

The role of amino acid metabolism in salt tolerance mechanism of *Medicago sativa* and *Medicago truncatula*

Sicong Shen

Yangzhou University

Ling Pan

Yangzhou University

Junhao Li

Yangzhou University

Jing Wang

Yangzhou University

Huhu Liu

Yangzhou University

Juncheng Yin

Yangzhou University

Yiwen Lu

Yangzhou University

Xiaoshan Wang (✉ xswang@yzu.edu.cn)

Yangzhou University

Research Article

Keywords: alfalfa, salt stress, amino acid metabolism, transcriptomics and proteomics

Posted Date: December 13th, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-3719416/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Background: Soil salinization is a major issue of global concern today. Under salt stress, the growth and development of plants are slowed down and the yield is reduced, so it is particularly important to understand the salt resistance mechanism of plants. Meanwhile, amino acid metabolism has been shown by many studies to effectively alleviate most abiotic stresses including salt stress.

Results: In this study, transcriptomics and proteomics were used to study the salt resistance mechanism of *Medicago sativa* and *Medicago truncatula* and the role of amino acid metabolism under salt stress. The results showed that the growth and development of the two alfalfa species were significantly inhibited under 50 mM NaCl stress, after KEGG enrichment analysis, it was found that most DEGs / DEPs were involved in amino acid metabolism. GSEA analysis showed that the two amino acid metabolism of Valine, leucine and isoleucine degradation and Tyrosine metabolism played important roles in the process of salt resistance. At the same time, leucine (Leu), isoleucine (Ile) and tyrosine (Tyr) were quantitatively determined. The results showed that under salt stress, the contents of the three amino acid increased significantly, and it was found that leucine had an important effect on the salt resistance of *Medicago sativa* while tyrosine effected more on the salt resistance of *Medicago truncatula*.

Conclusion: This experiment revealed that the role of amino acid metabolism in *Medicago sativa* and *Medicago truncatula* under salt stress, meanwhile some amino acids can significantly improve the damage of plants to salt stress, which provides some help and theoretical basis for the growth and development of alfalfa spp. plants in saline-alkali land and the increase of yield in the future.

1. Introduction

In recent years, land salinization has become a global problem for crop cultivation^[1]. In many regions, land salinization has become increasingly serious due to misuse, overuse of groundwater or over-exploitation of land^[2]. Salinization is accompanied by a loss of soil fertility, which has an impact on the cultivation and growth of plants and, consequently, on the development of national agriculture and the environment^[3-5]. Salinization is mainly caused by the high content of sodium salts in the soil, which causes a difference in infiltration between the soil and the plants, making it difficult for the plants to absorb free water and nutrients from the soil, as well as limiting their growth and development due to ion poisoning and a number of other reasons, ultimately leading to the death of the plants^[6].

Amino acid metabolism is a very important part of the plant metabolic pathway. Moreover, in recent years, amino acid metabolism has been shown to be involved in many types of abiotic stresses. Proline, for example, is effective in protecting plants from osmotic stress and its role in storing plant energy under drought stress^[7-8]. At the same time, its content is one of the most important indicators of abiotic stress in plants^[7]. In addition to proline, other free amino acids such as Glu have been shown to rise in their accumulation in plants under drought stress^[9-10]. Besides drought stress, amino acids are also involved in abiotic stresses such as dehydration stress, light stress, and salt stress in plants. This shows that

amino acid metabolism has a very important relationship with plant response to stress ^[11-13]. Amino acids are also the important components of the plant body, and it is closely related to the synthesis of secondary metabolites and signaling molecules. For example, aromatic amino acids, one of the essential amino acids, are precursors for the synthesis of many hormones and have an important impact on the regulation of plant growth and development ^[14]. It is also known to promote the production of a variety of secondary metabolites that are beneficial to plants ^[15]. In terms of signaling in plants, immune signals require lysine as a raw material for transformation ^[16], Arginine can synthesize polyamines to participate in signal transduction ^[17]. TOR signaling in plants, on the other hand, is activated by 15 amino acids and is regulated by individual amino acids (e.g., leucine, etc.) ^[18-19]. Leucine, isoleucine and tyrosine are strongly associated with each other, and in previous studies, they were found to act together on abiotic stresses such as heavy metal stress, osmotic stress and biological signaling ^[20-22].

Alfalfa (*Medicago*.spp.) is an annual or perennial herb of the legume family and is the most cultivated forage grass in the world ^[23-24]. It is the most important source of protein in animal feed ^[25-26] and has the ability to fix nitrogen with rhizobia ^[27], which helps to improve soil fertility and plant growth environment. *Medicago sativa* L. is a perennial alfalfa crop that is grown on more than 6 million hectares in China ^[28] and is one of the most important crops in China's agriculture and livestock industry ^[29-30]. *Medicago truncatula* L. is a very important legume with a small genome, self-pollination, short life cycle, high fruit set and a stable genetic transformation system, which has been favoured by many scholars in recent years ^[31]. In previous studies, we have found that different varieties of alfalfa have great differences in their salt tolerance. Among the species of alfalfa, *Medicago arborea* L. is one of the oldest species and it has been confirmed by previous data to have good salt tolerance ^[32-33]. *Medicago falcata* L. is questioned as a common alfalfa specie and has also been argued by scholars to be extremely resistant to salt stress ^[34]. Four annual varieties of alfalfa were analyzed by M. Karami et al ^[35]. It was found that under salt stress, salt resistance genes were significantly expressed in *Medicago polymorpha* and *Medicago laciniata*, so their salt tolerance was better than *Medicago truncatula* Karaj and *Medicago truncatula* Qom whose genes were not significantly expressed.

In this study, we used transcriptomics and proteomics to investigate the response of amino acid metabolism during salt stress in *Medicago sativa* and *Medicago truncatula* under salt stress. The results provide new findings for the study of differences in salt resistance mechanisms in alfalfa and contribute to the subsequent revelation of salt resistance mechanisms in alfalfa spp.

2. Materials and methods

2.1. Plant growth conditions and stress treatment

Chifeng F2(*M. sativa*) and r108(*M. truncatula*) were used as test materials. The *M. sativa* varieties were obtained from the Chifeng region of Inner Mongolia through self-propagation, and the *M. truncatula* varieties were taken from the test material of Yangzhou University(32.38°N, 119.42°E).

The seeds of *M. sativa* and *M. truncatula* were treated with 50 mM NaCl and a blank control (0 mM NaCl) was used in this study. The different alfalfa species and salt concentration gradients were divided into four groups of S0, T0, S50 and T50 samples, with three additional replicates for each sample. A thin sponge and a layer of filter paper were placed inside the germination box to ensure that the seeds were kept at the corresponding NaCl concentration for germination tests, and then placed in an artificial incubator at a temperature of 25°C during the day and 20°C at night, with the number of germinations counted daily for the first seven days and the growth index measured after seven days.

2.2. Transcriptome sequencing and data analysis

In this study, each sample group was made in three independent biological replicates, and RNA was extracted from alfalfa seedlings using the Tiangen kit method. 1 µg of RNA was used for library construction for each sample, and the quality control method was mainly by Agilent 2100 bioanalyzer, and the insert size of the library was detected, and after the insert size met the expectation. qRT-PCR was used to accurately quantify the effective concentration of the library (above 2nM) to ensure the quality of the library.

The sequencing platform used for this study was illumina NovaSeq 6000 (illumina, USA), S4 Suite kit, provided by NovoZero Beijing. The reference genome and gene model annotation file is <ftp://ftp.ensemblgenomes.org/pub/plants/release-55>. The reference genome was indexed using HISAT2 v2.0.5 and paired-end clean reads were aligned to the reference genome using HISAT2 v2.0.5. featureCounts (1.5.0-p3) was used to calculate the number of reads mapped to each gene. The FPKM for each gene was then calculated based on the length of the gene and the number of reads mapped to that gene was calculated. Genes with adjusted P-values ≤ 0.05 and fold change ≥ 2 were found to be DEGs by DESeq2. GSEA analysis was performed on the GO and KEGG datasets for this species separately using the native version of the GSEA analysis tool <http://www.broadinstitute.org/gsea/index.jsp>.

2.3. Proteome profiling and data analysis

In this experiment, TCA-acetone extraction was used to extract total protein. 0.1g sample of each of the roots of *M. sativa* and *M. truncatula* was ground in liquid nitrogen, added to the lysate, treated with ultrasound on an ice box for 5min and then centrifuged at 12,000g for 15min at 4°C. The supernatant was collected. The supernatant was mixed with 5 times the volume of pre-cooled 10% TCA-acetone and shaken thoroughly. After natural precipitation at -20°C for 2h, the precipitate was collected by centrifugation at 12,000g for 10min at 4°C. An appropriate amount of pre-cooled acetone was added and the precipitate was collected by centrifugation at 12,000g for 15min at 4°C. After the precipitate was dried, it was dissolved in the sample lysate at 30°C for 1h, then centrifuged at 12000g for 10min at room temperature, and the supernatant was taken after 3 repetitions.

The total proteins obtained were subjected to TMT proteomics analysis using the database 929452-merge.fasta and the library search software Proteome Discoverer 2.4. To improve the quality of the analysis results and reduce the false positive rate, the Proteome Discoverer software further filtered the search results: peptides with a confidence level of Peptide Spectrum Matches (PSMs) with 99% or more

confidence were considered as plausible PSMs, proteins containing at least one unique peptide (unique peptide) were considered as plausible proteins, only plausible spectral peptides and proteins were retained, and FDR verification was done to remove peptides and proteins with FDR greater than 1%.

