Impacts of shale gas wastewater leaks on neighboring crops: Physiological and morphological responses of tomatoes

Impacts of shale gas wastewater leaks on neighboring crops: physiological and morphological responses of tomatoes

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Abstract: As shale gas extraction expands, the volume of flowback and produced water (FPW) from hydraulic fracturing increases, raising concerns about the potential ecological risks of leaks. In this work, we mixed tap water with different proportions of FPW to simulate various leak scenarios. Tomatoes were irrigated using these mixtures or using FPW treated with multi-stage pre-treatment and advanced membrane technologies to assess the effects on tomato seed germination, plant vigor, element accumulation, and fruit gene expression. Compared to tap water irrigation, all three dilution ratios of FPW inhibited seed germination and growth to varying degrees, significantly reduced tomato biomass and fruit yield, and caused the fruit to exceed safe limits for Pb and Cr. In the lowest dilution group, the germination rate was only 17%, and total biomass decreased by 55%. Even in the highest dilution group, fruit yield was 11% lower than the control. Additionally, the dilution showed high salt accumulation toxicity, with yellowing at the edges of the leaves. Applying treated FPW accelerated tomato growth and increased the yield of tomato hairs, with total biomass rising by 38%. Finally, a large number of differentially expressed genes were detected in the fruits irrigated with FPW. These genes are irelated to carbon and nitrogen metabolism, affecting the synthesis of carbohydrates and proteins. These findings provide insights into the risks associated with shale gas FPW leakage and offer guidance for the reuse of treated FPW.

Keywords: Shale gas wastewater, heavy metals, tomato growth, gene expression, food safety

1. Introduction

The combined use of conventional and clean energy is a key strategy for balancing energy supply and environmental protection during the current energy transition. As natural gas prices rise, interest in shale gas resources is growing. The level of hydraulic fracturing technology is advancing, and industrial exploration and development of shale gas are rapidly expanding (Serrano-Areválo et al., 2022; Sun et al., 2019; Zhong et al., 2023). Upon hydraulic fracturing, fracturing fluids and formation water return to the surface in the form of flowback and produced water (FPW). Based on current annual FPW emissions in the Sichuan Basin, studies predict that by 2030, China will produce about 5 to 73 million cubic meters of FPW (Zou et al., 2018). This wastewater contains high concentrations of total dissolved solids, metals, organics, chemical additives, and naturally occurring radioactivity (Liang et al., 2022; Zhou et al., 2022). In recent years, there has been growing concern among communities and regulatory agencies about the potential impacts of FPW on agricultural activities, particularly on crop and soil quality, and it has become increasingly important to understand the interactions between FPW and agricultural systems (Ni et al., 2022; Peng et al., 2024; Zhou et al., 2023a).

Currently, FPW is treated by deep well reinjection, reused following some treatment, or discharged (Estrada and Bhamidimarri, 2016; Zhang et al., 2016). The deep well injection method involves treating FPW and reinjecting it into the same geological formation. However, this method faces issues such as limited injection well capacity, high uncertainty in injection risks, and increasingly strict water quality standards(Sun et al., 2019). Studies have shown that treating FPW through deep well injection can cause elevated concentrations of Cl, Br, Sr, and

Li in nearby streams (Akob et al., 2016). The internal recycling method for treated FPW involves processing the wastewater to meet the quality standards for drilling or fracturing fluids, then reusing it in operations. This method offers benefits such as water conservation, low environmental impact, and low treatment costs. However, while repeated internal reuse has been shown to lead to enrichment of salinity and some pollutants in the recycled stream, causing pipeline corrosion and negatively affecting the production of gas wells (Zhou et al., 2022). After FPW is treated by physical or chemical methods to meet discharge standards, discharging it into surface water is the most widely used treatment approach. Current methods for treating shale gas wastewater include membrane separation, adsorption, advanced oxidation, activated sludge, and biofilm methods. Membrane-based technologies are considered a promising solution for treating such wastewater (Liu et al., 2024). Due to the complexity of shale gas wastewater composition, even after treatment, it may impact ecosystems in water used for crop irrigation and other purposes. (Song et al., 2024; Wu et al., 2023).

Large volumes of FPW require consideration of the ecological risks of its leakage, which will inevitably result from storage pit and pipeline leaks, transportation accidents, or mishandling of surface discharges. The U.S. Environmental Protection Agency reported 457 hydraulic fracturing-related spills across 11 states between January 2006 and April 2012, with FPW spills being the most common type. Studies estimate that FPW transportation spills occur once every 19 well pads developed, and onsite spills occur once every 16 well pads (Clancy et al., 2018; EPA, 2015). Leaked FPW may enter streams through surface runoff and groundwater. Na and Cl are the two most concentrated substances in leaked FPW, and spills of saline wastewater can impact nearby streams for over six months. Studies monitoring FPW spills into rivers found high levels of Na downstream even six months after the incident (Agarwal et al., 2020). In January 2015, 11.4 million liters of natural gas wastewater spilled into a local river in the Williston Basin. Elevated concentrations of chlorides and bromides were detected downstream, with lithium, boron, and strontium levels 5–10 times higher than upstream. Radium activity in downstream sediments reached up to 15 times that of upstream levels (Cozzarelli et al., 2021; Cozzarelli et al., 2017). Even treated FPW poses risks to receiving water bodies. Organic pollutants persist in treated FPW and can be transported downstream (Zhou et al., 2024). Researchers found a significant increase in the relative abundance of 5 antibiotic resistance gene (ARG) subtypes and 7 virulence factor genes (VFGs) at downstream sites near discharge points, indicating wastewater discharge impacts microbial communities (Mumford et al., 2020; Peng et al., 2024).

Plants can absorb and accumulate complex organic molecules, such as polycyclic aromatic hydrocarbons, endocrine disrupting chemicals, personal care products, and pharmaceuticals from soil and irrigation water (Dodgen et al., 2013; González García et al., 2019; Zhang et al., 2017). While FPW does not commonly contain any pharmaceuticals or personal care products, the salinity, alkalinity, and ionic composition of FPW may affect normal plant development and soil quality. Wheat plants have been shown to absorb and accumulate organic compounds from FPW, in turn affecting crop growth and causing wheat failure (Sedlacko et al., 2022; Shariq et al., 2021; Yang et al., 2024). FPW has also been shown to suppress plant immune responses and affect plant disease resistance (Miller et al., 2019).

