



## Article

# Effects of Hydrogen-Rich Water on Postharvest Physiology in Scales of Lanzhou Lily during Storage

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**Abstract:** Hydrogen gas ( $H_2$ ) is considered as a signaling molecule and plays multiple roles in plant growth. However, the effect of  $H_2$  on postharvest physiology in lily scales during storage has not been reported. In this study, the regulatory roles of hydrogen-rich water (HRW, a  $H_2$  donor, a concentration of 0.45 mM for 100% HRW) in water status, ion balance, and nutrients in Lanzhou lily (*Lilium davidii* var. *unicolor*) scales were investigated. The scales were soaked in HRW for 12 d, and sampling was performed every 3 d for a total of 5 times. The results show that HRW (0, 10, 50, and 100%) increased the fresh weight, dry weight, relative water content, and water loss rate in lily scales, with maximum biological response at 50% HRW. Treatment with 50% HRW significantly increased the  $K^+$  content and  $K^+/Na^+$  ratio in lily scales and decreased  $Na^+$  content. The  $Na^+ K^+$ -ATPase, and PM  $H^+$ -ATPase activities were also increased by 50% HRW treatment. Meanwhile, 50% HRW up-regulated the expression of *AKT1* and *HA3* genes and down-regulated the expression of *NHX2* and *SOS1* genes. In addition, 50% HRW treatment significantly increased the expression level of *PIP1;5*, *PIP2A*, *TIP1;3*, and *TIP2;2* genes. Treatment with 50% HRW significantly increased the content of water-soluble carbohydrate, sucrose, glucose, and fructose in lily scales, and decreased the content of starch. In addition, 50% HRW treatment significantly increased the activity of  $\alpha$ -amylase,  $\beta$ -amylase, total amylase, sucrose synthase, and sucrose phosphate synthase. Collectively,  $H_2$  might enhance the water retention capacity and nutrient content in lily scales by maintaining ion balance, regulating aquaporin, and increasing sugar-metabolizing enzyme activity, thereby prolonging the storage period of postharvest scales of Lanzhou lily.

**Keywords:** hydrogen-rich water (HRW); lily scales; water loss; ionic equilibrium; aquaporin; nutrients; storage life



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## 1. Introduction

Hydrogen sulfide ( $H_2S$ ) and methane ( $CH_4$ ) are important gas transfer molecules in many physiological and developmental processes, including adventitious root development, postharvest preservation of horticultural products, stomatal movement, seedling growth, and seed germination [1]. In recent years, it has been discovered that hydrogen, as a novel beneficial gas signaling molecule, can respond to physiological processes [2]. Hydrogen ( $H_2$ ) has an unmistakable color and taste and is the simplest gas in nature. The regulation mechanism of  $H_2$  mainly involves seed germination, seedling growth, adventitious root development, cut flower preservation, and postharvest storage [3,4]. For example, 100% hydrogen-rich water (HRW) could effectively increase the germination efficiency and the concentration of bioactive phytochemicals in black barley [5]. Treatment with 75% HRW promoted the germination of cucumber seeds by increasing the metabolism of sugar and starch [6]. HRW (75%) induced the tolerance of maize seedlings by maintaining redox balance and nutrient balance [7]. Exogenous 50% HRW induced adventitious root development in cucumber through heme oxygenase-1/carbon monoxide dependence [8]. The application of 1% HRW extended the lifespan of reducing ethylene production and

alleviating ethylene signaling in cut flower, thus improving cut flower quality [9]. HRW (80%) enhanced kiwifruit firmness by relieving the dissolution of pectin and reducing the respiration intensity [10]. Furthermore, H<sub>2</sub>, as an important signaling regulator, may induce plant tolerance to various abiotic stresses, including salt stress [11,12], heavy metal stress [13], drought stress [14], and heat stress [15]. Thus, it was found that the commonly used concentrations of HRW were 1%, 50%, 75%, 80%, and 100%.

Water is an important part of the plant body. All life activities in plants are carried out with the participation of water, such as photosynthesis [16], respiration [17], transpiration [18], absorption of mineral nutrients [19], and transport and synthesis of organics [20]. The water potential in horticultural products will be reduced after harvest, thus accelerating the water loss. Aquaporins are a group of highly conserved membrane proteins that assist water molecules to pass through the plasma membrane of plant cells through protein channels formed by members of the aquaporin superfamily [21]. Aquaporins play an important role in plant water use efficiency and water balance [22]. PIPs and TIPs are the most abundant aquaporins in most plants and are essential for transcellular and intracellular water transport [23].

Potassium ion (K<sup>+</sup>) has strong mobility in most plants. K<sup>+</sup> has a strong regulatory role in plant cell ion balance, cytoplasmic pH homeostasis, osmotic potential, stomatal activity, enzyme activation, starch synthesis, photosynthate transport, and sugar uptake [24]. K<sup>+</sup> is also associated with programmed cell death and senescence in plants [25]. Under abiotic stresses, K<sup>+</sup> deficiency and sodium (Na<sup>+</sup>) toxicity also occur in plants, and K<sup>+</sup> adsorption is affected due to excessive Na<sup>+</sup> concentration [26,27]. The excessive accumulation of Na<sup>+</sup> may lead to the interruption of K<sup>+</sup> acquisition in plants, which further leads to the serious reduction of product quality [28]. Simultaneously, the accumulation of Na<sup>+</sup> inhibited plant metabolism, enhancing leaf wilting and plant death [29]. A study found that Na<sup>+</sup> causes premature leaf senescence by reducing chlorophyll content and photosynthetic efficiency [30]. Under low K and stress conditions, the homeostasis of ions in plants can be maintained by K<sup>+</sup>, thus maintaining cell turgor and normal life activities. The key to plant survival under stress is maintaining a high K<sup>+</sup>/Na<sup>+</sup> in cells. [31]. Allu et al. [30] found that ABA and cytokinin could regulate Na<sup>+</sup> excess-induced cotton leaf senescence. In addition, the regulation of K<sup>+</sup> and/or Na<sup>+</sup> membrane transporter expression is used by plants to respond to K<sup>+</sup> deficiency or Na<sup>+</sup> accumulation [32]. The *AKT1*, *NHX2*, and *SOS1* are involved in the homeostasis of membrane transporters for K<sup>+</sup> and Na<sup>+</sup> during abiotic stress [33]. In addition, plasma membrane H<sup>+</sup>-ATP enzyme is not only a key enzyme to maintain plant plasma membrane potential, but also participates in K homeostasis under drought stress [34]. Under salt stress, the special ion environment of low Na<sup>+</sup> and high K<sup>+</sup> in cells was maintained by Na<sup>+</sup> K<sup>+</sup>-ATPase activity, and the transport driving force of some substances was provided [35].

Starch is a common storage polysaccharide in plants and the main component in *Lilium* bulbs. Starch supplies the required energy and substances for *Lilium* bulbs during storage [36]. Starch changes in plants are closely related to enzymes. The  $\alpha$ -amylase and  $\beta$ -amylase play an important role in starch hydrolysis. A previous study has shown that  $\alpha$ -amylase and  $\beta$ -amylase degrade starch into dextrin, which is then converted into maltose and glucose [37]. During cucumber seed germination, HRW improved the content of glucose and sucrose by activating the activity of  $\alpha$ -amylase,  $\beta$ -amylase, and total amylase, thereby promoting seed germination [38]. Therefore, plants may accumulate a large amount of water-soluble carbohydrate, glucose, and fructose nutrients with the degradation of starch. Reported that the degradation of starch would accumulate more sucrose content in lily bulbs [39]. Sucrose synthase and sucrose phosphate synthase are key enzymes in the sucrose metabolic pathway, which catalyze the catabolism of sucrose into glucose and fructose in two directions, providing nutrition and energy for plant activities. In addition, water-soluble carbohydrate is also an osmotic regulator and accumulates more monosaccharides under drought stress, thereby increasing intracellular water content [40]. It was reported that HRW increased the content of glucose, sucrose, and water-soluble

carbohydrate by increasing the activity of sucrose synthesis-related enzymes and glucose-related enzymes during the occurrence of adventitious roots in cucumber [41].

