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Deciphering the role of sugar transport genes in modulating seed protein content in Chickpea

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Abstract

This study examines the impact of genes unique to sugar metabolism in regulating seed protein content by comparing them across two genotypes, FG212, 20 % (low protein content, LPC) and ICC8397, 30 % (high protein content, HPC) of (*Cicer arietinum* L.). Genes specific to sugar transport, which promote glycolysis and energy-intensive activities like development and stress responses, are more highly expressed in FG212 despite its low protein content. On the other hand, ICC8397 supports its high protein content by prioritising nitrogen assimilation over carbohydrate metabolism and by expressing more genes linked to nitrogen absorption, such as glutamine synthetase and nitrate reductase. The analysis revealed 17 sugar transport-specific genes, predominantly belonging to the SWEET family, with enhanced expression in FG212, these genes prioritise stress tolerance and glucose metabolism above protein synthesis. Gene ontology and KEGG pathway analysis revealed important biological processes such as hexose transport and carbohydrate metabolism, with genes related to energy balance and sugar distribution showing differential expression. While DNA repair proteins interacted with SWEET genes, suggesting their developmental significance, interaction studies showed that SWEET transporters and transcription factors such as MYB played important roles in stress. The findings of this research are useful in breeding new chickpea cultivars with enhanced SPC and higher nutritional values.

Keywords

KEGG analysis; seed protein content; SWEET transporters

Introduction

Legumes are an essential component of world agriculture and human nutrition, valued especially for their high protein content. Legumes such as soybean, chickpea, lentil and pea are important dietary protein sources that contribute to food security and the prevention of protein deficiency (1). The protein content of legume seeds is a complicated characteristic that is affected by genetic, environmental and metabolic variables. Among them, metabolic chemicals are emerging as important factors of seed protein production and accumulation, highlighting the complex relationship between metabolism and seed development (2). A wide range of biomolecules are classified as metabolic substances, including amino acids, carbohydrates, lipids and secondary metabolites. These chemicals function as precursors, regulators, or facilitators of protein synthesis during seed development.

Chickpea (*Cicer arietinum* L.) is an important pulse crop that provides nutritional protein, iron, phosphorus, folic acid and bioactive compounds, particularly in Africa, Asia, and South America (3, 4). It contributes to the nutritional value of a cereal-only diet by providing twice as much protein (5). Chickpeas are a major source of protein for the Indian subcontinent's vegetarian population. To assure food and nutritional security in an era of rapidly changing global climate conditions, the availability of chickpea cultivars with improved nutritional profiles is critical (6). To achieve this goal, it is critical to identify the key genes that control both seed protein content (SPC) and related post-transcriptional gene regulatory processes. Nitrogen-containing substances, such as amino acids and ureides, are essential for protein production. Legumes have a unique ability to fix atmospheric nitrogen through symbiotic interactions with rhizobia and the nitrogen absorbed during this process goes into metabolic pathways that synthesise amino acids, the building blocks of proteins (7-11). The availability and distribution of these nitrogenous metabolites are thus crucial in determining seed protein content. Additionally, carbon metabolism is critical in stimulating seed protein production. Carbohydrates, such as sucrose, supply the energy and carbon skeletons needed for amino acid synthesis and protein building. The balance of carbon and nitrogen metabolism has a considerable impact on seed protein content, with metabolic pathways tightly regulated to maximise resource utilisation during seed filling (12-14).

Understanding the control of these processes is critical for increasing protein production in legumes. Secondary metabolites, including as polyphenols, flavonoids and alkaloids, indirectly contribute to seed protein content by influencing physiological processes like stress response and nutrient transport. These chemicals regulate the efficiency of nitrogen fixation, assimilation, and transport, changing the pool of precursors available for protein synthesis (15-17). Furthermore, hormonal signals produced from metabolic chemicals, including as auxins and cytokinins, control seed development and protein deposition by orchestrating cellular and molecular processes during seed maturation (18-20). The protein content of legume seeds is influenced by environmental factors, such as soil fertility, water availability, and climate conditions. The nutritional makeup of grain legumes reflects the negative effects of climate change. In a study it was demonstrated how climate change significantly affects the amount of protein and micronutrients in grain legumes (C3 crops) as opposed to cereals (C4 crops). Similarly, abiotic conditions like as drought or high temperatures can alter metabolic fluxes, resulting in alterations in protein accumulation. For instance, exponential rise in atmospheric temperature (eT) poses a major risk to the biochemical properties and metabolism of nodules. By controlling C-allocation, increasing the risk of oxidative stress, blocking normal N-metabolism and finally exposing the bacteroid to oxygen (O_2), the eT interferes with nodule metabolism and taken together, impairs Nase function (21-23). Additionally, eT reduces the amount of total soluble protein in leaves, inhibits carboxylase activity, causes water stress and aggressively shuts guard cells, all of which reduce the photosynthetic rate.