Moreover, FPW may contribute to the accumulation of metals in soil and crops, thus affecting soil quality and posing risks to human health. A related study found that FPW caused significant accumulation of chloride and sulfate ions in soil, increased conductivity, and altered microbial communities, particularly near wastewater discharge sites (Zhou et al., 2023b). Another study analyzed 96 soil samples from shale gas extraction areas in China for toxic metals, revealing severe contamination with As, Cd, and Ni (Li et al., 2023). Overall, our understanding of the ability of plants to take up substances in FPW, the formation of toxic metabolites through plant metabolism, and the potential for these organic compounds to synergize with pesticides or inorganic compounds is limited (Cooper et al., 2022).

In this study, tap water is mixed with different ratios of FPW to simulate different scenarios of potential leakage. The growth of tomatoes, fruit gene expression, and element accumulation levels are discussed upon irrigation with tap-FPW mixtures, treated FPW from wastewater treatment plants, condensate from the mechanical vapor recompression system, and tap water as control solution. This study aims to strengthen our understanding of the effects of FPW leakage on soil and crops, as well as to provide guidance for the safe reuse of treated FPW.

2. Materials and methods

2.1. Water samples and water quality analytical methods

Seven types of aqueous solutions were used for irrigation, with the following denominations: 1) a “TW” tap water control; 2) “TFPW”: treated FPW from a shale gas wastewater treatment plant; 3) “MVR”: condensate from the mechanical vapor recompression system at the shale gas wastewater treatment plant; 4) “FPW”: undiluted flowback and produced wastewater; 5) “FPW5” (intense leakage scenario): FPW diluted in a ratio of 1:4 with TW; 6) “FPW10” (medium leakage scenario): FPW diluted in a ratio 1:9 with TW; 7) “FPW20” (weak leakage scenario): FPW diluted in a ratio of 1:19 with TW.

The raw shale gas FPW used in this study was obtained from a shale gas well in Yibin， Sichuan, China. The TFPW and MVR were collected from a shale gas wastewater treatment plant in Yibing, Sichuan, China. The treatment plant comprises a multistage pretreatment combined with biotreatment and membrane-based separation (**Fig S1)** (Supporting Information), and the treated effluent meets the first-level standard for integrated wastewater discharge (GB8978-1996). The MVR system mainly consists of the following components: steam compressor, evaporative condenser, preheater, and various pumps for supplying power to the feed and discharge. The system operates at an evaporation temperature of 75.4℃, a condensation temperature of 85.5℃, and produces 12 m^3 of condensate per hour. Description of the analytical methods for the determination of total dissolved solids (TDS), total nitrogen (TN), dissolved organic carbon (DOC), ions and elements can be found in **Text S1**.

2.2. Greenhouse experimental design

Tomato, which is widely grown in the Sichuan Basin, was chosen as the focus crop of this work. The tomato seeds and planting soil were purchased from Lanxiang Horticultural Seed Co., Ltd (Gansu, China).

The greenhouse investigation was conducted in a laboratory of Sichuan University, Yibin Campus, using full-spectrum fill-in lamps for supplemental illumination, and with controlled ambient temperature values between 18°C and 25°C. First, a ten-day seed germination experiment was conducted in Petri dishes. Specifically, germination experiments were conducted in a greenhouse with 30 seeds per treatment group, equally divided into three Petri dishes, each with a qualitative filter paper and 8 mL of corresponding irrigation water, which was changed every 24 h. The number of seeds germinated in each group was recorded every 48 h and the germination percentage was calculated. The length of the embryonic axis and root were measured using a centimeter ruler, and the fresh weight of the seeds was weighed on the tenth day using an electronic balance.

After 10 days of cultivation in the Petri dishes, four tomato seedlings were selected from each group and transferred into rectangular seedling boxes for cultivation, with the length and width of each rectangle being 3 cm, and the depth being 5 cm. The seedlings were incubated in rectangular boxes for 30 days and irrigated every 7 days with 50 mL of solution. On the thirtieth day, the seedlings entered the four-leaf stage and were moved into 3-gallon plastic pots for stabilized culture, irrigated using 100 mL of solution every 72 h for 90 days in total. Each tomato plant used approximately 3.5 L of corresponding irrigation water during the whole cultivation period **(Fig S2 and Fig S3)**.

2.3. Morphological measurements and harvesting

The height of the seedlings above the soil surface was measured every 15 days after the seedlings were planted. On days 60 and 120, the leaf area of each tomato plant was measured. When the tomatoes were ripe, the plants were stripped from the soil and washed with ultrapure water, then dried in a freeze-dryer for 36 h. The above and below-ground biomass of each plant was determined. Soil samples were collected from the roots of mature tomato plants, along with a small amount of soil that was not used for the cultivation of the plants. The soil samples were crushed with a mortar and pestle, filtered through a 100-mesh nylon sieve, and finally dried with a freeze dryer for 36 h.

2.4. Soil and tomato tissue extraction and analysis via ICP-MS

100 mg of dried samples were added to polytetrafluoroethylene (PTFE) digestion tubes, followed by 70% nitric acid (6mL) and hydrogen peroxide (1 mL), then the digestion was conducted using a microwave dissolver. The digestion temperature program was the following: ramp from room temperature to 120 ℃ in 5 min, hold for 1min, ramp to 160 ℃ at 8 ℃/min rate, hold for 5 min, ramp from 160 ℃ to 180 ℃ at 5 ℃/min rate, hold for 10 min. After digestion, samples were cooled, diluted to 50mL with ultrapure water, and finally filtered using a 0.22 μm PTFE filter into glass bottles that had been pre-washed with acid. The heavy metal concentrations were quantified with an inductively coupled plasma mass spectrometer (NexION 1000GPerkinElmer, Inc., MA, USA).

2.5. Gene expression of tomato fruit

Fresh fruits weighing slightly more than 1 g were taken from each irrigated group during the fruiting stage. These fruits were then washed with DEPC water, packed into enzyme-free freezing tubes, immediately frozen in liquid nitrogen, and stored at −80 °C until RNA extraction and transcriptome sequencing. Detailed information about RNA extraction, sequencing, read mapping, differential expression analysis, and functional enrichment were summarized in **Text S2**. Raw sequencing data were uploaded to the NCBI database under the biological project number PRJNA1197360.

2.6. Statistical analysis.

Data analysis was performed using Microsoft Excel 2010 and graphing was done using Origin 2022 (OriginLab, Hampden, MA). Statistical significance was analyzed through one-way analysis of variance (ANOVA, $p < 0.05$).