The lily is divided into three major categories: medicinal lilies, edible lilies, and ornamental lilies [42]. Lanzhou lily (*L. davidii* var. *unicolor*) is a variety of *L. davidii* Duchartre. It is a unique “medicinal and food” sweet lily in China [43]. Lanzhou lily is mostly distributed in Gansu Province, and it is a famous vegetable with local characteristics [36]. In addition, Lanzhou lily is known as “vegetable ginseng”. However, after being harvested from the plant, lily bulbs begin to gradually lose water, energy, and nutrients, causing the bulbs to wither and eventually ending their longevity. Therefore, delaying the senescence of lily bulbs is an indispensable means to realize the medicinal and edible use of lily. Preservation technology plays an important role in the preservation of postharvest quality of horticultural products. Therefore, it is urgent to apply new technologies to improve the postharvest quality of lily bulbs.

Thus, H<sub>2</sub> might regulate plant ion homeostasis under abiotic stress [44]. However, the roles of H<sub>2</sub> in ion homeostasis and aquaporins in postharvest horticultural products were not clear. Therefore, the purpose of this study is to investigate whether H<sub>2</sub> maintains water content and nutrients in lily scales during postharvest storage, accordingly affording technological support for enhancing the postharvest quality of Lanzhou lily.

## 2. Materials and Methods

### 2.1. Plant Material

In this study, fresh mature Lanzhou lily bulbs were purchased from the West Orchard of Qilihe District, Lanzhou City, Gansu Province, China. Healthy Lanzhou lily bulbs with a single head, uniform size, and were free of pest diseases and physical damage were selected. The 3rd, 4th, and 5th layers of scales were collected, disinfected with 3% sodium hypochlorite for 5 min, and then rinsed with distilled water.

### 2.2. Preparation of Hydrogen-Rich Water and Treatment

Hydrogen-rich water (HRW) as a H<sub>2</sub> donor was prepared and analyzed from a hydrogen gas generator (QL-300, Saikesaisi Hydrogen Energy Co., Ltd., Shandong, China) and a dissolved hydrogen portable meter (ENH-1000, Trustlex Co., Led, Tokyo, Japan) in reference to Hou et al. [45]. In our experiments, a solution with H<sub>2</sub> concentration of 0.45 mM and maintained at a relative constant level at room temperature for at least 12 h was defined as 100% HRW [6]. The prepared HRW was immediately diluted to various concentrations (10%, 50%, and 100%, [v/v]). The washed lily scales were immersed in distilled water (control), 10%, 50%, and 100% HRW for 3 h. Each treatment contained three biological replicates and each replicate consisted of 225 scales. Next, lily scales were allowed to dry at room temperature and placed in 14 cm petri dishes with filter paper at the bottom with 5 lily scales per dish. Changes of Lanzhou lily scales on 0, 3, 6, 9, and 12 days were observed. Furthermore, samples were taken every three days for the determination of relevant indicators. Only the optimum concentration HRW, which could be selected from 10%, 50%, and 100%, was used for the analysis of physiological and biochemical parameters.

### 2.3. Measurement of Fresh Weight, Dry Weight, and Relative Water Content

The 3 lily scales were weighed, and fresh weight (FW) was recorded. Then, the lily scales were soaked in distilled water for 4 h and turgid weight (TW) was recorded. Then, they were dried for 24 h at 80 °C, and dry weight (DW) was recorded. The relative water content (RWC) was calculated as follows [46]:

$$\text{RWC (\%)} = \left[ \frac{(\text{FW} - \text{DW})}{(\text{TW} - \text{DW})} \right] \times 100$$

#### 2.4. Measurement of Water Loss Rate

The water loss rate in lily scales was measured according to the method of gravimetrically with slight modifications [47]. The lily scales were stored at room temperature for 12 d and weighed in 3-day intervals. Three scales were weighed each time, and the experiment was repeated 3 times. The rate of water loss (WLR) was determined as the ratio of the initial weight (day 0) of the lily scale to the weight of the lily scales at each time period. WLR was calculated with the following formula as given by:

$$\text{WLR (\%)} = \frac{(X_0 - X_n)}{X_0} \times 100$$

where,  $X_0$  = Initial weight,  $X_n$  = The per time period weight.

#### 2.5. Measurement of $K^+$ and $Na^+$ Contents

The total  $Na^+$  and  $K^+$  content was assayed as described by Li et al. [26], with modifications. Lily scales were killed at 105 °C for 2 h and dried at 80 °C for 48 h. Dried samples (0.5 g) were incubated overnight in a conical flask with 5 mL concentrated  $H_2SO_4$ . After samples were digested on a digester until clear and transparent, the digestion samples were diluted with distilled water. Finally, F-300 Flame Photometry (Shanghai Metash Instruments Co., Ltd., Shanghai, China) was used to determine  $Na^+$  and  $K^+$  contents.

#### 2.6. Measurement of Starch, Water-Soluble Carbohydrate, Sucrose, Glucose, and Fructose Content

Iodine colorimetric method was applied to determine starch content [36]. Lily scales (0.5 g) were ground thoroughly in 2 mL distilled water, followed by 3.2 mL 60% perchloric acid for another 10 min. The grinding fluid was centrifuged at  $5000 \times g$  at room temperature for 5 min and supernatant was filtered to obtain a starch extract. The extract (0.5 mL) was added with 3 mL distilled water and 2 mL iodine reagent and stood for 5 min, and then diluted with distilled water to 10 mL. The absorbance value was measured at 660 nm.

The water-soluble carbohydrate content was determined according to the method of Wei et al. [48] with slight modifications. Lily scales (0.2 g) were ground into a fine powder placed in a 15 mL test tube. The test tube was placed in a boiling water bath for 30 min and cooled, and the extraction could be obtained by filtration and dilution. Adding 0.5 mL extraction solution, 1.5 mL distilled water, 0.5 mL anthraquinone-ethyl acetate reagent and 5 mL concentrated  $H_2SO_4$  to the test tube, we then shook the tubes in a boiling water bath for 1 min. Finally, the absorbance value at 630 nm was measured.

The sucrose content was determined by anthrone spectrophotometry [36]. Briefly, the fresh sample (1 g) was ground into homogenate by adding 80% ethanol (5 mL). The homogenate was bathed in water at 80 °C for 45 min, and the reaction solution was obtained by filtration after cooling. Following that, the reaction solution (0.4 mL) added with 200  $\mu$ L sodium hydroxide solution ( $2 \text{ mol} \cdot \text{L}^{-1}$ ) was placed in a boiling water bath for 5 min. After the reaction solution was cooled, 2.8 mL of hydrochloric acid solution and 0.8 mL of 1% m-diphenol were added to the solution and placed at 80 °C for 10 min. The absorbance value was measured at 480 nm after the solution was cooled.

Glucose content was determined according to Zhao et al. [41]. Lily scales (0.5 g) were thoroughly ground with distilled water. The homogenate was diluted to 50 mL and placed in a constant temperature water bath at 50 °C for 10 min. Following that, the centrifuge tube was centrifuged at  $5000 \times g$  at room temperature for 5 min, and the supernatant was collected. The supernatant (2 mL) was added with 1.5 mL of 3,5-dinitrosalicylic acid (DNS) in boiling water bath for 5 min. After the test solution was cooled, it was diluted to 25 mL and the absorbance value was measured at 540 nm.

The fructose content was determined by referring to the method of Ozaki et al. [49] with slight modifications. The fresh sample (0.2 g) was fully ground by adding 80% ethanol (0.5 mL) to the extract. The grinding fluid was quickly transferred to a centrifuge tube, and the supernatant was obtained in a water bath at 80 °C for 10 min. Activated carbon (2 mg) was added to supernatant at 80 °C for 30 min for decolorization, and then 0.5 mL

extract was added. The centrifuge tube was centrifuged at  $4000\times g$  at  $25\text{ }^{\circ}\text{C}$  for 10 min. Next, 30% hydrochloric acid solution (200  $\mu\text{L}$ ) and 0.1% resorcinol (60  $\mu\text{L}$ ) were added to the supernatant. The test tube was placed at  $95\text{ }^{\circ}\text{C}$  water bath for 30 min, and the absorbance value was measured at 480 nm after the solution was cooled.