Recent advancements in metabolomics and systems biology have shed light on these connections, suggesting new targets for crop enhancement. Efforts to increase seed protein content in legumes are increasingly centred on the metabolic networks that support this feature (24, 25). Genetic engineering and breeding efforts that modulate major metabolic pathways have showed promise for increasing protein production. For instance, a previous study has demonstrated that improving amino acid redistribution in soybean, overexpression of GmAAP6a increases adaptation to low nitrogen and improves seed nitrogen status (26). Integrating metabolic engineering with traditional breeding procedures has enormous potential for generating high-protein legume varieties that meet the nutritional requirements of a growing population. Finally, metabolic compound has an important role in regulating seed protein content in legumes, serving as both substrates and regulator of protein production. A thorough understanding of their roles and interconnections will help to advance sustainable agriculture techniques and improve the nutritional value of legume crops.

Materials and Methods

Collection and preprocessing RNA-Seq data

For estimation of seed protein content 200 mg of the sample from both chickpea genotypes ICC8397 (high protein content) and FG212 (low protein content) was placed in the digestion tubes after being wrapped and weighed on Whatman filter paper. It was mixed with 3 g of the digesting mixture ($CuSO_4$: K_2SO_4; 1:10). Shortly before digestion, 10 mL of concentrated sulfuric acid were added. The Kjeldahl digestor (KELPLUS KES 20L VA DLS TS) was used to perform the digestion (27). The estimated protein content the seed protein content was found ICC8397 (30 %) and FG212 (20 %) respectively. The RNA seq data of these genotypes was study used in-house RNA sequencing data from these chickpea genotypes, cultivated at the Indian Agricultural Research Institute (IARI) in New Delhi. The data was submitted with project ID (PRJNA926788) (11). The initial step in sorting sugar-specific genes is to preprocess the raw RNA-Seq data. High-quality reads are obtained by trimming adapter sequences and filtering low-quality reads with tools like trimmomatic version 0.33 (28). These filtered reads are then matched with a reference genome or transcriptome using techniques such as HISAT2 (29). The number of reads mapped to each gene is used to quantify gene expression levels using software feature Counts.

Mapping and assembly of the RNAseq data

The Kabuli chickpea reference genome was obtained from NCBI (30). The genome (v1.0) was aligned using the BWA-MEM algorithm (v0.7.5, http://bio-bwa.sourceforge.net/) with default parameters, which used filtered high-quality reads from all samples (31). Samtools (v0.1.19) was used to count gene read mapped on the reference genome (32). Cufflinks (v2.0.2, https://github.com/cole-trapnell-lab/cufflinks) was used to assemble reference annotation-based transcripts (RABT) using the mapped reads for each sample and the genome GFF file. Transfrags were then eliminated from the Cufflinks assemblies, and a consensus assembly was

constructed for further analysis by comparing and combining these assemblies using Cuffmerge (http://cole-trapnell-lab.github.io/cufflinks/cuffmerge/) (33).

Identification of differentially expressed genes and annotation

The differential gene expression of the confirmed genes was determined using the DESeq "R" program (34). The DESeq programme includes techniques for evaluating differential expression using the negative binomial distribution, as well as an increase estimate for the distribution variance. Expression plots such as heatmaps were built with the use of TBTools (35).

Functional annotation of the DEGs

Further, DEGs are annotated to better understand their biological functions. The Gene Ontology (GO) and Kyoto Encyclopaedia of Genes and Genomes (KEGG) databases are commonly utilised for this stage. Genes showing differential expression pattern were annotated using the KEGG database and Uniprot. The gene ontology (GO) was visualised by ShinyGO (v 2.0; https://wego.genomics.cn/) (36). Pathway enrichment analysis detects sugar-related pathways such as glycolysis, sucrose metabolism, starch biosynthesis and hexose transport, among others.

