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ANALYSIS OF KEY BIOLOGICAL PATHWAYS FOR SUGAR ACCUMULATION DURING KERNEL DEVELOPMENT IN DIFFERENT GENOTYPES OF SWEET CORN

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Abstract

Sweet corn as a nutritious, grain and vegetable crop plays a key role in the structural adjustment of the plant industry. The analysis of metabolic pathways of sugar accumulation during sweet corn kernel development is important for sweet corn breeding and efficient development. In this study, we used super sweet corn (T) and non-sweet corn (NT) as screened materials to carry out transcriptome analysis involved phenotypic parameters and physiological characteristics under two key periods of kernel development. Results showed that the key stage for the formation of sugar accumulation differences between super T and NT was 12 days after filling (GP12) to 20 days after filling (GP20), the proportion of soluble sugars of T decreased significantly compared with NT during this stage, while the proportion of sucrose and glucose of T increased significantly compared with NT. In terms of phenotypes, analysis showed that there was no significant difference in sugar accumulation between the two genotypes of sweet corn seeds at the two filling stages of GP12 and GP20. Transcriptome analysis revealed that 3175 genes were up-regulated and 3189 genes were up-regulated in the T and NT genotypes of sweet corn at the GP12 filling stage, while 3966 genes were up-regulated and 2809 genes were up-regulated at the GP20 filling stage, and clustering analysis indicated that LOC100382789, Zm00001d027703, and LOC1003043343 were significantly different from others. Meanwhile, GO analysis showed that seven biological processes, including Cyanoamino acid metabolism and Alanine aspartate, were the key regulatory pathways for sugar accumulation in sweet corn at GP12 and GP20 stages, among which Linoleic acid metabolism was significantly expressed in the middle and late stages of filling. In addition, KEGG enrichment differential analysis revealed that the key gene metabolic pathways Circadian rhythm-plant and Fatty acid metabolism were the key metabolic pathways for metabolic regulation of sugar accumulation in sweet corn. The study also mapped the target genes involved in metabolic pathways found by the KEGG enrichment analysis at the BP level, of which revealed 15 key metabolic pathways that are likely to be intrinsic to the differences in sugar accumulation between the two genotypes sweet corn. Sweet corn of super sweet genotype (T) could complete sugar accumulation through three types of key gene regulation, seven metabolic pathways and 15 metabolic pathways during the key stages of 12 days after filling (GP12) to 20 days after filling (GP20).

Key words: Sweet corn, Seed development, Sugar accumulation, Transcriptome, Metabolic pathway.

Introduction

Sweet corn, also known as fruit and vegetable crop, is a sweet subspecies of corn that has been widely developed in many countries around the world. It plays an important irreplaceable role in improving the dietary structure of people and promoting the restructuring of the cropping industry (Kelsey *et al*., 2017; Lakshmi *et al.*, 2020). Sweet corn can be classified as normal sweet corn, super sweet corn, and enhanced sweet corn, and currently, the most predominant on the market is super sweet, it was controlled by the endosperm mutation gene *sh2* (Kraiprom & Tumwasorn, 2017; Guo *et al*., 2018; Iedan & Alag, 2021). Sweet corn is a class of specialty corn in which soluble sugars such as fructose, sucrose and reducing sugars accumulate in large amounts in the kernels due to the presence of one or several mutant genes that block the synthesis pathway of seed starch (Promkhambut *et al*., 2010; Khan *et al*., 2017). The composition and content of carbohydrates in sweet corn kernels are important factors in determining quality (Iedan & Alag, 2021). Carbohydrates are mainly sugars, and the main forms of sugar accumulation in sweet corn kernels are sucrose, fructose and glucose, which constitute more than 90% of soluble sugars, and their sweetness in the order are fructose > sucrose > glucose, with sucrose content being the highest, accounting for 62% to 77% of total sugars, and showing a highly significant positive correlation with total soluble sugars (Khan *et al*., 2013; Naik *et al*., 2020; Zedan *et al*., 2020).

The mechanism of biological regulation of sugar accumulation during sweet corn kernel development is not very deep. Some scholars have shown that sugar accumulation in sweet corn during the filling period is related to betaine metabolism (Williams, 2017; Wang *et al*., 2018) some scholars also pointed out that Amino sugar key metabolic pathway is closely related to sugar accumulation in sweet corn (Priyatam *et al*., 2020). In addition, more scholarly studies generally agree that sweet corn sugar accumulation is closely related to variety type, that sugar metabolic pathways vary greatly among genotypes, and that sweet corn sugar accumulation varies with the developmental process of the kernel (Cheah *et al*., 2020; Milagres *et al*., 2021).

In this context, this paper investigates the phenotypic parameters, physiological characteristics and key biological metabolic pathways of sugar accumulation in two key periods of kernel development of sweet corn of two different genotypes, based on two near-isogenic genotypes of super-sweet (T) and non-sweet (NT), with the aim of investigating the characteristics of sugar accumulation in sweet corn, analyzing the key biological pathways of sugar accumulation in sweet corn, and then providing theoretical support for sweet corn variety selection, quality and efficient production. The aim was to

investigate the characteristics of sweet corn sugar accumulation and to analyze the key biological pathways of sweet corn sugar accumulation, so as to provide theoretical support for the selection and breeding of sweet corn varieties and the production of high quality and efficiency.

Materials and Methods

Test materials: Two materials, super sweet (T) and non-sweet (NT), were selected, from two hundred fifty-seven different sweet corn materials, both of this two genotypes sweet corn had similar growing period and were very similar in some traits such as plant height and plant shape, but differed greatly only in kernel sugar content.

Test site and conditions: The experiment was conducted in 2021 at the experimental site, affiliated Liaoning Academy of Agricultural Sciences (Shenyang, Liaoning Province, China. 123°22′ E, 41°71′ N). The average temperature in this experimental area during the growing season (May-October) was 20.1°C, rainfall 593.6 mm, and sunshine hours 6.8 h. The soil nutrient status was 0.12% total nitrogen, 0.17% total phosphorus, 1.91% total potassium, 64.17 mg· kg^{-1} hydrolytic nitrogen, 12.36 mg·kg^{-1} effective phosphorus, 117.65 mg·kg^{-1} effective potassium, and pH 6.9.

Experimental design: The experiment was conducted in a randomized group design with 6 rows, row length 3 m, row width 0.6 m, plot area 10.8 m^2 with three replications, planting density is set at 52,500 plants per ha with four protection rows around the experimental site.In terms of fertilizer application, 225 kg per ha (N 46.6%), 75 kg per ha of phosphorus P_2O_5 (calcium superphosphate, containing P_2O_5 12%) and 75 kg per ha of potassium K_2O (potassium chloride, containing K_2O 60%) application as a base fertilizer. Sweet corn(T) and non-sweet corn (N) were sampled at 12 (R1) and 20 (R2) days after pollination, respectively, and sequencing data were analyzed by eliminating the effects of genes involved in agronomic traits and resistance parameters between the two materials in an R2-R1 manner. Water supply disease, insect and weed pest field management was consistent with the normal level of the local field.