### 2.7. Measurement of Enzyme Activities

The sample (about 0.1 g) was placed in 1 mL extraction solution and homogenized in an ice bath. The solution was centrifuged at  $8000\text{ }g$  at  $4\text{ }^{\circ}\text{C}$  for 10 min, and the supernatant was placed on ice for detection. The subsequent specific operation steps were determined according to the instructions of the test  $\text{Na}^+ \text{K}^+$ -ATPase activator kit (Suzhou Keming Biotechnology Co., Ltd.).

PM  $\text{H}^+$ -ATPase activity was determined by ELISA kit (Yuanmu Biotechnology, Shanghai, China). The sample enzyme solution was extracted as follows: the sample (1 g) was homogenized in 9 mL of 0.01 MPBS (pH 7.4) on ice, which was loaded into the test tube. To further lyse the tissue cells, the homogenate was sonicated (or repeatedly freeze-thawed). Afterwards, the solution was centrifuged at  $5000\times g$  for 10 min at  $4\text{ }^{\circ}\text{C}$ . Then, standards of different concentrations (50  $\mu\text{L}$ ) were added to the standard wells of the microtiter plate. The test solution (10  $\mu\text{L}$ ) and the diluent (40  $\mu\text{L}$ ) were added to the sample wells. Detection antibody-HRP (100  $\mu\text{L}$ ) was added to each well and incubated at  $37\text{ }^{\circ}\text{C}$  for 60 min. After washing the plate, 50  $\mu\text{L}$  each of substrate A and B was added to the plate wells and incubated at  $37\text{ }^{\circ}\text{C}$  for 15 min in the dark. The reaction in the above operation was stopped with termination solution. The absorbance at 450 nm was measured with a microplate reader (Cmax Plus, Migu Molecular Instruments) within 15 min. The enzymatic activity in the sample was calculated by comparison with the standard curve.

The enzyme activity of  $\alpha$ -amylase,  $\beta$ -amylase, total amylase, sucrose synthase, and sucrose phosphate synthase were determined by the ELISA method (ELISA kit: Yuanmu Biotechnology Co., Ltd., Shanghai, China) [38]. The enzyme solutions were extracted according to the instructions as follows: fresh sample (0.5 g) was quickly ground in liquid nitrogen and transferred to the test tube. The grinding fluid was kept constant for 10 min, and then the solution was fully shaken and extracted at room temperature. Meanwhile, PBS (pH 7.4) was added to the test tube for rinsing. Then, the grinding solution was centrifuged at  $4000\times g$  at  $4\text{ }^{\circ}\text{C}$  for 15 min and the supernatant was collected. The collected liquid was centrifuged at  $3000\times g$  at  $25\text{ }^{\circ}\text{C}$  for 10 min. Distilled water was added to the centrifuge tube to make the supernatant volume 10 mL, and the solution was mixed evenly. Enzyme solution (10  $\mu\text{L}$ ) and HRP coupling reagent (50  $\mu\text{L}$ ) were added to the plate wells with antibodies. The plate was washed after incubation at  $37\text{ }^{\circ}\text{C}$  in the dark for 30 min. Color reagent solution (100  $\mu\text{L}$ ) was added to the plate well and incubated at  $37\text{ }^{\circ}\text{C}$  for 30 min. The absorbance was measured at 450 nm after the termination of the reaction.

### 2.8. Extraction of RNA and Real-Time Reverse Transcription-PCR Assay

Total RNA was extracted according to the Trizol method of Li et al. [36]. Briefly, healthy scales (0.5 g) were ground in liquid nitrogen and quickly transferred to a sterile centrifuge tube, and then Trizol (1 mL) was added and shaken well. After standing at room temperature for 10 min, chloroform (200  $\mu\text{L}$ ) was added and mixed. Next, tubes were centrifuged at  $4\text{ }^{\circ}\text{C}$   $12,000\times g$  for 15 min after standing on ice for 5 min. The supernatant was collected and an equal volume of isopropanol was added, then it was incubated at  $-20\text{ }^{\circ}\text{C}$  for 1 h. The liquid was centrifuged and washed twice with 75% ethanol. Finally, the RNA was dissolved with RNase-free ddH<sub>2</sub>O and collected by centrifugation. The expression of genes was determined according to the method of Hou et al. [45]. The real-time RT-PCR (RT-PCR) analysis method and statistical data analysis were referenced from Wu et al. [50], with LoTIP1 (F: CGAAGCCAGAAACGGAGAAGAAT, R: GGGTAGGGTGGATTGGGAAGA) as the internal reference gene. The primers used for RT-PCR (F: 50-ACG GTT TCT GAA AGG ACT GCT ACA C-30 and R: 50-GCA CCC TGA AGA CCT GAT GAA TAC G-30)

were designed using prime5 software. The relevant primer sequences are shown in Table 1. All experiments were set up in triplicate biological replicates.

**Table 1.** Primers for real-time fluorescent quantitative PCR assays.

Gene Name	Gene ID	Annotation	Primer Sequence (Forward/Reverse)
AKT1	transcript_HQ_Ld3_vu_transcript5092/f3p0/2924	Inward rectifier potassium channel	F: GATGACAACAAGCAGA-GACGGAGAG R: CAACGAACCTGGTAG-GAGCACAAG F: TCACCAC-CATTCCAGGCTCTCC
NHX2	transcript_HQ_Ld3_vu_transcript10872/f16p0/2127	Sodium/hydrogen exchanger family	R: AATCCTCGCTGAACAC-CAAGATGC F: TGCGACTG-GAAGGGATTGAATGC
SOS1	transcript_HQ_Ld3_vu_transcript2231/f3p0/3731	Sodium/hydrogen exchanger family	R: TCTTAACACACGGAG-GACCTGAGG F: AAGAACTCTG-CACGGGCTTCAAC
HA3	transcript_HQ_Ld3_vu_transcript27371/f2p0/604	Plasma membrane H <sup>+</sup> -transporting ATPase P Inorganic ion transport and metabolism	R: CAGACTGTGTAGTGCT-GCTGGATC F: GGAGGGCAAAGAAGAA-GATGTGAGG
PIP1;5	transcript_HQ_Ld3_vu_transcript20905/f18p0/1042	Aquaporin G Carbohydrate transport and metabolism	R: ACGGTGAGGATGGTGAT-GTAGAGG F: ACAAGCACCAATCCGA-CACCAC
PIP2A	transcript_HQ_Ld3_vu_transcript19036/f4p0/1288	Aquaporin G Carbohydrate transport and metabolism	R: ACAAGCACGAAGGTTC-CGATGATC F: TCATCCGTGGCGTCCCT-TACTG
TIP1;3	transcript_HQ_Ld3_vu_transcript22649/f4p0/950	Aquaporin G Carbohydrate transport and metabolism	R: TCCGACTATGAAAC-CAATGGCGATC F: TTATTGTTTCGT-GTTTGCGGGTGTG
TIP2;2	transcript_HQ_Ld3_vu_transcript19036/f4p0/1288	Aquaporin G Carbohydrate transport and metabolism	R: AGCCGAGAAGTTGTG-CAATCCAG