2.4. Determination of amino acids

Leaves and roots of *M. sativa* and *M. truncatula* were separated from plants for amino acid assay and 3 biological replicates were done for samples from each treatment. The experimental method was as follows: weigh about 50 mg of the sample, add 1 steel bead and 10 ceramic beads, homogenise for 1 min, add 1 mL of 50% methanol aqueous solution, and extract by vortexing and shaking for 1 h. Then place the centrifuge tube in a low-temperature centrifuge and centrifuge for 10 min at 4°C and 12000 rpm, take 10 µL of supernatant, put it in a 1.5 mL centrifuge tube, add 10 µL of ultrapure water, 5 µL of internal standard and 40 µL of isopropanol (0.1% formic acid), and vortex for 2 min. After that, place the tube in a low-temperature centrifuge and centrifuge for 10 min at 4°C and 12000 rpm, take 10 µL of the supernatant, put it in a 1.5 mL centrifuge tube, add 70 µL of boric acid buffer salt, 20 µL of AccQ Tag derivatisation reagent (Kairos amino acid kit, USA), and immediately shake for 10 s. After 1 min, the excess derivatisation agent was hydrolysed, and the derivatisation reaction was finished; put the centrifuge tube in a centrifuge tube and heat it up at 55°C for 10 min; then, add 400 µL of water to dilute it for measurement.

For the preparation of standards, the gradient dilution method was used in this study, in which the solutions of each amino acid with concentrations of 400, 200, 100, 40, 20, 10, 4, 2 and 1 µmol/L were prepared sequentially, and an equal volume of isotope internal standard was added, mixed and left to be measured. The chromatographic separation was performed by Waters ACQUITY UPLC I-CLASS ultra performance liquid chromatography, followed by mass spectrometry analysis using Waters XEVO TQ-S tandem quadrupole mass spectrometry system. The positive ion source voltage was 3 kV and the cone-well voltage was 10 V; the desolventisation temperature was 500 °C, and the desolventisation gas flow rate was 1000 L/h; the cone-well gas flow rate was 10 L/h. The peak areas were calculated by using the MassLynx quantitative software for data processing, and the retention time was allowed to have an error of 15 s. The quantitative results were obtained by the standard curve method.

2.5. Integrated analysis of transcriptome and proteome

Venn diagrams of transcriptome and proteome were performed, and correlation analysis was also performed for the differential plicity of genes (proteins) identified jointly by transcriptome and proteome in the two histologies, and finally GO and KEGG enrichment analysis was performed for the collected data.

2.6. Assays of antioxidant enzyme activities

In order to be able to better observe the differences in salt resistance between the two alfalfa species, oxidase activity was measured in this experiment. SOD (Superoxide Dismutase) and POD (Peroxidase) are widely existed in plants and animals, SOD is the main enzyme of H₂O₂ generation, which can

scavenge anions, while POD can catalyse the oxidation of phenolic and amine compounds by H_2O_2 and has the effect of eliminating the toxicity of peroxides. POD can catalyse the oxidation of phenolic and amine compounds by H_2O_2 and can eliminate the toxicity of peroxides. Both of them were positively correlated with salt stress in alfalfa. In this study, alfalfa seedlings were collected and homogenised in an ice bath, and then the enzyme activity was determined by the kit method (Suzhou Keming Bio).

2.7. Statistical analysis

All data from this study were collated using Excel 2007, and statistical analysis was performed using SPSS 18.0 for ANOVA, with $p < 0.05$ as the criterion for significance.

3. Results

3.1. Physiological responses of alfalfa to NaCl stress

To test whether two species of alfalfa have distinct abilities to deal with saline conditions, The optimal test conditions such as salt treatment time and salt stress concentration were determined by the preliminary test. The seedlings were harvested after 7 days of salt stress treatment and subsequent tests were carried out. The experimental results showed that the growth of two alfalfa species was significantly inhibited and not lethal after being treated with 50 mM NaCl (Fig. 1). Because *M. truncatula* has been reported that its salt tolerance is lower than that of *M. sativa*, this experiment studied the physiological and biochemical indexes of two alfalfa species under salt stress to compare the differences of index and observe their salt tolerance mechanism.

Compared with the control, the root length, seedling length and germination index of *M. sativa* were reduced by 44.97%, 35.12% and 25.5%, respectively, while the root length, seedling length and germination index of *M. truncatula* were reduced by 55.43%, 42.03% and 41.32%, respectively under NaCl stress (Table S1). Na^+ , MDA, H_2O_2 , CAT, SOD and POD activities were significantly higher in both *M. sativa* and *M. truncatula* seedlings after 50 mM NaCl treatment (Fig. 2 A-F). Na^+ content and MDA content of *M. sativa* seedlings were significantly lower than those of *M. truncatula* seedlings treated with after 50 mM NaCl treatment ($P < 0.05$) (Fig. 2 A-B). While the CAT and H_2O_2 contents and SOD and POD activities of *M. sativa* seedlings were significantly higher ($P < 0.05$) than those of *M. truncatula* seedlings treated with 50 mM NaCl (Fig. 2 C-F). The results proved that *M. sativa* was more salt resistant than *M. truncatula* during seed germination, while *M. truncatula* exhibited sensitivity to NaCl stress.

3.2. Amino acid metabolism during stress

To further assess the importance of amino acids in enhancing plant tolerance in alfalfa species, Transcriptome sequencing was performed after 50 mM NaCl treatment of *M. sativa* and *M. truncatula* seedlings, and a total of 75.76 Gb of pure data were obtained. The results showed that the sequencing results of *M. sativa* and *M. truncatula* seedlings after 50 mM NaCl treatment were highly correlated with the sequencing results of the respective controls ($R^2 > 0.8$), indicating reliable biological

replicates (Fig. S1). The results of principal component analysis (PCA) showed that PCA values were distributed more discrete between *M. sativa* and *M. truncatula* species and separated mainly in the PC1 direction (Fig. S2), indicating that compared with S0 and T0, S50 and T50 showed significant differences in transcript levels.

The KEGG heatmap of transcriptional sequencing was drawn based on the expression of DEGs in control and experimental groups of *M. sativa* and *M. truncatula*. From Fig. 3, we can know that most of the DEGs are involved in the metabolism of other amino acids and lipid metabolism these 2 KEGG pathways. Compared with the control groups, the expression of DEGs contained in metabolism of other amino acids and carbohydrate metabolism of *M. sativa* and *M. truncatula* subjected to salt treatment did not change significantly, the expression of DEGs in the amino acid metabolism pathway showed a decreasing trend, while the expression of DEGs in the lipid metabolism pathway showed a significant increasing trend.

In this experiment, genes with adjusted *P* value < 0.05 and absolute fold changes ≥ 2 were considered significant DEGs. The differentially expressed amino acids involved in the KEGG pathway in this study were statistically analysed and the results are shown in Table 1. The results showed that after 50 mM NaCl treatment, there were no significantly expressed amino acid metabolic pathways in *M. sativa* (S0 vs S50), while valine, leucine and isoleucine degradation was significantly expressed in *M. truncatula* (T0 vs T50) and most of the DEGs which participate in the pathways were down-regulated. When comparing the control group of *M. sativa* and *M. truncatula* (S0 vs T0), tyrosine metabolism was found to be significantly expressed and most DEGs were down-regulated. When comparing the treatment groups of *M. sativa* and *M. truncatula* (S50 vs T50), not only tyrosine metabolism but also glutathione metabolism was significantly expressed, with the difference that the majority of DEGs involved in tyrosine metabolism were down-regulated, while those involved in glutathione metabolism were up-regulated.

Table 1 Summary of amino acid metabolic pathways involved in DEGs of the transcriptome in each groups

Groups	Name of KEGG pathways	up-regulation	down-regulation	Total
S0 vs S50	-	-	-	-
T0 vs T50	Valine, leucine and isoleucine degradation	3	16	19
S0 vs T0	Tyrosine metabolism	1	5	6
S50 vs T50	Tyrosine metabolism	3	7	33
	Glutathione metabolism	20	3	

Note: S0 stands for *M. sativa* control group; T0 stands for *M. truncatula* control group; S50 stands for *M. sativa* under 50 mM NaCl treatment; T50 stands for *M. truncatula* under 50 mM NaCl treatment;

The GSEA analysis was used to analyze whether the amino acid metabolism was the important pathway which many DEGs participate in. The results showed that a total of 24,460 genes were detected in the S0

and S50 groups, 28,444 genes in the T0 and T50 groups, and 27,968 genes in the S50 and T50 groups. A total of 2 KEGG pathways which were acid metabolism with significant differences were detected in this experiment, namely valine, leucine and isoleucine degradation (NES=1.887, FDR=0.045, P=0.000) detected in T0 and T50 groups, another is tyrosine metabolism (NES=1.769, FDR=0.085, P=0.043) which was found in S50 and T50 groups (Fig. 4). Meanwhile ,the significantly expressed amino acid metabolic pathways were expressed as shown in Fig. 5, which shows that the metabolism of leucine, isoleucine and valine showed an increasing and then decreasing trend, while tyrosine and glutathione showed a decreasing trend.

The 50 mM NaCl treatment of *M. sativa* and *M. truncatula* seedlings for proteome sequencing yielded a total of 266,819 spectra, including 71,265 matched spectrum, 42,350 peptides, 7,192 proteins identified, and 7,154 total proteins for all quantifiable proteins (Table 2). The results of principal component analysis (PCA) showed that the PCA values of T50 were discrete in the PC2 direction compared with the control, while the PCA values between *M. sativa* and *M. truncatula* species were discrete in the PC1 direction (Fig. S3), indicating that there was species were significantly different from each other at the protein level.

Table 2. General overview of protein identification

Total spectra	Matched spectrum	Peptide	Identified protein	ALL
266819	71265	42350	7192	7154

We also used the expression data of DEPs to draw the heatmap of proteomics and find whether there were many amino metabolism involved in, from the results of proteomic analysis, the expression of most of the DEPs in the KEGG pathway was significantly increased in *M. sativa* compared to *M. truncatula*. Meanwhile, after 50 mM NaCl treatment, the expression of the majority of DEPs which involved in metabolism of other amino acids and carbohydrate metabolism did not change significantly, as can be seen from Fig. 6. Compared with the control, the expression of the majority of DEPs involved in lipid metabolism showed an upward change, and the expression of DEPs involved in amino acid metabolism showed a downward trend, which was consistent with the changes in the results of transcriptomics analysis(Fig. 3).

