Multi-omics analysis of the effects of soil amendment on rapeseed (Brassica napus L.) photosynthesis under drip irrigation with brackish water

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Abstract

Drip irrigation with brackish water increases the risk of soil salinization while alleviating water shortage in arid areas. In order to alleviate soil salinity stress on crops, polymer soil amendments are increasingly used. But the regulation mechanism of polymer soil amendment composed of polyacrylamide polyvinyl alcohol, and manganese sulfate (PPM) on rapeseed photosynthesis under drip irrigation with different types of brackish water is still unclear. In this field study, PPM was applied to study the responses of rapeseed (*Brassica napus* L.) phenotype, photosynthetic physiology, transcriptomics, and metabolomics at the peak flowering stage under drip irrigation with water containing 6 g · L$^{-1}$ NaCl (S) and Na$_2$CO$_3$ (A).

The results showed that the inhibitory effect of A treatment on rapeseed photosynthesis was greater than that of S treatment, which was reflected in higher Na$^+$ content (73.30%) and lower photosynthetic-fluorescence parameters (6.30-61.54%) and antioxidant enzyme activity (53.13–77.10%) of A-treat plants. The application of PPM increased the biomass (63.03–75.91%), photosynthetic parameters (10.55%-34.06%), chlorophyll fluorescence parameters (33.83–62.52%), leaf pigment content (10.30-187.73%), and antioxidant enzyme activity (28.37-198.57%) under S and A treatments. However, the difference is that under S treatment, PPM regulated sulfur metabolism, carbon fixation and carbon metabolism pathways in rapeseed leaves. And it regulated the photosynthesis-, oxidative phosphorylation-, and TCA cycle-related metabolic pathways in rapeseed leaves under A treatment. This study will provide new insights for the application of polymer materials to tackle the salinity stress on crops caused by drip irrigation with brackish water, and solve the difficulty in brackish water utilization.

1. Background

Freshwater is scarce in arid regions, so drip irrigation with brackish water is an important measure to alleviate the contradiction between agricultural production and water scarcity [1]. At present, countries around the world mainly use brackish and fresh water mixed irrigation, brackish and fresh water rotation irrigation, and filtration technology to increase the use of brackish water [2]. However, these methods still do not meet freshwater needs due to freshwater shortages, great domestic and industrial freshwater needs, and high costs in most arid regions, and also leads to surface accumulation of a large amount of salt, which increases the risk of salinization. Particularly, irrigation with brackish water could significantly alter many physiological processes of plants, especially photosynthesis, resulting in retarded plant growth and great yield reduction [3, 4].

The possible ways of salt stress affecting plant photosynthesis mainly include: ion poisoning, osmotic stress, and sugar accumulation-induced feedback inhibition [5]. Previous studies have shown that high soil salinity could destroy the stomatal structure and chloroplast structural integrity of plants [6], reduce net photosynthesis, photosynthetic pigment content, $F_v/F_m$ ratio, stomatal conductance, transformation rate, gas exchange, PSII efficiency, and water potential, and cause osmotic stress and nutrient deficiency in plants [7, 8]. Salt stress can also inhibit the carbon assimilation of plants, resulting in reduced accumulation of photosynthates [9]. In addition, salt stress also impacts the activities of key enzymes
related to photosynthesis, such as RuBPCase (ribulose-1, 5-bisphosphatecarboxylase), leading to reduced photosynthetic carbon assimilation and photosynthetic rate [10].

At present, there are many methods used to improve the tolerance of crops to salt stress. Biochar, a commonly used solid soil amendment, can reduce the negative effects of drought and salt stress on plants, and improve soil physical structure, water holding capacity, and fertility and plant photosynthesis [11]. However, biochar is insoluble in water and cannot be used through drip irrigation system that is widely applied by farmers in arid areas [12]. Artificial and mechanical application of biochar are time-consuming and high-cost. Other soil amendments widely studied by scholars such as zeolite, bentonite and gypsum are also insoluble in water [13]. Bioactive compounds and organic amendments such as plant growth-promoting bacteria and biofertilizers can enhance crop salt tolerance and yield by maintaining ionic homeostasis and reducing oxidative damage, but such amendments are expensive and have a slow effect and low utilization rate. Especially, high pH environment always causes inactivation of most bacteria [14]. Therefore, bioactive compounds and organic amendments are also not suitable for large-scale application in arid areas. Therefore, it is urgent to find a soil amendment suitable for drip irrigation system.