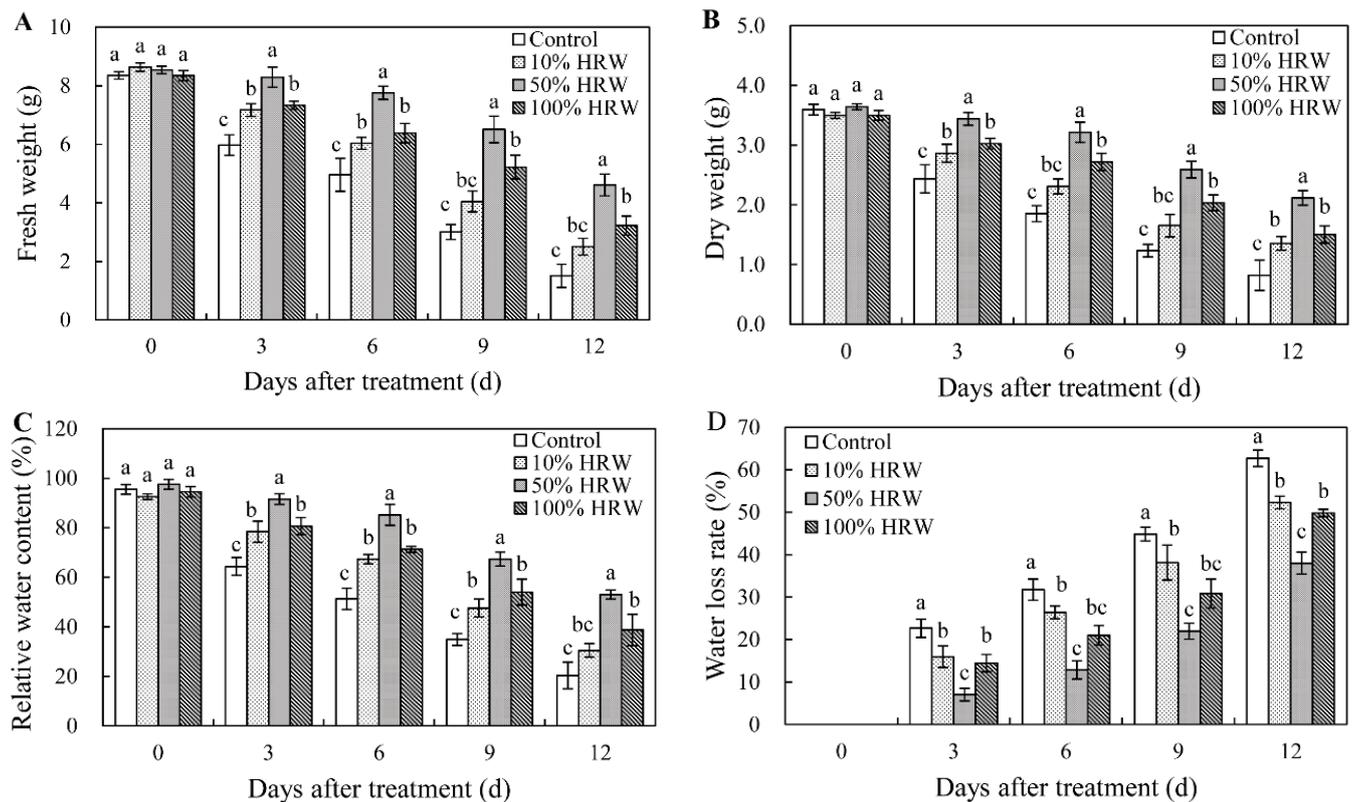
### 2.9. Statistical Analysis

SPSS 22.0 (SPPS Inc. Chicago, IL, USA) was used to perform ANOVA in this study. Statistical differences were tested among treatments using Tukey's multiple range test or *t*-test ( $p < 0.05$ ). Three biological replicates were set in all treatments.

## 3. Results

### 3.1. Effects of Different Concentrations of HRW on Water Content of Lanzhou Lily Scales

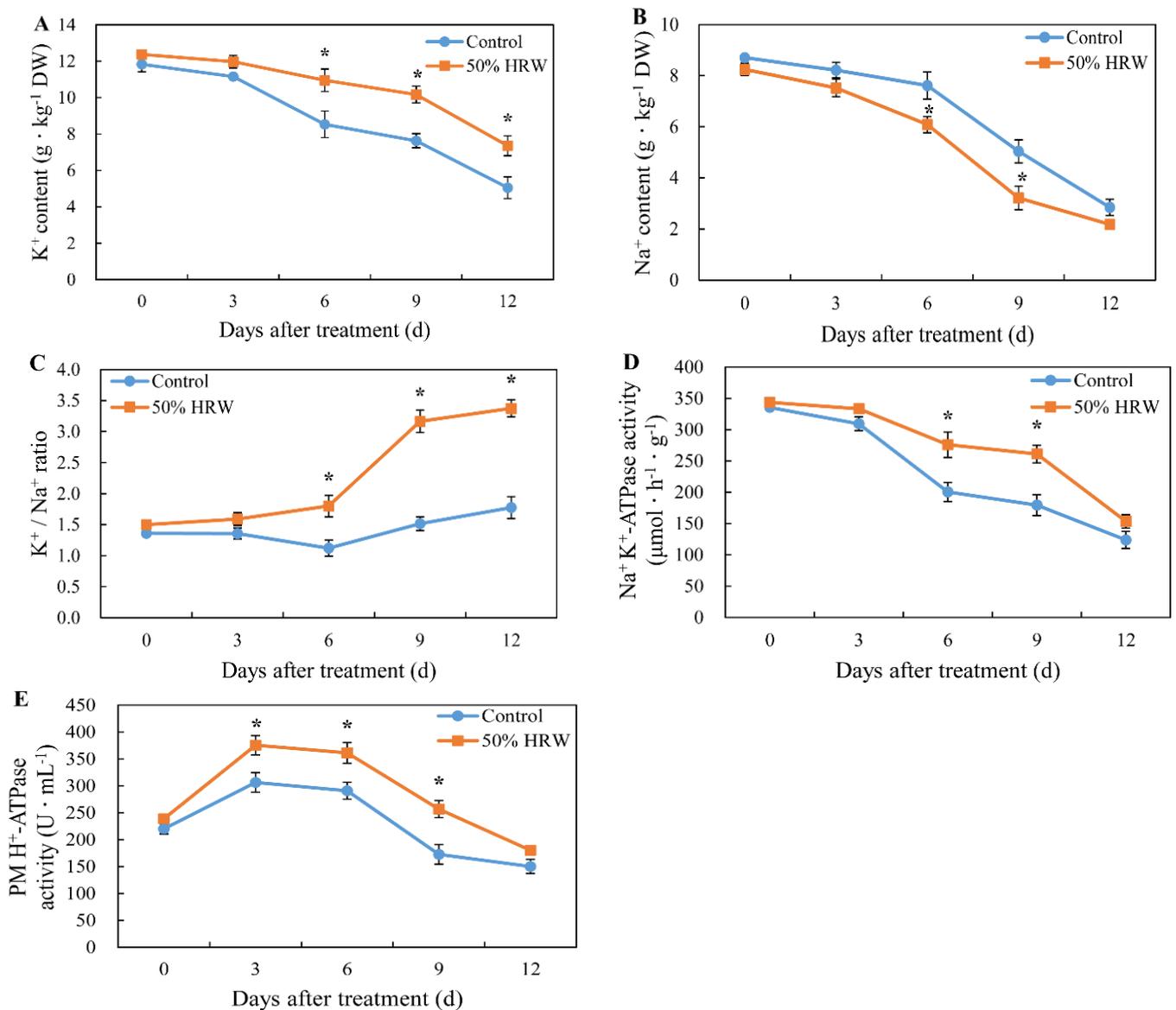
In order to understand the roles of HRW in the storage of Lanzhou lily scales, scales were treated with 0 (distilled water), 10%, 50%, and 100% HRW (a donor of H<sub>2</sub>, Figure 1). With the increase of treatment time, the fresh weight, dry weight, and relative water content gradually decreased (Figure 1A–C). The fresh weight, dry weight, and relative water content in different concentrations of HRW treatments were higher than those in the control. Among them, treatment with 50% HRW obtained the highest fresh weight, dry weight, and relative water content. Due to the gradual extension of storage time, the water loss rate of lily scales also increased accordingly (Figure 1D). Compared with the control, HRW treatments delayed the water loss rate. Among different concentrations of HRW, 50% HRW showed the lowest water loss rate, especially on days 9 and 12. Thus, 50% HRW treatment was used for subsequent experiments.



**Figure 1.** Effects of different concentrations of HRW treatments on fresh weight (A); dry weight (B), relative water content (C), and water loss rate (D) in Lanzhou lily scales during storage at room temperature for 12 days. Values are the mean  $\pm$  SE of three independent experiments. Variance analysis was performed according to Tukey's multiple range test. Different lowercase letters indicate the significant difference under different treatments on the same day ( $p < 0.05$ ). HRW: hydrogen-rich water.