In this experiment, we selected to screen up-regulated expression proteins when $FC \geq 2.0$ while $P\text{value} \leq 0.05$, and down-regulated expression proteins when $FC \leq 0.50$ while $P\text{value} \leq 0.05$. The results showed that there were 0 differentially expressed DEP which participate in amino acid metabolism detected in *M. sativa* after 50 mM NaCl treatment compared with the control (S0 vs S50). Only 1 DEP which was down-regulated was detected in seedlings of *M. truncatula* treated with 50 mM NaCl compared with the control in amino acid metabolism (T0 vs T50). Comparing *M. sativa* and *M. truncatula* control-treated seedlings, a total of 21 DEPs were detected in amino acid metabolism, of which 14 were up-regulated and 7 were down-regulated (S0 vs T0). Comparing 50 mM NaCl-treated *M. sativa* and *M. truncatula* seedlings, there were 3 significant amino acid metabolism expressed (S50 vs T50), among these 3 pathways, DEGs in

tryptophan metabolism and tyrosine metabolism were predominantly down-regulated, while DEGs in glutathione metabolism were predominantly up-regulated (Table 3).

Table 3. Summary of amino acid metabolic pathways involved in DEGs of the proteomics in each groups

Groups	Name of KEGG pathways	up-regulated	down-regulated	Total
S0 vs S50	-	-	-	-
T0 vs T50	Valine, leucine and isoleucine degradation	0	1	1
S0 vs T0	Glutathione metabolism	14	7	21
S50 vs T50	Tryptophan metabolism	2	4	49
	Tyrosine metabolism	1	8	
	Glutathione metabolism	24	10	

To investigate the function of amino acid metabolism in *M. sativa* and *M. truncatula* after 50 mM NaCl treatment, a combined transcriptomics and proteomics analysis was performed. The results showed that *M. sativa* and *M. truncatula* obtained a few differentially expressed DEGs/DEPs after treatment with 50 mM NaCl compared with the control, respectively. While there were a total of 563 DEGs/DEPs were obtained with differential expression at the gene and protein levels comparing between the seedlings of *M. sativa* and *M. truncatula* treated with 50 mM NaCl. At the same time, the KEGG enrichment analysis showed that there were 19 KEGG pathways were detected as co-enriched by *M. sativa* and *M. truncatula* after treatment with 50 mM NaCl. After analyzing the 563 shared proteins, these proteins were found to be predominantly enriched in 3 categories, including amino acid metabolism, metabolism of other amino acids and lipid metabolism (Table S2). Among these pathways, we found that proteins which participate in glutathione metabolism are most, following the pathway linoleic acid metabolism, and finally are tryptophan metabolism. The results of the correlation analysis showed that amino acid also played an important role in 2 alfalfa species, which is consistent with heatmap analysis and GSEA analysis.

3.3. Quantitative analysis of amino acids

Based on the results of transcriptome and proteomics, this experiment selected Tyr, Leu and Ile this 3 amino acids to determine the contents of in leaves and roots of *M. sativa* and *M. truncatula* were detected respectively. The results are shown in Fig.7. From Fig. 7, it can be seen that the content of three amino acids in leaves was significantly higher than that in roots. The Leu content in *M. sativa* and *M. truncatula* is the highest, followed by the Ile content, and the Tyr content is the lowest. After 50 mM NaCl treatment, the contents of three amino acids in leaves and roots of *M. sativa* and *M. truncatula* were significantly higher than those of their respective control groups ($P < 0.05$). At the same time, this study found that in the whole plant (root + leaves), the Leu content of *M. sativa* was always higher than that of *M. truncatula*,

while the Tyr content was always lower than that of *M. truncatula*, indicating that Leu and Tyr may be important amino acids that affect the salt resistance of determine the 2 species of alfalfa.

3.4. Epitaxial experimental validation

In order to verify the effect of Leu, Ile and Tyr on the salt tolerance of *M. sativa* and *M. truncatula*, a series of epitaxial experiments were done in this experiment. At the same time, due to the Pro has been shown to have a mitigating effect on salt stress, it was used as a marker to assess whether the other three amino acids have a positive effect on alfalfa salt stress. From Fig. 8, it can be seen that after adding three amino acids, compared with the salt treatment groups, all biochemical indexes of *M. sativa* and *M. truncatula* showed a decreasing trend. After adding Leu, the chlorophyll content of the two alfalfa was significantly higher than that of other amino acids ($P < 0.05$), and the CAT and POD content of alfalfa was significantly lower than that of Pro ($P < 0.05$). Interestingly, after adding Tyr, the content of SOD and H_2O_2 in *M. truncatula* was significantly lower than that of Pro group ($P < 0.05$). While after adding Ile treatment, most of its indicators are between the two. In conclusion, the three amino acids all improved the salt resistance of the two alfalfa species, and Leu had a better effect on *M. sativa* while Tyr on *M. truncatula*, which was consistent with the quantitative results of amino acids, indicating that Leu and Tyr may be important factors affecting the salt resistance mechanism of the 2 species of alfalfa.

4. Discussion

4.1. Changes in reactive oxygen species (ROS)

Reactive oxygen species (ROS) are a class of reactive molecules derived from oxygen and more active than oxygen molecules (O_2)^[36], which play an important role in plant response to adversity stress and during growth and development. In plants, subcellular basal metabolism is the main pathway for ROS production, which includes aerobic respiration in mitochondria, photosynthesis in chloroplasts, respiration in peroxisomes and NADPH oxidase^[37], and studies have shown that the abundance of ROS increases under biotic and abiotic stresses^[38]. Therefore, in plant cells, excessive ROS can cause damage, so it is particularly important to maintain the stability of ROS-active molecules between cells. MDA, whose main component is unsaturated fatty acids, is one of the markers indicating whether plant cellular conserved matter is damaged^[39]. H_2O_2 is a type of ROS, which can be cleared by CAT^[40]. while SOD and POD are oxidoreductases, which protect major plant cellular components and remove excess O_2^- ions.

In the present study, after 50 mM NaCl treatment, the concentrations of MDA, CAT, H_2O_2 , SOD and POD of *M. sativa* and *M. truncatula* showed an increasing trend, indicating that both alfalfa species showed changes in their physiological and biochemical functions under salt treatment. Liu ZB^[41] et al. found that the model plant *Arabidopsis thaliana* under NaCl stress showed an increase in SOD and MDA. Yang Hongbing^[42] et al. found that the SOD activity, MDA content and photosynthetic rate of wheat increased under salt stress, and the SOD activity and photosynthetic rate increased faster compared to the MDA

content. Long Ruicai et al. [6] found that SOD, H₂O₂, POD, Pro, ABA and MDA concentrations contained under 20 mM salt stress increased, which is consistent with the results of the present study. The results of this study showed that, compared with the control, the concentrations of H₂O₂, CAT, SOD and POD increased more in *M. sativa* seedlings after 50 mM NaCl treatment than in *M. truncatula*, while the increase in MDA content was lower than in *M. truncatula*, indicating that there are differences in the regulatory mechanisms of *M. sativa* and *M. truncatula* to NaCl stress, and *M. truncatula* showed more sensitive characteristics to NaCl compared with *M. sativa*.

4.2. Relationship between amino acid metabolism and salt resistance mechanism of alfalfa

In this experiment, after sequencing the salt stress treatment groups of *M. sativa* and *M. truncatula* by transcriptomics and proteomics, the significantly up-regulated / down-regulated pathways were screened by KEGG enrichment analysis. The results showed that most of the pathways belonged to amino acid metabolism, so this experiment speculated that it had a key effect on the salt resistance mechanism of alfalfa. Amino acid metabolism, as one of the important metabolic pathways in plants, has been shown to improve a variety of abiotic stresses^[43–44]. Under salt stress, the metabolism of serine and threonine in *Leymus chinensis* was significantly increased^[45], and the metabolism of alanine and glutamic acid in *Leymus chinensis* was significantly increased^[46].

The results of this study show that glutathione metabolism is one of the main pathways involved in the differential proteins / genes after salt stress in the KEGG pathway analysis of *M. sativa* and *M. truncatula*. Meanwhile, Hernández^[47] et al. found that because of the increase of glutathione reductase activity, glutathione was confirmed to be involved in the salt resistance mechanism of *Arabidopsis thaliana*, this finding is consistent with the results of this study. Bao et al. studied Suaeda salsa plants through metabolomics and found that under salt stress, the concentrations of many metabolites amino acids including Leu and Tyr increased^[48], and the pathways in which they were located also became significantly enriched, confirming that these amino acid-involved pathways may play an important role in plant salt stress^[49]. Interestingly, GSEA analysis of this experiment found that two amino acid metabolic pathways, Valine, leucine and isoleucine degradation and Tyrosine metabolism, were significantly expressed, indicating that these two pathways are also important factors affecting salt stress. In summary, amino acid metabolism can improve the resistance of *M. sativa* and *M. truncatula* to salt stress to some extent.