PPM is an independently developed soil amendment with inorganic and organic polymers as the main components. It has high biodegradability and high-water solubility, and can be applied through drip irrigation. Our previous study found that PPM had a variety of functional groups, such as carboxyl, alcohol hydroxyl, and silicone [15, 16]. The application of PPM could enhance the specific surface area and pore structure of soil, promote the formation of soil aggregates, increase soil nutrients [17], thereby improving the resistance of cotton, wheat, and other crops to salt stress [15]. B. napus is a salt-tolerant plant. The cropping of B. napus combined with drip irrigation with brackish water can improve the utilization of brackish water, alleviate freshwater crisis, and ensure agriculture production in arid areas. However, previous study showed that when soil salinity reached 5 g·L$^{-1}$, the growth of B. napus was significantly inhibited [18].

Therefore, in this study, the molecular regulatory mechanism of PPM on the photosynthesis of B. napus under drip irrigation with neutral (NaCl) and alkaline (Na$_2$CO$_3$) water was determined by the integrated transcriptomic and metabolomic analysis. The objectives were to reveal (1) the differences in the effects of drip irrigation with neutral and alkaline water on the physiological and photosynthetic parameters of B. napus, and (2) the differences in the mechanisms of PPM improving photosynthesis of B. napus under drip irrigation with neutral and alkaline water. This study will have guiding significance for brackish water drip irrigation and crop yield increase in arid areas.

2. Methods

2.1 Experimental site and materials
This experiment was conducted in Shihezi, Xinjiang, China (44°32′44.6″N, 85°99′877″E). The region is a continental dry climate, with annual sunshine hours of 2300–2700 h, annual average rainfall of 220 mm and annual evaporation of 1000–1500 mm. Soil amendment (PPM), a mixture of polyacrylamide, polyvinyl alcohol, and manganese sulfate prepared at 90 °C, were used in this experiment. Before application (22 July 2020), the PPM was mixed with inorganic fertilizer (polyvinyl alcohol: polyacrylamide: manganese sulphate: inorganic fertilizer = 1:3: 6: 50).

2.2 Experimental design

On June 15, 2020, 120 kg of soil (0–60 cm soil layer, pH: 8.25, cation exchange capacity: 17.32 cmol/kg; alkaline hydrolyzable nitrogen content: 56 mg/kg; available phosphorus content: 10.7 mg/kg; available potassium content: 226 mg/kg) was collected from the experimental site. Then, the soil was transferred into barrels (0.3 m × 0.6 m × 0.6 m) and the original soil layers were kept. After that, the barrels were buried back to the field. This experiment adopted a randomized complete block design with four groups, namely (1) SCK group (no soil amendment and water containing 6 g·L\(^{-1}\) NaCl (neutral salt) was used for drip irrigation), (2) ACK group (no soil amendment and water containing 6 g·L\(^{-1}\) Na\(_2\)CO\(_3\) (alkaline salt) was used for drip irrigation); (3) SPPM group (water containing 6 g·L\(^{-1}\) NaCl was used for drip irrigation and 12 g·L\(^{-1}\) of PPM was applied); (4) APPM treatment (water containing 6 g·L\(^{-1}\) Na\(_2\)CO\(_3\) was used for drip irrigation and 12 g·L\(^{-1}\) of PPM was applied). Each group had three replicates.

*B. napus* seeds (variety Huayouza 82) were sown after mixing with triple superphosphate (1: 15) on 15 July, 2020. After emergence, six seedlings were retained in each barrel. According to the experimental design, brackish water was irrigated at 10 d intervals throughout the growth period, and PPM was dissolved in irrigation water and applied through the drip irrigation system during the first irrigation. Six leaves were collected from the plants in each group at the full flowering stage (20 October), stored in liquid nitrogen.

2.3 Measurement methods

2.3.1 Determination of photosynthetic parameters, chlorophyll fluorescence parameters and plant fresh weight

At the full flowering stage (20 October), photosynthetic parameters including net photosynthesis rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), and intracellular CO\(_2\) concentration (Ci) were measured with a Li-6400 portable photosynthesis instrument (LI-COR, USA) [19]. Then, the maximum fluorescence after dark adaptation (F\(_{\text{v}}\)), minimum fluorescence after dark adaptation (F\(_{\text{0}}\)), minimum fluorescence under light (F\(_{\text{0}'}\)), and steady-state fluorescence after light adaptation (F\(_{\text{m}'}\)) were determined with a PAM-2100 modulated chlorophyll fluorometer (WALZ, Germany) [20]. Each measurement was repeated four times.

\[
F_{\text{v}}/F_{\text{0}} = (F_{\text{m}'}-F_{\text{0}})/F_{\text{0}} 
\] (1)
\[ \frac{F_v}{F_m} = \frac{(F_m - F_0)}{F_m} \] (2)

\[ \Phi = \frac{(F_m - F_s)}{F_m} \] (3)

\[ qP = \frac{(F_m - F_s)}{(F_m - F_0)} \] (4)

On 23 October, three plants were collected from each group. The roots, leaves and stems were weighed after rinsing with distilled water.