3. Results and discussion

3.1. Water quality analysis

Table 1 summarizes the general water quality of the seven irrigation solutions. Total dissolved solids (TDS) and various metals at substantial concentrations are found in FPW, which may have negative effects on plant health and disrupt the ecological balance of soil and water (Golding et al., 2022; Isayenkov and Maathuis, 2019; Nikalje and Suprasanna, 2018; Sun et al., 2021; Xie et al., 2022). The raw FPW used in the experiments, as well as FPW5, FPW10, and FPW20, did not comply with Chinese and FAO irrigation standards. Except for elemental Sr, the concentrations of heavy metals in FPW5, FPW10, and FPW20 were within the FAO limits for heavy metals, but the salinity of all three types of aqueous mixtures was high. Specifically, the salinity of FPW20, the most diluted solution, was 1520 mg/L and exceeded the Chinese standard for irrigation water for agricultural fields, namely, 1000 mg/L. Note that even low concentrations of metals in irrigation solutions may lead to substantial accumulation in plants (Oetjen et al., 2018; Sedlacko et al., 2020). The water quality of TW, TFPW, and MVR met instead Chinese and FAO irrigation standards. TFPW had lower heavy metal concentrations than TW, except for Ba (0.35 mg/L) and Co (0.024 mg/L). The concentration of total nitrogen (71.4 mg/L) in MVR was significantly higher than that in TW (1.28 mg/L); note that a large amount of nitrogen could increase tomato fruit yield (Bénard et al., 2009; Trandel et al., 2018). The water quality analysis in this study was limited to two inorganic families of constituents, salinity and metals, while toxic organic matter in FPW can also pose a threat to crop safety and would require further study.

Table 1. Comparison of water quality of 7 irrigation solutions (TW, TFPW, MVR, FPW, FPW5, FPW10, FPW20) with national and international irrigation guidelines

Parameter	TW	TFPW	MVR	FPW	FPW5	FPW10	FPW20	water quality for irrigation (China)	water quality for irrigation (UN)
Turbidity (NTU)	0.17	0.65	1.62	198	54.9	10.0	7.58	/	/
DOC (mg/L)	1.52	2.37	6.67	181	34.4	16.9	8.39	/	/
TN (mg/L)	1.28	0.518	71.4	56.2	11.2	5.94	3.33	/	/
UV_{254} (cm^{-1})	0.011	0.006	0.012	1.42	0.132	0.323	0.596	/	/
TDS (mg/L)	251	392	272	24500	4890	2400	1520	1000	2000
pH	7.78	8.04	8.36	7.91	7.17	7.07	7.09	5.5~8.5	6.5~8.4
K (mg/L)	2.58	0.936	0.187	135	29.7	13.8	7.96	/	/
Ca (mg/L)	30.4	3.31	1.24	577	119	60.1	33.2	/	400
Na (mg/L)	2.14	9.70	1.10	6780	1380	695	351	/	920
Mg (mg/L)	27.0	2.51	0.687	104	22.4	10.1	4.07	/	60
Ba (mg/L)	0.222	0.351	0.054	33.6	7.12	3.86	1.83	/	/
Sr (mg/L)	0.137	0.075	0.046	59.3	22.7	10.6	4.98	/	0.2
Fe (μg/L)	438	37.8	/	1340	258	127	57.8	/	5000
Mn (μg/L)	6.47	1.26	2.45	506	103	53.4	29.2	300	200
Cu (μg/L)	0.288	0.283	/	77.4	15.9	8.70	5.48	500	200
Zn (μg/L)	14.7	3.42	1.79	9.45	10.3	11.5	13.1	2000	2000
Ni (μg/L)	4.96	2.01	0.264	8.48	7.14	6.26	5.93	100	200

Cr (μg/L)	0.790	0.012	0.064	21.8	3.62	1.64	0.834	100	100
Pb (μg/L)	0.020	0.020	/	2.67	0.593	0.314	0.175	200	5000
Cd (μg/L)	0.013	0.010	/	0.416	0.097	0.062	0.035	10	10
Co (μg/L)	0.003	0.024	/	0.310	0.057	0.023	0.012	1000	50
NH_4^+(mg/L)	0.012	/	77.9	55.6	5.67	2.59	0.903	2	6
F^- (mg/L)	0.129	0.075	/	9.88	2.34	0.981	0.398	2	1
Cl^- (mg/L)	51.1	155	20.2	31800	6602	4190	2660	350	350
Br^-(mg/L)	2.42	0.006	0.247	94.5	72.5	54.6	42.0	/	/
NO_3^-(mg/L)	3.67	0.891	0.395	45.3	27.9	15.2	8.92	/	10

Note: NA: not available; TFPW: treated flowback and produced water; TW: tap water; FPW5: untreated FPW diluted 1:4 with TW; FPW10: untreated FPW diluted 1:9 with TW; FPW 20: untreated FPW diluted 1:19 with TW; DOC: dissolved organic carbon; TN: total nitrogen; TDS: total dissolved solids.

3.2. Effects on the germination and growth of tomato seeds

We conducted a 10-day experiment to evaluate the effect of different irrigation solutions on tomato seed germination. The results are summarized in **Fig 1**, including the germination number, hypocotyl length, root length, and seed fresh weight. At the end of the ten-day germination experiment, the MVR group had a comparable germination rate to TW group, both exceeding 80%, while the other solutions containing raw FPW at different ratios produced lower germination numbers. In particular, FPW5 and FPW10, the two mixed solutions containing the larger fractions of raw FPW, consistently provided worse results, with germination rate as low as 17% and 20%, respectively (**Fig 1A**). The experimental results were similar to previous results of FPW used for plant irrigation (Chang et al., 2020; Novichkova et al., 2021).

The poor germination performance of seeds in the TFPW group may be related to the higher levels of Na and Cl in TFPW compared to TW. These ions can affect the seed's ability to absorb water and nutrients, thereby impacting seed physiological functions. The low germination rate in the FPW groups can be attributed to their high conductivity and salinity. During germination, salinity may cause irreversible toxic effects. High conductivity and salinity disrupt the osmotic relationship between seeds and water, reducing the amount of water absorbed for germination (Jacob et al., 2024). On the other hand, Yang et al found that untreated FPW inhibits seed germination and growth due to its high toxicity, which also limits nutrient absorption by the seeds. (Yang et al., 2022). Compaoré et al showed that distilled water, which contains few minerals and very little salt, accelerates the germination and growth of tomato seeds (Compaoré et al., 2024). MVR irrigation not only results in a germination rate similar to that of TW, but also promotes seedling hypocotyl length, root length, and weight. This is likely because MVR contains fewer minerals and small amounts of salt, which are more easily absorbed by the seeds. Although germination mainly depends on water quality, environmental factors and the inherent conditions of the seeds may also influence germination.

