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TRANSCRIPTOMIC CHARACTERIZATION AND ANALYSIS OF KEY BIOLOGICAL PATHWAYS ASSOCIATED WITH APHID RESISTANCE IN SORGHUM (*SORGHUM BICOLOR* L. MOENCH)

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Abstract

Aphid damage is one of the most critical biotic stresses limiting sorghum production, worldwide. In this study, two sterile sorghum lines having aphid-resistant (APT) and aphid-sensitive (APS) phenotypes, along with eight hybrid lines were used to study the infestation and natural attack rates of aphids in three ecological zones. The results show that sorghum has a dominant genetic effect on aphid resistance, with aphid incidence ranging from 93.2% to 96.6% in the F_1 generation of crosses mated to APT sorghum sterile lines. APT vs APS transcriptome analysis suggested the ATP sorghum genotype may be able to stimulate defenses against aphid infestation, experienced less change in sugar and chlorophyll content than the APS genotype. The APT genotype also exhibited improved in vivo carbohydrate binding and endopeptidase inhibitor activity, which are two key biological metabolic pathways which are involved in inhibiting aphid feeding and growth, by converting glucose in organs to pyruvate in the cytoplasm via the glycolysis metabolic pathway, thereby reducing glucose content in tissues and creating an inhospitable environment for aphid growth.

Key words: Aphid, Biotic stresses, *Aphis sacchari zehntner*, Sorghum

Introduction

The sorghum aphid (*Aphis sacchari zehntner*) belongs to the order Homoptera, family Aphididae, and is distributed throughout many parts of Asia, Africa, and the Americas Khan *et al*., 2013; Bacaha *et al*., 2015; Calvin *et al*., 2020. It is a serious threat in areas such as Liaoning, Shandong, and Hebei of China, where it is one of the most critical biotic stresses limiting growth of the sorghum industry and sustainable agricultural development Wang *et al*., 2013. In addition to direct detrimental effects caused by sorghum aphid feeding, such as impaired nutrient transport and reduced photosynthate production capacity, aphids also transmit nearly 100 different viruses, which can lead to even greater yield losses Tolmay Vicki *et al*., 2020. While the sorghum aphid primarily feeds on sorghum, it can also infest sugarcane, wheat, barley, millet, corn, among other crops. It is a highly destructive, rapidly reproducing pest, causing about 15% reductions in crop yield in a typical year, and failure to control the aphid in high pressure years may cause extremely severe yield reductions and even complete crop failure Vieira *et al*., 2019.

Plant resistance to aphids can be controlled by single or multiple genes. In many plant species, aphid resistance is typically controlled by dominant single genes Dogimont *et al*., 2010; Porras Mitzy *et al*., 2018, such as wheat resistance to *Diuraphis noxia*, barley and rye resistance to *Aphis glycines*, and the soybean genes *Rag l*, *Rag 2 and Rag 3* which are responsible for resistance to *Aphis glycines* Liu *et al*., However, resistance to aphids can also be controlled by recessive single genes, such as the wheat *dn3* gene resistance to the wheat bobtail aphid, peanut resistance to the cowpea aphid (*Aphis craccivora*), and maize resistance to the maize aphid (*Rhopalosiphum maidis*) Nkongolo *et al*.,1991; Herselman *et al*., 2004; Catena & Glogoza, 2004. The resistance of sorghum to aphids varies according to the species of aphids affecting sorghum. Currently, sorghum aphids have the greatest impact on sorghum growth, but despite this there are fewer on aphid resistance in sorghum to aphids than there are on wheat resistance to wheat dinoflagellate aphids Yang *et al*., 2021. A genetic study of sorghum aphid resistance using the aphid-resistant line TAM 428 found that sorghum aphid resistance was controlled by a pair of genes that showed incomplete dominant inheritance and stable resistance Wenqing *et al*., 1985, while the results of Chang *et al*., found sorghum aphid resistance controlled by a pair of dominant single genes Jinhua *et al*., 2006.

Plants generally use two defense mechanisms to defend against pathogens and insects, namely constitutive and inducible defenses, and under healthy conditions constitutive defenses predominate Du *et al*.,. Li and Liu S Q. made crosses of the sorghum varieties BTAM 428 (highly resistant) and ICS-12 B (highly susceptible), and using the F_2 generation, found that the number of leaf stomata in aphid-resistant sorghum was higher than that of aphid-susceptible lines, while the diameter of vascular bundles was smaller than that of aphid-susceptible lines Xianying & Shiqiang, 2004. Paudyal *et al*., found that leaf sugar content and chlorophyll content were key factors contributing to aphid resistance through a study on resistance of different sorghum genotypes to the sorghum aphid, and their relationship to physical traits of sorghum Paudyal *et al*., 2019. It has also been noted that leaves with high nitrogen, sugar, and chlorophyll content are more susceptible to aphids, while those with high phosphorus, potassium, and polyphenol contents are less susceptible to aphids (Tao *et al*., 2020).

Currently, bioinformatics analysis of transcriptome data remains one of the most effective means of studying the molecular mechanisms of aphid resistance in sorghum, and the development of high-throughput sequencing technologies has allowed comprehensive resolution of gene expression changes that occur during plant-insect interactions Wang *et al*., 2018. In potato, soybean, and wheat, gene expression profiles in resistant and susceptible varieties before and after aphid feeding have been analyzed using genetic techniques Park *et al*., 2006. Wang *et al*., studied differences in gene expression profiles of sorghum before and after feeding by the wheat bifurcation aphid and found that gene expression relating to defense, mechanical damage, phytohormone signaling pathways, secondary metabolism, transmembrane transport and cooperation was closely associated with sorghum aphid resistance Wang *et al*., 2018. Uchimiya *et al*., used cDNA microarray technology to compare the metabolic pathways of resistant and susceptible sorghum varieties after infestation with wheat bifurcation wax and suggested that the resistance response of sorghum requires coordinated control of plant hormones such as salicylic acid, jasmonic acid, abscisic acid, growth hormone, and gibberellin Uchimiya *et al*., 2019. The above studies have laid the foundation for further studies on the molecular mechanisms of aphid resistance in crop plants, but they have generally been conducted on the wheat dinoflagellate aphid, with very few studies having been reported on the molecular mechanisms of resistance to the sorghum aphid.