2.3.2 Determination of chlorophyll and carotenoids in plant leaves

Leaves were cut into small pieces. Then, 0.2 g of leaf sample and 20 mL of extract (absolute ethanol: acetone = 1: 1) were mixed evenly in a test tube, sealed, and placed in the dark until the sample turned white. After that, the chlorophyll solution was transferred into a cuvette, and the extract was used as a blank. Finally, colorimetry was conducted at 645 nm, 652 nm, 663 nm, and 440 nm [21].

2.3.3 Determination of leaf antioxidant enzyme activity and malondialdehyde (MDA) content

Leaf Superoxide dismutase (SOD), Peroxidase (POD), Catalase (CAT) activities and malondialdehyde (MDA) content were determined by NBT photochemical reduction method, guaiacol absorbance method, ultraviolet spectrophotometry and thiobarbituric acid method, respectively [22].

2.3.4 Determination of Na\(^+\), K\(^+\) content and relative electrical conductivity in leaves

The Na\(^+\) and K\(^+\) content and REC were determined as previous method by AP1200 flame spectrophotometer (AP1200, Shanghai, China) and DDSJ-219L conductivity meter [23, 24].

2.3.5 Transcriptomic and metabolomic assays

12 leaf samples rapidly stored in liquid nitrogen and stored by Beijing Biomic Biotechnology Co., Ltd. for transcriptomic and metabolomic analysis. The transcriptional sequencing platform was Illumina HiSeq, and the metabolome was determined by UPLC-MS/MS [25].

2.4 Statistical analysis

The data was processed by Excel 2016 software. SPSS 23.0 (SPSS Inc., Chicago, IL, USA) was used for one-way variance (ANOVA) analysis (\( \alpha = 0.05 \)). Figures was drawn using Origin 8.0 (Origin Lab, Massachusetts, USA). The co-occurrence relationships were visualized using Gephi 0.9.2 (https://Gephi.org/). Fastp was used to remove low-quality sequences in adapters and reads [26]. The filtered reads were aligned to the reference genome of \( B. \ napus \) (https://www.cottongen.org/species/Gossypium_hirsutum/ZJU-AD1_v2.1). Gene expression level was calculated in units of FPKM (Fragments Per Kilo bases Per Million Fragments Mapped). Differential analysis of gene expression was performed using the DESeq package in R software, and differentially expressed genes (DEGs) were selected based on \(|\log_{2}\text{FoldChange}| > 1\) and \( p < 0.05 \). Differentially
expressed genes and transcription factors were subjected to GO and KEGG enrichment analysis using the clusterProfiler package in R software [27].

3. Result

3.1 Effects of application of PPM on biomass and leaf physiological parameters of *B. napus*

Comparing the ACK group with the SCK group, the root, stem, and leaf fresh weight decreased by 25.37% percent, 25.37%, and 25.37%, respectively (*p* < 0.05). A decrease of 17.96% was observed in the leaf K+ content, a rise of 51.32% in the K+/Na+ ratio, and a decrease of 40.52% in the REC in the ACK group (*p* < 0.05), while the Na+ content increased by 73.30% (*p* < 0.05), compared with those in the SCK group. The application of PPM obviously increased the fresh weight, leaf K+/Na+ ratio, K+ content, and REC content of *B. napus* under brackish water irrigation. The root, stem, and leaf fresh weight in the SPPM group increased by 69.03%, 63.03%, and 72.04%, respectively (*p* < 0.05) compared with those in the SCK group. The leaf K+/Na+ ratio and K+ content in the SPPM group increased by 29.54% and 12.22%, respectively (*p* < 0.05), while leaf Na+ content and REC decreased by 12.22% and 64.88%, respectively (*p* < 0.05), compared with those in the SCK group. The root, stem, and leaf fresh weight in the APPM group increased by 75.11%, 66.59%, and 75.91%, respectively (*p* < 0.05) compared with those in the SPPM group. The leaf K+/Na+ ratio and K+ content in the APPM group increased by 36.14% and 26.55%, respectively (*p* < 0.05), while leaf Na+ content and REC decreased by 7.05% and 22.23%, respectively (*p* < 0.05), compared with those in the ACK group (Fig. 1).