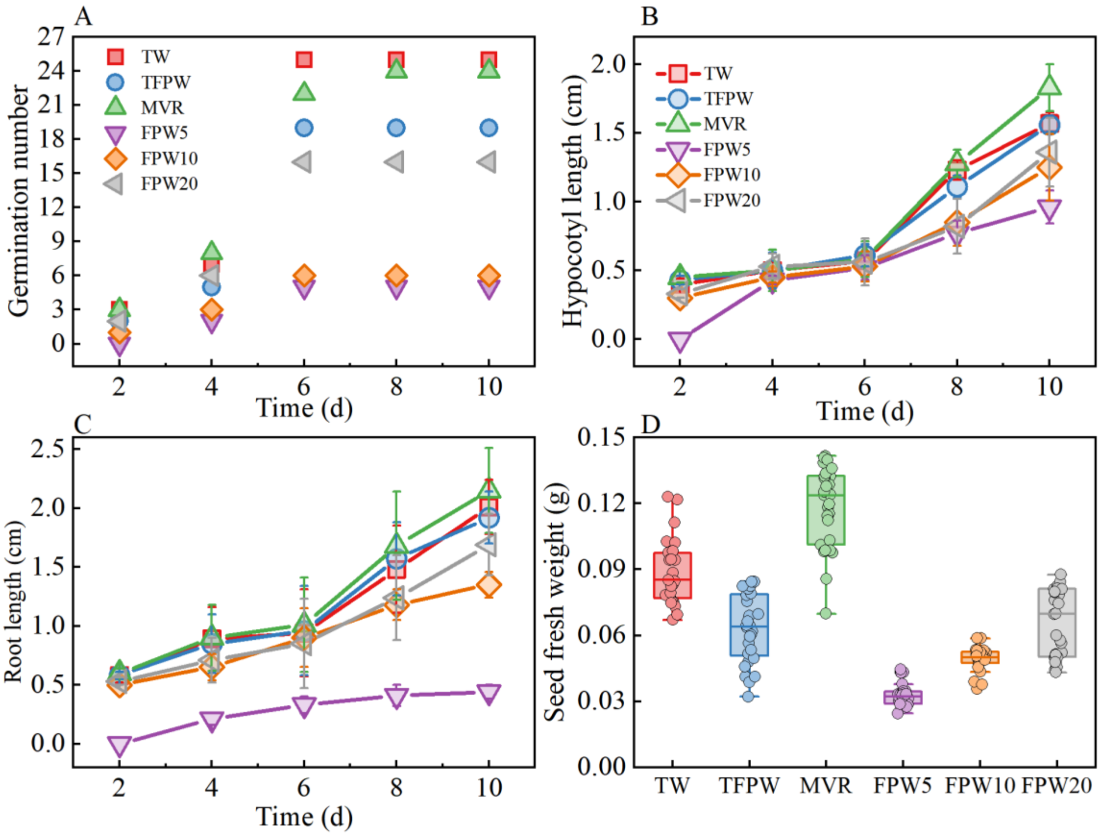

Fig 1. Germination of tomato seeds in different irrigation groups. (A) Number of germination seeds; (B) Hypocotyl length; (C) Root length; (D) Seed fresh weight at the end of germination experiment.

3.3. Effects on tomato growth and harvesting

Tomatoes were cultivated for 120 days in 3-gallon plastic pots before being harvested. Among the six irrigation groups, using MVR marginally increased the average plant height and leaf area, while tomatoes irrigated with TFPW and FPW20 showed no significant difference in plant height compared to the TW control group (**Fig 2**). Compared to TW, FPW5 and FPW10 irrigation significantly decreased plant biomass and fruit yield (Figure 3). Due to higher dilution, FPW20 had similar biomass levels to TW, but its fruit yield was 11% lower than the control. Irrigating with TFPW and MVR increased total biomass by 38% and 46%, respectively. Interestingly, the TFPW group performed much better in the pot experiment than in the petri dish germination test, suggesting that treated shale gas wastewater can be reused for irrigation without severely affecting plant health. The differences in seed germination and plant growth may be due to varying cultivation conditions, which aligns with previous reports on crop cultivation (Yang et al., 2022). Nitrogen is one of the most important nutrients for plant growth, and adequate nitrogen supply promotes leaf growth in tomatoes, increasing overall photosynthetic capacity(Batelli et al., 2024). MVR had a total nitrogen content of 71.4 mg/L, and the highest biomass in the MVR group may be due to the high nitrogen content in the water.

The reduction in tomato biomass and fruit yield in the three FPW treatment groups may result from the combined effects of high salinity and ion concentration in FPW. In FPW5, FPW10, and FPW20, the concentrations of most cations, except Sr and Na, were within irrigation standards. However, compared to TW, the concentrations of ions like K, Ca, and Na were generally higher. High ion concentrations can cause competition between different ions, which affects the plant's nutrient uptake(Rodrigues et al., 2016). Some ions, such as Cu and Mn, can be toxic at high concentrations and may inhibit physiological processes like photosynthesis and cell division. Under salt stress, the plant's ability to absorb nutrients through the roots decreases, which affects root growth(Ma et al., 2020). The leaves may suffer from burning, yellowing, and growth stagnation, leading to reduced biomass. FPW5 had a salinity of 4890 mg/L, and at such high salinity, clear developmental setbacks were observed, with a 55% reduction in tomato biomass. In FPW10, tomato leaves showed signs of burning (**Fig S3**).

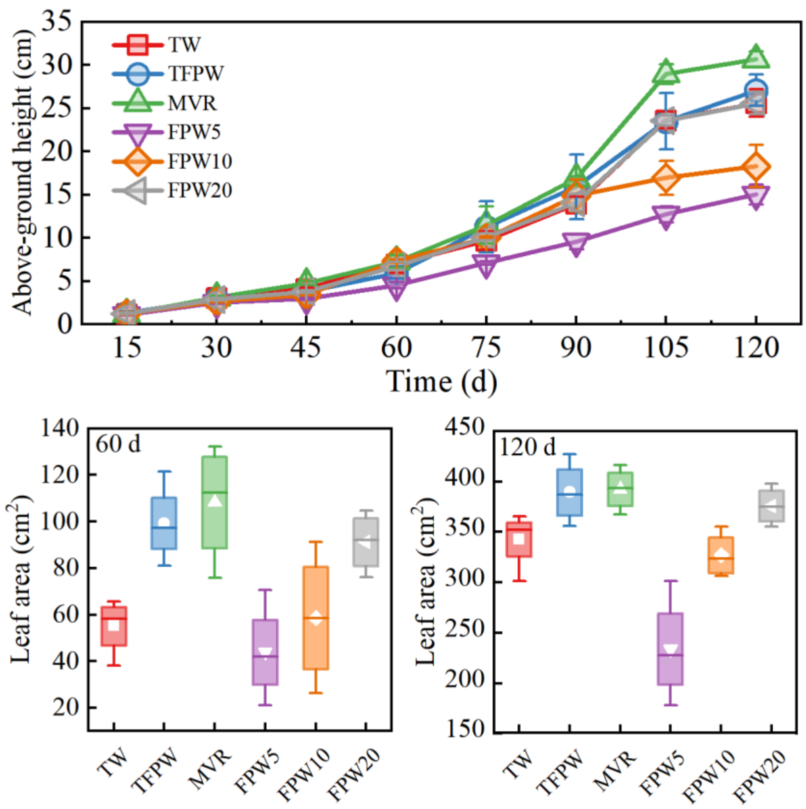

Fig 2. (A) Above-ground height of the tomato irrigated with different waters in the soil.