### 3.2. Effects of HRW on $K^+$ Content, $Na^+$ Content, and ATP Enzyme Activity

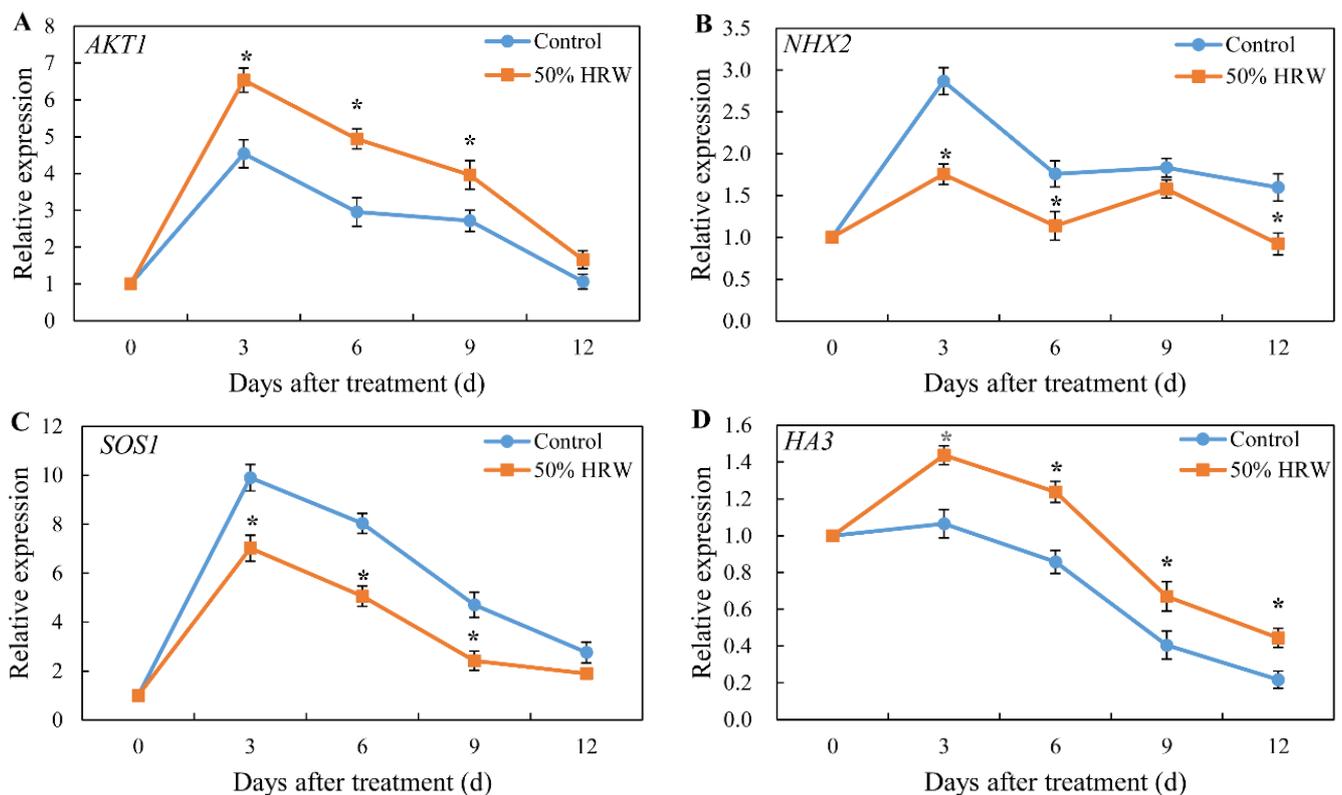
The  $K^+$  content,  $K^+/Na^+$  ratio,  $Na^+$   $K^+$ -ATPase, and PM  $H^+$ -ATPase activity in Lanzhou lily scales were increased by 50% HRW treatment (Figure 2A,C–E). Figure 2A,D showed a decreasing trend of the  $K^+$  content and  $Na^+$   $K^+$ -ATPase activity throughout the test period. Compared to the control, the  $K^+$  content was improved approximately 28%, 33%, and 46% by 50% HRW treatment at 6, 9, and 12 days, respectively. Simultaneously, compared with the control, 50% HRW treatment increased the  $Na^+$   $K^+$ -ATPase activity by 38% and 46% at 6 and 9 days, respectively.  $K^+/Na^+$  ratio and PM  $H^+$ -ATPase activity in scales treated with 50% HRW increased gradually and reached a maximum on days 9 and 3, respectively. It gradually decreased with the extension of time (Figure 2C,E). Compared with the control, 50% HRW significantly increased the  $K^+/Na^+$  ratio by 61%, 109%, and 90% at days 6, 9, and 12, respectively. Additionally, in comparison with the control, the PM  $H^+$ -ATPase activity was significantly improved by 23%, 24%, and 49% by 50% HRW treatment from day 3 to day 9. Compared to the control, 50% HRW treatment significantly decreased the  $Na^+$  content by 20 and 36% at days 6 and 9 (Figure 2B).



**Figure 2.** Changes in K<sup>+</sup> content (A); Na<sup>+</sup> content (B); K<sup>+</sup>/Na<sup>+</sup> ratio (C); Na<sup>+</sup> K<sup>+</sup>-ATPase activity (D) and PM H<sup>+</sup>-ATPase activity (E) of Lanzhou lily scales with or without 50% HRW treatment during storage at room temperature for 12 days. Values are the mean ± SE of three independent experiments. The asterisks denote *t*-test significance between the control and 50% HRW treatment on the same day: \* *p* < 0.05. HRW: hydrogen-rich water.

### 3.3. Effect of HRW on the Expression of Genes Related to K<sup>+</sup>/Na<sup>+</sup> Homeostasis

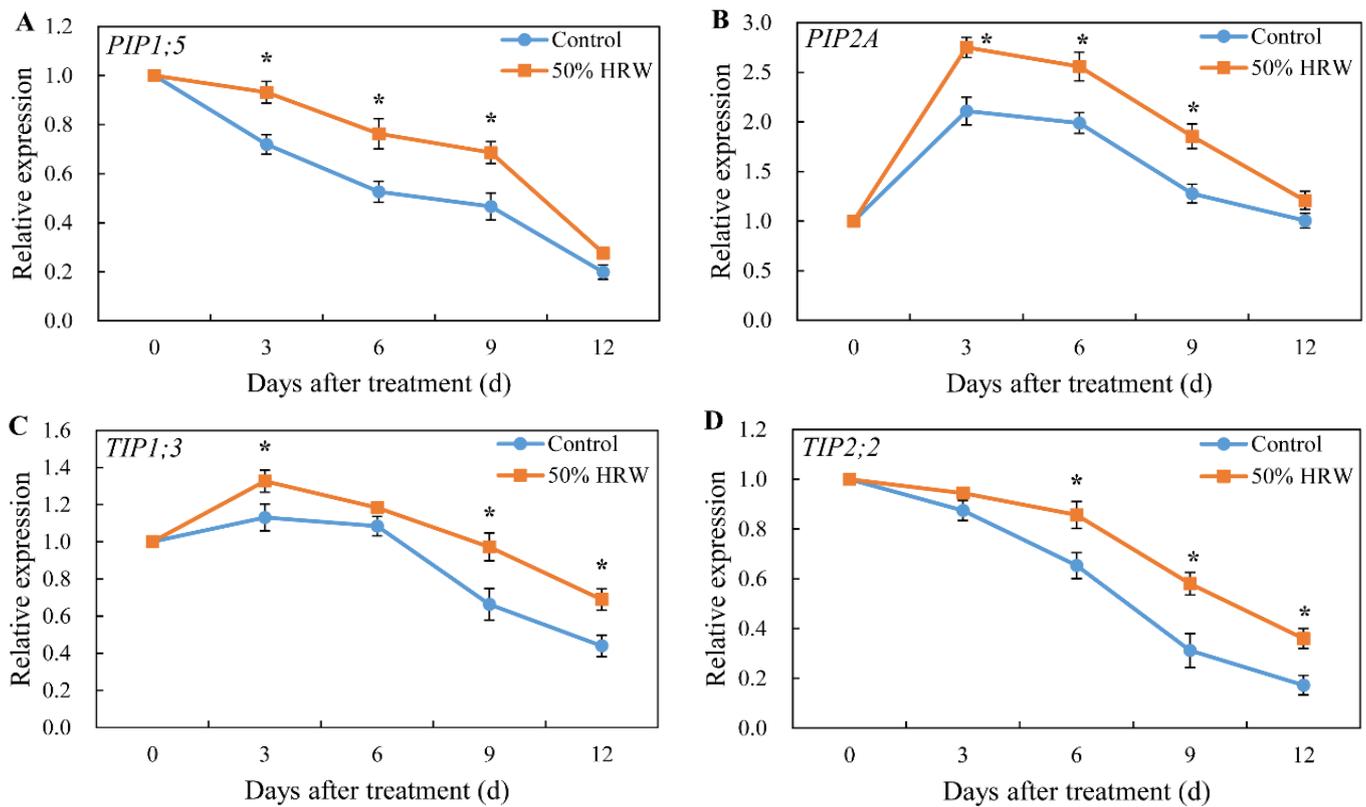
The expression of *AKT1*, *NHX2*, *SOS1*, and *HA3* genes in lily scales increased first and then decreased during the whole experiment (Figure 3). Compared with the control, 50% HRW treatment up-regulated the expression of *AKT1* and *HA3* genes, obtaining the maximum at 3 day (Figure 3A,D). However, compared with the control, 50% HRW treatment significantly down-regulated *NHX2* gene expression on days 3, 6, and 12 (Figure 3B). In addition, the expression of *SOS1* gene in 50% HRW treatment was significantly lower than that in the control on days 3, 6, and 12 (Figure 3C).