4.3. Effects of Leu, Tyr and Ile on salt stress of alfalfa

Leucine and isoleucine belong to branched-chain amino acids, and they have a strong correlation with glucose metabolism. It has been reported that the addition of glucose to green algae is mainly involved in photosynthesis, biosynthesis of Leu and Ile, etc^[50]. Meanwhile Scarponi^[51] found that exogenous addition of Leu and Ile to soybean can effectively reduce the synthesis of glucose and starch. Tyrosine is an aromatic α -amino acid, which has a positive effect on plant leaves and photosynthesis. Previous

studies have shown that Tyr plays an important role in the metabolism of photosynthetic system in plants, which can promote the transition of plants from photosystem I to II ^[52], and the metabolism of tyrosine can enhance photosynthesis in carrots ^[53]. Secondary metabolites have also been proved to be a key factor in plant salt tolerance. Cui ^[54] et al. found that the content of secondary metabolites of amino acids increased significantly under salt stress, and these secondary metabolites have a significant improvement effect on salt stress ^[55]. Leu and Ile accumulate in radish after abiotic stress and are positively correlated with the number of secondary metabolite precursors ^[56]. As aromatic amino acids, Tyr can accelerate the stimulation of the meridian to produce many kinds of phenolic secondary metabolites ^[57], indicating that these three amino acids have an important relationship with the synthesis of secondary metabolites. The results of this study showed that Leu was related to secondary metabolism such as Terpenoid backbone biosynthesis, and acetyl-CoA carboxylase and methylcrotonyl-CoA carboxylase beta chain were up-regulated. Ile is associated with biosynthesis of 12-, 14- and 16-membered macrolides, and the up-regulated proteins are 3-ketoacyl-CoA thiolase 2, peroxisomal-like protein and acyl-CoA dehydrogenase family protein. At the same time, Isoquinoline alkaloid biosynthesis is associated with Tyr, and the protein tyrosine / DOPA decarboxylase is up-regulated.

In order to better observe the effects of Leu, Ile and Tyr on salt stress in *M. sativa* and *M. truncatula*, the contents of three amino acids were determined in this experiment. The results showed that the content of the three amino acids in the roots of the plant was lower than which in the leaves, which may be that the amino acids were transported from the roots to the leaves during plant growth. Many studies have shown that the accumulation of amino acids in the cytoplasm of plants can alleviate the cell water potential to a certain extent, thereby reducing the harm of osmotic stress to plants ^[58]. As an osmotic regulator ^[59], Pro accumulates more than other common amino acids ^[60], and it mainly alleviates salt stress by balancing osmotic pressure ^[61]. By studying the salt resistance mechanism of soybean, Yang et al. found that Tyr, Leu, Ile and many other amino acids were accumulated in soybean seedling leaves after salt stress ^[62]. When studying the salt tolerance of Arabidopsis seedlings, Huang et al. found that the accumulation of branched-chain amino acids (Leu, Ile and Val) increased, and even the accumulation of some amino acids exceeded that of Pro ^[63]. After transcriptome and metabolomics analysis of *Zygophyllum*, Wang et al. ^[64] found that the accumulation of Leu and Ile increased significantly, and proved that the metabolic pathways they participated in could affect their salt tolerance. Interestingly, the quantitative results of the three amino acids in this study showed that the content of Leu was the highest, followed by Ile and Tyr, which was consistent with the previous research results. In addition, in alfalfa, the activities of POD and CAT in the salt treatment group after adding Leu were significantly lower than those in the Pro treatment group ; after adding Tyr in the salt treatment group of *Medicago truncatula*, it was found that the SOD enzyme activity and H₂O₂ content were significantly lower than those in the Pro treatment group, indicating that the accumulation of Leu and Tyr was significantly higher than that of Pro, and the metabolism of these two amino acids was the key to affecting alfalfa. In general, the accumulation of Leu, Ile and Tyr increased significantly after salt treatment, while Leu and Tyr had better effects on the corresponding alfalfa.

5. Conclusion

After the analysis of transcriptome and proteomics results, we found that amino acid metabolism was significantly enriched, and Leu, Ile and Tyr were accumulated after salt stress. The results showed that amino acid metabolism could well alleviate the salt stress damage of alfalfa, and Leu could better exert its resistance to salt stress on *M. sativa* and Tyr on *M. truncatula*. This study puts forward new insights on the salt tolerance and amino acid metabolism of alfalfa plants, and provides a theoretical basis for future research on the salt resistance of alfalfa spp.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

CSS performed the experiment, CSS, HHL, YWL and JCY collected the data. CSS, JHL and JW performed data analysis. LP supervised the research. LP and XSW advised the research and revised the manuscript critically. All authors read and approved the final manuscript.

Funding

This work was supported by the National Key P & D Program of China NO. 2022YFE0113400 .

Availability of data and materials

The transcriptome datasets generated and analysed during the current study are available in the NCBI repository, the accession number is PRJNA1050396. The proteome datasets generated and analysed during the current study are available in the iProX repository, and the the accession number is PXD047690. The datasets generated for this study are available on request to the corresponding author.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that there are no conflicts of interest.

Author details

References

1. Chen, J.; Zhang, H. Q.; Zhang, X. L.; Tang, M. Arbuscular Mycorrhizal Symbiosis Alleviates Salt Stress in Black Locust through Improved Photosynthesis, Water Status, and K⁺/Na⁺ Homeostasis, *Front Plant Sci.* 2017, 8, 1739.
2. Jiao, S. Y.; Li, Y. Q.; Jia, J. W.; Xu, Z. Y. Soil C, N, and P distribution as affected by plant communities in the Yellow River Delta, China, *PLoS One.* 2019, 14, doi: 10.1371/journal.pone.0226887.
3. Kristin, M. R.; Noah, F.; Daniel, V. M.; Johannes, R. Linking bacterial community composition to soil salinity along environmental gradients, *ISME J.* 2019, 13, 836–846.
4. Zhang, P.; Duo, T. Q.; Wang, F. D.; Zhang, X. Z.; Yang, Z. Z.; Hu, G. F. De novo transcriptome in roots of switchgrass (*Panicum virgatum* L.) reveals gene expression dynamic and act network under alkaline salt stress, *BMC Genomics.* 2021; 22, 1197-1210.
5. Cheng, T. L.; Chen, J. H.; Zhang, J. B.; Shi, S. Q.; Zhou, Y. W.; Lu, L.; Wang, P. K.; Jiang, Z. P.; Yang, J. C.; Zhang, S. G.; Shi, J. S. Physiological and proteomic analyses of leaves from the halophyte Tangut *Nitraria* reveals diverse response pathways critical for high salinity tolerance, *Front Plant Sci.* 2015, 6, 30.
6. Long, R. C.; Gao, Y. L.; Sun, H.; Zhang, T. J.; Li, X.; Li, M. N.; Sun, Y.; Kang, J. M.; Wang, Z.; Ding, W.; Yang, Q. C. Quantitative proteomic analysis using iTRAQ to identify salt-responsive proteins during the germination stage of two *Medicago* species, *Scientific Reports.* 2018, 8, 1-11.
7. Szabados, L.; Savouré, A. Proline: a multifunctional amino acid, *Trends in Plant Science.* 2010, 15, 89–97.
8. Heinemann, B.; Künzler, P.; Eubel, H.; Braun, H. P.; Hildebr, T. M. Estimating the number of protein molecules in a plant cell: protein and amino acid homeostasis during drought, *Plant Physiology.* 2021, 185, 385–404.
9. Martinelli, T.; Whittaker, A.; Bochicchio, A.; Vazzana, C.; Suzuki, A.; Masclaux-Daubresse, C. Amino acid pattern and glutamate metabolism during dehydration stress in the 'resurrection' plant *Sporobolus stapfianus*: A comparison between desiccation-sensitive and desiccation-tolerant leaves, *Journal of Experimental Botany.* 2007, 58, 3037–3046.
10. Ferreira Júnior, D. C.; Gaion, L. A.; Sousa Júnior, G. S.; Santos, D. M. M.; Carvalho, R. F. Drought-induced proline synthesis depends on root-to-shoot communication mediated by light perception, *Acta Physiologiae Plantarum.* 2018, 40, 363.
11. Hildebrandt, T. M. Synthesis versus degradation: Directions of amino acid metabolism during *Arabidopsis* abiotic stress response, *Plant Molecular Biology.* 2018, 98, 121–135.
12. Pires, M. V.; Pereira Júnior, A. A.; Medeiros, D. B.; Daloso, D. M.; Pham, P. A.; Barros, K. A.; Fernie, A. R. The influence of alternative pathways of respiration that utilize branched-chain amino acids

- following water shortage in *Arabidopsis*, Plant, Cell and Environment. 2016, 39, 1304–1319.
13. Kopriva, S.; Malagoli, M.; Takahashi, H. Sulfur nutrition: impacts on plant development, metabolism, and stress responses, Exp. Bot. 2019, 70, 4069–4073.
 14. Vogt, T. Phenylpropanoid biosynthesis, Mol. Plant. 2010, 3, 2-20.
 15. Tzin, V.; Galili, G. New insights into the shikimate and aromatic amino acids biosynthesis pathways in plants, Mol. Plant. 2010, 3, 956–972.
 16. Hartmann, M.; Zeier, T.; Bernsdorff, F.; Reichel-Deland, V.; Kim, D.; Hohmann, M.; Zeier, J. Flavin monooxygenase-generated N-hydroxypipecolic acid is a critical element of plant systemic immunity, Cell. 2018, 173, 456–469.
 17. Alcázar, R.; Altabella, T.; Marco, F.; Bortolotti, C.; Reymond, M.; Koncz, C.; Carrasco, P.; Tiburcio, A. F. Polyamines: molecules with regulatory functions in plant abiotic stress tolerance, Planta. 2010, 231, 1237–1249.
 18. Liu, Y.; Duan, X.; Zhao, X.; Ding, W.; Wang, Y.; Xiong, Y. Diverse nitrogen signals activate convergent ROP2-TOR signaling in *Arabidopsis*, Developmental Cell. 2021, 9, 1283-1301.
 19. O’Leary, B. M.; Oh, G. G. K.; Lee, C. P.; Millar, A. H. Metabolite regulatory interactions control plant respiratory metabolism via Target of Rapamycin (TOR) kinase activation, The Plant Cell. 2020, 32, 666–682.
 20. Dong, Q.; Chen, M. J.; Zhang, Y. R.; Song, P. P.; Yang, H. L.; Zhao, Y.; Yu, C. X.; Zha, L. Integrated physiologic and proteomic analysis of *Stropharia rugosoannulata* mycelia in response to Cd stress, Journal of Hazardous Materials. 2023, 441, 23-36.
 21. Kazem, G.G.; Samira, S. A. Exogenous Cytokinin and Salicylic Acid Improve Amino Acid Content and Composition of Faba Bean Seeds Under Salt Stress, Gesunde Pflanzen. 2022, 74, 935-945.
 22. Hiroshi, O.; Ruben, C. A.; David, Y.; Daisuke, T.; Takashi, S.; Juan, S. Bonifacino. The Medium Subunits of Adaptor Complexes Recognize Distinct but Overlapping Sets of Tyrosine-based Sorting Signals, Journal of biological chemistry. 1998, 40, 25915-25921.
 23. Cao, Q.; Wang, X. G.; Zhang, S. X.; Liu, B. W.; Jin, M. Y.; Ning, Y. M. Effect of soil moisture content on cold resistance of alfalfa before cold exercise. Chinese Journal of Grassland, 2021, 03, 1-9.
 24. Zheng, W. M. Effect of adding cellulose complex enzymes to alfalfa grass meal diets on growth and feed utilization of broiler poult, Journal of Henan Institute of Science and Technology, Natural Science Edition. 2006, 01, 29-31.
 25. Daryanto, S.; Wang, L.; Jacinthe, P. A. Global synthesis of drought effects on food legume production, PLoS One. 2015, 10, doi:10.1371/journal.pone.0127401.
 26. Vance, C. P.; Graham, P. H.; Allan, D. L. “Biological nitrogen fixation: phosphorus - a critical future need?,” in Nitrogen Fixation, Molecules to Crop Productivity. 2015, 38, 509–514.
 27. Graham, P. H.; Vance, C. P. Legumes: importance and constraints to greater use, Plant Physiol. 2003, 131, 872–877.