Determination of phenotypic parameters

Soluble sugars, sucrose and glucose: High performance liquid chromatography (HPLC) was used to determine the total soluble sugars, sucrose and glucose contents of super sweet corn SY01 and SY02. The chromatographic conditions were carried out as followed, the column was a Sepax HP-Amino column (4.6 mm × 250 mm, 5 μm),the detection cell temperature was 35℃, the column temperature was 28 ℃,the mobile phase was acetonitrile-water (80:20, V/V), the injection volume was 10 μL,the flow rate was 1.5 ml/min, and the detection cycle was 20 min for a single sample. The standard solutions of sucrose and glucose were prepared in ultrapure water at the concentrations of 0.5, 1, 2, 4 and 6 mg per ml, respectively.

Determination of the transcriptome on the mechanism of sugar metabolism

Selection of test materials: Twenty representative plants were selected for self-crossing with the date of pollination, and the middle seeds of corn were taken 12 days (R1) and 20 days (R2) after pollination in three replicates. Each sample was packed in 1.5 ml RNase free centrifuge tubes after sampling, put into liquid nitrogen and cooled rapidly at -80°C to prepare for total RNA extraction.

Extraction of total RNA from corn seeds: TaKaRa reagent for total RNA extraction was used. 0.1g of sweet corn seeds were ground under liquid nitrogen, the powder was transferred into a centrifuge tube, 1ml of RNAiso-mate was added to cover the sample and mixed well, and the sample was centrifuged at 4°C for 5 minutes at 12000×g, and the supernatant was taken into a new centrifuge tube. Add an equal volume of RNAiso Plus, shake to emulsify, and let stand at room temperature for 5 minutes. After that, add 1/5 volume amount of chloroform of the supernatant, shake for 15 seconds, let it stand at room temperature for 5 minutes, centrifuge at 12000×g for 15 minutes at 4°C, transfer the colorless supernatant to a new centrifuge tube, add equal amount of isopropanol, mix well and let it stand at room temperature for 10 minutes, centrifuge at 12000×g for 10 minutes at 4°C until a precipitate will appear at the bottom of the tube. Finally, take the supernatant, add 1 ml of 75 percentage ethanol, repeat three times, and discard the ethanol after instantaneous centrifugation at 12000×g at 4°C. After the ethanol evaporates (do not let the precipitate be too dry), dissolve in an appropriate amount of RNase-free ddH_2O, put the centrifuge tube with open cap on an ultra-clean table, leave it for 5 minutes, and put the RNA in -80℃ to avoid repeated freezing and thawing.

Transcriptome sequencing analysis: Illumina sequencing was used for bioinformatics and analysis. Among them, gene expression quantification was performed using Htseq software to extract the number of reads of genes, and the RPKM (Reads Per Kilo bases per Million reads) method was used to calculate gene expression.

The formula is:

$$RPKM = \frac{\text{ReadsTotal exon reads}}{\text{Mapped reads (millions)}*\text{exon length}}$$

and RPKM values were used to represent gene expression levels in transcriptome sequencing, and differentially expressed genes were screened by comparing data between samples, followed by clustering analysis of differential gene expression patterns, and differential gene statistics and analysis using the DESeq method. The differentially expressed gene enrichment analysis and

functional annotation involved the Cluster of Orthologous Groups (COG) nucleotide (Nt), Swiss-Prot, GO database, non -redundant (Nr), the Kyoto Encyclopedia of Genes and Genomes (KEGG).

The qRT-PCR analysis was used to detect and validate the detected differentially expressed genes. The quantitative primers were designed using primer 3.0 software, with product length fragments between 100bp and 300bp. Quantitative PCR experiments were performed using a Roche quantitative PCR instrument, Light Cycler® 480II (Roche) and SYBR Premix EX Taq reagent was used. The data of gene expression in qRT-PCR were generally consistent with the results of transcriptome sequencing.

Statistical data and analysis

Data were statistically analyzed using GraphPad Prism 5 and Microsoft Excel 2003 software, and all data were replicated at least three times.

Results and Analysis

Comparison of sugar accumulation at different kernel filling periods: Sweet corn (T) and non-sweet (NT) genotypes of kernel differed significantly in sugar accumulation at different stages of development (Table 1). Both T and NT genotypes of sweet corn kernels exhibited higher soluble sugars, sucrose and glucose in T than NT genotypes at five filling stages including GP8, GP12, GP16, GP20 and GP24. The percentage increase of soluble sugars decreased significantly in T compared with NT with the continuation of the filling time, while the percentage increase of sucrose and glucose in T increased significantly compared with NT. It is noteworthy that sugar accumulation increased was most rapid at the filling stage from GP12 to GP20, while the difference in sugar accumulation between T and N genotypes of sweet corn also remained at a high level. Therefore, the two key stages, GP12 and GP20, can be selected for analysis of gene regulation and metabolic pathways for sugar accumulation to resolve the intrinsic differences in sugar accumulation between T and NT genotypes of sweet corn. In addition, statistical analysis revealed that there were significant differences in kernel soluble sugars, sucrose and glucose between varieties and kernel filling periods, and significant differences in soluble sugars between varieties × kernel filling periods intercropping, while the differences in sucrose and glucose were not significant.

Analysis of phenotypic parameters for sugar accumulation in GP12 and GP20: In view of the significant differences in sugar accumulation between sweet (T) and non-sweet (NT) genotypes of sweet corn at the GP12 to GP20 filling stage, two periods including GP12 and GP20 were selected for transcriptome analysis of kernel sugar accumulation. At the filling stage (GP12 and GP20), both sweet (T) and non-sweet (N) kernels were faint and it was difficult to see differences by phenotypic (Figs. 1A, B). Moreover, T-M kernels accumulating less dry matter and showing dryness, while NT-M kernels were appearances full and accumulated significantly more dry matter than T-M (Fig. 1C). It indicated that sweet corn genotypes (T) could accumulate more kernels sugar in the mid-filling and early-filling stages compared with non-sweet genotypes (NT).

Quality testing of transcriptome data: The results of clean data analysis of two genotypes, two filling periods, and three replicates for a total of 12 samples from 12 libraries indicated the reliability of sequencing the data (Table 2, Fig. 2). Clean reads were higher than 98.0% for all samples. This indicates that clean data meets the requirements of transcriptome sequencing (Table 2). In addition, FPKM distribution and consistency heat map analysis of sweet corn samples of sweet (T) and not sweet (NT) genotypes showed that the data of biological replicates were uniform and the correlation between samples of the same group was high, indicating good consistency and reproducibility between biological replicates and the data were suitable for in-depth analysis (Fig. 2).