3.2 Effects of PPM application on photosynthetic parameters of *B. napus* leaves

The Pn, Gs, and Tr of *B. napus* leaves in the SPPM group increased by 10.55%, 17.01%, and 34.06%, respectively (*p* < 0.05), but the Ci decreased by 3.36% (*p* > 0.05), compared with those in the SCK group. The Pn, Gs, and Tr in the APPM group increased by 17.01%, 23.74%, and 42.34%, respectively (*p* < 0.05), but the Ci decreased by 18.83% (*p* < 0.05), compared with those in the ACK group. In addition, there are no difference in Pn, Tr, and Gs between NaCl (SCK and SPPM) groups and Na$_2$CO$_3$ (ACK and APPM) groups (*p* > 0.05) (Fig. 2a). The leaf chlorophyll a (Chl a) in the ACK group increased by 10.00% (*p* < 0.05), but the chlorophyll b (Chl b) and carotenoids (Car) reduced by 14.49% and 16.74%, respectively, compared with those in the SCK group. The application of PPM enhanced photosynthetic pigment contents of *B. napus* leaves under drip irrigation using brackish water. The leaf Chl a, Chl b, Chl a + Chl b, and Car in the SPPM group increased by 56.34%, 40.05%, 51.68%, and 33.83%, respectively (*p* < 0.05) compared with those in the SPPM group, and those in the APPM group increased by 35.00%, 43.25%, 36.97%, and 62.52%, respectively (*p* < 0.05) compared with those in the ACK group. Besides, the Chl a in the SPPM group decreased by 6.08% (*p* < 0.05) compared with that in the APPM group (Fig. 2b). As compared to the SCK group, the F$_{v}$/F$_{0}$, Fv/Fm, and qP of *B. napus* leaves in the SPPM group increased by 49.91%, 10.30%, and 14.29%, respectively (*p* < 0.05). In comparison to the ACK group, the F$_{v}$/F$_{0}$, Fv/Fm, and qP of *B. napus*
leaves in the APPM group increased by 183.73%, 48.07%, and 57.62%, respectively ($p<0.05$). PSII photochemistry ($\Phi_{\text{PSII}}$) did not differ among the four groups (Fig. 2c).

### 3.3 Effects of PPM application on antioxidant enzyme activity

A comparison between the ACK and SCK treatments revealed that SOD, POD, and CAT activity increased by 53.13%, 54.25%, and 77.10% ($p<0.05$). The activity of SOD, POD, and CAT in the SPPM group increased by 29.21%, 140.17%, and 198.57%, respectively compared with those in the SCK group ($p<0.05$), and the activity of SOD, POD, and CAT in the APPM group increased by 28.37%, 70.33%, and 77.87%, respectively compared with those in the ACK group ($p<0.05$). The content of MDA in the SPPM group decreased by 15.42% compared with that in the APPM group ($p<0.05$) (Fig. 2d). RDA analysis (Fig. 2e, f) showed that the leaf photosynthetic parameters ($\text{Tr}$, $\text{Ci}$, $\text{qP}$, and $\Phi_{\text{PSII}}$), antioxidant enzyme activities, and photosynthetic pigment content were significantly positively correlated with stem and leaf fresh weight, and negatively correlated with leaf REC, $\text{K}^+$ and $\text{Na}^+$ content. Meanwhile, $F_v/F_m$, $F_v/F_0$, and $\text{qP}$ were positively correlated with root fresh weight, and negatively correlated with $\text{K}^+$ and $\text{Na}^+$ content. However, MDA content and $\text{Gs}$ were negatively correlated with the fresh weight of plant organs and positively correlated with $\text{K}^+$ content and REC.

### 3.4 Transcriptomic and metabonomic analysis

#### 3.4.1 Integrated metabolomic and transcriptomic analysis

Principal component analysis (PCA) was performed to assess the correlation of the transcriptomes and metabolomes. The results showed that samples of the SCK and ACK group were separated on PC2, and those of the control (SCK and ACK) and PPM (SMMP and AMMP) groups were separated on PC1 based on the metabolite count (Fig. 3A). Samples of the NaCl and Na$_2$CO$_3$ groups were separated on PC2, and those of the control and PPM groups were separated on PC1 based on the gene counts (Fig. 3D). Moreover, there were 83 shared DAMs and 7 shared DEGs for the four groups (Fig. 3B, E). The number of DEGs and DAMs in the SPPM and APPM groups were more than those in the SCK and ACK groups. In addition, the up-regulated DEGs were more than the down-regulated, while the down-regulated DAMs were more than the up-regulated (Fig. 3C, F).

