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Morphology and metabolism of storage substances contribution to alkali stress responses in two contrasting barley cultivars during germination stage

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Abstract: Barley (*Hordeum vulgare* L.) is a globally significant crop and serves as a pioneer crop for improving saline-alkaline soils due to its salt-alkali tolerant properties. However, the response mechanism of barley to alkali stress remains unclear. In this study, two barley genotypes with contrasting performance under alkali stress were selected: the alkali-tolerant SCMS and the alkali-sensitive QT9919. The morphological and physiological mechanisms of these two barley cultivars in response to alkali stress were elucidated. Results showed that alkali stress inhibited germination in QT9919, leading to significant decreases in germination vigor, plant height and root length. On the other hand, SCMS presented stronger water absorbing capacity than QT9919 under all circumstances. Furthermore, starch degradation and sugar metabolism were suppressed by alkali stress in both barley cultivars through down-regulation of genes expression and reduction of enzymes activities involved in the processes of EMP (Embden-Meyerhof-Parnas), tricarboxylic acid cycle (TCA), and pentose phosphate pathway (PPP). Notably, enzyme activity of hexokinase (HK) and malic dehydrogenase (MDH) showed significant changes under alkali stress between the two barley genotypes. This study provides insights into screening barley germplasm with strong alkali-tolerance and holds significant implications for improving and utilizing of saline-alkaline soils.

Index terms: alkali stress, barley, morphological mechanism, physiological mechanism.

Resumo: A cevada (*Hordeum vulgare* L.) é uma cultura globalmente significativa e serve como uma cultura pioneira para melhorar solos salino-alcalinos devido às suas propriedades de tolerância aos sais e à alcalinidade. No entanto, o mecanismo de resposta da cevada ao estresse alcalino permanece desconhecido. Neste estudo, foram selecionados dois genótipos de cevada com desempenho contrastante sob estresse alcalino: o SCMS tolerante e o QT9919 sensível à alcalinidade. Os mecanismos morfológicos e fisiológicos destas duas cultivares de cevada em resposta ao estresse alcalino foram elucidados. Os resultados mostraram que o estresse alcalino inibiu a germinação em QT9919, levando a reduções significativas no vigor da germinação, na altura da planta e no comprimento da raiz. Por outro lado, o SCMS apresentou maior capacidade de absorção de água que o QT9919 em todas as circunstâncias. Além disso, a degradação do amido e o metabolismo do açúcar foram suprimidos pelo estresse alcalino em ambas as cultivares de cevada através da regulação negativa da expressão genética e da redução das atividades enzimáticas envolvidas nas vias de EMP (Embden-Meyerhof-Parnas), ciclo do ácido tricarboxílico (TCA) e via das pentoses fosfato (PPP). Claramente, as atividades enzimáticas de hexoquinase (HK) e desidrogenase málica (MDH) mostraram alterações significativas sob estresse alcalino entre os dois genótipos de cevada. Este estudo fornece informações sobre a triagem de germoplasma de cevada com forte tolerância à alcalinidade e tem implicações significativas para a melhoria e utilização de solos salino-alcalinos.

Termos para indexação: estresse alcalino, cevada, mecanismo morfológico, mecanismo fisiológico.

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INTRODUCTION

Soil salinization and/or alkalization is a major environmental factor that hampers initial crop growth and productivity worldwide, posing a global challenge (Guo et al., 2010). It is estimated that approximately 8.31×10^8 hm^2 of soil worldwide are strongly affected by saline-alkali stress, with half of this land being alkaline soil (Zou et al., 2021). Northeast China possesses one of the three largest soda saline-alkaline lands globally; its saline-alkali soil covers approximately 3.70×10^7 hm^2, with around 70% being alkalized soil (Li et al., 2017). Due to the frequent co-occurrence of soil salinization (NaCl and Na_2SO4) and alkalinization ($NaHCO_3$ and Na_2CO_3) (Yang et al., 2009), natural saline-alkalinized soils are highly complex. Alkali stress, caused by extra high pH, has been shown to be more destructive than salt stress (osmotic stress and ion injury) in various plants (Yang et al., 2007; Guo et al., 2010). However, only a few alkali-resistant plant can survive in these soils. Therefore, it is crucial to improve alkalized soil, elucidate the mechanisms of plant response to alkali stress, and breed alkali-resistant plant varieties.