Table 1. Changes in kernel sugar accumulation in sweet (T) and non-sweet (N) genotypes of sweet corn at different kernel filling periods.

filling Period	Different genotypes of corn	Soluble sugar	Increase in T over N (%)	Sucrose	Increase in T over N (%)	Glucose	Increase in T over N (%)
GP8	T	85.3 ± 2.1a	289.5a	42.5 ± 1.2a	40.7e	27.4 ± 1.4a	13.7e
	NT	21.9 ± 1.3b		30.2 ± 1.5b		24.1 ± 1.2b	
GP12	T	86.9 ± 2.3b	169.0b	52.6 ± 2.3a	78.9d	25.3 ± 1.8a	18.2d
	NT	32.3 ± 1.6b		29.4 ± 0.9b		21.4 ± 1.6b	
GP16	T	88.3 ± 3.2b	122.4c	61.3 ± 2.1a	122.1c	23.6 ± 0.9a	26.9c
	NT	39.7 ± 2.4b		27.6 ± 1.5b		18.6 ± 1.1b	
GP20	T	93.6 ± 3.7b	98.3d	68.7 ± 2.5a	182.7b	21.4 ± 1.4a	54.0b
	NT	47.2 ± 1.9b		24.3 ± 3.3b		13.9 ± 1.3b	
GP24	T	93.9 ± 1.8b	83.4e	71.4 ± 2.8a	214.5a	19.7 ± 1.2a	75.9a
	NT	51.2 ± 2.1b		22.7 ± 4.7b		11.2 ± 0.8b	
Varieties - P-value		0.0002**	—	0.0001**	—	0.0004**	—
filling period - P-value		0.0062**	—	0.0029**	—	0.0047**	—
Variety × filling period- P-value		0.0371*	—	0.1338	—	0.2312	—

Note: Lower case letters indicate the comparisons between T and NT at the same filling Period (0.05 level of significance)

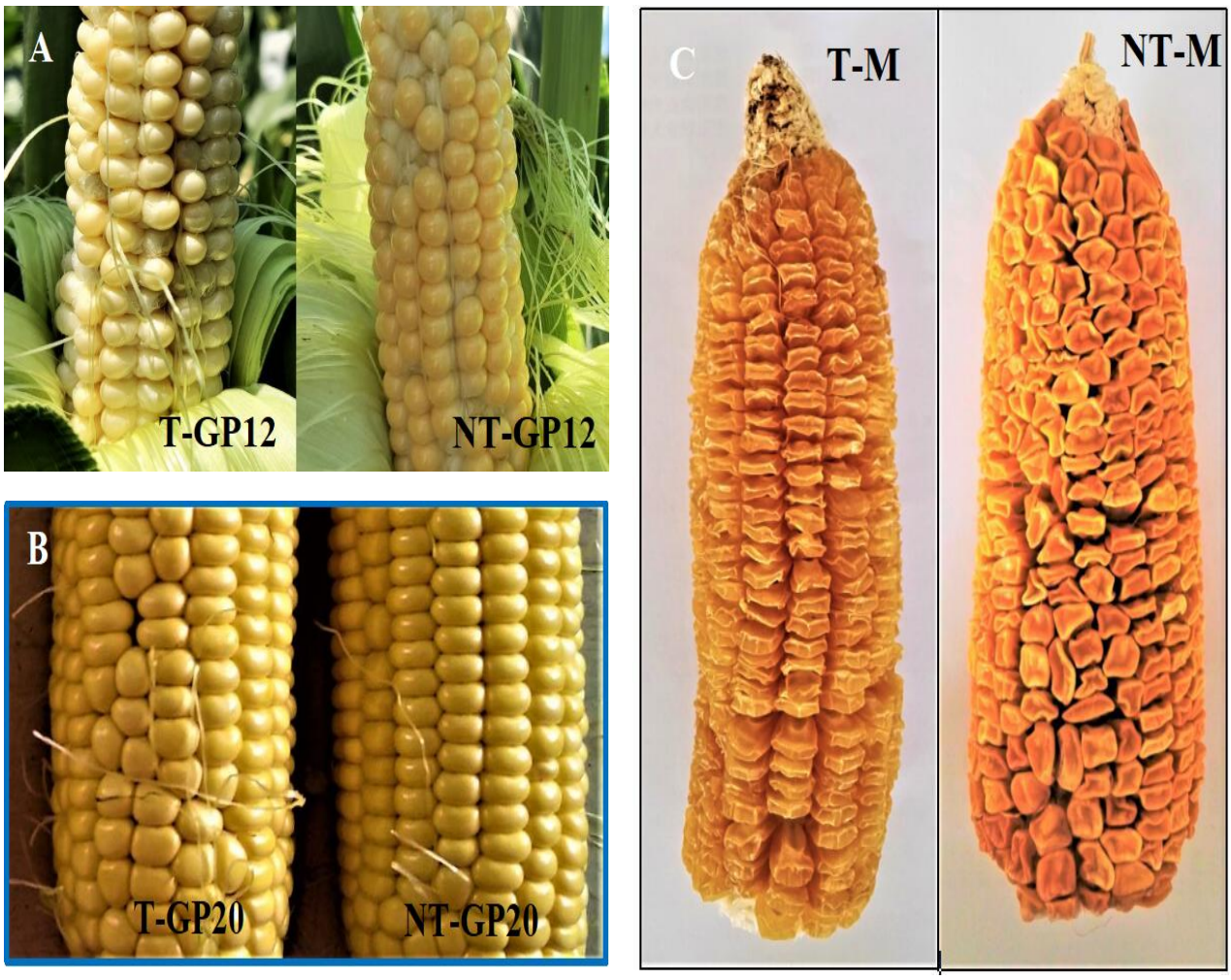


Fig. 1. Analysis of phenotypic parameters of kernels sugar accumulation at GP12 and GP20.