28. Guo, T.; Bai, J.; Wang, J. Research on the present situation and countermeasures of alfalfa grass industry in China, *Grassl.* 2018,40, 111–115.
29. Raiesi, F. The conversion of overgrazed pastures to almond orchards and alfalfa cropping systems may favor microbial indicators of soil quality in central Iran; *Agricult. Ecosyst. Environ.* 2007, 121, 309–318.
30. Su, Y. Soil carbon and nitrogen sequestration following the conversion of cropland to alfalfa forage land in northwest China, *Soil Tillage Res.* 2007, 92, 181–189.
31. Tadege, M.; Ratet, P.; Mysore, K. S. Insertional mutagenesis: a Swiss Army knife for functional genomics of *Medicago truncatula*, *Trends in Plant Science.* 2005, 10, 229-235.
32. Boughalleb, F.; Denden, M.; Tiba, B.B. Anatomical changes induced by increasing NaCl salinity in three fodder shrubs, *Nitraria retusa*, *Atriplex halimus* and *Medicago arborea*, *Acta Physiol. Plant.* 2009, 31, 947–960.
33. Lefi, E.; Conesa, M.À.; Cifre, J.; Gulías, J.; Medrano, H. Dry matter allocation in *Medicago arborea* and *Medicago citrina* in response to drought and defoliation, *Crop Pasture Sci.* 2012, 63, 179–189.
34. Liu, M.; Wang, T. Z.; Zhang, W. H. Sodium extrusion associated with enhanced expression of SOS1 underlies different salt tolerance between *Medicago falcata* and *Medicago truncatula* seedlings, *Environmental and Experimental Botany.* 2014, 110, 46-55.
35. Karami, M. ; Rafiei,F.; Shiran, B.; Khodambashi, M. Comparative response of annual *Medicago* spp. to salinity, *Russian Journal of Plant Physiology.* 2015, 62, 617–624.
36. Mittler, R. ROS Are Good, *Trends in Plant Science.*2017, 22, 11–19.
37. Waszczak, C.; Carmody, M.; Kangasjärvi, J. Annual review of plant biology reactive oxygen species in plant signaling, *Annual Reviews of Plant Biology.* 2018, 69, 209–236.
38. You, J.; Chan, Z. L. ROS Regulation During Abiotic Stress Responses in Crop Plants. *Front Plant Sci* , 2015. 6, 36-50.
39. Davey, M. W.; Stals, E.; Panis, B.; Keulemans, J.; Swennen, R. L. High-throughput determination of malondialdehyde in plant tissues, *Analytical Biochemistry.* 2005, 347, 201–207.
40. Masuta, C.; Inaba, J. I.; Shimura, H. The 2b proteins of Cucumber mosaic virus generally have the potential to differentially induce necrosis on *Arabidopsis*, *Plant Signal Behav.* 2012, 7, 43–45.
41. Liu, Z. B.; Zhang, W. J.; Gong, X. D.; Zhang, Q.; Zhou, L. R. A Cu/Zn superoxide dismutase from *Jatropha curcas* enhances salt tolerance of *Arabidopsis thaliana*, *Genetics and molecular research.* 2015. 14, 2086-2098.
42. Yang, H. B. Effects of osmotic and salt stress on some physiological indexes of wheat seedlings, *Research on crops.* 2013, 14, 404-408.
43. Zörb, C.; Schmitt, S.; Neeb, A.; Karl, S.; Linder, M.; Schubert, S. The biochemical reaction of maize (*Zea mays* L.) to salt stress is characterized by a mitigation of symptoms and not by a specific adaptation, *Plant Sci.* 2004, 167, 91–100.

44. Shi, H. T.; Ye, T. T.; Chen, F. F.; Cheng, Z. M.; Wang, Y. P.; Yang, P. F.; Zhang, Y. S.; Chan, Z. L. Manipulation of arginase expression modulates abiotic stress tolerance in *Arabidopsis*: effect on arginine metabolism and ROS accumulation, *Exp. Bot.* 2013, 64, 1367–1379.
45. He, Y. Study on the Enhancement of Drought Tolerance of Ryegrass by Introduction of the Glycine-Methylation Biosynthetic Pathway of Glycinebetaine. Ph.D. Thesis, Shandong University, Jinan, China, 2010.
46. Yan, G.; Shi, Y. J.; Mu, C. S.; Wang, J. F. Differences in Organic Solute and Metabolites of *Leymus chinensis* in Response to Different Intensities of Salt and Alkali Stress, *Plants*. 2023, 12, 78-92.
47. Hernández, J. A.; Jiménez, A.; Mullineaux, P.; Sevilla, F. Tolerance of pea (*Pisum sativum* L.) to long-term salt stress is associated with induction of antioxidant defences, *Plant Cell Environ.* 2000, 23, 853–862.
48. Obata, T.; Fernie, A. R. The use of metabolomics to dissect plant responses to abiotic stresses, *Cellular and Molecular Life Sciences*. 2012, 69, 3225–3243.
49. Bao, J. B.; Liu, Z. Y.; Ding, Z. J.; Gulbar, Y.; Wang, Q. Y.; Tian, X. M.
50. Metabolomic analysis reveals key metabolites and metabolic pathways in *Suaeda salsa* under salt and drought stress, *Functional Plant Biology*. 2023, 9, 701-711.
51. Qiu, Wen.; Chen, R. F.; Wang, X. X.; Liu, J. Y.; Lv, W. G. Quantitative Proteomics of *Chromochloris zofingiensis* Reveals the Key Proteins Involved in Cell Growth and Bioactive Compound Biosynthesis, *Plants*. 2022, 11, 756-773.
52. Scarponi, L.; Martinetti, L.; Nemat Alla, M. M. Growth response and changes in starch formation as a result of imazethapyr treatment of soybean (*Glycine max* L.), *Journal of Agricultural and food chemistry*. 1996, 44, 1572-1577.
53. Jens, F.; John, F. A. Protein tyrosine phosphorylation in the transition to light state 2 of chloroplast thylakoids, *Photosynthesis Research*, 2001, 68, 71-79.
54. Wang, B.; Wang, Y. H.; Deng, Y. J.; Yao, Q. H.; Xiong, A. S. Effect of betanin synthesis on photosynthesis and tyrosine metabolism in transgenic carrot, *BMC Plant Biology*. 2023, 23, 425-437.
55. Cui, G. C.; Zhang, Y.; Zhang, W. J.; Lang, D. Y.; Zhang, X. J.; Li, Z. X.; Zhang, X. H. Response of Carbon and Nitrogen Metabolism and Secondary Metabolites to Drought Stress and Salt Stress in Plants, *Journal of Plant Biology*. 2019, 62, 387-399.
56. Jitendra, M.; Tahmish, F.; Naveen, K. A. Role of Secondary Metabolites from Plant Growth-Promoting Rhizobacteria in Combating Salinity Stress, *Plant Microbiome: Stress Response*. 2018, 5, 127-163.
57. Tatjana, M. H. Synthesis versus degradation: directions of amino acid metabolism during *Arabidopsis* abiotic stress response, *Plant Molecular Biology*. 2018, 98, 121-135.
58. Pavel, F.; Liubov, S.; Anastasiia, R.; Artem, P.; Elina, T.; Pavel, M.; Galina, C. Phenylalanine and Tyrosine as Exogenous Precursors of Wheat (*Triticum aestivum* L.) Secondary Metabolism through PAL-Associated Pathways, *Plants*. 2020, 9, 1177-1195.