**Figure 3.** Expression of *AKT1* (A); *NHX2* (B); *SOS1* (C); *HA3* (D) genes in Lanzhou lily scales with or without 50% HRW treatment during storage at room temperature for 12 days. Values are the mean  $\pm$  SE of three independent experiments. The asterisks denote *T*-test significance between the control and 50% HRW treatment on the same day: \*  $p < 0.05$ . HRW: hydrogen-rich water. *AKT1*: Inward rectifier potassium channel, *NHX2*: Sodium/hydrogen exchanger family, *SOS1*: Sodium/hydrogen exchanger family, *HA3*: Plasma membrane  $H^+$ -transporting ATPase P Inorganic ion transport and metabolism.

### 3.4. Effect of HRW on the Expression of Aquaporin Genes

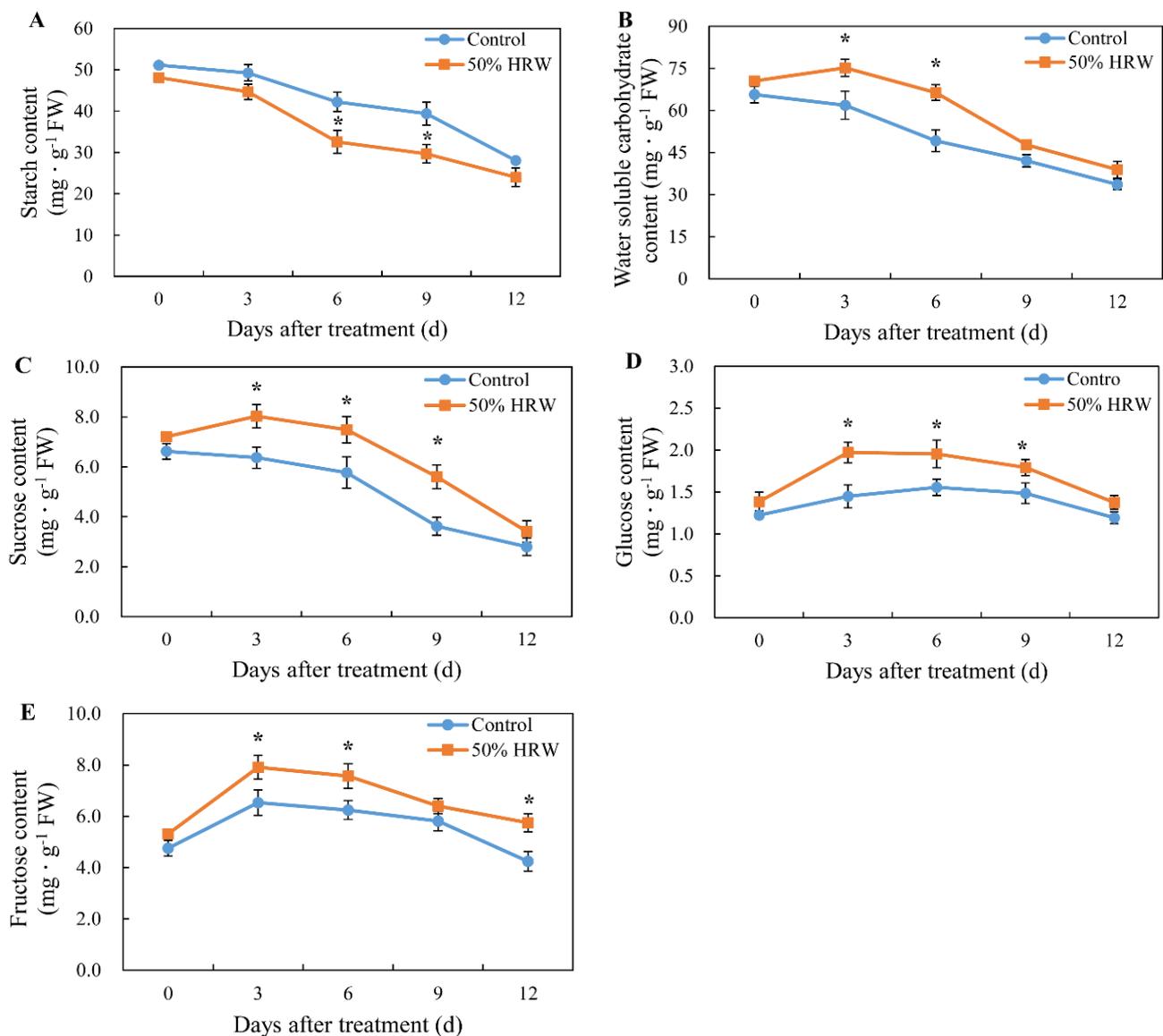
To further understand the effect of  $H_2$  on water status, the expression levels of *PIP1;5*, *PIP2A*, *TIP1;3*, and *TIP2;2* genes were determined at 0, 3, 6, 9, and 12 days after treatment (Figure 4). Compared to the control, the expression of *PIP1;5* gene was improved approximately 30%, 45% and 47% by 50% HRW treatment at 3, 6, and 9 days, respectively (Figure 4A). Similar to *PIP1;5* gene, the expression of *TIP2;2* gene showed a decreasing trend (Figure 4D). Additionally, 50% HRW treatment significantly up-regulated *TIP2;2* gene expression at days 6, 9, and 12 in comparison with the control. The expression level of *PIP2A* and *TIP1;3* in scales treated with 50% HRW increased gradually and reached the maximum on day 3, followed by a gradual decrease with time (Figure 4B,C). Meanwhile, compared with the control, 50% HRW significantly up-regulated the expression of *PIP2A* and *TIP1;3* genes from day 6 to day 12.



**Figure 4.** Expression of *PIP1;5* (A), *PIP2A* (B), *TIP1;3* (C), and *TIP2;2* (D) genes in Lanzhou lily scales with or without 50% HRW treatment during storage at room temperature for 12 days. Values are the mean  $\pm$  SE of three independent experiments. The asterisks denote *t*-test significance between the control and 50% HRW treatment on the same day: \*  $p < 0.05$ . HRW: hydrogen-rich water. *PIP1;5*: Aquaporin G Carbohydrate transport and metabolism, *PIP2A*: Aquaporin G Carbohydrate transport and metabolism, *TIP1;3*: Aquaporin G Carbohydrate transport and metabolism, *TIP2;2*: Aquaporin G Carbohydrate transport and metabolism.