Sorting of sugar-specific genes from DEGs data

Sugar-specific genes were selected from RNA-Seq data of the seed protein content contrasting chickpea genotypes. Genes involved in sugar metabolism are filtered based on their annotations and pathways. For example, sugar transporter genes, such as those from the SWEET, mainly found in majority and two highly differentially expressed genes (LOC101498095 and LOC101509872) were selected for protein-protein interaction prediction using STRING database

Results and Discussion

Comparative analysis of sugar metabolism specific genes chickpea genotypes FG212 (LPC) and ICC8397 (HPC) and their role in seed protein content regulation

Chickpea is an important legume crop known for its nutritional value, particularly its protein content. Protein content differences between genotypes are regulated by genetic variables and metabolic pathways, particularly those related to sugar metabolism. Two contrasting genotypes, FG212 (low protein content) and ICC8397 (high protein content), provide an ideal model for investigating the link between sugar metabolism and protein synthesis (Fig. 1).

This study focusses on the differential expression of sugar metabolism-specific genes between these genotypes and the implications for their metabolic and nutritional profiles. From the detailed analysis we have been able to find 17 sugar transport specific genes and were showing almost similar types of expression pattern i.e., high in FG212 (LPC) and low expression in ICC8397 (HPC) (Table 1, Fig. 2). FG212 is classified as a low-protein genotype, whereas ICC8397 has a high protein profile. The variations in protein composition between these genotypes reflect different metabolic priority and genetic regulation. Sugars serve as both metabolic precursors and signalling molecules in legumes, making storage protein production inextricably related to carbohydrate metabolism. Understanding how sugar metabolism genes are expressed differently in these genotypes provides insights into the metabolic trade-offs that drive protein accumulation.

Sugar metabolism in FG212: enhanced gene expression

Despite its low protein composition, FG212 shows considerably higher expression of sugar metabolism-specific genes than ICC8397. These genes produce enzymes and regulatory proteins involved in important processes like glycolysis, the tricarboxylic acid (TCA) cycle, and starch degradation. This increased expression shows that FG212 prioritizes glucose metabolism over nitrogen absorption, which could explain its low protein composition. Upregulated expression of genes encoding enzymes such as hexokinase (HXK), phosphofructokinase (PFK) and pyruvate kinase was observed

Fig. 1. Significant variation was found in the size and seed protein content of chickpea genotypes i.e., ICC8397 (high protein content) and FG212 (low protein content).

Table 1. List of 17 sugar transporter genes and their roles

SL No	Genes	Protein names	Biological process
1	LOC101498095	Bidirectional sugar transporter SWEET	Carbohydrate transport
2	LOC101509872	Bidirectional sugar transporter SWEET17 isoform X2	Carbohydrate transport
3	LOC101505723	Bidirectional sugar transporter SWEET	Carbohydrate transport
4	LOC101497351	Bidirectional sugar transporter SWEET	Carbohydrate transport
5	LOC101511936	Bidirectional sugar transporter SWEET	Carbohydrate transport
6	LOC101512270	Bidirectional sugar transporter SWEET	Carbohydrate transport
7	LOC105851942	Sugar transport protein 10-like	Carbohydrate transport
8	LOC101497415	Sugar transport protein 14-like	Carbohydrate transport
9	LOC101508142	Probable sugar phosphate/phosphate translocator At3g11320	NA
10	LOC101510607	Bidirectional sugar transporter SWEET	Carbohydrate transport
11	LOC101515250	Bidirectional sugar transporter SWEET	Carbohydrate transport
12	LOC101499800	Bidirectional sugar transporter SWEET	Carbohydrate transport
13	LOC101504615	Sugar transport protein 14-like	Carbohydrate transport
14	LOC101491054	Bidirectional sugar transporter SWEET	Carbohydrate transport
15	LOC101511170	Probable sugar phosphate/phosphate translocator At3g17430 isoform X1	NA
16	LOC101510416	Sugar transporter ERD6-like 16	Carbohydrate transport
17	LOC101490920	Sugar carrier protein C-like	NA