In this study, we used sorghum aphid resistant and sorghum aphid salt-sensitive sorghum sterile lines and their hybrids as materials to determine the aphid infestation and natural attack rates in different ecological zones. Additionally, the physiological and molecular mechanisms of aphid resistance in sorghum were investigated in order to determine key metabolic and regulatory pathways that may be involved in aphid resistance in sorghum, and provide theoretical support for sorghum aphid resistance breeding programs.

Materials and Methods

Test materials

Male sterile lines (A) of sorghum having two different aphid resistance genotypes: The aphid-resistant sorghum male sterile line LA34 and aphid-sensitive sorghum male sterile line P03A were used as parental source materials, in which both LA34 and P03A harbored several materially excellent genes, with excellent general compatibility, high specific compatibility, resistance to leaf spot, drought, flooding, and other agriculturally beneficial characteristics, and a number of hybrids were grouped and cultivated respectively.

Six sorghum recovery lines (R): Six grain restoration sorghum lines, LR2381, LR415, LNR-4, LNR-6, NK1, LR9198, were grown in groups with LA34 and P03A.

Eight F_1 generation hybrids: Four hybrids of aphid-resistant male sterile sorghum lines, LA34 with LNR-4, LNR-6, NK1, and LR9198, LN 11 (LA34/NK1), LN 10 (LA34//LNR-4), LN 7 (LA34//LNR-6), and L 3498 (LA34/LR9198), were made. Four hybrids of aphid-sensitive male sterile sorghum lines, P03A and LR2381, LNR-4, LNR-6, and LR415 were mated, LZ37 (P03A/LR2381), LXL1 (P03A/LNR-4), LZ79 (P03A/LNR-6), and LZ23 (P03A/LR415) were made.

Experimental design and conditions: The experiment was conducted in Shenyang (SY, 123°38′E, 41°8′N), Liaoning Province of China, for 3 consecutive years from 2018 to 2020 to identify and analyze the incidence of aphids in the F_1 generation of two sorghum sterile lines, six restorer lines, and eight crosses of their groupings after infestation with aphids. Additionally, the incidence of aphids in the F1 generation of LN 11 (LA34 grouping with aphid-resistant genotype) and LZ79 (P03A grouping with aphid-sensitive genotype) was determined and analyzed in natural settings of three different ecological zones, including Shenyang (SY, medium-temperature humid zone), Chaoyang (CY, high-temperature arid zone, 120°42′E, 41°58′N) and Jinzhou (JZ, medium-temperature semi-arid zone, 121°15′E, 41°13′N). A randomized group design was employed, with 6 row zones, 3m row length, 0.6m row, 10.8m^2 plot area, three replications, and field management conducted normally as in local fields.

Sorghum aphid infestation & resistance identification: After the growth of sorghum spikes (about 70 days after sowing), test plants were manually infested with aphids to identify aphid resistance associated with the genotypes of sorghum parental lines (sterile and restorative lines) and hybrid F_1 lines. Aphids collected from natural field populations and maintained as indoor live plant cultures were used for the study. On the second day of the survey, aphids were applied the cultivation of aphids to sorghum leaves as a carrier using staples in sorghum upper number 7 leaves, the specific site is 1/3 of the distance from the back of the leaf stalk, applying 50 aphids per leaf. 20 plants per treatment were surveyed for aphid incidence, and the average number of aphids per plant were calculated; surveys were repeated three times. The score of aphid resistance was determined with reference to the sorghum aphid resistance grading standard developed by the Chinese Crop Variety Resources Identification Professional Group [Weiyu J, 1985], which is classified into five levels according to the number of aphids in the field. Specifically, level 1 is highly resistant, level 2 is resistant, level 3 is moderately resistant, level 4 is susceptible, and level 5 is highly susceptible.

Leaf soluble sugar content and chlorophyll content: Soluble sugars and chlorophyll content were determined in new leaves taken from the middle of the seventh upper leaf where the aphids were inoculated, 2 days before and 4 days after aphid inoculation. Soluble sugar and chlorophyll contents were determined following the methods described by Wang X Wang *et al*., 2019.

Transcriptome analysis of aphid-resistant and aphid-sensitive genotypic sterile male sorghum lines: RNA was extracted from tissue sampled from the middle of the upper seventh leaf 2 days before and 4 days after aphid inoculation, and subjected to Illumina sequencing and the resulting data was bioinformatically analyzed. Gene expression quantification was performed using Htseq software to extract the number of reads of genes, and gene expression was calculated using the RPKM (Reads Per Kilo bases per Million reads) method.

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

The formula is, and gene expression levels in the transcriptome sequencing data were expressed as RPKM values. Data between different samples was compared to screen for differentially expressed genes (DEGs), and subsequent analysis of DEG expression patterns was performed by clustering analysis using DESeq, with Fold Change ≥ 2 and FDR < 0.01 used as screening criteria. Functional annotation and enrichment analysis of DEGs was performed using the non-redundant (Nr), nucleotide (Nt), Swiss-Prot, Kyoto Encyclopedia of Genes and Genomes (KEGG), the Cluster of Orthologous Groups (KEGG), the GeneNet, The Cluster of Orthologous Groups (COG) and the GO databases.

To confirm the accuracy of the sequencing results, RT-qPCR analysis was performed to validate the accumulation of differentially expressed genes. Primer design was performed using primer 3.0 software, with product length fragments between 100 and 300 bp. Quantitative PCR experiments were performed using a Roche quantitative PCR Light Cycler® 480II (Roche), using SYBR Premix EX Taq reagents. The gene expression data based on RT-qPCR were generally consistent with the sequencing results, showing that the transcriptome results were accurate.

Statistical analysis of data: DPS 7.05 and Excel 2007 software were used for statistical analysis of the data.

Results and Analysis

Phenotypic effects of aphid infestation in sterile sorghum lines =: Aphid populations on APT (LA34) and APS (P03A) sorghum lines differed significantly after aphid infestation (Fig. 1). Of the 20 infested (50 aphids/plant) sorghum plants measured, APT (LA34) had mean values of 2.2, 3.2 and 2.4 in 2018, 2019 and 2020, respectively, while APS (P03A) had 37.6, 34.9 and 37.8, respectively, with significant differences between the two lines (Test 0.05 = 3.82*). It is worth noting that in 2019 the APT (LA34) line had one strain (No. 5) with a significantly higher number of aphids than the other samples, which could be related to genotypic variation in this strain or small environmental changes in the field. Additionally, phenotypically we can see that at the same trial site APT (LA34) had very low shoot susceptibility and bright green leaves, while APS (P03A) was significantly affected by aphid infestation and developed yellow leaves which adversely affected tasseling (Fig. 2).