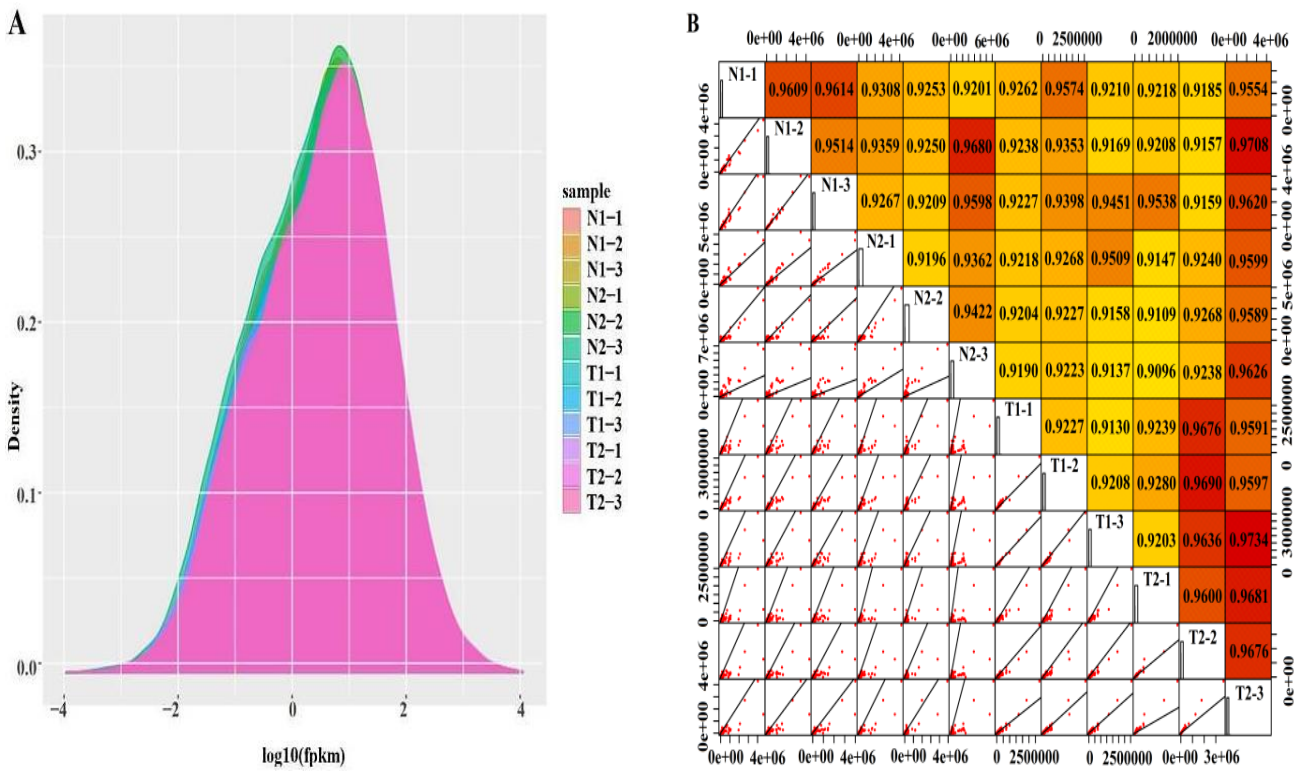


Fig. 2. FPKM distribution and consistency heat map analysis of sweet (T) and non-sweet (NT) genotypes of sweet corn samples.

Table 2. Quality analysis of transcriptome sequencing data for sweet (T) and non-sweet (N) genotypes of sweet corn.

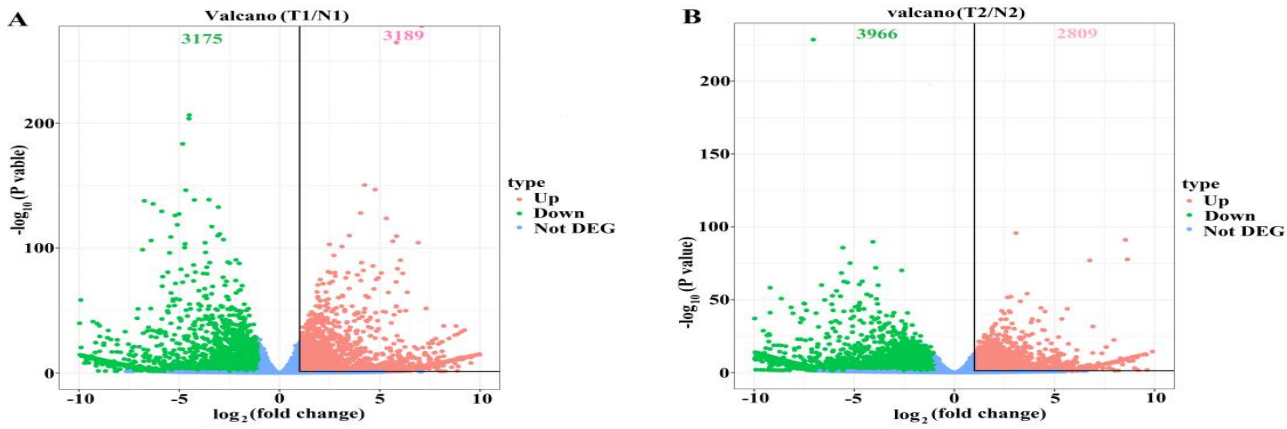
Different genotypes of corn	Sample	Clean data								Clean reads (%)
		R1				R2				
		Reads (Millions)	Bases (Millions)	Q20 (%)	Q30 (%)	Reads (Millions)	Bases (Millions)	Q20 (%)	Q30 (%)	
T	T1-1	25.7	3811.5	99.0	96.6	25.7	3793.4	97.3	93.1	98.3
	T1-2	29.9	4438.5	99.0	96.5	29.9	4417.8	97.4	93.4	98.4
	T1-3	27.4	4071.2	99.0	96.6	27.4	4051.5	97.3	93.1	98.5
	T2-1	26.5	3927.3	99.0	96.5	26.5	3910.2	97.6	93.8	98.5
	T2-2	23.6	3504.4	99.0	96.6	23.6	3487.4	97.3	93.2	98.4
	T2-3	23.2	3436.6	99.0	96.6	23.2	3421.3	97.5	93.5	98.3
	T平均	26.1	3864.9	99.0	96.6	26.1	3846.9	97.4	93.4	98.4
N	N1-1	25.0	3657.6	99.0	96.6	25.0	3645.1	98.0	94.7	98.7
	N1-2	24.2	3587.3	99.0	96.6	24.2	3571.2	97.3	93.2	98.6
	N1-3	24.6	3657.4	99.0	96.6	24.6	3640.3	97.3	93.0	98.3
	N2-1	26.6	3945.3	99.0	96.7	26.6	3926.3	97.3	93.1	98.1
	N2-2	24.6	3660.4	99.0	96.5	24.6	3643.2	97.4	93.3	98.1
	N2-3	32.7	4850.3	99.0	96.8	32.7	4828.4	97.4	93.4	98.3
	N平均	26.3	3893.1	99.0	96.6	26.3	3875.8	97.5	93.5	98.4

Note: Clean Reads(R1): number of R1-end Reads from QC data; Clean Bases(R1): number of R1-end bases from QC data; Clean Q20(R1): percentage of Q20 bases from R1-end Reads from QC data; Clean Q30(R1): percentage of Q30 bases from R1-end Reads from QC data Clean Bases(R2): number of bases on the R2 side of the data after QC; Clean Bases(R2): number of bases on the R2 side of the data after QC; Clean Q20(R2): percentage of bases on the Q20 side of the R2 side of the data after QC; Clean Q30(R2): percentage of bases on the Q30 side of the R2 side of the data after QC Clean Reads Ratio: percentage of Reads that passed QC

Differential gene expression: Analysis of the detected differential mRNA expression scatter, clustering heat map and volcano plot for sweet (T) and non-sweet (NT) genotypes revealed that there were differences in the number of up-regulated and down-regulated genes and the types of gene aggregation in both GP12 and GP20 filling stages (Fig. 3). The analysis found that 3175 genes were up-regulated and 3189 genes were up-regulated in T vs NT at GP12 filling stage, while 3966 genes were up-regulated and 2809 genes were up-regulated at GP20 filling stage,meanwhile,3966 genes were up-regulated and 2809 genes were up-regulated at the GP20 filling stage. The clustering analysis of these genes revealed that the genes were clustered into five categories at the GP12 filling stage, with significant variation in three categories of sweet (T) and non-sweet (NT) genotypes of sweet corn varieties, LOC100382789, Zm00001d027703, and LOC100304334, etc. The genes were clustered into four categories at the GP20 filling stage, AZS22-16, Zm00001d027703, and Zm100304334. LOC100273695, Zm00001d016318 and other genes differed significantly. In addition, the differential clustering analysis of the genes detected at the GP20 and GP12 filling stages showed that the genes detected in the GP12 and GP20 screening clusters were basically closely related to kernels accumulation.