### 3.5. Effects of HRW on Starch, Water-Soluble Carbohydrate, Sucrose, Glucose, and Fructose Content

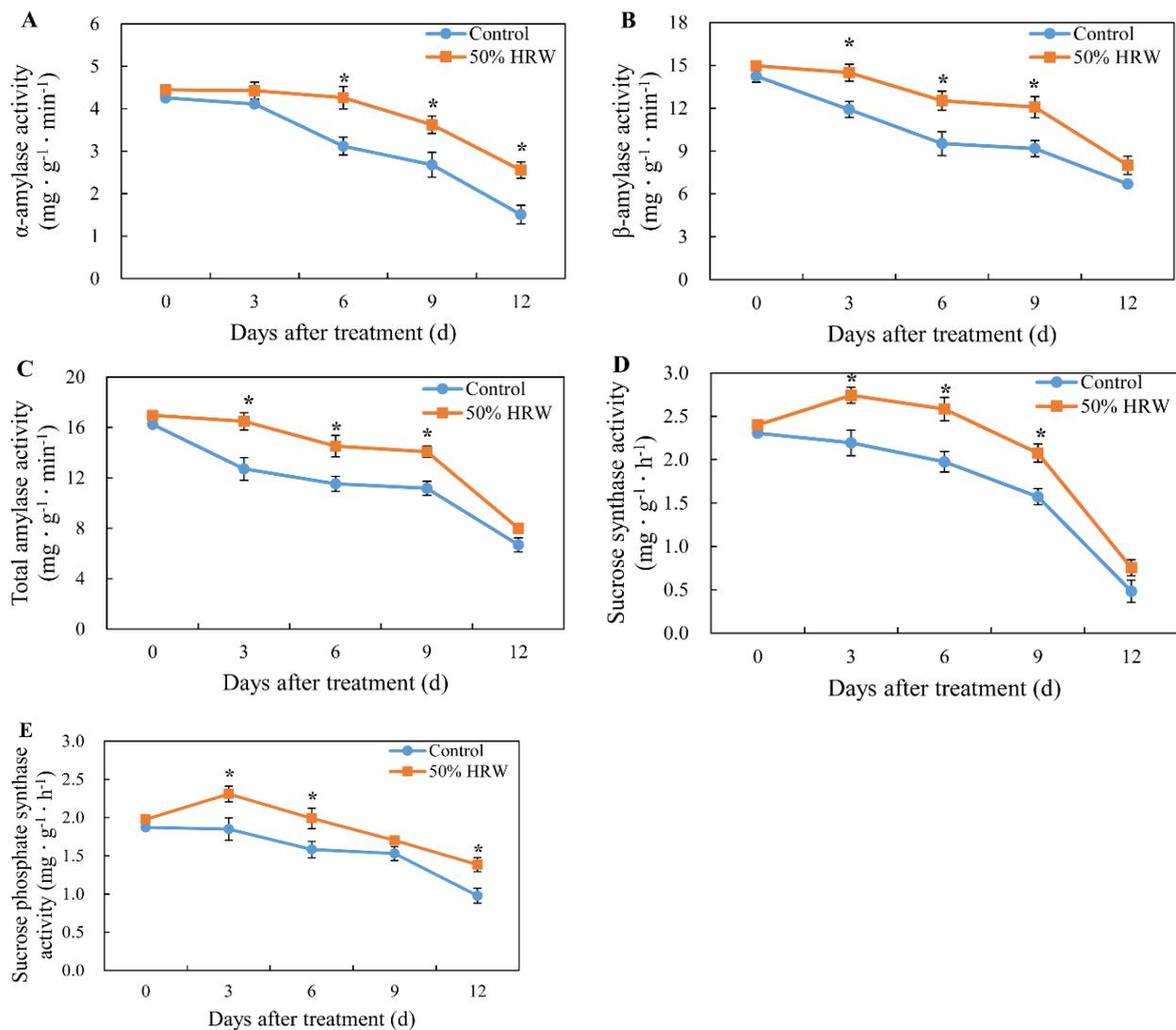
The content of water-soluble carbohydrate, sucrose, glucose, and fructose in lily scales was increased first and then decreased during the whole experiment, attaining the maximum value at day 3 (Figure 5B–E). Compared with the control, 50% HRW treatment significantly increased water-soluble carbohydrate content on days 3 and 6 (Figure 5B). Compared with the control, 50% HRW treatment significantly increased sucrose and glucose content on days 3, 6, and 9 (Figure 5C,D). The fructose content in 50% HRW treatment was significantly higher than that in the control on days 3, 6, and 12 (Figure 5E). Conversely, the starch content in lily scales treated with 50% HRW was significantly lower than that in the control on days 6 and 9 (Figure 5A).



**Figure 5.** Changes in the content of starch (A); water-soluble carbohydrate (B); sucrose (C); glucose (D) and fructose (E) in Lanzhou lily scales with or without 50% HRW treatment during storage at room temperature for 12 days. Values are the mean  $\pm$  SE of three independent experiments. The asterisks denote *T*-test significance between the control and 50% HRW treatment on the same day: \*  $p < 0.05$ . HRW: hydrogen-rich water.

### 3.6. Effects of HRW on the Activity of Total Amylase, $\alpha$ -Amylase, $\beta$ -Amylase, Sucrose Synthase, and Sucrose Phosphate Synthase

The activity of  $\alpha$ -amylase,  $\beta$ -amylase, and total amylase in lily scales decreased gradually throughout the test period (Figure 6A–C). Compared with the control, 50% treatment significantly increased the enzyme activity of  $\alpha$ -amylase,  $\beta$ -amylase, and total amylase from day 3 to day 12. Compared with the control, sucrose synthase activity was significantly increased by 50% HRW treatment from day 3 to day 9 (Figure 6D). Similar to the sucrose synthase activity, the tendency of sucrose phosphate synthase activity increased first and then decreased, obtaining the maximum at 3 days (Figure 6E). Compared with the control, 50% HRW treatment resulted in higher sucrose phosphate synthase activity on days 3, 6, 12.



**Figure 6.** Changes in sugar-metabolizing enzyme activity in Lanzhou lily scales with or without 50% HRW treatment during storage at room temperature for 12 days. (A)  $\alpha$ -amylase activity; (B)  $\beta$ -amylase activity; (C) Total amylase activity; (D) Sucrose synthase activity; (E) Sucrose phosphate synthase activity. Values are the mean  $\pm$  SE of three independent experiments. The asterisks denote *T*-test significance between the control and 50% HRW treatment on the same day: \*  $p < 0.05$ . HRW: hydrogen-rich water.

#### 4. Discussion

As a new type of multifunctional beneficial gas,  $H_2$  is involved in the postharvest preservation processes of plants and induces plant tolerance to various abiotic stresses [15]. Our study found that different concentrations of HRW could increase the fresh weight, dry weight, relative water content, and reduce the water loss rate in Lanzhou lily scales. Among them, 50% HRW had the most obvious effect on these parameters (Figure 1). A previous study has shown that 50% HRW treatment significantly increased the fresh weight and relative water content in cut lilies and roses, resulting in the high quality of cut flowers [51]. Yu et al. [52] also found that HRW obviously improved the dry weight of *Cucumis sativus* L. under salt stress. In addition, it was also reported that HRW reduced the decay rate and delayed the postharvest senescence of kiwifruit [10]. Therefore, our results indicate that HRW could prolong the postharvest storage time of Lanzhou lily scales by reducing water loss. At present, the common storage strategy of Lanzhou lily scales is low temperature storage [43]. As a storage method, HRW treatment has the advantages of low cost, easy

implementation, and simple operation compared with low temperature storage. However, H<sub>2</sub> is highly flammable and poses a safety hazard.

Na<sup>+</sup> K<sup>+</sup>-ATPase (sodium pump) and PM H<sup>+</sup>-ATPase are necessary for limiting K<sup>+</sup> efflux and retaining K<sup>+</sup>, which is essential for maintaining ion balance in cells [53,54]. In the study, 50% HRW significantly increased K<sup>+</sup> content, K<sup>+</sup>/Na<sup>+</sup> ratio, Na<sup>+</sup> K<sup>+</sup>-ATPase, and PM H<sup>+</sup>-ATPase activities in Lanzhou lily scales, while 50% HRW decreased Na<sup>+</sup> content (Figure 2). This may be due to the fact that lily scales were soaked in HRW solution, and ions may exchange in aqueous solution. It has been reported that H<sub>2</sub>S regulated the Na<sup>+</sup>/K<sup>+</sup> homeostasis in *Malus hupehensis* roots by increasing K<sup>+</sup> content and decreasing Na<sup>+</sup> content [26]. Zhu et al. [55] found that the accumulation of Na<sup>+</sup> inhibited the uptake of K<sup>+</sup> and disrupted the K<sup>+</sup>/Na<sup>+</sup> homeostasis, thus producing toxic effects on plant cells. It was also found that hydrogen molecules increased K<sup>+</sup> content in the field rice grains [56]. Moreover, Li et al. [57] showed that NO increased H<sub>2</sub>-induced H<sup>+</sup>-ATPase activity in cucumber adventitious roots. It was reported that Na<sup>+</sup> K<sup>+</sup>-ATPase increased the drought resistance in Tibetan wild barley [58]. Therefore, our results suggest that HRW might maintain the ion balance in lily scales by increasing K<sup>+</sup> content, Na<sup>+</sup> K<sup>+</sup>-ATPase, and PM H<sup>+</sup>-ATPase activity and reducing Na<sup>+</sup> content, thereby maintaining the water content in Lanzhou lily scales.