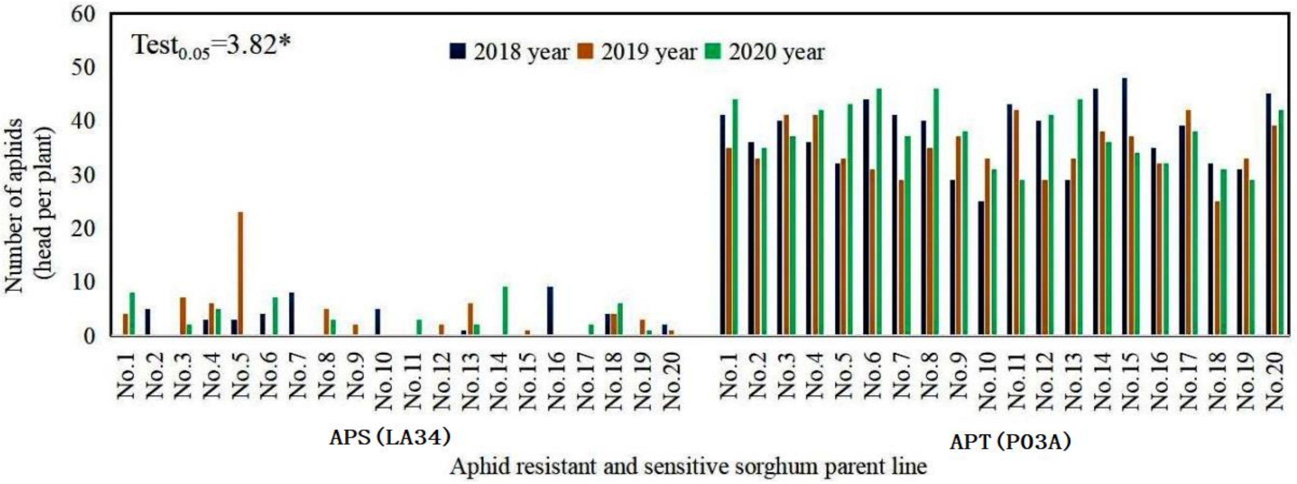

Fig. 1. Changes in aphid numbers in aphid-resistant and aphid-sensitive sorghum sterile lines after aphid inoculation.
Note: Data were obtained from a random sample of 20 aphid-resistant and aphid-sensitive sterile sorghum lines.

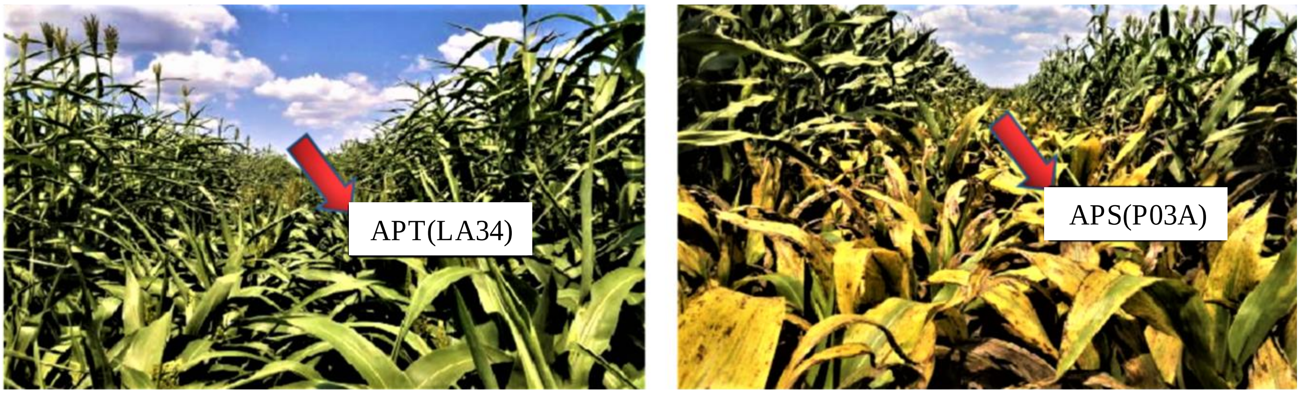

Fig. 2. Comparison of phenotypes of aphid-resistant and aphid-sensitive sorghum sterile lines after aphid inoculation.

Table 1. Genetic effects of aphid incidence in the F_1 generation of two sterile lines APT (LA34) and APS (P03A) and hybrids after infestation with aphids.

Hybrids	Hybrid combinations (♀×♂) Cross	Aphid incidence (%)			Average aphid incidence in F_1 (%)	F_1 aphid incidence coefficient CV (%)	F_1 aphid incidence correlated with parental			F_1 Aphid resistance grade
		♀	♂	Average			Biased maternal effect	between two parents	Biased paternalism effect	
LN11	LA34/NK1	0.4a	96.4a	48.2a	1.2c	12.4a	+	-	-	Level 1
LN10	LA34//LNR-4	0.9a	15.8b	7.9b	3.6b	7.7b	+	-	-	Level 1
LN7	LA34//LNR-6	0.5a	3.8c	1.9c	1.3c	2.1c	+	-	-	Level 1
L3498	LA34/LR9198	1.0a	98.2a	49.1a	4.8a	15.3a	+	-	-	Level 1
	Average	0.7	52.6	26.3	2.7	9.4	+	-	-	Level 1
LZ37	P03A/LR2381	91.3a	3.1c	47.2b	65.7a	11.5a	-	+	-	Level 4
LXL1	P03A/LNR-4	91.3a	15.8b	53.6ab	73.2a	13.2a	-	+	-	Level 4
LZ79	P03A/LNR-6	91.3a	3.8c	45.7b	66.6a	8.7b	-	+	-	Level 4
LZ23	P03A/LR415	91.3a	43.6a	67.5a	69.7a	6.9b	-	+	-	Level 4
	Average	91.3	15.6	53.5		10.1	-	+	-	Level 4
T-test for the mean of 2 parental lines		*	*	ns	**	ns				