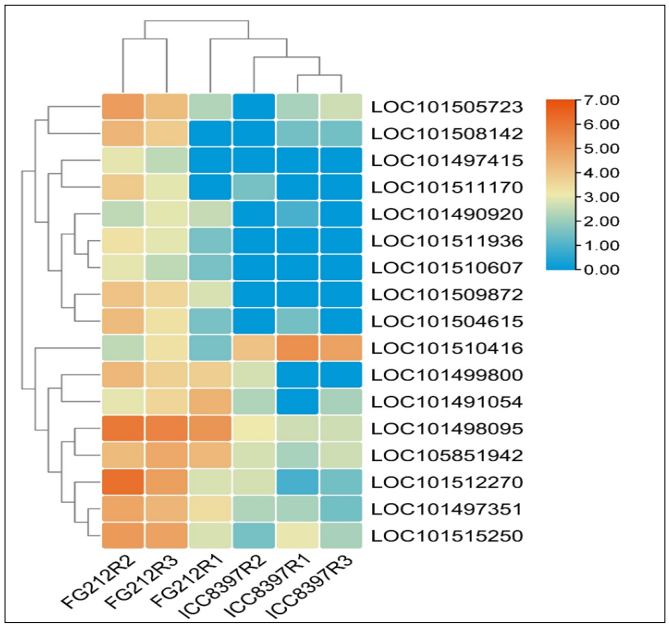

Fig. 2. This figure illustrates the expression pattern variation of sugar transporter genes in contrasting chickpea genotypes, FG212 (LPC) and ICC8397 (HPC). R1, R2 and R3 represents the triplicates of RNAseq data for both genotypes.

in FG212. These enzymes are responsible for driving the glycolytic pathway, which breaks down glucose into pyruvate and produces ATP (37). The increased glycolytic activity in FG212 most likely supports energy-intensive processes such as development and stress responses at the cost of nitrogen storage in the form of proteins. As we have observed most of the genes belongs to bidirectional sugar transporter SWEET family and was highly expressed in low protein content genotype (FG212). Instead of favouring the synthesis of proteins and amino acids, this enhanced flow through the TCA cycle can favour the production of structural carbohydrates or secondary metabolites (38). Earlier studies proposed the role of Sweet transporters in abiotic stress tolerance. High concentrations of soluble sugars, including glucose, sucrose, trehalose and sugar alcohols, operate as antioxidants and osmoprotectants, stabilising membrane structures through interactions with the lipid bilayer and reducing abiotic stress (39-41). These sugars high concentrations may act as signalling molecules, encouraging pathways that give priority to the metabolism of carbohydrates over the absorption of nitrogen.

ICC8397 exhibits a metabolic profile that is more focused on protein synthesis than FG212, with somewhat lower expression of genes related to sugar metabolism. A metabolic shift towards nitrogen absorption and amino acid production is suggested by the decreased glycolytic and TCA cycle activity in ICC8397 (41-42). Therefore, based on RNAseq study, ICC8397 had higher expression levels of genes linked to nitrogen absorption and assimilation, such as glutamine synthetase (GS) and nitrate reductase (NR). Its high protein concentration is explained by the fact that nitrogen is a necessary component of proteins and amino acids. The metabolism of carbohydrates may suffer if resources are diverted to the metabolism of nitrogen. ICC8397 maintains a baseline level of activity in glycolysis and starch metabolism, despite the fact that sugar metabolism genes are not as highly expressed as in FG212. This prevents too much carbon flux into secondary pathways and guarantees a consistent supply of energy and precursors for the production of amino acids (43-44). The decisions to be made between nitrogen and carbohydrate metabolism in chickpea genotypes are highlighted by the divergent metabolic profiles of FG212 and ICC8397. The improved sugar metabolism of FG212 probably aids in physiological functions like growth, stress tolerance and reproductive development in addition to protein synthesis. However, the high protein content of ICC8397 is supported by its metabolic concentration on nitrogen assimilation, which makes it a useful genotype for enhancing the nutritional value of chickpeas (11).

Gene ontology and KEGG pathway analysis

The functional roles of differentially expressed genes (DEGs) in the genotypes under study were revealed by the gene ontology (GO) analysis. Numerous important biological processes, such as hexose transmembrane transport (GO:0008645), protein homo-oligomerization (GO:0051260), and glucose import (GO:0046323), have been linked to these DEGs (Fig. 3 A and B). The control of energy balance and carbohydrate metabolism in the plant system depends on these mechanisms.