(B) Statistics of leaf area of tomato plants in different irrigation groups on days 60 and 120. (After transferring from the Petri dishes into soil for cultivation, four plants from each group).

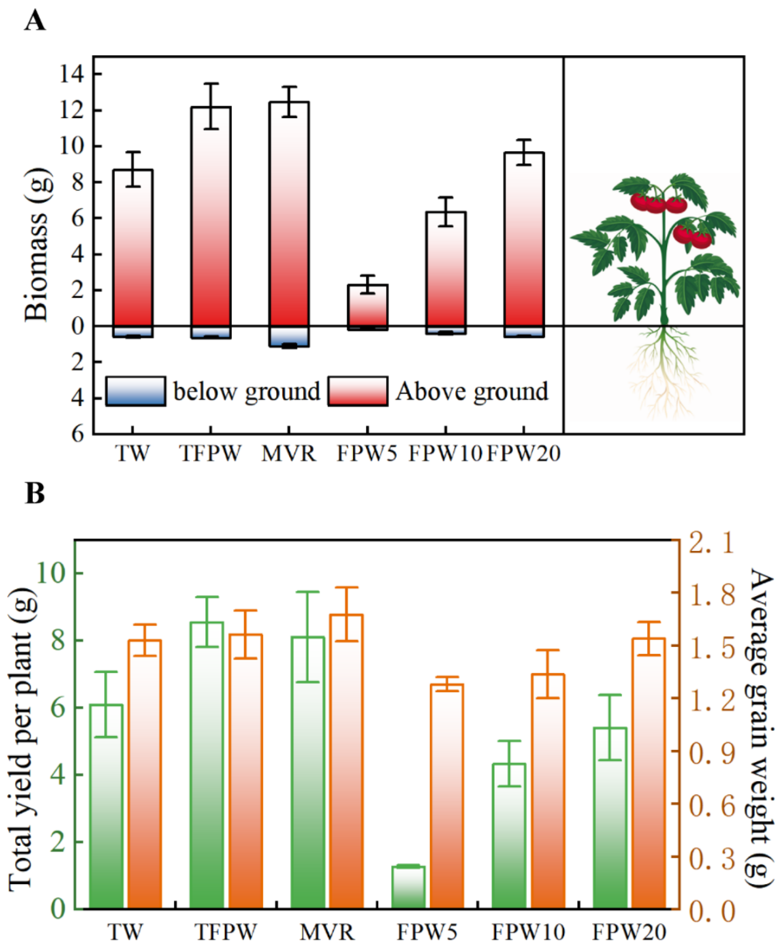

Fig 3. (A) Aboveground biomass (red) and belowground biomass (blue) of different irrigation groups after harvesting. (B) Total tomato fruit yield per plant (green) and average fruit weight (orange).

3.4. Effects on element accumulation in soil

As irrigation time increases, constituents in the irrigation water may accumulate in the soil and alter soil quality. To investigate the possible accumulation of elements in soil from irrigation solutions, we measured the content of 12 elements (K, Ca, Mg, Cd, Sr, V, Zn, Cr, Ni, Pb, Cu, Co) in the original soil and in the soil after plant harvest. As shown in **Fig S4**, elemental concentrations increased to varying degrees in each irrigation group compared to the original soil, and the growth trend was related to the elemental concentrations in the irrigation water. No significant differences in soil heavy metal concentrations were observed with TW or MVR. Except for Zn and Mg, FPW5, FPW10, and FPW20 had higher elemental accumulation than TW. For example, V and Sr in soils irrigated with FPW5 were 41% and 18% higher than TW, respectively. Although irrigation with diluted FPW resulted in higher heavy metal soil content, none of the values exceeded the maximum permissible limits set by the Chinese Soil Environmental Quality Standards and FAO (Bakari et al., 2022; Yang et al., 2022). However, if FPW leaks over a long period, the concentration of heavy metals in the soil may gradually accumulate. This could negatively impact soil microbial structure and diversity. Additionally, these metals may be absorbed by plants or consumed by animals, leading to contamination of the soil, food chain, and groundwater, ultimately affecting public health. (Li et al., 2024; Ren et al., 2024).

3.5. Effects on element accumulation in tomato tissues.

The results in terms of elemental content in tomato roots, stems, leaves, and fruits are shown in **Fig 4** and **Fig S5**. The trend of accumulation of different elements in different parts of the tomato varied, but irrespective of the type of irrigation, Ba, Co, Cr, Mn, Zn, and Ni accumulated more in tomato roots than in other parts of the tomato. All other elements accumulated in stems and leaves, and the transport of these elements to the fruits seemed limited.

Irrigation solutions containing FPW at different proportion were associated with the most important elemental accumulation in various parts of the plants. In detail, tomatoes irrigated with FPW5, FPW10, and FPW20 accumulated higher levels of Cd, Cu, Pb, Co, Cr, Mn, and Ni, with Cd, Cu, and Pb accumulating in large quantities in the stems and leaves, and the other elements in the tomato root system. Although FPW5 had the highest levels of each substance, tomato tissues irrigated by FPW5 did not show the highest elemental concentrations among the six groups. These results align with previous findings and other studies suggesting that high aqueous concentrations may poison plants and affect the uptake and transport of elements (Kumari et al., 2015; Sedlacko et al., 2019; Yang et al., 2022). Interestingly, TFPW-irrigated plants contained lower elemental content than TW, except for Co, likely owing to the lower levels of elements in treated FPW, again suggesting the potentially safe reuse of this stream for irrigation. MVR-treated plants contained instead higher levels of Ca, Mg, and Zn compared to plants irrigated with TW. Specifically, the high ammonia-nitrogen content of MVR promoted tomato plants growth, resulting in more robust root systems and enhanced nutrient absorption.