Note: Data are mean values for 2018-2020, "**" indicates $p<0.01$; "*" indicates $p<0.05$, "ns" indicates difference are not significant. "+" indicates in this range, "-" indicates not in this range. Lowercase letters indicate APT (LA34) & APS (P03A) compared with the four restoration lines and hybrids they were mated with, respectively. F_1 resistance to aphids was graded into five levels: level 1 (highly resistant) to level 5 (highly susceptible)

Genetic effects of parental lines and F_1 generation hybrids on aphid incidence after infestation: The averaged data from the three year study (2018 to 2020) showed that the two male sterile sorghum lines APT (LA34) and APS (P03A) were significantly different from the F_1 generation hybrids mated to the four restoration lines, with respect to aphid resistance (Table 1). The variation in aphid incidence in the F_1 generation hybrids mated to the four restoration lines of APT (LA34) was 2.1-15.3%, while aphid incidence in the F_1 hybrids of the sorghum hybrids mated with APS (P03A) restoration lines ranged from 93.2-96.6%, with the mean value of APT (LA34) being 92.1% higher than that of APS (P03A). In contrast, the F_1 hybrids of the APS (P03A) group showed a slightly higher maternal, than paternal, effect. In general, the genetic association with aphid resistance of the maternal (sterile) line in the F_1 generation of hybrids showed a dominant genetic effect.

Genetic effects of the F_1 generation of sorghum parental lines crossed with hybrids on aphid incidence under natural infestation conditions: The results of three consecutive years of trials from 2018 to 2020 showed that there was a large difference in the natural incidence of aphids in the F_1 generation of hybrids crossed with APT (LA34) and APS (P03A) lines and their groupings under natural aphid infestation conditions (Table 2). Under natural conditions, the aphid incidence of APT (LA34) in the three ecological zones of SY, CY and JZ was 0, exhibiting high resistance (level 1), while the aphid incidence of the F_1 generation hybrid LN11 was extremely low, at only 0.5% to 7.3%, thus also exhibiting high resistance. APS (P03A) had 39.1% to 68.1% aphid incidence in SY, CY, and JZ zones, which varied from year to year, exhibiting moderate (level 3) to severely susceptible (level 5) resistance, and its F_1 generation hybrid LZ79 had higher aphid resistance than APS (P03A). Additionally, statistical analysis revealed that the natural occurrence of aphids on F1 generation hybrids differed significantly among varieties (*P value=0.002***), locations (*P value=0.035**) and years (*P value=0.043**), indicating that in addition to genetic factors, environmental factors played key roles in influencing aphid occurrence.

Changes in sugar and chlorophyll content before and after aphid infestation: As can be seen in (Table 3), the differences in sugar and chlorophyll content between APT (LA34) and APS (P03A) genotypes of sorghum before and after aphid infestation were significant. Soluble sugar content was lower in APT (LA34) than in APS (P03A) before aphid infestation, and the increase in soluble sugar content after aphid infestation was significantly higher in APS (P03A) than in APT (LA34). This suggests that the high sugar content of the leaves of APS-type sorghum varieties may be an important factor in their susceptibility to shoot, and that the transfer of sugars to the leaves is intensified after susceptibility to shoot, which in turn affects the transport of nutrients to the spike. The results of chlorophyll analysis showed that differences in chlorophyll content between APT (LA34) and APS (P03A) lines before bud sensing was not significant, but after bud sensing APS (P03A) chlorophyll content decreased rapidly, which in turn limited photosynthetic material production.

Table 2. Aphid incidence in the F1 generation of hybrids crossed with APT (LA34) and APS (P03A) sorghum lines and their groupings under natural aphid infestation conditions.

Hybrids	Test location	Year	Parent (sterile line)			F_1 generation hybrids		
			Natural incidence of aphids (%)	Coefficient of variation CV (%)	Aphid resistance grade	Natural incidence of aphids (%)	Coefficient of variation CV (%)	Aphid resistance grade
LN11	SY	2018	0a	-	Level 1	5.9a	2.1a	Level 1
		2019	0a	-	Level 1	0.7c	1.8a	Level 1
		2020	0a	-	Level 1	2.3b	1.3b	Level 1
	CY	2018	0a	-	Level 1	3.8b	1.6a	Level 1
		2019	0a	-	Level 1	0.2c	0.3c	Level 1
		2020	0a	-	Level 1	4.9a	0.7b	Level 1
	JZ	2018	0a	-	Level 1	7.3a	2.0a	Level 1
		2019	0a	-	Level 1	0.5c	0.6c	Level 1
		2020	0a	-	Level 1	1.3b	1.1b	Level 1
LZ79	SY	2018	63.2ab	5.9b	Level 5	35.3a	11.6a	Level 4
		2019	68.1a	4.8c	Level 5	22.9b	5.4c	Level 3
		2020	49.8b	12.3a	Level 4	24.7b	7.8b	Level 3
	CY	2018	45.3a	4.7b	Level 4	28.4b	3.3b	Level 3
		2019	41.3ab	6.3a	Level 4	35.6c	8.4a	Level 4
		2020	49.7b	2.5c	Level 4	38.2a	8.2a	Level 4
	JZ	2018	72.7a	9.7a	Level 5	23.9b	6.5c	Level 3
		2019	56.8b	11.2a	Level 5	35.2a	12.3a	Level 4
		2020	39.1c	10.4a	Level 3	23.7b	8.9b	Level 3
P-value between locations			-	-	-	0.035*	-	-
P-value between years			-	-	-	0.043*	-	-
P-value between hybrids			-	-	-	0.002**	-	-

Note: "**" indicates *p<0.01*; "*" indicates *p<0.05*, "ns" indicates non-significant difference. Lowercase letters indicate comparisons among the three years 2018, 2019, and 2020 in the same ecological zone. F1 resistance to aphids is classified into five levels: level 1 - highly resistant, level 2 - resistant, level 3 - moderately resistant, level 4 - susceptible, and level 5 - highly susceptible. "-" indicates that no analysis was done

Table 3. Changes in sugar and chlorophyll content of aphid-resistant and aphid-sensitive sterile sorghum lines before and after aphid infestation.