Many DEGs and DAMs for the four groups were enriched in pathways related to Energy metabolism (including Sulfur metabolism and Oxidative phosphorylation) and Carbohydrate metabolism. Besides, DEGs were also enriched in Nitrogen metabolism, Carbon fixation in photosynthetic organisms, Photosynthesis-antenna proteins, and Photosynthesis pathways, and DAMs were also enriched in the Amino sugar and nucleotide sugar metabolism, Tricarboxylic acid (TCA) cycle, Carbon metabolism, and Carbon fixation pathways in photosynthetic organisms. The DEGs for the SCK vs ACK were significantly enriched in Nitrogen metabolism and Photosynthesis -antenna proteins pathways, and those for the SCK
vs SPPM were significantly enriched in Sulfur metabolism pathway. The DEGs for the ACK vs APPM were significantly enriched in Photosynthesis - antenna proteins and Photosynthesis pathways, while those for the SPPM vs APPM were significantly enriched in Citrate cycle pathway (Fig. 3G, H).

3.4.2 PPM' impacts on energy metabolism and carbohydrate metabolism pathways in B. napus

DEGs and DAMs with a high photosynthesis-related content were enriched in pathways such as energy metabolism, carbohydrate metabolism, photosynthesis-antenna proteins, and photosynthesis (Fig. 4A). Many genes (BanA09G0675700ZS, BanC04G0172900ZS, and BanA09G0666300ZS) were highly expressed in the four groups, but the expression of genes such as BanA09G0675700ZS, BanC04G0172900ZS, and BanA09G0666300ZS were at a low level. Compared with the CK (ACK and SCK), the expression of BanC02G0540200ZS and BanC02G0555600ZS in the PPM groups (APPM and SPPM) were up-regulated, while that of BanA03G0578600ZS, BanC01G0424400ZS, and BanC07G0195800ZS were down-regulated. Different treatments had different mechanisms in regulating the carbon metabolism pathway (Fig. 4B, C). The expression of ALDO and FBP were significantly down-regulated in the Calvin cycle, while that of RPIA was significantly up-regulated. Besides, the expression of ACLY, FH, and SDHB were significantly down-regulated in the TCA cycle. Compared with the CK, the relative abundance of metabolites 2-Oxoglutaric acid and Citric acid were significantly down-regulated, while that of Fumarate and L-malic acid were significantly up-regulated. The abundance of metabolites related to Amino acid metabolism and Carbohydrate metabolism were down-regulated.

RDA analysis results (Fig. 4D,E) showed that the expression of photosynthesis-related transcription factors psb.O, psb.W, psa.O, and psa.N and the abundance of Fumaric acid, L-Malic acid, and 5'-Deoxy-5'- (methylthio)adenosine were positively correlated with the fresh weight of plant organs and negatively correlated with REC, K\(^+\) content, and Na\(^+\) content. while the expression of psb.D, psb.W, psa.H, and psa.N and the abundance of Fumaric acid, L-Malic acid, and 5'-Deoxy-5'-(methylthio) adenosine were negatively correlated with the fresh weight of plant organs and positively correlated with REC, K\(^+\) content, and Na\(^+\) content.

4. Discussion

Irrigation using brackish water with high salt concentration produces ionic toxicity on B. napus and inhibits its photosynthesis and cell growth [8]. This study found that photosynthetic characteristics and antioxidant enzymes activities of B. napus showed differences when subjected to different types of brackish water drip irrigation. The leaf photosynthetic parameters (Pn, Tr, and Gs), fluorescence parameters (Fv/Fo, Fv/Fm, and qP), and Chl b under alkaline water drip irrigation condition were lower than those under neutral water drip irrigation condition. This indicates that alkaline salts have greater inhibition on the leaf photosynthesis of B. napus, especially on the net photosynthetic rate and fluorescence parameters. This ultimately inhibits the dry matter accumulation in B. napus [28, 29].
Besides, long-term brackish water drip irrigation could also lead to massive accumulation of Na\(^+\) and other salt ions in plants, disrupting osmotic balance, and competitively inhibiting the uptake of other ions [30]. This study found that more Na\(^+\) and less K\(^+\) were accumulated in leaves under alkaline water drip irrigation compared to neutral water drip irrigation. High Na\(^+\) content could cause high osmotic pressure in \textit{B. napus}, resulting in the closing of leaf stomata and inhibited photosynthesis, metabolism, and biomass accumulation. Besides, excessive Na\(^+\) can also cause a deficiency of phosphorus and nitrogen, restricting the synthesis of chloroplasts [31, 32]. The less K\(^+\) content under alkaline water drip irrigation may hinder starch synthesis in \textit{B. napus} and accelerate the decomposition of starch into sugar. However, carbohydrates in leaves cannot be transported smoothly, which causes massive accumulation of photosynthesis-synthesized sugars in cells. This ultimately causes feedback inhibition and reduced accumulation of dry matter by photosynthesis. This study also found that alkaline water irrigation had a greater effect on the chlorophyll content of \textit{B. napus} than neutral water irrigation. Chloroplasts are the main sites of plant photosynthesis [33, 34]. So, in this study, alkaline water irrigation led to reduced leaf photosynthates and plant biomass.