GO analysis of differentially expressed genes: GO analysis of differentially expressed genes in sweet (T) and non-sweet (NT) genotypes of sweet corn at 2 filling stages(GP12 and GP20) revealed differences in the types and amounts of genes enriched in sorghum at different filling stages (Fig. 4). At the GP12 filling stage, tetrapyrrole binding, transferase activity, transferring hexosyl groups, hydrolase activity, hydrolyzing O-glycosyl compounds. At the MF level, the enrichment of four genes, hydrolase activity, acting on glycosyl bonds were the highest at the CC level, the enrichment of cofactor catabolic process genes were significantly higher than the number of other genes.The enrichment of three genes, cellular lipid process and lipid metabolic process, were higher at the MF level, but significantly lower than the number of more genes at the BP and CC levels. At the GP20 filling stage, Cyanoamino acid metabolism, Alanine,aspartate and glutamate metabolism, Porphyrin and chlorophyll metabolism, Nitrogen metabolism, and Riboflavin metabolism, and the overall number of genes was significantly higher than that of the GP12 filling stage. At the CC level, the enrichment of Linoleic acid metabolism genes tended to be consistent with the trend of the GP12 stage, while at the MF level, the number of Linoleic acid metabolism genes was higher than that of the GP12 filling stage. The number of Linoleic acid metabolism genes was significantly higher than that of GP12 stage, and was significantly higher than that of other species. It indicates that Cyanoamino acid metabolism, Alanine, aspartate and glutamate metabolism, Porphyrin and chlorophyll metabolism, Nitrogen metabolism, Riboflavin metabolism, Linoleic acid metabolism and Linoleic acid metabolism are the key regulatory genes for sugar accumulation in sweet corn at GP12 and GP20 stages, among which Linoleic acid metabolism was significantly expressed in the middle and late stages of filling.

In order to deeply analyze the characteristics of sugar accumulation gene regulation in sweet corn during the filling period, a differential analysis of the genes detected in GP20 and GP12 during the filling period was performed (Fig. 4C). The results of the analysis were basically consistent with the analysis of the graph 4 (Fig. 4A, B), further indicating that these seven genes play a key role in sugar accumulation, and also suggesting that the regulation of the BP biological process (five genes involved) is likely to be the key biological process in sugar regulation in sweet corn.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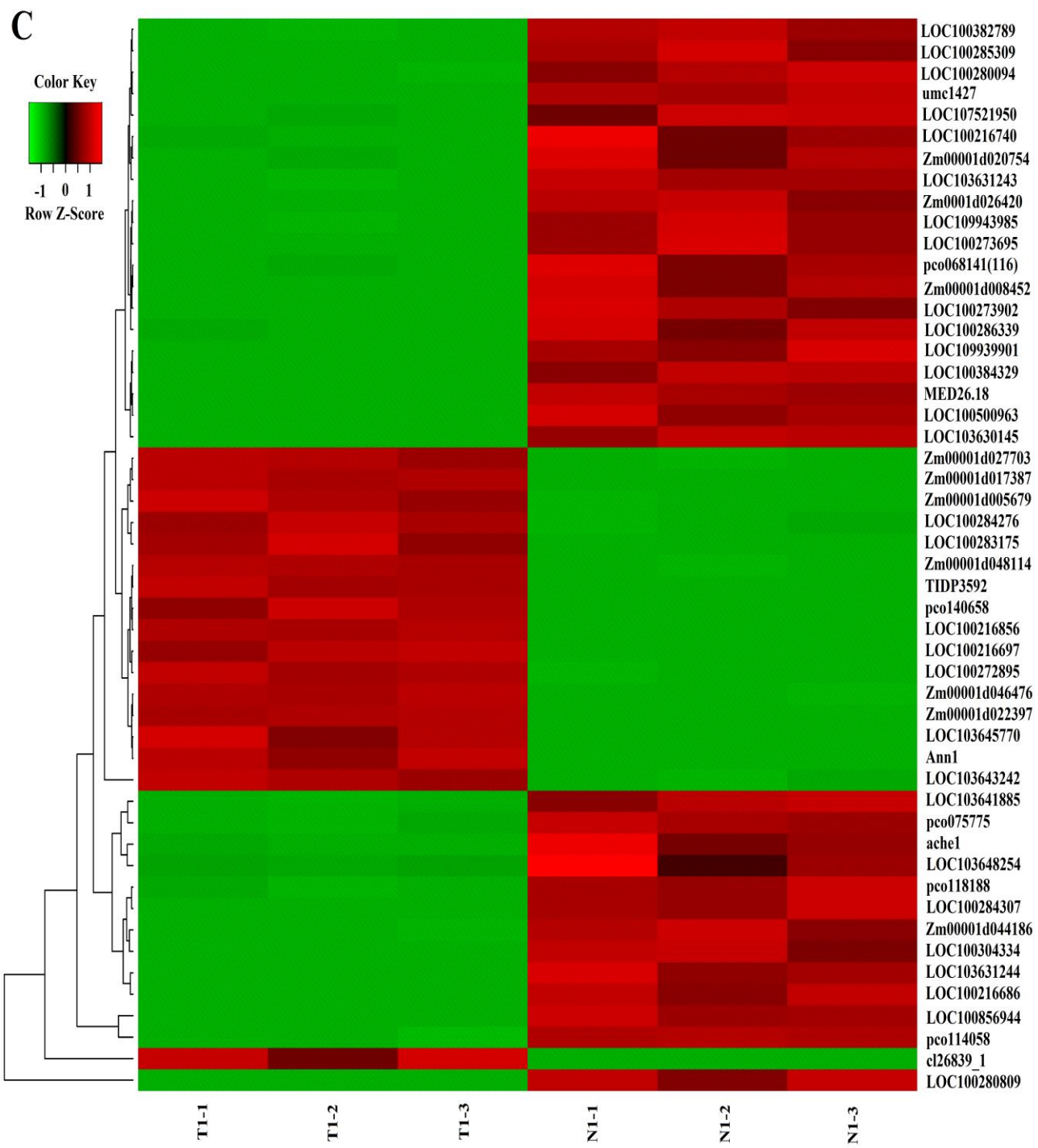
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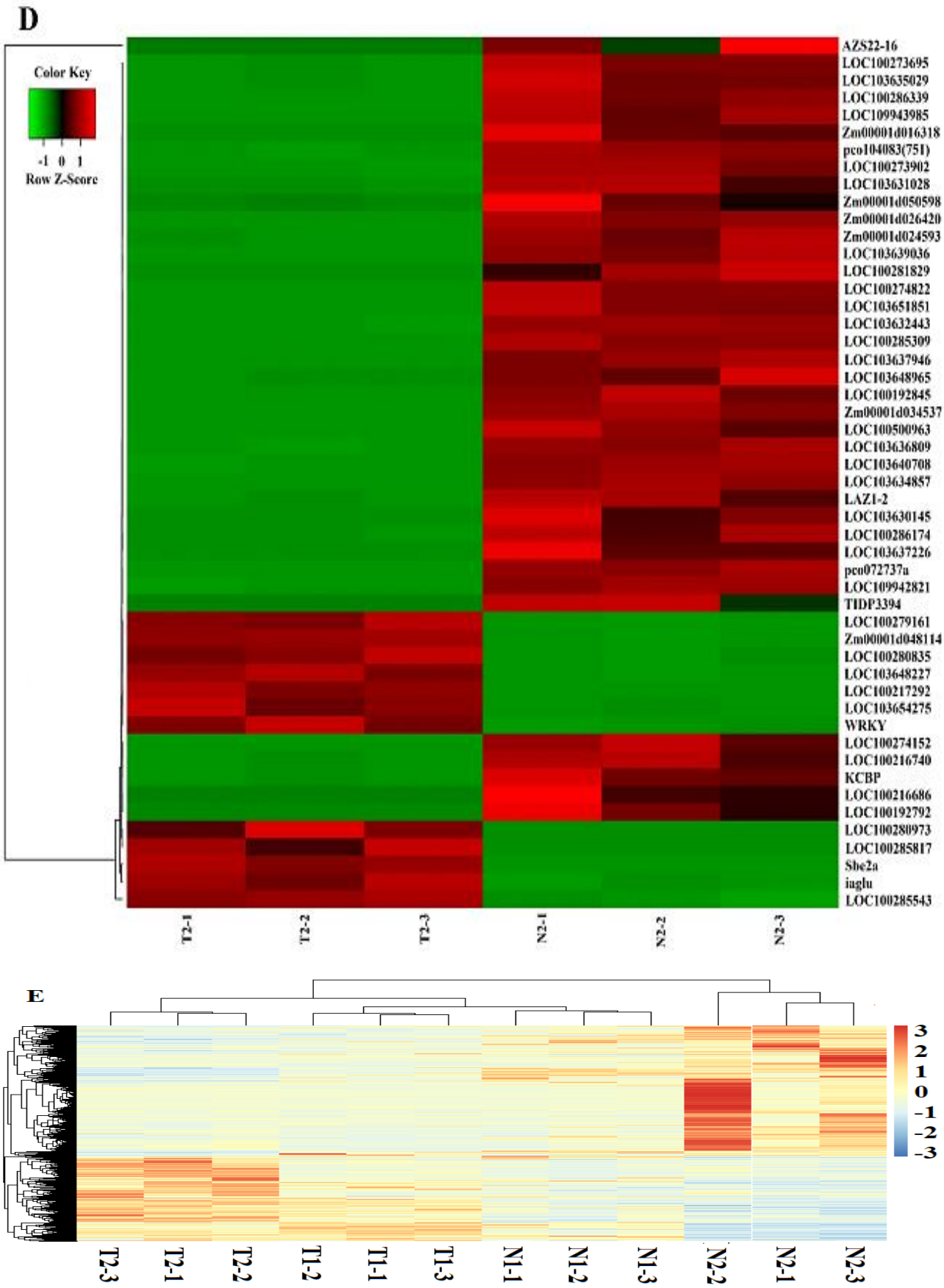