Hybrids	Lines	Sugar content (%)			Chlorophyll content (mg.g^{-1}.FW)		
		Before aphid infection	**After aphid infection**	**Increase (%)**	**Before aphid infection**	**After aphid infection**	**Increase (%)**
APT (LA34)	2018	0.27a	0.27a	0.27a	1.48a	1.47a	0.68b
	2019	0.23b	0.23b	0.23b	1.53a	1.52a	0.65b
	2020	0.25ab	0.25ab	0.25ab	1.52a	1.50a	1.32a
	Average	0.25	0.25	0.25	1.51	1.50	0.88
APS (P03A)	2018	0.31b	0.36a	16.1b	1.47a	0.98b	33.33a
	2019	0.28c	0.35a	25.0a	1.49a	1.23a	17.45b
	2020	0.32b	0.38a	18.7b	1.55a	1.06b	31.61a
	Average	0.29	0.36	24.14	1.50	1.09	27.49
P-value between hybrids		0.871ns	0.003**	0.001**	0.746ns	0.008**	0.004**
P-value between years		0.264 ns	0.037*	0.135ns	0.619ns	0.045*	0.031*
Hybrids * Year P value CHRN**CHENGYI CHENGYI		0.593ns	0.142ns	0.031*	0.318ns	0.041*	0.561ns

Note: "**" indicates $p<0.01$; "*" indicates $p<0.05$, and "ns" indicates non-significant difference. Lowercase letters indicate comparisons of APT (LA34) and APS (P03A) lines between years 2018, 2019, and 2020

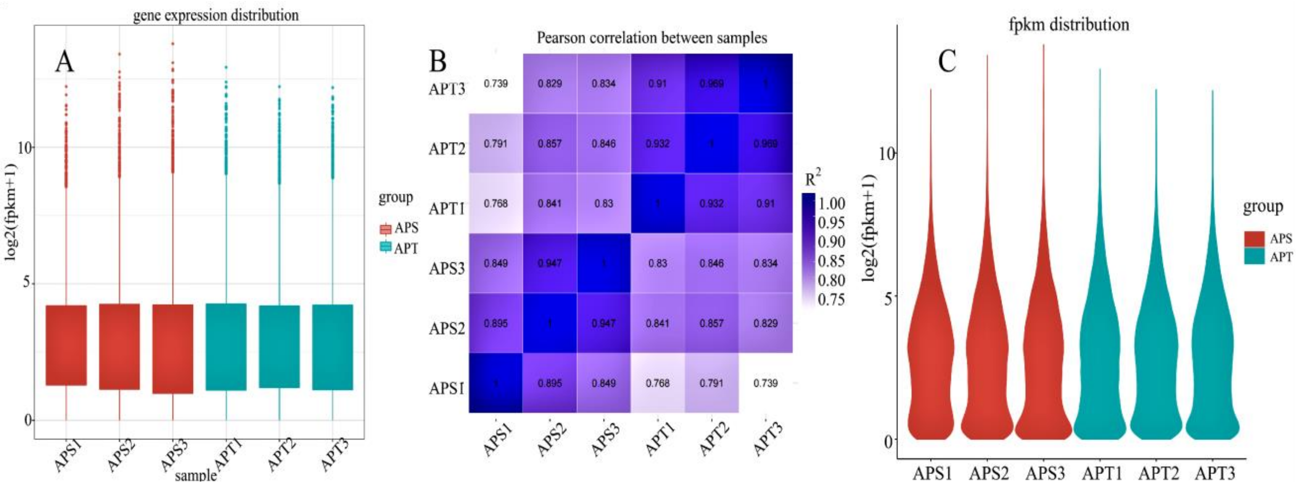

Fig. 3. (A, B, C) Quality check of transcriptome data of APT he APS samples.

Metabolic mechanism of aphid resistance in sorghum

Quality testing of transcriptome data: The results of the sample assays showed that the gene length distribution pattern was consistent and the gene expression distribution was uniform (Fig. 3A). In addition, for the measured data of six samples (APT and APS 2 sorghum lines, 3 replicates each) the filtered Pearson correlation between samples were in the range of 0.739 to 0.989 (Fig. 3B), which fully met the requirements of the assay. The results of the FPKM distribution analysis table also confirmed high accuracy of the tested samples and good quality of the data, which facilitated downstream the analysis of the data (Fig. 3C).

Differential gene expression associated with aphid resistance: Differentially expressed genes identified by comparison between APT (LA34) and APS (P03A) sorghum lines were analyzed in depth, the results of the DESeq assay were screened according to the differential significance criteria (more than 2-fold change in differential gene expression and FDR<=0.05), and the up- and down-regulation of significantly differentially expressed genes was counted. A total of 3535 differentially expressed genes were detected, of which 1834 were up-regulated and 1701 were down-regulated (Fig. 4A, B). Clustering analysis classified these genes into 2 main categories.

GO analysis of differentially expressed genes: Gene ontology (GO) analysis revealed that aphid resistance in APT (LA34) and APS (P03A) genotypes of sorghum is apparently regulated by genes falling into three categories, "Biological process' (BP), 'Cellular components' (CC), and Molecular function (MF) (Fig. 5). Analysis of the 10 associated biological processes that best fit within the three Categories (BP, CC, MF) showed that APT (LA34) was more resistant than the APS (P03A) sorghum genotype. In the BP category, genes relating to 'DNA integration', 'response to biotic stimulus', 'defense response three biological metabolism -log10 (padi)' were more highly expressed than others; 'drug transport metabolism -log10 (padi)' was highest in CC, and 'ADP binding', 'Carbohydrate binding Pattern binding', and 'Polysaccharide binding' were highest in the MF category. This indicates that the APT (LA34) genotype may stimulate resistance and detoxification responses through bio-stress response regulation more efficiently than the APS (P03A) sorghum genotype, and those activities such as DNA integration, carbohydrate, binding, Polysaccharide binding may also be involved in the resistance response to aphids.