These genes participate in hexose transport emphasizes their function in preserving sugar availability for cellular processes, which may have an impact on resource allocation and metabolic partitioning. Interestingly, it was shown that the majority of these genes are essential parts of the plasma membrane (GO:0005887, GO:0016021), indicating that they actively participate in transmembrane transport activities. Their molecular functions, which mostly consist of hexose transmembrane transporter activity (GO:0015149), sugar transmembrane transporter activity (GO:0051119) and carbohydrate transmembrane transporter activity (GO:0015144), are consistent with this localisation. Furthermore, certain responsibilities like sucrose transmembrane transporter activity (GO:0008515) and carbohydrate proton symporter activity (GO:0005351) emphasise their unique involvement in sugar distribution and transport (45-46). GO ontology, results highlight the significance of differentially expressed genes related to sugar metabolism in regulating the transport and utilisation of sugars, which may have a direct effect on the phenotypic and nutritional distinctions between genotypes with low and high protein content.

Interaction analysis of the differentially expressed sugar transport specific genes

To elucidate the interacting partners of two highly differentially expressed genes we have carried out the protein-protein interaction analysis using STRING database (Fig. 4 A and B). LOC101498095, found to interact with Aspartic proteinase, Transcription factor MYB-like proteins, bidirectional sugar transporter SWEET, glycosyltransferase kind of proteins. The results obtained from protein-protein interaction revealed that SWEET transporter found to interact with each other, which might help to active their functionality (47-48).

As we also know, MYBs transcription factor plays important role in stress tolerance to plants, interaction with SWEET in FG212 genotypes leads to activates its functionality and hence might provide stress tolerance (49). Similarly, gene LOC101509872 found to interact with some of the DNA repair proteins, ligases and polymerases, which indicates the critical roles of SWEET genes in plant development.

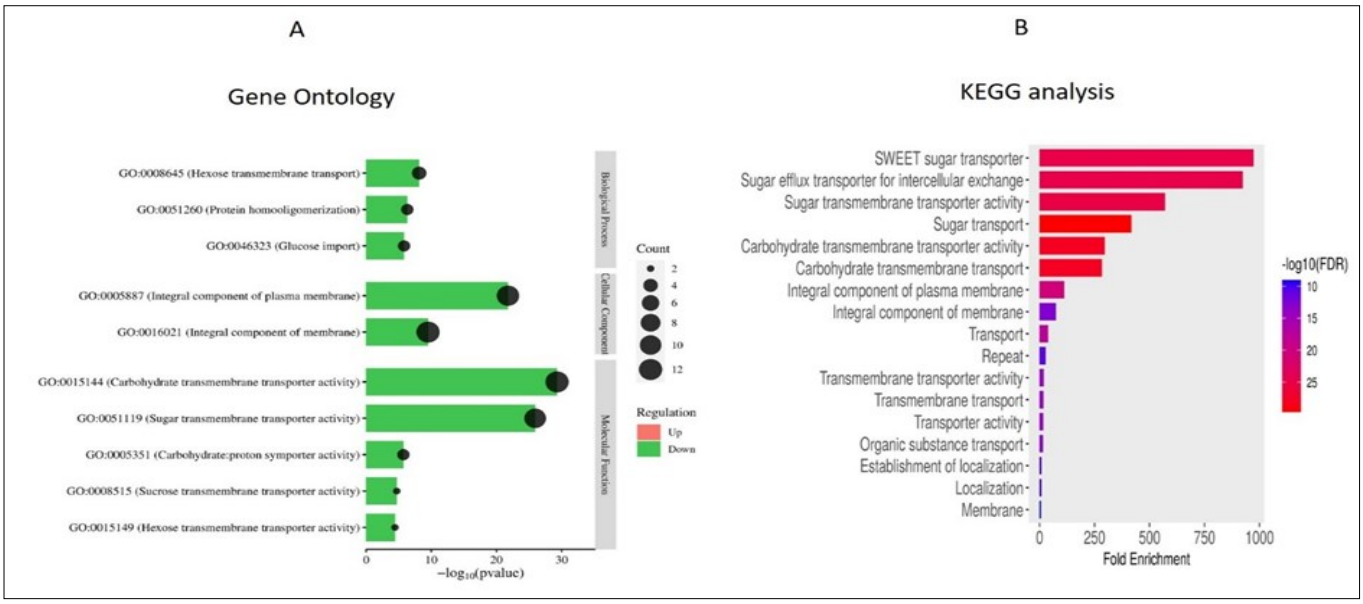

Fig. 3. A,B. Here we represented the involvement of sugar transporters in various pathways using KEGG database and their functionality using Shiny GO.