Fig. 3. Scatter, cluster heat map and volcano plot analysis of differential mRNA expression in T and NT genotypes of sweet corn GP12 and GP20.

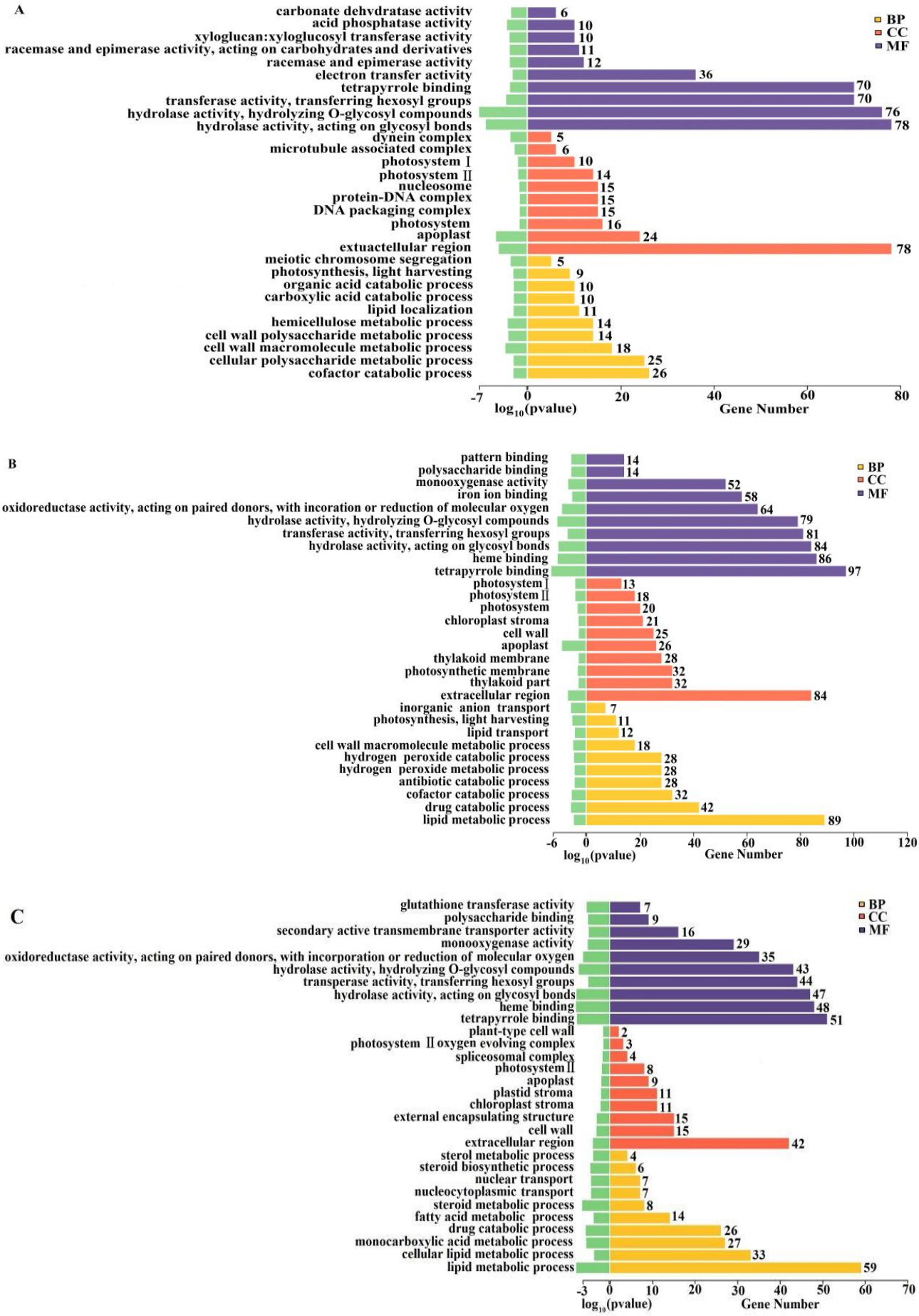

Fig. 4. GO enrichment analysis of differentially expressed genes in sweet corn of different genotypes at different filling periods.