The tomato root system plays a crucial role in the chelation and adsorption of heavy metals, with metal ions being transported through the root cell membranes into plant tissues. (Page and Feller, 2015). High salinity water generally increases the uptake of heavy metals in plants, and in phytoremediation, salinity is often used to enhance metal bioavailability, while salinity inhibits the uptake of nutrients (Munns and Tester, 2008; Nikalje and Suprasanna, 2018; Sedlacko et al., 2020). However, in this work, tomatoes irrigated with FPW20 exhibited higher nutrient content than the control (TW). This may be explained by the fact that, under salinity stress, an imbalance in water concentration across the plant cell membranes disrupts ionic balance, and the plant may tend to increases the uptake through the root system to resist the osmotic pressure difference caused by the salinity stress, in which case the plant is forced to selectively absorb more nutrients (Acosta-Motos et al., 2017). Additionally, the transport of many heavy metals is regulated by the same transporter proteins, creating competitive relationships in metal accumulation: e.g, Cd, Ca, Mg, Cu, and Zn are absorbed through the same transporter proteins (Ghuge et al., 2023; Ismael et al., 2019; Zhou et al., 2016). The complex interrelationships between metals, nutrients, and salt need to be followed up with further, in-depth studies.

Plants, as the first consumers in the food chain, absorb metal elements through their roots and accumulate them in their tissues. These metals can then transfer up the food chain, affecting other organisms that consume the plants, such as insects, small animals, and humans. Elevated levels of Cr and Pb, exceeding food safety standards, were detected in the tomato fruits from the three FPW-containing groups. Consuming tomatoes exposed to FPW could cause serious acute and chronic health risks for humans. Cr may lead to chronic health issues, such as affecting glucose metabolism and cardiovascular health. Pb toxicity increases with each generation, and even low-level, long-term exposure can accumulate and cause health problems(Chowdhury et al., 2024).

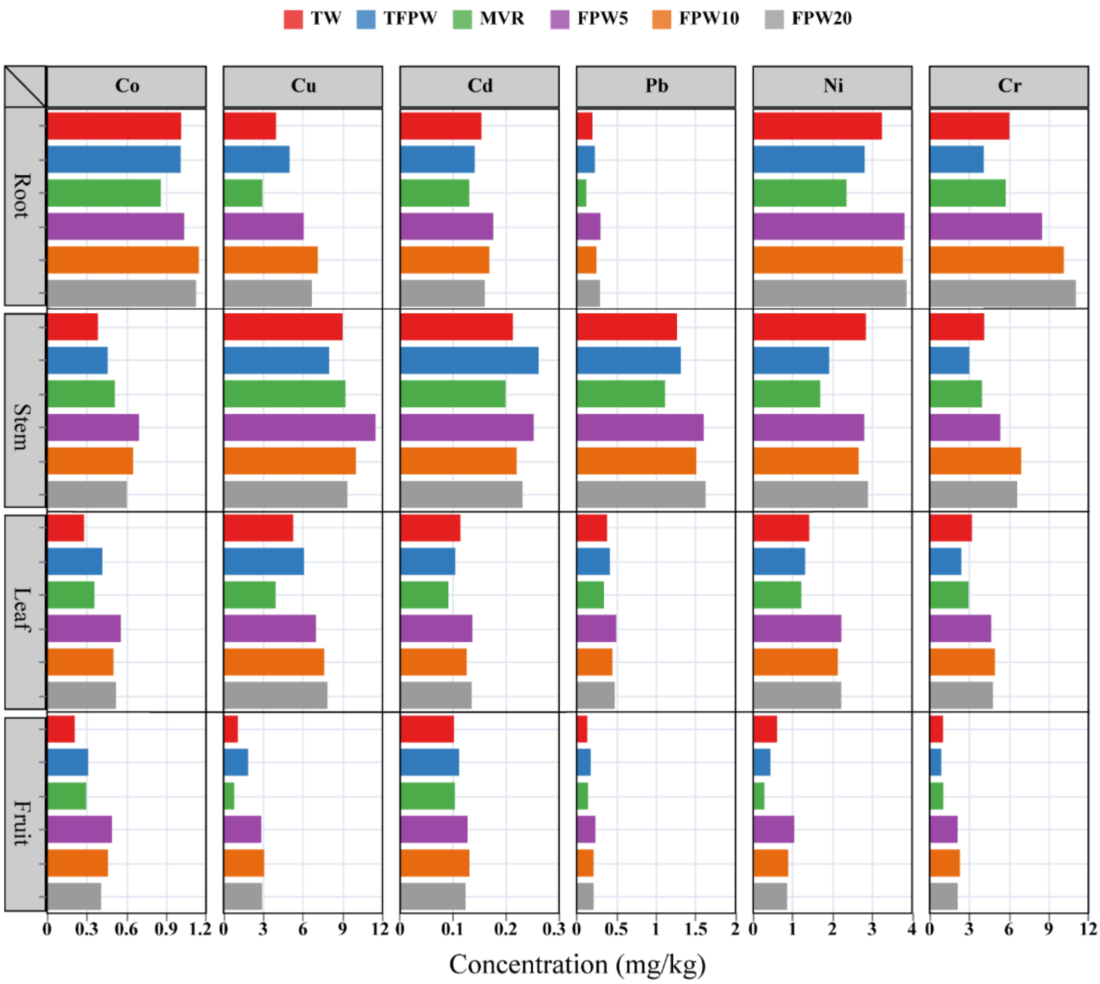

Fig 4. Concentrations of Co, Cu, Cd, Pb, Ni, and Cr in various tissues of tomatoes irrigated with different solutions.

3.6. Effects of gene expression in tomato fruits

To better understand the effects of different treatments on the physiological basis traits of tomatoes, fruits during the irrigation period were used as materials to analyze gene expression. The obtained RNA was free of pigments, proteins, sugars, and other impurities. Detailed sequencing quality control data are presented in **Tables S1 and S2**. Comparing the genes expressed in tomato fruits from different irrigation groups, a total of 25,299 expressed genes were detected in this analysis, of which 23,663 were known genes and 1,636 were new genes. A large number of co-expressed genes were identified in all the detected samples (12,617), accounting for 64.76% of the total detected genes (**Fig 5A**). DEGs, i.e., genes unique to each treatment group, may play an important role in producing differences in physiological characteristics. **Table S3** reports the number of genes that were up-regulated and down-regulated in the five experimental groups, compared to the control TW group. Specifically, the FPW20 group had the highest number of DEGs (9108), FPW5 group had the highest number of down-regulated genes (6257), MVR group had the highest number of up-regulated genes (3550). Key genes among up- and down-regulated ones may play important roles in tomato growth. To further understand and clarify the functional categories of differentially expressed genes, we used Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases for enrichment analysis of the identified DEGs.