In this study, application of PPM regulated \textit{B. napus} growth and photosynthesis during the full flowering stage under brackish water drip irrigation conditions. Photosynthesis and antioxidant defense play key roles in the response of \textit{B. napus} to salt stress [35]. The present study showed that PPM application reduced the ionic toxicity and oxidative stress to \textit{B. napus} under brackish water drip irrigation conditions. This may be due to that the acidic functional groups such as COOH-, C = O, -SH, and -CHO in PPM could combine with the salt ions brought by brackish water drip irrigation, and improve the structure of soil aggregates and soil structure [36]. This could reduce the uptake of salt ions by \textit{B. napus}, alleviate ionic toxicity and oxidative stress, and ensure normal photosynthesis and biomass accumulation. Besides, PPM can also regulate osmotic potential in \textit{B. napus} leaves by regulating the accumulation of compatible solutes such as soluble sugars, organic acids, amino acids, choline, and betaine, which could protect the photosynthase system [37], thus improving the photosynthetic rate and antioxidant defense system of \textit{B. napus} [38]. Therefore, PPM can maintain the intracellular stability and normal physiological activities of \textit{B. napus} leaves under salt stress, and protect photosynthesis and other physiological processes from serious impact.

\textit{B. napus} can adapt to high salinity environments by molecular and metabolic regulations. The differences in \textit{B. napus}'s self-regulation under different types of brackish water drip irrigation were reflected in pathways related to nitrogen metabolism and photosynthesis. This study (Fig. 5A, 5B) found that under neutral water irrigation, transcription factors \textit{psaN} and \textit{atpF} and metabolites malic acid, glycolyneural, and fumaric were the main responders in the photosynthesis-related pathways and played positive regulatory roles. Previous study [39] has shown that salt stress could inhibit the transcription and translation of \textit{psbA} encoding D1 protein and the expression of light-induced genes, so that these genes could not be transcribed, translated, and repaired to form active PSII reaction center. However, the expression of \textit{psbW} and \textit{psbN} were negatively correlated with root fresh weight, leaf fresh weight, and the abundance of Oxoglutaric, and the leaf and root fresh weight and photosynthetic parameters were
negatively regulated by Chla, Fv/Fo, and Oxoglutaric abundance. This indicates that \textit{psbW} and \textit{psbN} could regulate plant growth and photosynthesis under neutral water irrigation conditions. In addition, in the ACK group, transcription factors \textit{psbW} and \textit{atpF} and metabolites Lactone, Malic, and Fumaric played a positive regulatory role on leaf and root fresh weight. In addition, the PSII and Chl a + b played a positive regulatory role on the internal homeostasis of \textit{B. napus} leaves. This indicates that these transcription factors and metabolites directly regulated the growth of \textit{B. napus} under alkaline water irrigation condition. Therefore, under different brackish water irrigation conditions, rapeseed can change the accumulation of metabolites in photosynthesis-related metabolic pathways by regulating the expression of photosynthesis-related genes and metabolites in rapeseed leaves, thereby maintaining the normal growth of plants in adverse environments. It should be noted that compared to neutral water irrigation, alkaline water irrigation had a greater impact on plant cell homeostasis.