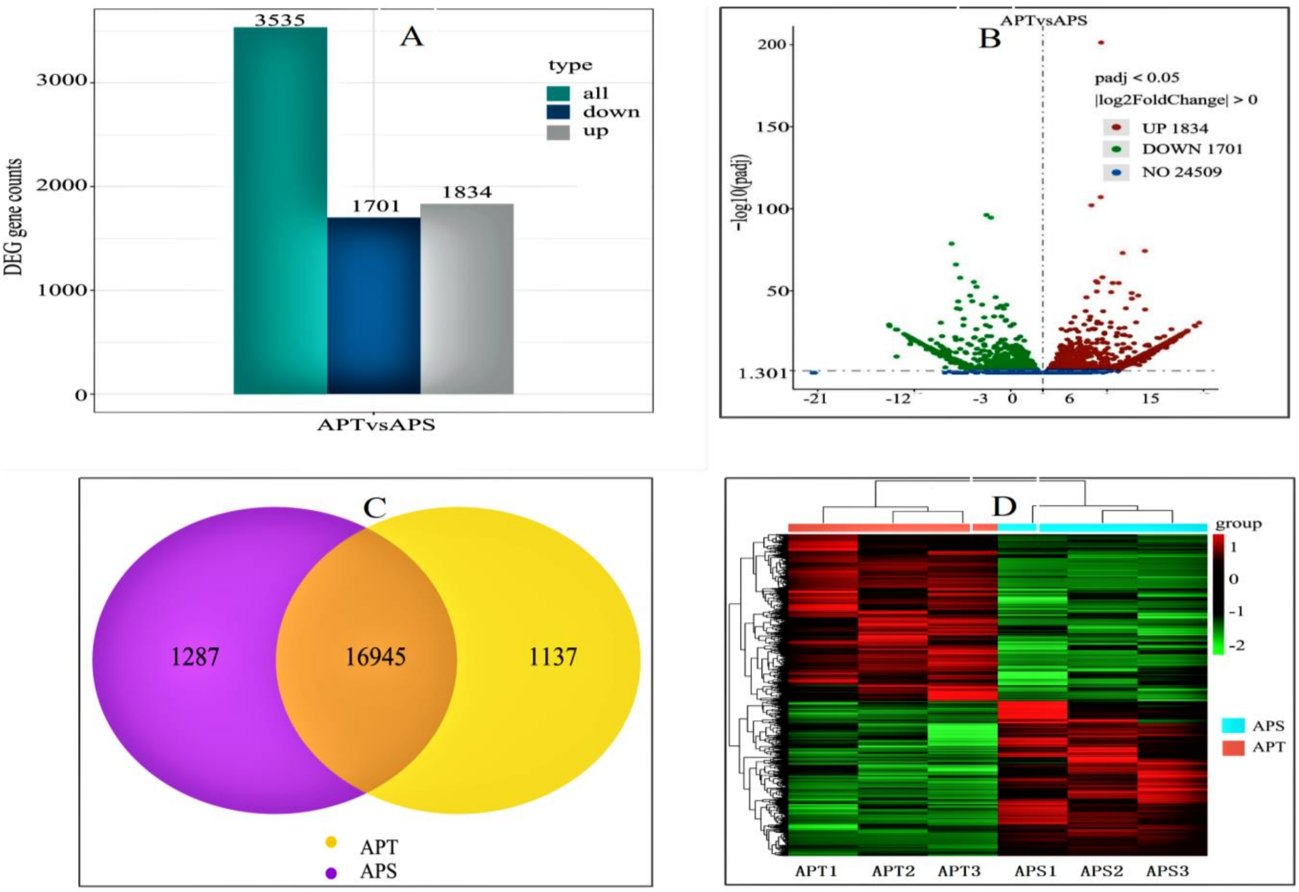

Fig. 4. (A, B, C, D) APT vs APS differential gene expression related to aphid resistance.

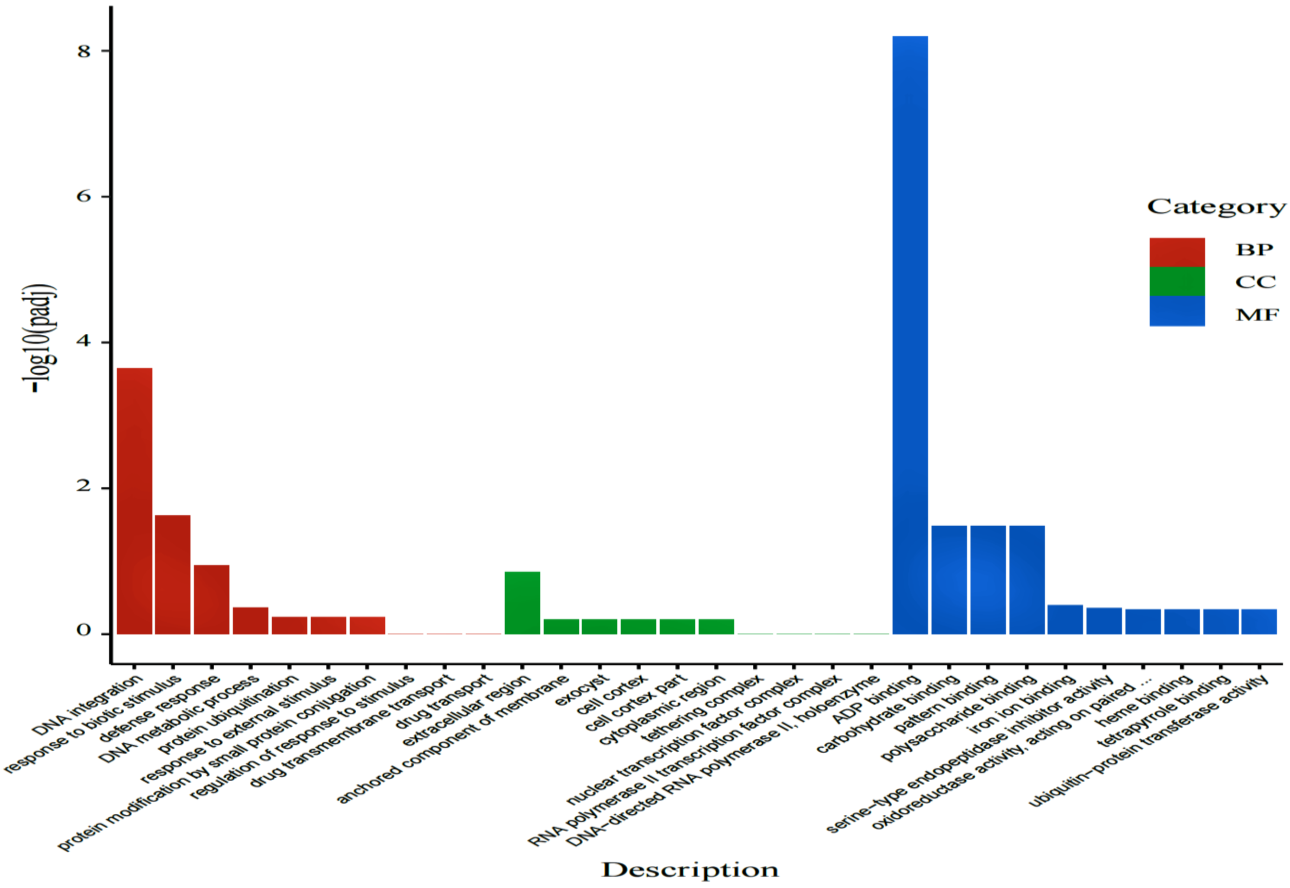

Fig. 5. APT vs APS differentially expressed gene GO (Gene ontology) analysis.