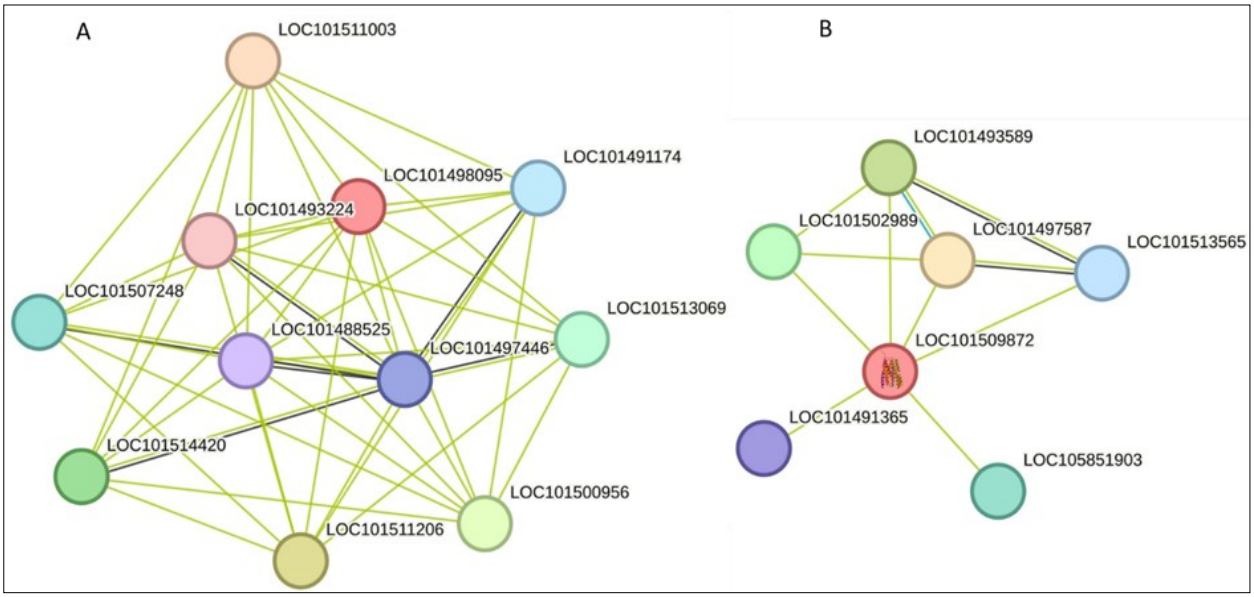

Fig. 4. A,B. The STRING database was used to create a network of protein-protein interactions for potential sugar transporter genes. The nodes indicate proteins, while the edges reflect protein-protein associations, colour of the node indicates that protein belongs to separate family.

Conclusion

The study of sugar metabolism-specific genes in chickpea genotypes FG212 (low protein content, LPC) and ICC8397 (high protein content, HPC) gave important insights into the metabolic and genetic pathways that influence protein content in legumes. Chickpea, a nutritionally valuable crop, has distinct protein profiles among genotypes due to sugar metabolic mechanisms. FG212 showed increased expression of sugar metabolism genes, including glycolysis, the TCA cycle and starch degradation. Enhanced activity of enzymes such as hexokinase, phosphofructokinase and pyruvate kinase in FG212 suggests a metabolic priority for energy production, carbohydrate synthesis and secondary metabolite creation. This emphasis shifts resources away from nitrogen uptake and protein synthesis. SWEET transporter family genes in FG212 further emphasize the role of sugar transport in stress tolerance and energy regulation, with soluble sugars acting as osmoprotectants under stress. On the other hand, ICC8397, with higher protein content, exhibited a metabolic shift preferred nitrogen absorption and amino acid biosynthesis. It can be assumed genes like glutamine synthetase and nitrate reductase were more active, reflecting a choice that prioritizes protein synthesis over carbohydrate metabolism. Instead of reduced sugar metabolism, ICC8397 maintained essential pathways to support energy production and precursor availability for protein synthesis. The metabolic profiles of FG212 and ICC8397 show a balance between sugar metabolism and nitrogen assimilation, offering valuable information for breeding strategies to improve protein content and stress resilience in legumes. Gene ontology and pathway analyses revealed processes like sugar transport and regulatory interactions, specifically the role of SWEET transporters and MYB transcription factors in resource allocation and stress response.

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Authors' contributions

GK conceptualized the study and designed the experiments. The experiments were conducted by GK, while data analysis was carried out by SD, PP, NMS, SK, SP and SY. The manuscript was prepared and edited by SD, GK, PP, SK, SY and PKJ. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare no conflict of interest.

Ethical issues: None

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