It was found that soil amendment could improve plant photosynthetic performance and biomass accumulation by changing the expression of genes, transcription factors and metabolites related to photosynthesis-related pathways under salt stress conditions (Fig. 4). However, the application of PPM could regulate transcription factors and metabolites in pathways related to Energy metabolism and carbohydrate metabolism in \textit{B. napus} leaves to regulate photosynthates. Under neutral water irrigation, the application of PPM mainly regulated Sulfur metabolism, Carbon fixation pathways in photosynthetic organisms, and carbohydrate metabolism pathways. The regulatory networks (Fig. 5C, D) showed that the application of PPM changed the internal homeostasis of \textit{B. Napus} leaves under brackish water drip irrigation, and \textit{atpF}, \textit{psbO}, \textit{psaN}, \textit{psaH}, malic, adenosine, and lactone were the main regulators. It has been reported that PsbO, PsbP, and PsbQ play a crucial role in maintaining the active site of PSII [40]. This indicates that in this study, PPM may improve the photosynthetic efficiency by maintaining the active site in PSII, thereby increasing the biomass. It was also found that under neutral water irrigation condition, \textit{atpF} and \textit{psaH} mainly regulated the dry matter. Among them, \textit{atpF} positively regulated the fresh weight and K+/Na+ ratio, while \textit{psaH} negatively regulated them (Fig. 5). \textit{PsbN} and \textit{psbO} mainly regulated the Pn, Tr, POD activity, SOD activity, and MDA content, while \textit{psbN} had an opposite effect on these parameters. Under alkaline water irrigation, the photosynthesis, oxidative phosphorylation, and TCA cycle metabolic pathways were mainly regulated. The regulatory mechanisms of \textit{atpF}, \textit{psaH}, and \textit{psbN} under alkaline water irrigation condition were similar to those under neutral water irrigation condition, but \textit{psbO} did not play a good regulatory role under alkaline water irrigation condition.

This study found that brackish water drip irrigation mainly led to changes in nitrogen metabolism, sulfur metabolism, and oxidative phosphorylation pathways in \textit{B. Napus} leaves at the full flowering stage. This is different from the self-regulation mechanism of \textit{B. Napus} under salt stress at the seedling stage. Wang reported that under salt stress, glucose metabolism, amino acid metabolism, and glycerol metabolism pathways were mainly regulated in \textit{B. Napus} at the seedling stage [25]. In this study, the application of PPM further changed the regulatory pathways of \textit{B. Napus} in the full flowering stage, with Calvin cycle and TCA cycle metabolism as the main metabolic pathways, and enhanced the regulatory effects of transcription factors (\textit{atpF}, \textit{psbO}) and metabolites (Fumarate, Citric acid, and Fumarate). Besides, after
the application of PPM, changes in transcription factors (such as ALDO, RPIA, and FH) and metabolites (such as Fumarate, Citric acid, and Fumarate) that were positively correlated with photosynthesis were more under neutral water drip irrigation than under alkaline water drip irrigation. This indicates that PPM has a better effect on alleviating neutral salt stress. It should be noted that under neutral and alkaline water irrigation conditions, the regulatory mechanism of *B. Napus* showed great differences (Fig. 5), the main factors were inapparent, and the regulatory effects of transcription factors (atpF, psaN) and metabolites (adenosine, malic) related to photosynthesis were weak. However, after the application of PPM, the regulatory networks under neutral and alkaline water irrigation conditions tended to be consistent. That is, the regulatory effects of transcription factors (atpF, psbO, psaH and psaN) and metabolites (Malic and adenosine) were enhanced, and the biomass and physiological indicators of *B. Napus* were positively regulated.

5. Conclusion

Irrigation using different types of brackish water cause different ionic toxicity to *B. Napus*, resulting in differences in photosynthetic characteristics. Alkaline water drip irrigation inhibited the photosynthesis of *B. Napus* greater, causing lower photosynthetic (Pn, Tr, and Gs) and fluorescence (Fv/Fo, Fv/Fm, and qP) parameters and chlorophyll b content in *B. Napus* leaves. Besides, there were differences in the expression of photosynthesis-related genes, transcription factors, and metabolites under different types of brackish water drip irrigation conditions, leading to differences in the photosynthesis-related metabolic pathways and photosynthate content. The pathways related to nitrogen metabolism and photosynthesis were changed by brackish water drip irrigation. However, the application of PPM reduced the ion toxicity and oxidative stress induced by brackish water drip irrigation, increased the photosynthetic pigment content and improved the chlorophyll fluorescence parameters, thereby enhancing the photosynthetic performance and biomass accumulation. The integrated transcriptomic and metabonomic analysis results showed that the application of PPM could regulate transcription factors and metabolites related to energy metabolism and carbohydrate metabolism-related pathways, to regulate photosynthates and increase biomass. However, these transcription factors and metabolites were different under alkaline and neutral water drip irrigation conditions. Under neutral water irrigation, the application of PPM mainly regulated Sulfur metabolism, Carbon fixation pathways in photosynthetic organisms, and Carbohydrate metabolism pathways in *B. Napus* leaves. However, under alkaline water irrigation, the photosynthesis, oxidative phosphorylation, and tricarboxylic acid (TCA) cycle metabolic pathways were mainly regulated (Fig. 6). This study provides technical guidance for the use of brackish water in agriculture by the application of polymer soil amendments in arid areas.

