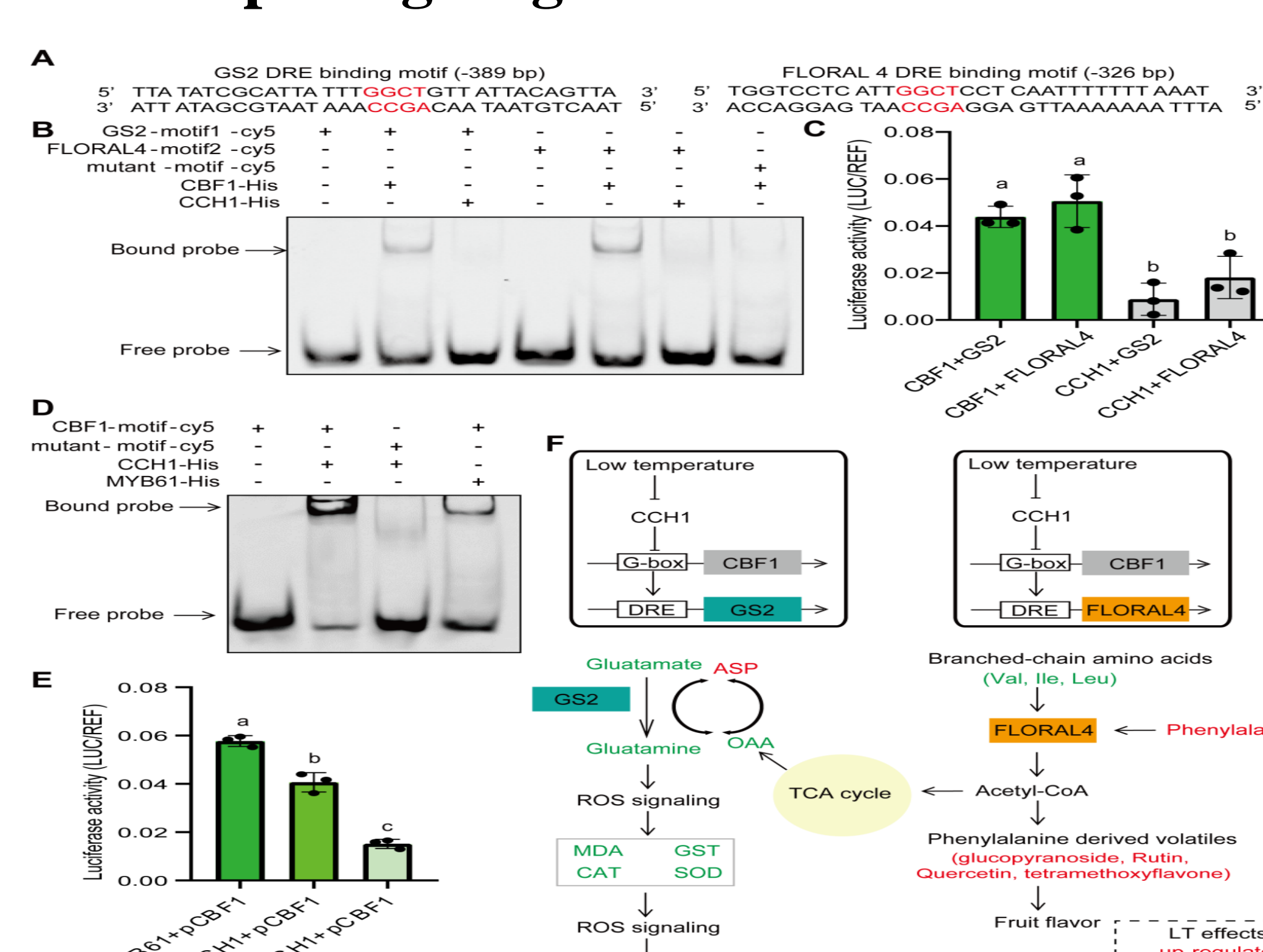
### 5. Non-targeted metabolites detected in tomato (AC)fruits exposed to different LT treatments.



### 6.Dynamic changes in the metabolic pathways in tomato fruits under low temperatures during ripening



### 7.CBF1 is inhibited by CCH1 gene under LT treatments at different ripening stages in AC.



## Acknowledgements

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## References

Aghdam MS, Luo Z, Jannatizadeh A, Sheikh-Assadi M, et al (2019) Employing exogenous melatonin applying confers chilling tolerance in tomato fruits by upregulating ZAT2/6/12 giving rise to promoting endogenous polyamines, proline, and nitric oxide accumulation by triggering arginine pathway activity, Food Chem, 275, 549-556.