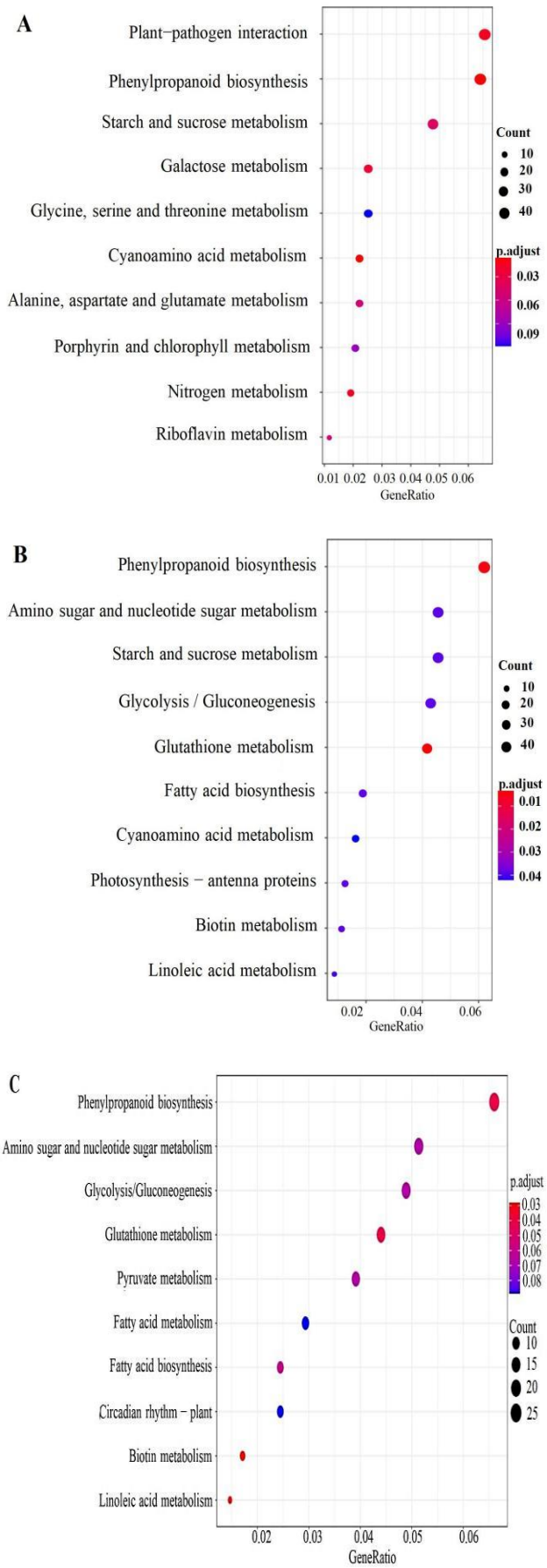

Fig. 5. KEGG enrichment analysis of differentially expressed genes in sweet (T) and non-sweet (NT) genotypes of sweet corn at different filling periods.

KEGG enrichment analysis of differentially expressed genes: KEGG enrichment analysis of the 10 most significantly differentially expressed genes revealed differences in the metabolic pathways of key genes between the two genotypes of sweet corn at the 2 filling stages of GP12 and GP20 (Fig. 5). The most significant differences in Glycine, serine and threonine metabolism were found at the GP12 stage of filling, but from the gene ratio analysis, Plant-pathogen interaction, Phenylpropanoid biosynthesis, and Starch and sucrose metabolism, Phenylpropanoid biosynthesis, Amino sugar and nucleotide sugar metabolism, Glycolysis/ Gluconeogenesis, and Glutathione metabolism and Pyruvate metabolism were enriched in a larger proportion of five genes, among which Amino sugar and nucleotide sugar metabolism, Glycolysis /Gluconeogenesis, and Glutathione metabolism. This indicates that Glycine, serine and threonine metabolism play a key role in sugar accumulation at the GP12 stage in sweet corn, while Plant-pathogen interaction, Phenylpropanoid biosynthesis, and Starch and sucrose metabolism are likely to play a key role in sugar accumulation in sweet corn. Sucrose metabolism is likely to play an important role in metabolic regulation,Amino sugar and nucleotide sugar metabolism, Glycolysis/ Gluconeogenesis, and Glutathione metabolism play a key role in GP20 stage sugar accumulation, and Phenylpropanoid metabolism is likely to play a key role in GP20 stage sugar accumulation. Phenylpropanoid biosynthesis and Pyruvate metabolism are also likely to play key roles in GP20 metabolism.

Analysis of the difference in KEGG enrichment of two genes at GP12 and GP20 during the perfusion period revealed that the key gene metabolic pathways were in general agreement with the results of previous (Fig. 5A, B) studies. It indicates that Circadian rhythm - plant and Fatty. Acid metabolism is a key metabolic pathway in the metabolic regulation of sugar accumulation in sweet corn, while Phenylpropanoid biosynthesis, Amino sugar and nucleotide sugar metabolism, Glycolysis/ Gluconeogenesis, and Glutathione metabolism, and Pyruvate metabolism are likely to be the main reasons for the differences in sugar accumulation between sweet (T) and non-sweet (NT) genotypes.

Analysis of differentially expressed mRNA-enriched metabolic pathways: The target genes involved in the metabolic pathways found by KEGG enrichment analysis were mapped to the corresponding KEGG pathways at the BP level, and 17 key metabolic pathways were identified (Fig. 6). Further analysis showed that among the 17 key metabolic pathways identified, GO: 0006629 lipid **metabolic process** 0.138 59/472 and GO:0008202 steroid metabolic pr... 0.332 8/272 metabolic pathway genes showed upregulation, GO: 0008610 lipid biosynthetic p... 1.000 29/251, GO:1901615 organic hydroxy comp... 1.000 9/61 and GO: 0044255 cellular lipid metab... 1.000 33/291 and 15 other metabolic pathway genes were down-regulated. It is suggested that these metabolic pathways are likely to be intrinsic to the differences in sugar accumulation between the two genotypes of sweet corn at the 2 filling stages of GP12 and GP20.