**Abbreviations**

*PPM*: A soil amendment composed of polyacrylamide, polyvinyl alcohol, and manganese sulfate

*Pn*: Leaf net photosynthetic rate
\( G_s \): stomatal conductance

\( T_r \): transpiration rate

\( C_i \): intracellular \( \text{CO}_2 \) concentration

\( F_v \): maximum fluorescence after dark adaptation

\( F_o \): minimum fluorescence value after dark adaptation

\( F_o' \): minimum fluorescence under light

\( F_m \): variable fluorescence

\( F_m' \): maximum fluorescence after light adaptation

\( F_s \): steady-state fluorescence after light adaptation

\( F_v/F_m \): maximum photochemical efficiency of photosystem

\( F_v/F_o \): potential activity of photosystem

\( q_P \): photochemical quenching coefficient

\( \text{Chl} \ a \): chlorophyll a

\( \text{Chl} \ b \): chlorophyll b

\( \text{Car} \): carotenoids

\( \text{SOD} \): superoxide dismutase

\( \text{POD} \): peroxidase

\( \text{CAT} \): catalase

\( \text{MDA} \): malondialdehyde

\( \text{REC} \): relative electrical conductivity

\( \text{DEGs} \): differentially expressed genes

\( \text{DAMs} \): differentially accumulated metabolites

\( \text{LC/MS} \): liquid chromatography-mass spectrometry

\( \text{PCA} \): principal component analysis
**RDA**: redundancy analysis

**FPKM**: fragments per kilobase of exon per million fragments mapped

## Declarations

### Ethics approval and consent to participate

We declare that experimental research on plants were done in compliance of institutional, national, and international guidelines and legislation.

### Consent for publication

Not applicable.

### Availability of data and materials

The raw reads of *Brassica Napus* Transcriptome and Gene expression (TaxID: 3708) are available under accession PRJNA772052 at NCBI Sequence Read Archive (SRA) repository. All data have been released.

### Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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### Authors' contributions

Z.W.L., W.K.Y., H.F., L.Y. and Y.T.L. designed the experiment; Z.W.L., S.W., D.S.H., S.Z.B. and S.C.L. collected the data; Z.W.L. and M.Z., W.D. wrote the manuscript. All authors contributed to the final version of the manuscript.

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## References


Figures
Figure 1

Effects of soil amendment (PPM) on the morphology (a), fresh weight (b), and leaf K$^+$ and Na$^+$ content (c) of *Brassica napus* under drip irrigation with water containing 6 g · L$^{-1}$ NaCl and 6 g · L$^{-1}$ Na$_2$CO$_3$. Different lowercase letters indicate significant difference between groups at $p < 0.05$. 
Figure 2

Effects of application of PPM on photosynthetic parameters (A), pigment content (b), fluorescence parameters (c), and antioxidant enzyme activities (b) of Brassica napus under drip irrigation with waters containing 6 g · L⁻¹ NaCl and 6 g · L⁻¹ Na₂CO₃ and RDA analysis of the parameters. Different lowercase letters indicate significant difference between groups at $p < 0.05$. 
Figure 3

PCA analysis (A, D), Venn diagram (D, E), up- and down- regulation statistics (C, F), and KEGG annotation (G, H) of DEGs (differentially expressed genes) and DAMs (differentially accumulated metabolites) in different groups. *, p < 0.05.
Figure 4

The expression of differentially expressed genes (DEGs) related to photosynthesis in different groups (A), metabolic pathway analysis (B), photosynthetic mechanism and energy metabolic pathway analysis (C), and RDA analysis of various parameters between groups (D, E).
Figure 5

Relationship networks of the yield/ion content (red), photosynthetic parameters (yellow), transcription factors (blue), and DAMs (green) of *B. Napus* under different treatments. The red lines indicate positive correlation, and the blue lines indicate negative correlation. Node size is positively correlated with the number of connections.
Figure 6

Regulatory mechanism of the effect of soil amendment on rapeseed (*Brassica napus* L.) photosynthesis under drip irrigation with brackish water.