The process by which plants maintain normal high K<sup>+</sup> and low Na<sup>+</sup> homeostasis under abiotic stress is complex [59]. Based on previous studies, K<sup>+</sup>/Na<sup>+</sup> homeostasis is mainly regulated by *AKT1*, *NHX2*, *SOS1*, and *HA3* genes [60,61]. We found that 50% HRW treatment significantly up-regulated *AKT1* and *HA3* genes in lily scales, especially on day 3. Meanwhile, 50% HRW treatment down-regulated *NHX2* and *SOS1* genes (Figure 3). It has been reported that plant K<sup>+</sup> uptake is mainly expressed through the potassium channel *AKT1* gene [62]. Zhao et al. [44] reported that HRW increased *NHX1* gene expression in *Brassica napus* L, indicating that H<sub>2</sub> has different effects on different plants. However, Yu et al. [52] found that HRW treatment increased the expression of *NHX1* and *SOS2* genes in cucumber seedlings under salt stress. Thus, H<sub>2</sub> may play an active role in ion homeostasis. Under non-stress conditions, we observed for the first time that H<sub>2</sub> reduced the expression of *NHX2* and *SOS1* genes in lily scales. Additionally, studies have found that H<sub>2</sub>S regulated Na<sup>+</sup>/K<sup>+</sup> homeostasis by reducing *SOS1* gene, thereby alleviating the damage caused by alkali-salt stress in the roots of *Malus hupehensis* [26]. It has also been reported that diphenylethylidonium significantly reduced the gene expression levels of *SOS1* and *NHX1* in strawberry plants under salt stress [63]. Zhang et al. [34] reported that drought stress inhibited plasma membrane H<sup>+</sup>-ATPase activity, leading to membrane potential depolarization, thereby enhancing K<sup>+</sup> loss. NO-H<sub>2</sub> induced up-regulation of *HA3* gene in cucumber adventitious roots [57]. Therefore, our results indicate that 50% HRW treatment may maintain the ion balance in Lanzhou lily scales during postharvest storage by regulating the expression of *AKT1*, *NHX2*, *SOS1*, and *HA3* genes.

The growth and development of plants depend on the tight regulation of water movement. Aquaporins promoted the absorption and flow of water on the cell membrane, which provides a means for plants to rapidly and reversibly change water permeability [64]. It has been reported that aquaporins adapted to variable living conditions through a wide range of selective profiles and regulatory characteristics throughout plant development [23]. In our study, we found that 50% HRW significantly up-regulated the expression of *PIP1;5*, *PIP2A*, *TIP1;3*, and *TIP2;2* in lily scales (Figure 4). It has also been reported that only *PIP2;5* gene expression was up-regulated and most *PIP* genes were down-regulated under cold stress [65]. This shows that plant cell membranes may be damaged by cold stress, resulting in water channels not transporting water properly. The inoculation of *Piriformospora indica* could increase the expression of *PIP1;2* and *PIP2;4* in tomato [33]. In addition, the expression levels of *TIP2;1* and *TIP5;1* in trifoliate orange roots were down-regulated by inoculation with *Arbuscular mycorrhizal* fungi [66]. Thus, the regulation of aquaporin family genes is complex. Simultaneously, different species have different response mechanisms to aquaporins. Therefore, our results indicate that 50% HRW treatment may maintain the

water content in lily scales by up-regulating *PIP1;5*, *PIP2A*, *TIP1;3*, and *TIP2;2* genes. Until now, there have been few studies on H<sub>2</sub>-regulated aquaporins in fresh cut agricultural products during storage, and its regulatory mechanism needs further study.

The accumulation of nutrients is closely related to the storage of postharvest horticultural products [50]. This study found that 50% HRW could reduce the starch content and increase the content of water-soluble carbohydrate, sucrose, glucose, and fructose in Lanzhou lily scales (Figure 5). HRW treatment promoted the occurrence of cucumber adventitious roots by increasing glucose, sucrose, and water-soluble carbohydrate content [41]. Huang et al. [38] found that HRW could accelerate starch degradation and increase sugar accumulation to promote seed germination in cucumber. Sugar always plays an energy source role in plant growth and development. In addition, exogenous hydrogen peroxides increased fructose content in melon leaves and fruits, thereby improving their quality [49]. Therefore, our results suggest that HRW may improve the nutritional quality of lily scales during storage by reducing starch content and increasing water-soluble carbohydrate, sucrose, glucose, and fructose content. It is generally believed that sucrose and starch provide essential nutrients for plant metabolism [50]. The activity of  $\alpha$ -amylase and  $\beta$ -amylase enzymes plays a key role in starch hydrolysis. As we know,  $\alpha$ -amylase stimulates osmotic potential by providing solute sugar to initiate starch mobilization. The  $\beta$ -amylase degrades starch into dextrin, which is then converted into maltose and glucose [37]. Sucrose synthase and sucrose phosphate synthase are involved in the synthesis and accumulation of sucrose [50]. In our study, 50% HRW significantly increased the activity of  $\alpha$ -amylase,  $\beta$ -amylase, total amylase, sucrose synthase, and sucrose phosphate synthase enzymes (Figure 6). This study has shown that 75% HRW increased the activity of  $\alpha$ -amylase,  $\beta$ -amylase, and total amylase, thereby promoting cucumber seed germination [6]. Zhao et al. [41] found that HRW regulated the occurrence of adventitious roots in cucumber by increasing the activity of sucrose synthase and sucrose phosphate synthase. Moreover, exogenous oxalic acid inhibited the activity of sucrose synthase in apricot fruit during cold storage [67]. This may be due to chilling damage to the plant oxidation system being threatened, thereby inhibiting the fruit sucrose content. Therefore, our results indicate that HRW may improve the level of soluble sugar in lily scales during storage by increasing the activity of  $\alpha$ -amylase,  $\beta$ -amylase, total amylase, sucrose synthase, and sucrose phosphate synthase.

## 5. Conclusions

In summary, HRW reduced water and nutrient loss in lily scales. Meanwhile, HRW could maintain ion balance by increasing K<sup>+</sup> content, K<sup>+</sup>/Na<sup>+</sup> ratio, Na<sup>+</sup> K<sup>+</sup>-ATPase, PM H<sup>+</sup>-ATPase activities, and decreasing Na<sup>+</sup> content, which was regulated by *AKT1*, *NHX2*, *SOS1*, and *HA3* genes, thereby maintaining the water content in lily scales during storage. HRW could also increase the water content by up-regulating the gene expression of *PIP1;5*, *PIP2A*, *TIP1;3*, and *TIP2;2*. Additionally, HRW could reduce starch content and increase water-soluble carbohydrate, sucrose, glucose, and fructose content by increasing the activity of  $\alpha$ -amylase,  $\beta$ -amylase, total amylase, sucrose synthase, and sucrose phosphate synthase, thereby maintaining the nutrients in lily scales during storage. Therefore, our results show that HRW maintained the water content of lily scales during postharvest storage by maintaining ion balance and regulating aquaporin gene expression. Meanwhile, HRW maintained the nutrients in lily scales during postharvest storage. This study provides a new direction for the role of H<sub>2</sub> in postharvest storage of horticultural products. However, future work will need to explore the molecular mechanism on H<sub>2</sub> maintaining ion balance and aquaporins during harvested horticultural product storage, including key gene expression analysis and protein–protein interaction.

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