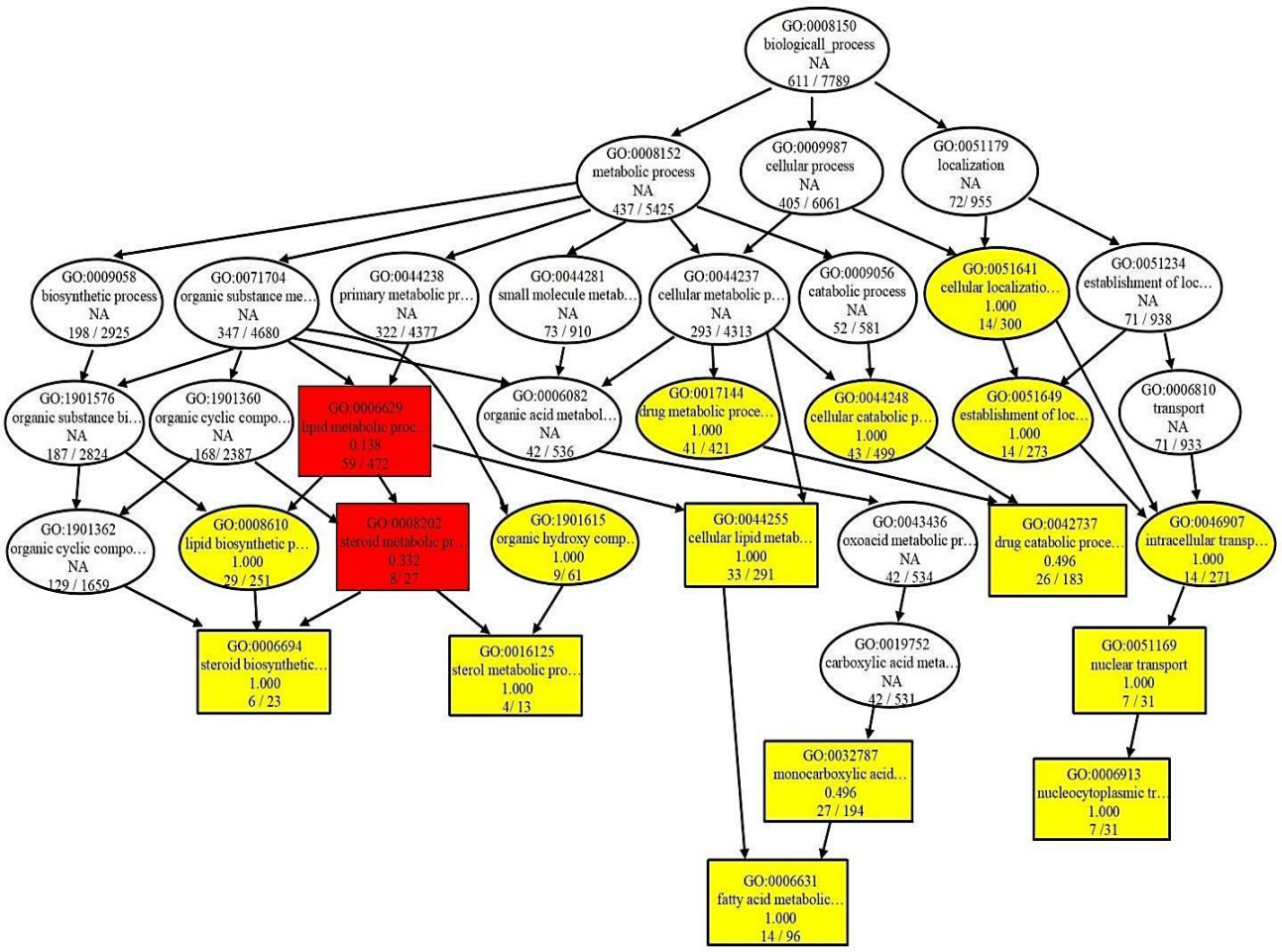

Fig. 6. Metabolic pathway of BP differentially expressed mRNA enrichment in sweet (T) and non-sweet (NT) genotypes of sweet corn at different filling periods.

Discussion

The filling period as a critical stage of sweet corn growth and development has been widely noticed by scholars (Fei *et al.*, 2016; Gavric & Omerbegovic, 2021). In this study, we found that the period from 12 to 20 days after filling was critical for sugar accumulation in super sweet (T) and non-sweet corn (NT), mainly in the form of a significant increase in the accumulation of soluble sugars, sucrose and glucose in super sweet (T) compared tonon-sweet corn (NT). The results of this study are in general agreement with those derived from studies such as Williams *et al*., 2017, and with those of numerous scholars who have studied the critical role of sugar accumulation in sweet corn during the grubbing period (Zainul *et al*., 2015; Zhu *et al*., 2021; Diao *et al*., 2021).

In the study of differential mechanisms of sugar accumulation in sweet corn, the results of this study showed that sweet (T) and non-sweet (NT) genotypes of sweet corn vary significantly in the filling stage LOC100382789, Zm00001d027703 and LOC1003043343 genes, which play a key role in the role of sky accumulation. The results of this study Yang *et al*., 2021, Gavric & Omerbegovic, 2021 have been similarly reported in the study of sugar sugar and nutrient accumulation mechanisms in sweet corn, but the differential genes were different and may be related to crop species differences Li *et al*., 2017, Chen *et al*., 2020. In terms of key regulatory pathways for sugar accumulation, the present study found that Cyanoamino acid metabolism, Alanine, aspartate and glutamate metabolism, Porphyrin and chlorophyll metabolism, Nitrogen metabolism, Riboflavin metabolism, Linoleic acid metabolism and Linoleic acid metabolism were found to play an important role in the accumulation of days at the GP12 and GP20 stages in sweet corn. Among them, Alanine, aspartate and glutamate metabolism, Porphyrin and chlorophyll metabolism, Nitrogen metabolism, Riboflavin metabolism, and Linoleic acid metabolism 5 pathways have been previously reported (Song *et al*., 2015; Zhang *et al*., 2016; Denwar *et al*., 2021; Cai *et al*., 2021), but Cyanoamino acid metabolism, Riboflavin metabolism have been rarely reported in previous studies. Therefore, the regulatory mechanisms of these 2 metabolic pathways remain to be explored and verified in depth. In the present study, we found that Linoleic acid metabolism was significantly expressed in the middle and late stages of perfusion by transcriptome Go analysis, which is in general agreement with the findings of Liu *et al*., *2005*, Rosa *et al.*, 2020, Naik *et al*., 2020, which may be related to the difference in test materials. In addition, the analysis of KEGG enrichment difference between super sweet (T) and non-sweet corn (NT) revealed that Circadian rhythm-plant and Fatty acid

metabolism are the key metabolic pathways for metabolic regulation of sugar accumulation in sweet corn, which plays an important reference for us to analyze the intrinsic mechanism of sugar accumulation in sweet corn. The results showed that Circadian rhythm-plant and Fatty acid metabolism are the key metabolic pathways for sweet corn sugar accumulation.

Conclusion

GP12 to GP20 is the critical stage in the formation of differences in sugar accumulation between super-sweet (T) and non-sweet corn (NT). There were no significant differences in sugar accumulation seeds between the two genotypes of sweet corn during this period, and the percentage of increase in soluble sugars was significantly lower in sweet (T) than in non-sweet (NT) genotypes of sweet corn, and sucrose and glucose increased significantly. Super-sweet (T) sweet corn could be affected by seven biological processes such as LOC100382789, Zm00001d027703, LOC1003043343 three types of genes, Cyanoamino acid metabolism, Alanine aspartate and Circadian rhythm -plant and Fatty acid metabolism key gene metabolic pathways to regulate sweet corn sugar accumulation, and through 17 key metabolic pathways to regulate the accumulation of sugar in sweet corn kernels at this filling stage, to achieve the accumulation of sugar in kernels at the key stage of filling.

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