59. Gorham, J. Mechanisms of salt tolerance in halophytes. In: Choukr-Allah R, Malcom CV, Hamdy A (eds) Halophytes and biosaline agriculture. Marcel Dekker, 1995, New York, 400.
60. Batista-Silva, W.; Heinemann, B.; Rugen, N.; Nunes-Nesi, A.; Araújo, W. L.; Braun, H. P.; Hildebrandt, T. M. The role of amino acid metabolism during abiotic stress release, Plant cell and environment. 2019, 5, 1630-1644.
61. Abraham, E.; Rigo, G.; Szekely, G.; Nagy, R.; Koncz, C.; Szabados, L. Light-dependent induction of proline biosynthesis by abscisic acid and salt stress is inhibited by brassinosteroid in *Arabidopsis*, Plant Mol Biol. 2003, 3, 363–372.
62. Yang, J.; Xu, X. Progress of research on accumulation of osmotic substance under salt-stress in plants, Ningxia Agr Coll. 2013, 4, 86–89.
63. Yang, D. S.; Zhang, J.; Li, M. X.; Shi, L. X. Metabolomics Analysis Reveals the Salt-Tolerant Mechanism in Glycine soja, Journal of Plant Growth Regulation. 2017, 36, 460-471.
64. Huang, T. F.; Georg, J. Absciscic acid-regulated protein degradation causes osmotic stress-induced accumulation of branched-chain amino acids in *Arabidopsis thaliana*, Planta. 2017, 4, 737-747.
65. Wang, J.; Jiang, X.; Zhao, C. F.; Fang, Z. M.; Jiao, P. P. Transcriptomic and metabolomic analysis reveals the role of CoA in the salt tolerance of *Zygophyllum* spp, BMC Plant Biology. 2020, 20, 122-135.

Figures

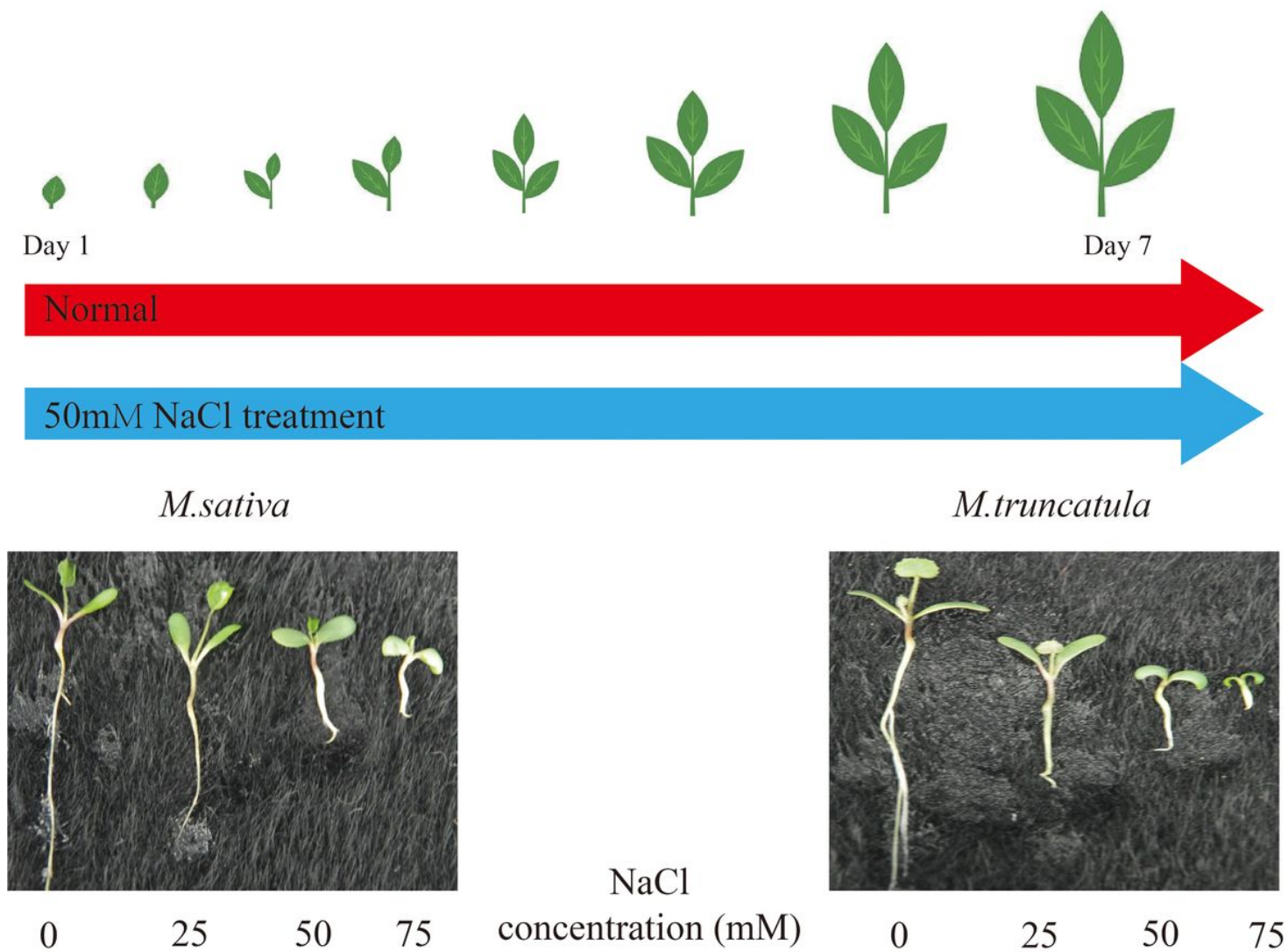


Figure 1

Experimental scheme and response to salt stress of two alfalfa species

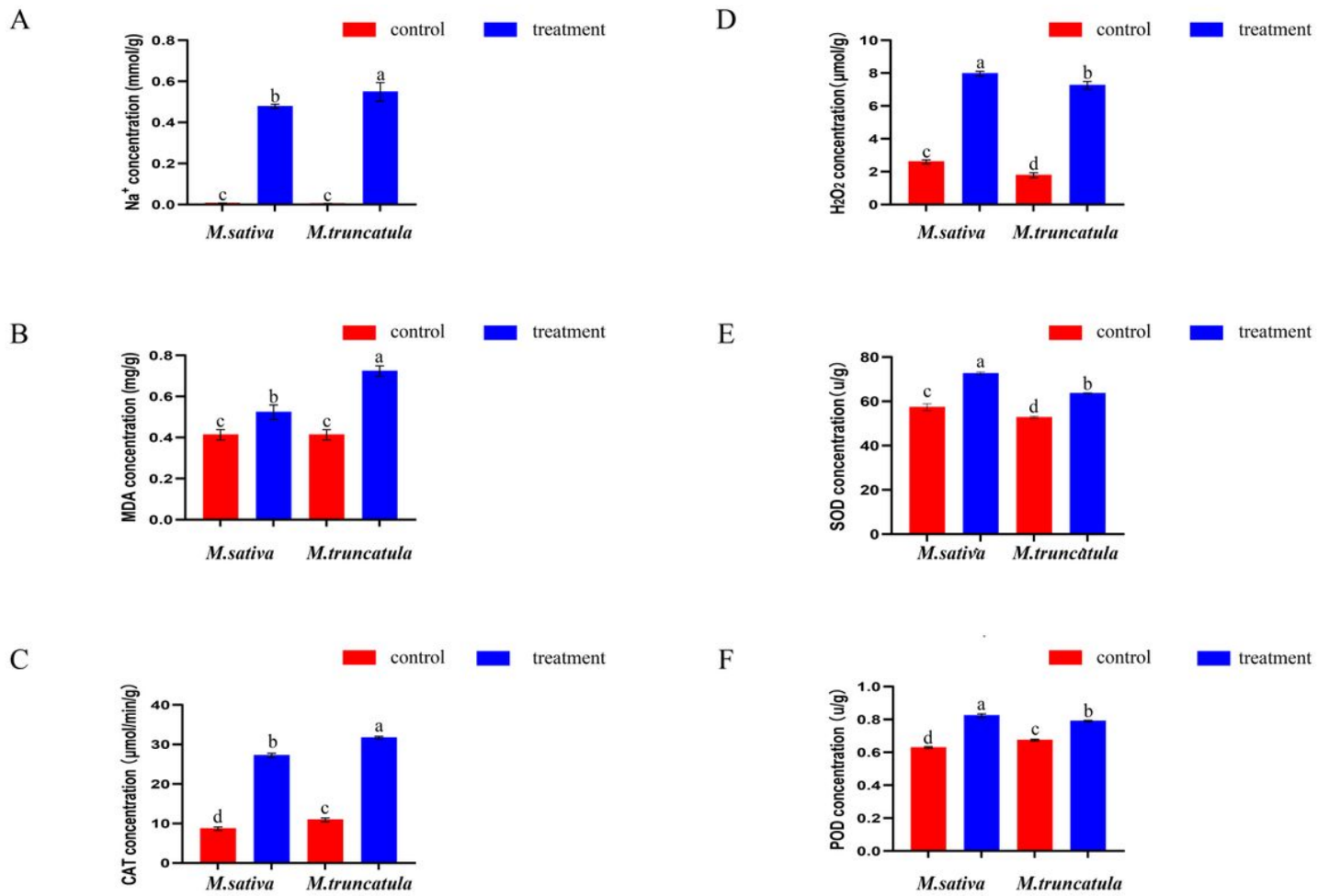


Figure 2

Morphological and physiological analysis of *M. sativa* and *M. truncatula* seedlings. (A-F) SOD, CAT, MDA, POD, H₂O₂, and Na⁺ concentrations of *M. sativa* and *M. truncatula* seedlings treated with 50 mM NaCl. Different letters indicate significant differences between the two groups. ($P<0.05$)