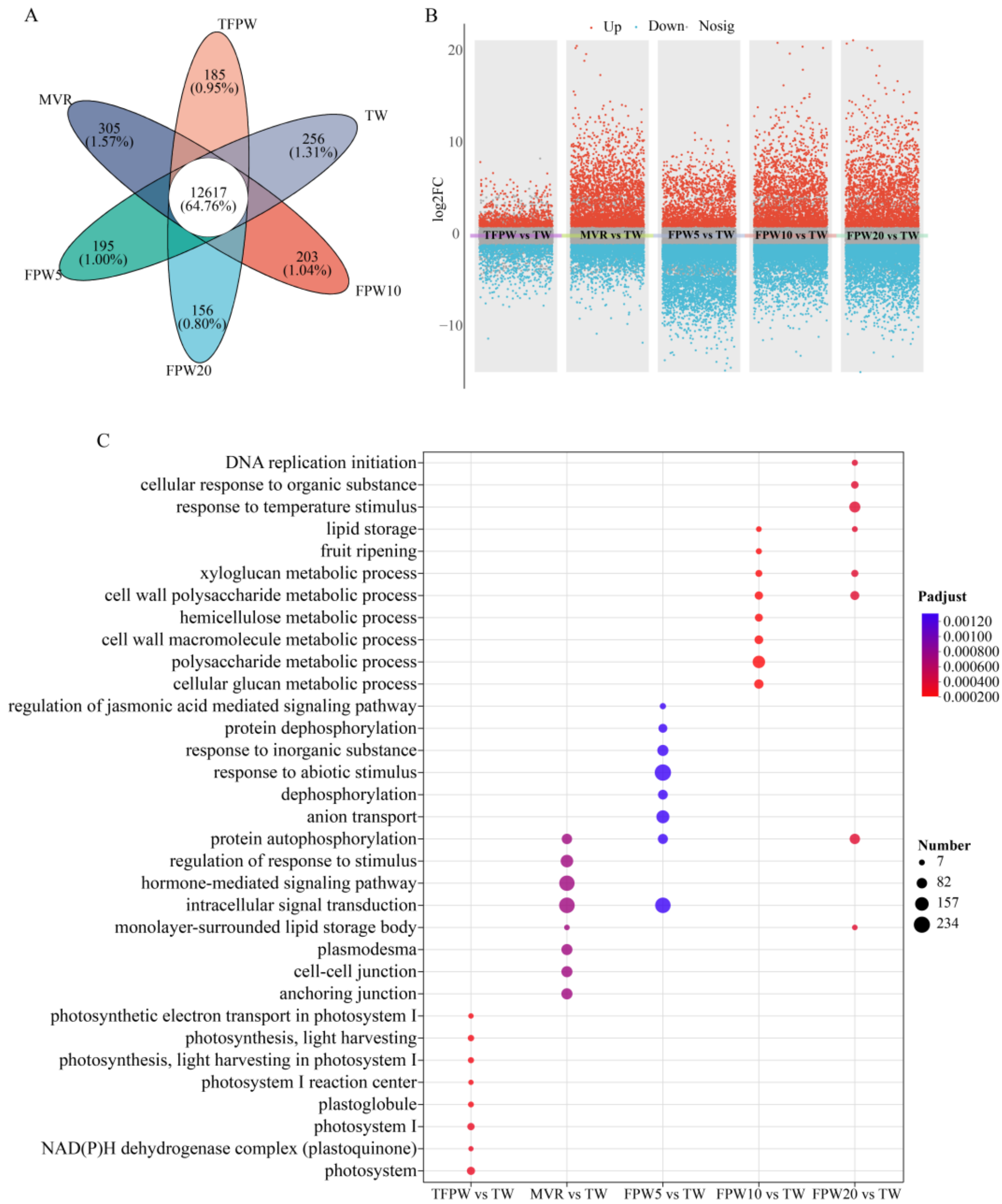

Fig 5. (A) Venn diagram of all expressed genes in different types of irrigation groups. The number shown in the outer non-overlapped portion indicates the number of genes found in only one group, and the overlap of the circles represents the common number of expressed genes. (B) Volcano plots of gene expression differences in TFPW vs. TW, MVR vs. TW, FPW5 vs. TW, FPW10 vs. TW, and FPW20 vs. TW analyses. Gray dots are not considered to be significantly differentially expressed. Red (up-regulated) and

green (down-regulated) points indicate DEGs (|log2 (fold change) | > 1, P adjust (FDR) < 0.05). (C) Bubble diagram of GO enrichment analysis of differentially expressed genes in tomato fruits (top 20 pathways based on enrichment factors).

3.6.1 GO enrichment analysis

Differential genes were analyzed for GO enrichment, and the top 8 enriched GO terms for each comparison group are presented in **Fig 5C**. At TFPW vs. TW, most of the significantly enriched GO terms were related to photosynthesis, such as photosystem, photosynthesis, and light harvesting. At MVR vs. TW, several key biological processes and molecular functions in tomato fruits were enriched, primarily involving the regulation of response to stimuli, signal transduction pathways, modulation of cellular structures, and reorganization of nutrient metabolism. Under high nitrogen conditions in MVR, tomatoes perceive and respond to environmental changes through complex signaling pathways, regulating protein activity and enhancing cell-to-cell interactions. In the high FPW treatment group（FPW5), some GO terms, such as response to inorganic substances, response to abiotic stimuli, and intracellular signal transduction, were enriched, reflecting metabolic disturbances in the plants. The plants initiated defensive mechanisms by regulating internal signaling pathways. In the medium and low FPW treatment groups(FPW10、FPW20), GO terms related to cell wall polysaccharide metabolic processes, cell wall macromolecule metabolic processes, and xyloglucan metabolic processes were significantly enriched, suggesting that components in FPW may induce restructuring of cell structures and enhance cell wall stability to adapt to environmental stress.

3.6.2 KEGG enrichment analysis

The KEGG enrichment results are shown in **Fig 6 and Fig S6**. At TFPW vs. TW, several pathways related to photosynthesis were enriched, such as Anthocyanin biosynthesis and Carotenoid biosynthesis. The expression of genes associated with light capture and conversion in tomato fruits was significantly enhanced under TFPW treatment. TFPW improved the photosynthetic efficiency of tomatoes, thereby promoting fruit growth and development, which is consistent with the GO enrichment results of TFPW. In MVR and FPW treatments, DEGs were mostly enriched in pathways related to amino acid metabolism, Plant hormone signal transduction, and MAPK signaling pathway - plant. The enriched amino acid metabolism pathways primarily affect carbon and nitrogen metabolism in plants, such as Arginine and proline metabolism, which influence chlorophyll synthesis. The enrichment of the Plant hormone signal transduction pathway indicates that tomatoes undergo stress responses to resist external influences. Carbon and nitrogen metabolism are the main pathways for synthesizing carbohydrates and proteins, respectively, and play a crucial role in plant growth and development. We hypothesize that MVR and FPW affect plant growth and fruit yield by influencing carbon and nitrogen metabolic pathways.