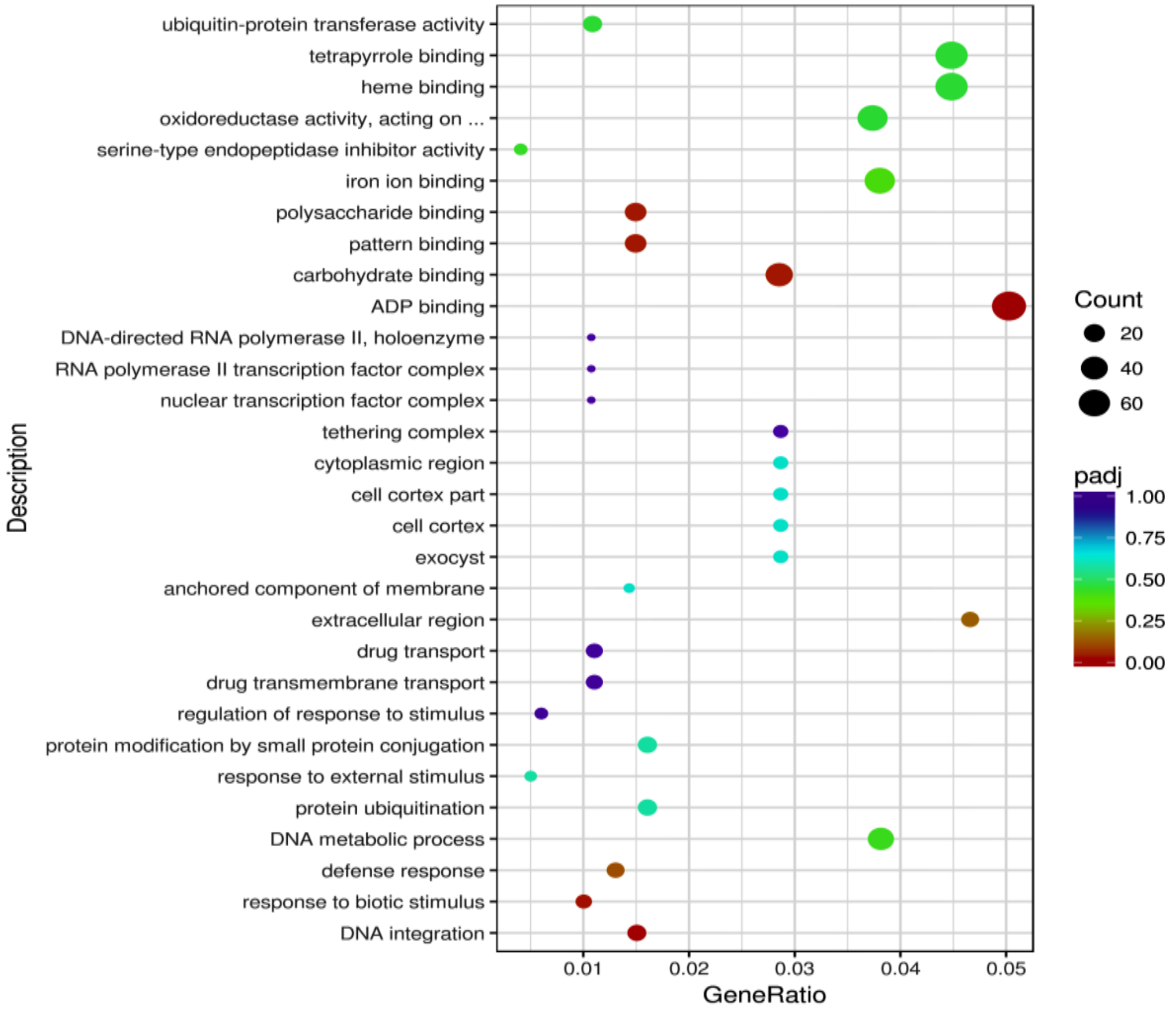

Fig. 6. KEGG analysis of differentially expressed genes in APT vs APS metabolic pathway.

KEGG analysis of metabolic pathways of differentially expressed genes: Analysis of differentially expressed genes based on the Kyoto encyclopedia of genes and genomes (KEGG) database revealed that APT (LA34) and APS (P03A) sorghum genotypes differed greatly gene expression of physiological metabolic pathways in response to aphid damage (Fig. 5). Analysis from a generational perspective revealed that ADP binding, tetrapyrole binding, heme binding, tetrapyrrole binding, oxidoreductase activity, serine-type endopeptidase inhibitor activity, iron ion binding, and carbohydrate binding were potentially critical in the regulation of aphid resistance in sorghum (Fig. 6).

Discussion

Genetics of aphid resistance in Sorghum: A great deal of work has been done on the inheritance of plant resistance to aphids, and in general, resistance can be attributed to either single or multiple gene control mechanisms Porras Mitzy *et al*., 2018. The results of this study showed that inheritance of aphid resistance in sorghum is typically controlled by dominant genes, and that the F_1 generation of sorghum hybrids mated with APT-type male sterile sorghum lines were highly resistant to aphids, thus demonstrating that sorghum varieties with improved aphid resistance can be developed by mating hybrids with the APT genotype (Fig. 1 & Tables 1-2). Similar research has been reported previously, however such genetic studies of resistance to aphids are made difficult by the fact that aphids attacking sorghum have multiple biotypes, and different results have been obtained using different resistant materials Tetreault *et al*., 2019. Kong *et al*., further confirmed the findings of this study Kong *et al*., 2018. Agrama and Nancy *et al*., searched for chromosomal segments harboring genes associated with resistance to type I and K wheat bifurcation aphids using recombinant selfed populations of the sorghum aphid-resistant variety GBIK and the aphid-susceptible variety, and found that resistance to each biotype was controlled by multiple QTL loci, again corroborating the conclusion that the inheritance of aphid resistance in sorghum is typically dominant Agrama *et al*., 2002; Nancy *et al*., 2020.