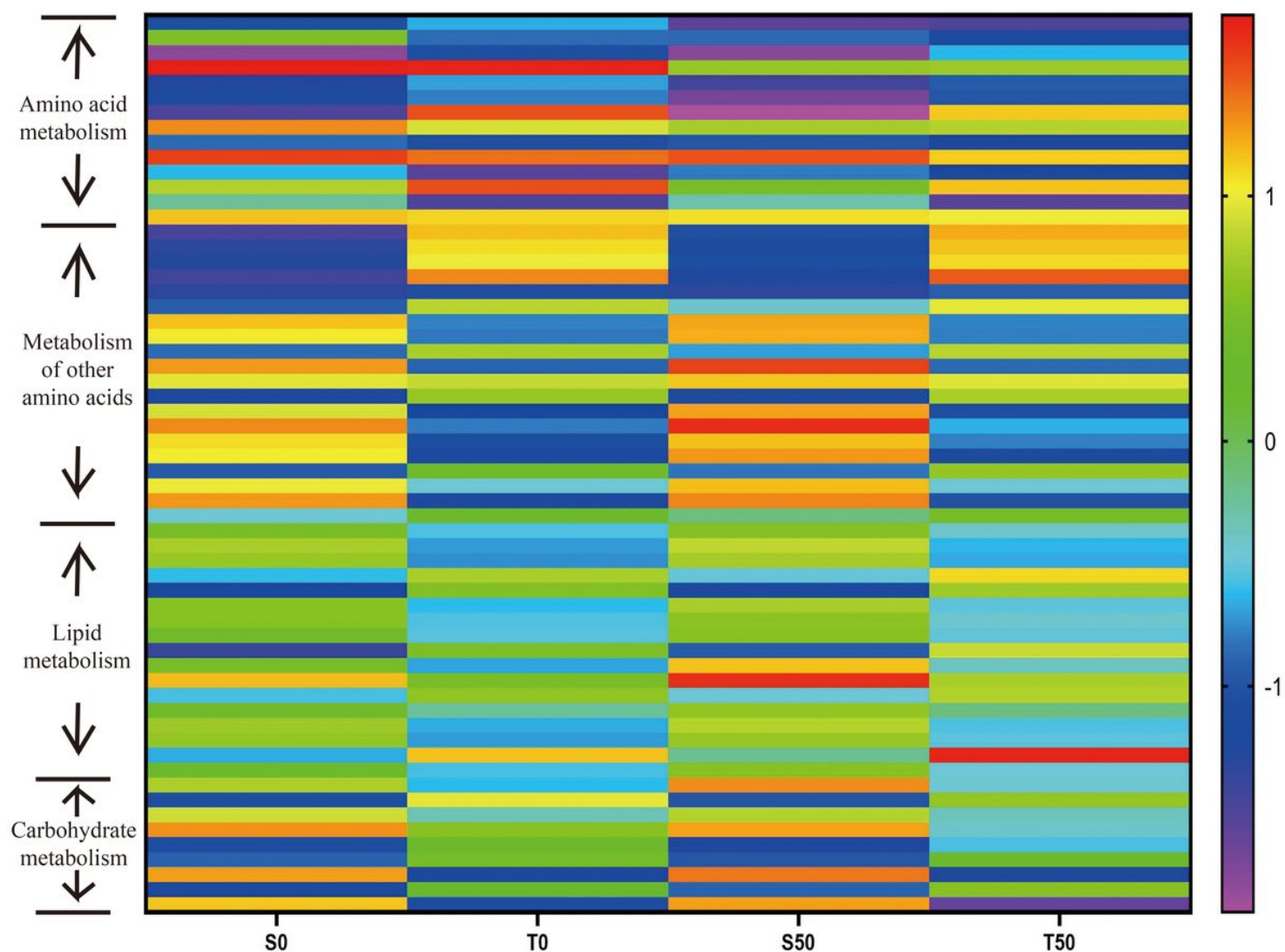
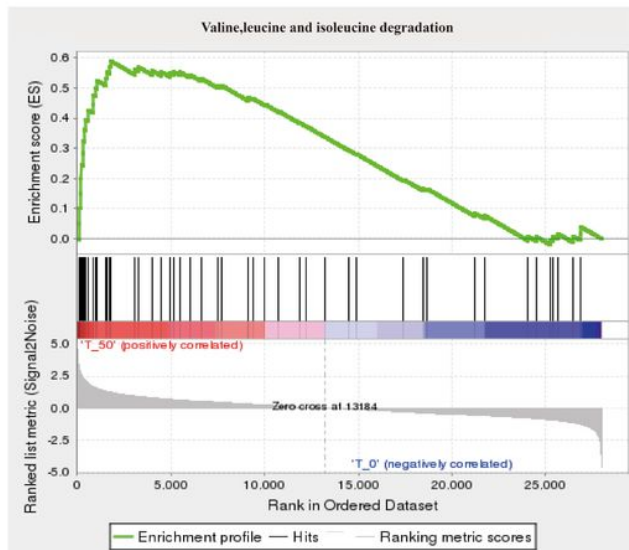


Figure 3

Heatmap of transcriptomics with each small plot representing the expression value of its gene, red for positive expression and blue for negative expression.

A



B

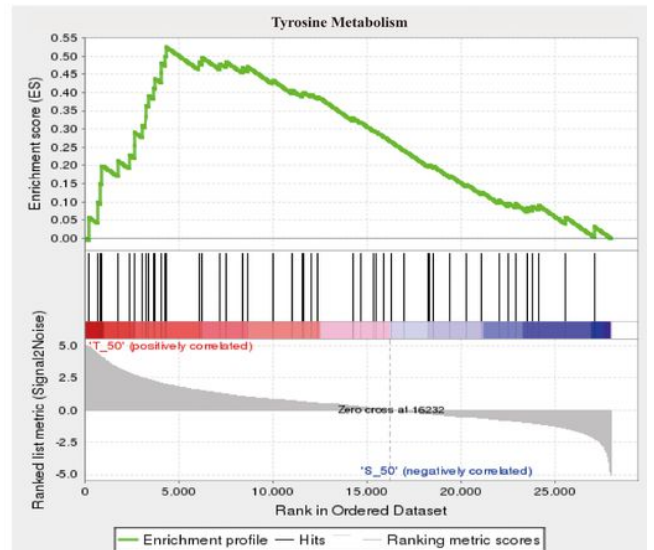


Figure 4

GSEA analysis (A)Valine, leucine and isoleucine degradation. (B)Tyrosine metabolism

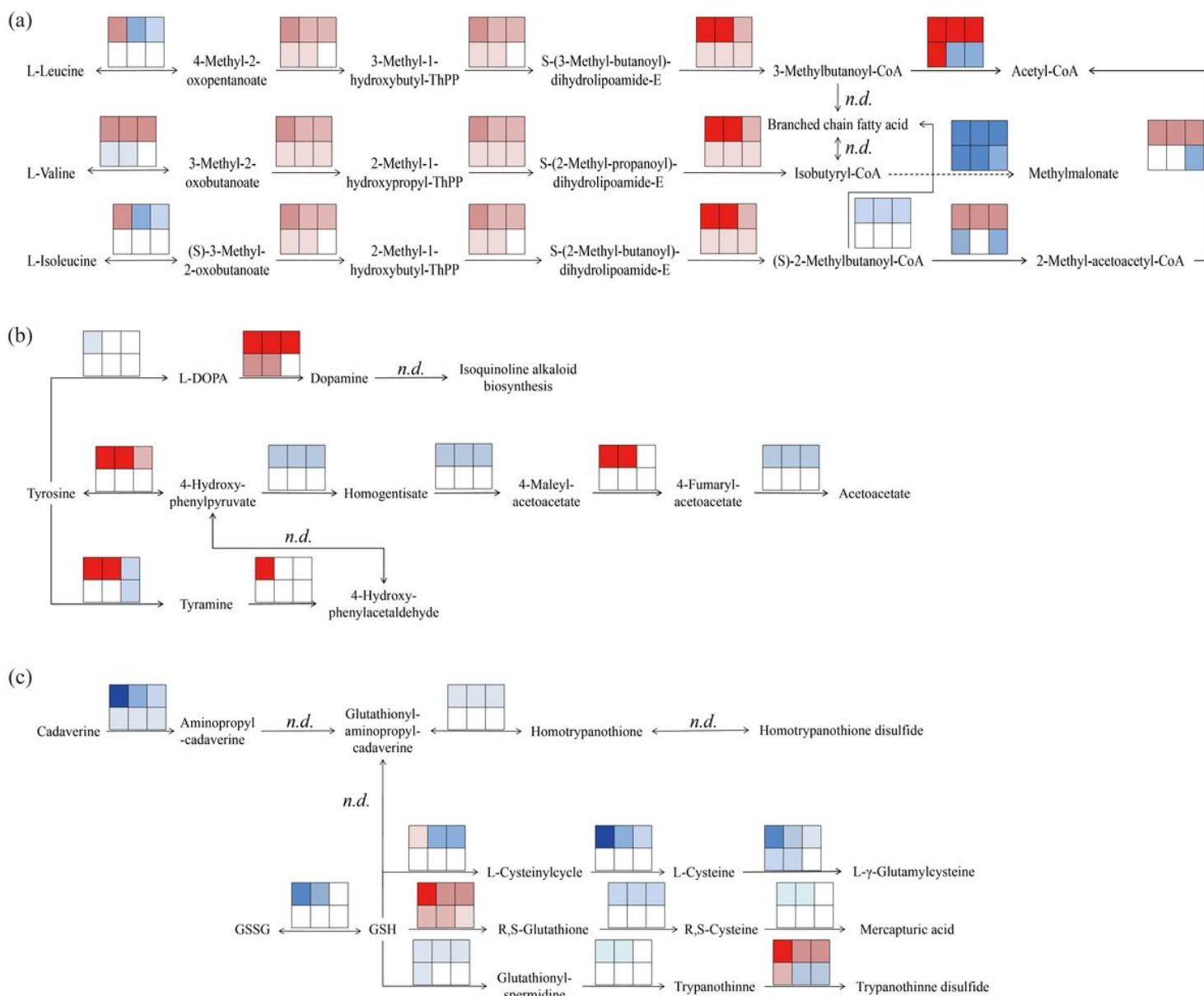


Figure 5

Significantly expressed amino acid metabolic pathways. The squares represent genes involved in this pathway, with red representing up-regulation and blue representing down-regulation.