To further analyze the impact of FPW on tomato carbon and nitrogen metabolism, we integrated the DEGs) from the three FPW groups and performed KEGG enrichment analysis. The enriched amino acid-related metabolic pathways were Alanine, Aspartate, and Glutamate metabolism. Analyzing the pathway diagram (**Fig S7**), the upregulated DEGs included Glutamate Pyruvate Transaminase (Solyc03g123600), Asparaginase-like protein 1 (Solyc06g069400, Solyc04g078450, Solyc04g078450), and Glutamate Transporter 1 (Solyc08g044270, Solyc03g083440), while the downregulated DEGs included Asparagine synthetase (Solyc04g055200), Nitrite Reductase 2 (Solyc07g041280), Nicotinate-nucleotide diphosphorylase (Solyc12g014530), and Glutamine Synthetase (Solyc01g080280). This suggests that FPW may negatively affect the metabolism of Alanine, Aspartate, and Glutamate in tomatoes.

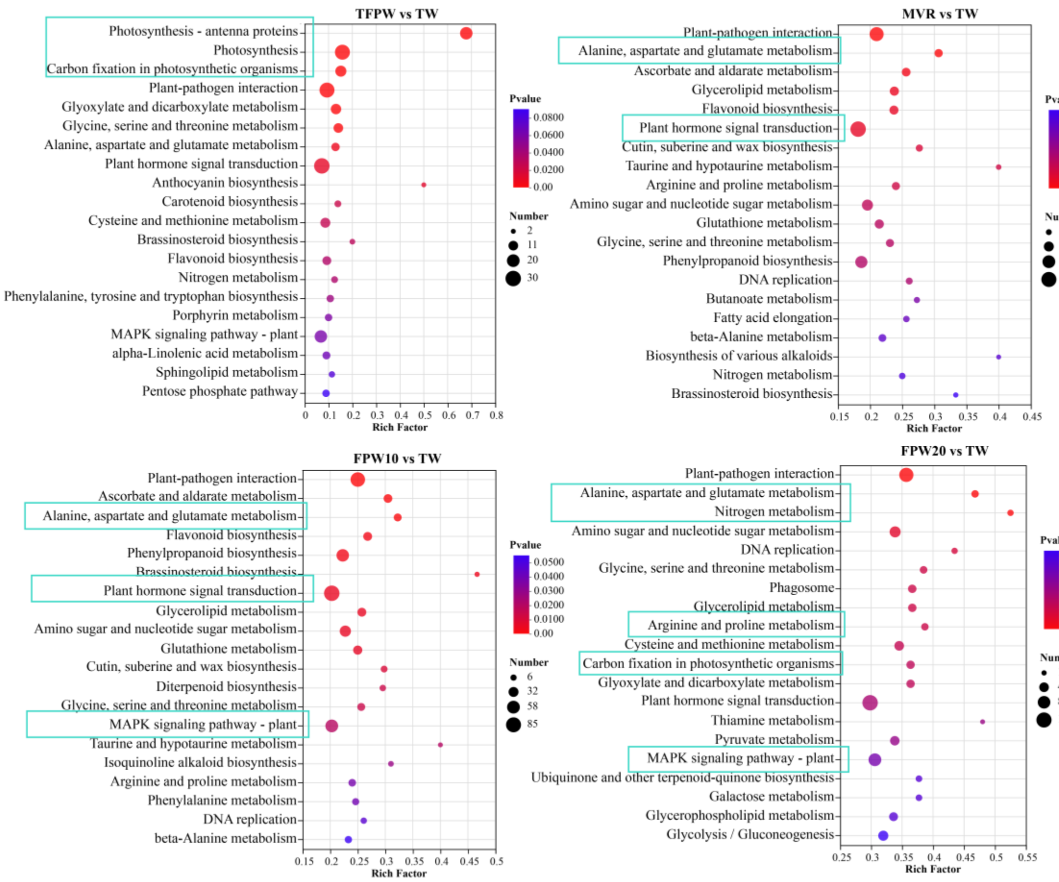

Fig 6. KEGG pathway assignment of the DEGs in different comparison groups (top 20

pathways according to enrichment factor).

4. Conclusions

This work focused on the effects of shale gas FPW, treated shale gas FPW, and condensate from the mechanical vapor recompression system at the shale gas wastewater treatment plant on tomatoes when used for irrigating this crop under different scenarios. Specifically, morphological traits, metal and salt accumulation in various tissues, and fruit gene expression in tomatoes were analyzed. Compared to tap water, FPW in the intense leakage scenario (FPW5) and in the medium leakage scenario (FPW10) inhibited tomato growth to varying degrees, while tomato biomass increased by 38% and 45% when treated FPW and when MVR solutions were used for irrigation, respectively. The latter solutions also resulted in lower levels of toxic element accumulation. In addition, a large number of DEGs were induced to be down-regulated by FPW5, FPW10, and FPW20, which play important roles in carbon and nitrogen metabolism in tomato, and consequently affect fruit yield. In conclusion, the leakage of shale gas FPW may affect the safety of the soil and lead to the accumulation of toxic elements in plants, posing a potential danger to human health. On the other hand, the results suggest that appropriate treatment of FPW is a better choice compared to its dilution, in terms of soil and plant health, when such treated stream is reused for irrigation. In this sense, this study provides insights into the impact of different water qualities on tomato development, toxicological properties, and fruit gene expression, but also guidance on the safe reuse strategy of shale gas FPW. Admittedly, this study has some limitations. Firstly, only one plant was studied in this paper. Secondly, FPW contains high concentrations of total dissolved solids, metals, organic matter, chemical additives, and natural radioactivity. This study evaluated the overall effects of FPW on the plant, and the toxicity of the individual components of FPW to the plant is unknown. In follow-up work, these issues will be explored in further experiments on a larger scale.

Supporting information

The supporting information is available free of charge.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (52270075, 52070134, and 52300101), Sichuan University and Yibin City People's Government Strategic Cooperation Project (2020CDYB-2), and Cultivation Program for Young and Middle-aged Leading Talents in Science and Technology (2023SCU17). A.T. acknowledges the support from the National Research Centre for Agricultural Technologies (Agritech) funded by the Italian National Recovery and Resilience Plan (NRRP). The views and ideas expressed herein are solely those of the authors and do not represent the ideas of the funding agencies in any form.

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