Physiological and molecular mechanisms of aphid resistance in sorghum: Induced defenses are formed when plants are damaged by pathogens and insects, and are the main defense strategy employed by plants in response to insect attacks as they consume less resources compared with continuously activated constitutive defenses, which can be further divided into direct and indirect defenses [Paudyal *et al*., 2019; Gong *et al*., 2021]. In this study, we found that the soluble sugar content of sorghum genotype APT (LA34) before aphid susceptibility was lower than that of genotype APS (P03A); after aphid infestation, the soluble sugar content of the APT (LA34) genotype was lower than that of the APS (P03A) genotype. However, the soluble sugar content changed very little after APT inoculation with aphid susceptibility, but APS increased substantially and was a direct defense (Table 3). The results of this study differ from results in wheat studies, in which the accumulation of polyphenolic substances was found to be closely related aphid resistance [Long *et al*., 2018], which may be due to differences in the metabolic mechanisms used in different crop species. Studies in different varieties of sorghum have also found that aconitic acid is effective against aphid infestation [Gordy *et al*., 2019], but the genes and pathways associated with aconitic acid metabolism were not identified in this study, which may be due to the lineage of sorghum species and/or the different periods and levels of aphid damage used in this study. In addition to direct defenses, aphid-infested plants also employ indirect defense mechanisms, including the production volatiles that attract aphid natural enemies, including lacewings, mosquito-eating flies, parasitic wasps, and lady beetles Francis *et al*., 2004; Hatano *et al*., 2008; Uchimiya & Knoll, 2019. However, no indirect defenses have been observed in the regulation of aphid resistance in sorghum in this study, a conclusion that can be speculated by the rapid decrease in chlorophyll content in the APS genotype after aphid infestation, but no sign of increased aphid natural enemy incidence.

Plant resistance to aphids can be divided into three categories: avoidance, resistance to growth, and pest tolerance, and it is important to gain insight into the mechanism of aphid resistance utilized APT sorghum by means of physiological metabolism Anders *et al*., 2015. Bioinformatics analysis of transcriptome data to resolve potential molecular mechanisms underlying aphid resistance in sorghum have also been reported Biruma *et al*., 2012; Le Nguyen *et al*., 2019. The development of high-throughput sequencing technologies have allowed us to comprehensively resolve gene expression changes in plant-insect interactions Anders *et al*., 2015; Kawahigashi *et al*., 2020. In wheat, soybean, and potato, gene expression profiles have been analyzed in resistant and susceptible varieties before and after aphid feeding using gene chip technology Fang *et al*., 2020. The present study also indicated that the APT sorghum genotype could increase expression of genes related to biotic stress response regulation, potentially stimulating resistance and detoxification through biological processes such as ‘response to biotic stimulus’, when subjected to aphid infestation more strongly than the APS genotype (Fig. 5). The results of this study are in general agreement with the findings of Felderhoff T J, who used cDNA microarray technology to compare the resistance responses of resistant and susceptible sorghum cultivars to wheat dinoflagellate aphid infestation and found that resistance required coordinated control of phytohormones such as salicylic acid, jasmonic acid, abscisic acid, growth hormone and gibberellin Felderhoff *et al*., 2016. However, there were slight differences in the plant hormone regulation pathways involved, which may be related to the different periods of aphid infestation or different species of aphid used. More importantly, it was found that the mechanism of APT sorghum resistance to aphids was primarily by means of antibiotic resistance. APT sorghum can reduce glucose accumulation and serine activity in the body through two key metabolic pathways, namely carbohydrate binding and endopeptidase inhibitor activity, thereby limiting aphid feeding and nutrient supply for growth and development. Such studies have been reported in kale, cotton, maize, wheat, among other plants Lin *et al*., 2010; Porras Mitzy *et al*., 2018; Gong *et al*., 2021 and have shown that amino acid metabolic pathways in kale plants are closely related to the aphid incidence, and that the concentration of asparagine (Asp) and glufosinate (Gin) is positively correlated with the average growth rate of infesting peach and kale aphids Lin *et al*., 2010. It has also been suggested that cotton aphid survival is correlated with the amino acid content of cotton plants, with higher amino acid content being associated with higher cotton aphid biomass Lin *et al*., 2010. Resistance of winter wheat varieties to wheat long-tube aphid and gram-winding aphid was negatively correlated with sucrose content in leaves, in contrast to resistance to maize aphids which was positively correlated with sucrose content in leaves Wang *et al*., 2020. The European maize borer (Ostrinianubilalis) requires glucose before the fourth larval instar and slight differences in glucose concentration in the host plant at this stage of insect development greatly impact the resistance response Carena & Glogoza, 2004. In contrast, our study found that, unlike cotton, in sorghum the endopeptidase inhibitor activity metabolic pathway may play an important role in the regulation of aphid resistance in sorghum, while the glycolysis metabolic pathway, may inhibit aphid growth and development by reducing glucose levels in plant tissues, which is likely similar to the mechanism of aphid resistance observed in maize plants.

Conclusion

The results of the three year (2018, 2019 and 2020) study in SY, CY and JZ ecological zones confirmed that the genetics of resistance to aphids in sorghum generally exhibit a dominant expression pattern and the incidence of aphids in F1 generation hybrids of sorghum mated with the aphid-resistant APT line were lower, demonstrating that sorghum varieties with improved aphid resistance could be obtained by mating hybrids with the APT line. APT plants infested with aphid exhibited less change in sugar and chlorophyll contents than the aphid-susceptible APS line. APT sorghum uses *In vivo* carbohydrate binding and endopeptidase inhibitor activity to influence aphid feeding by reducing the glucose content in plant tissues through the glycolysis metabolic pathway, which breaks glucose down into pyruvate in the cytoplasm thereby inhibiting aphid growth and development.

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