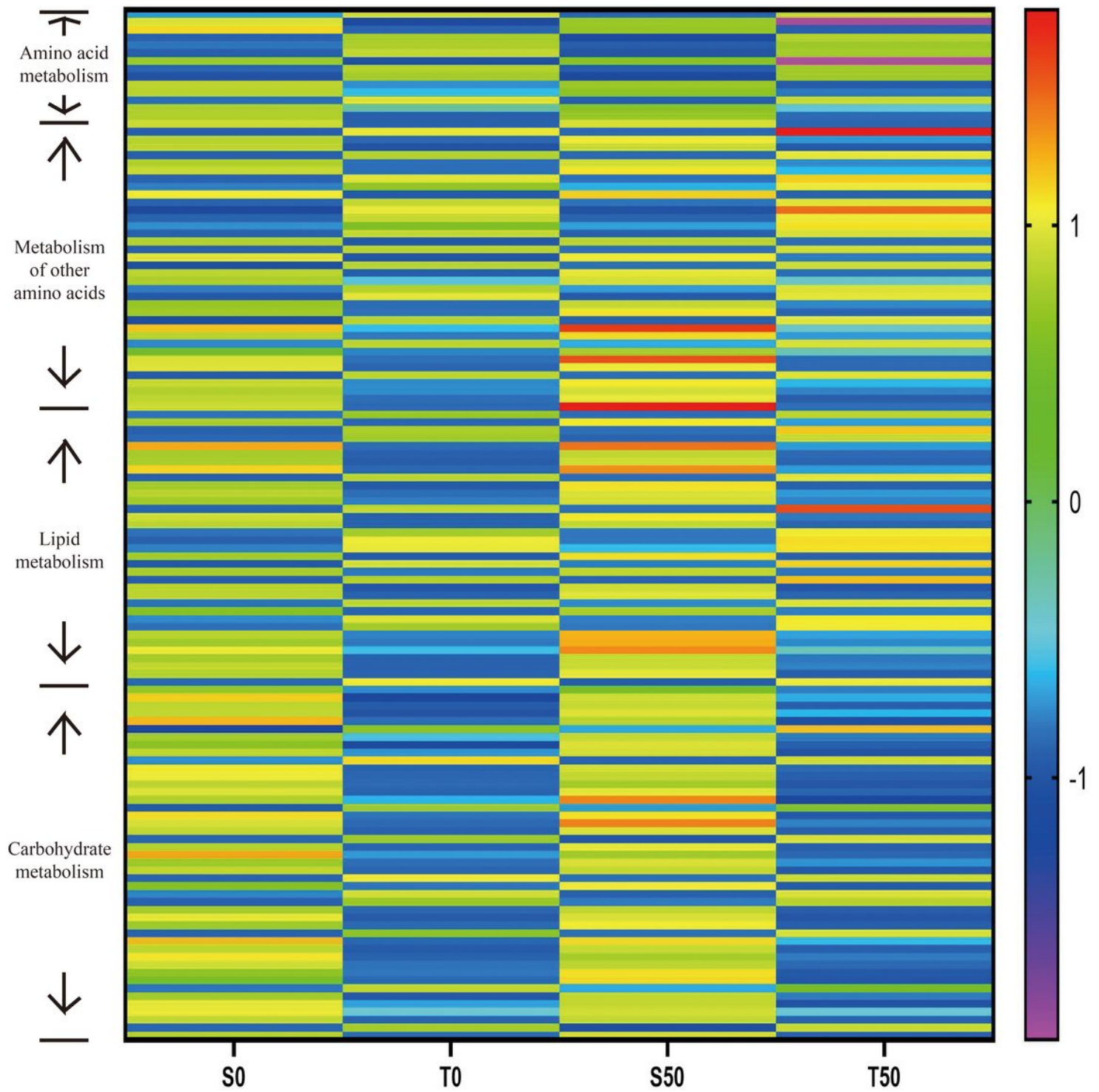


Figure 6

Heatmap of proteomics with each small plot representing the expression value of its protein , red for positive expression and blue for negative expression.

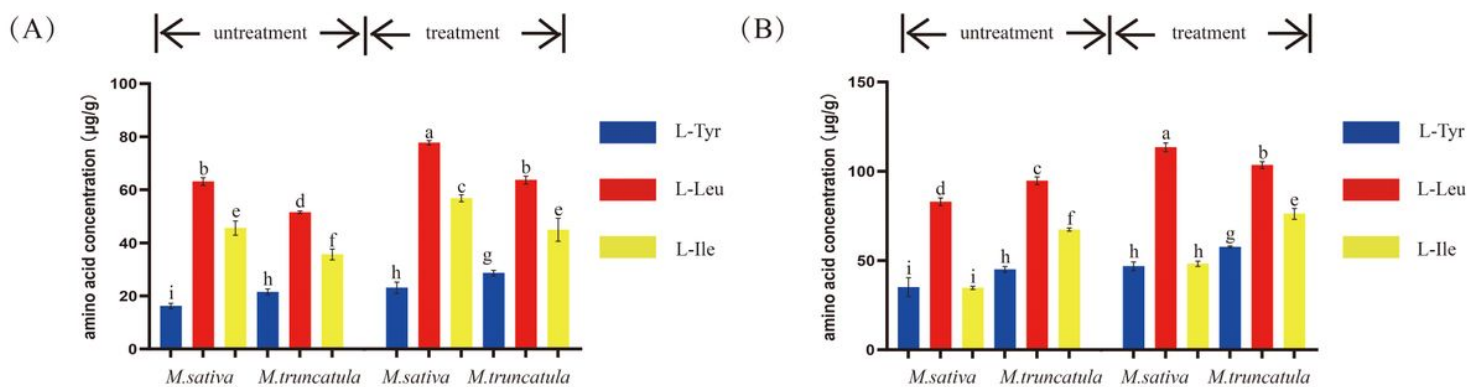


Figure 7

Determination of amino acid content. (A) Contents of three amino acids in roots (B) Contents of three amino acids in leaves. Different letters indicate significant differences between the two groups. ($P < 0.05$)

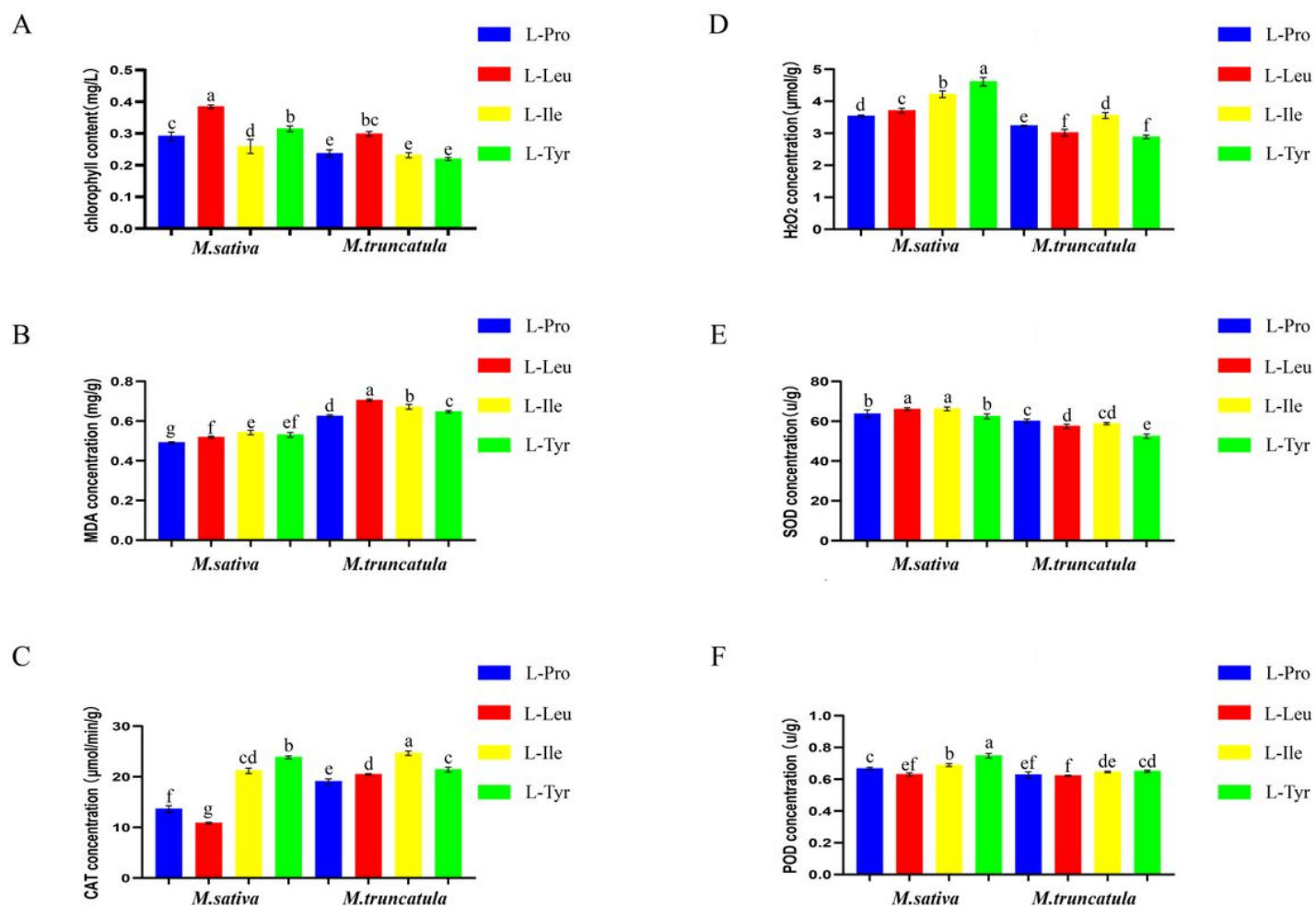


Figure 8

Physiological and biochemical indexes of epitaxial experiment.(A-F) SOD, CAT, MDA, POD, H₂O₂, and Na⁺ concentrations of *M. sativa* and *M. truncatula* seedlings treated with 50 mM NaCl. Different letters indicate significant differences between the two groups. ($P<0.05$)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [FigureS1.jpg](#)
- [FigureS2.jpg](#)
- [FigureS3.jpg](#)
- [TableS1.xlsx](#)
- [TableS2.xlsx](#)