Plants under alkali stress experience adverse effects that involve multiple processes such as decreased germination rate, depressed plant growth, reduced setting percentage, and disturbance of key metabolisms (Fang et al., 2021). Several studies have investigated the morphological, physiological, and biochemical characteristics associated with alkali stress. After exposure to alkali stress, plants' germination is severely disrupted while their morphology changes to better adapt to the environment (Fang et al., 2021). For instance, reports revealed that the growth state of alkali-tolerant *Puccinellia tenuiflora* was better with only a few yellow leaves after treatment than alkali-sensitive *Oryza sativa* with curled and yellow leaves (Ye et al., 2019). Other typical changes observed in plants' morphology when responding to alkalinity include lower water potential, relative germination potential and relative germination rate, decrease in plant height, green leaf area and biomass, root weight, and others (Ma et al., 2021). To mediate these environmental stresses on wheat (*Triticum aestivum* L.), researchers found that its physiological characteristics enable it address intracellular ion imbalance through accumulating osmotic substances and synthesizing organic acids (Guo et al., 2010). Researchers further presented that excessive levels of bicarbonate, citrate and malate could lead to early growth reduction in *Vicia faba* L. under alkali stress (Sagervanshi et al., 2021). Recently, five indices including antioxidant enzyme activity, soluble substances, malondialdehyde (MDA) content, electrolyte leakage rate and leaf structure were selected to access the alkali tolerance of 296 *Broomcorn millet* genotypes during germination and seedling stages (Ma et al., 2021). Moreover, metabolic processes such as carbohydrate degradation also play a role in plant responses to alkali stress. This process is slowed down under mild alkali stress, but promoted under moderate and severe stress to accumulate relatively higher amounts of carbohydrates (Ye et al., 2021). Therefore, investigating the physiological responses of plants to alkali stress may contribute to improving alkaline soils.

Seed germination is the initial developmental process in the plant life cycle and ideal seed germination is crucial for establishing healthy seedlings and achieving high crop yield (Nerling et al., 2022). High germination rates under alkali stress are widely recognized criteria used for selecting tolerant genotypes (Ma et al., 2021). The process of seed germination involves numerous complex physiological and biochemical reactions such as mobilizing stored substances in the endosperm and transporting and reusing nutrients in embryos (Sagervanshi et al., 2021). Starch serves as the main component of cereal endosperm, with starch degradation being a key substance metabolism during germination stage providing essential nutrients and energy for seed germination (Morohashi and Shimokoriyama, 1972). Studies have demonstrated that saline-alkali stress significantly decreased starch degradation along with inhibiting decomposition and transformation of stored substances through suppressing α-amylase and β-amylase (Liu et al., 2019). Research on the resistance between two rice cultivars with different stress tolerance has confirmed this idea. Under saline-alkali stress, the activity of α-amylase in tolerant Dongdao-4 rice was found to be higher than that in sensitive Jigeng-88 rice seeds (Li et al., 2019). Once the radicle penetrates the seed coat, aerobic respiration becomes the primary metabolic process for providing nutrition for growth (Han and Yang, 2015). Glucose derived from starch degradation further undergoes degradation through pathways such glycolysis (Embden-Meyerhof-Parnas; EMP), tricarboxylic acid cycle (TCA cycle), pentose phosphate pathway (PPP), and others. The abilities to respond to stress are determined by the activity of key enzymes and expression levels of related genes involved in these pathways (Zhang et al., 2011). Researchers have discovered that wild soybean with strong saline-alkali tolerance exhibits more active synthesis of organic and amino acids, as well as glycolysis and TCA cycle compared to cultivated soybean (Zhang et al., 2016). Therefore, it is crucial to explore the morphological, physiological and molecular mechanisms underlying plants responses to alkali stress during seed germination in order to improve crop alkali-resistance and minimize yield loss.

Barley (*Hordeum vulgare* L.) is a significant cereal crop widely used in agriculture and husbandry for food production, animal feed, beer brewing and medicine purposes (Cheng and Wu, 2007). Barley mainly distributed in alpine regions at high-altitude or latitudes with severe saline-alkali conditions. After undergoing long-term selection and evolution under extreme environmental conditions, barley has developed unique tolerance mechanisms along with rich genetic resources specifically adapted for saline-alkali resistance (Dai et al., 2014). Hence, barley serves as exemplary model plant species for studying genetics related to stress resistance (Colmer et al., 2006). Currently, research on salt and alkali tolerance in barley primarily focuses on the former. Therefore, it is imperative to investigate the mechanisms of barley under alkali stress. Additionally, the extent of crop damage caused by alkali stress depends on factors such as alkali concentration, development stage, and crop genotypes (Xue et al., 2009). Resistance levels also vary among different plant species and even within different varieties of the same species. However, there has been no identification or study conducted on alkali-tolerant and alkali-sensitive genotypes of barley. In this study, two contrasting barley germplasms with varying degrees of alkali-tolerance were selected as the materials to examine their morphological and physiological responses during germination stages under alkaline stress conditions. The findings from this study will provide a theoretical foundation for improvement alkaline soils through the application of alkali-tolerant barley.

MATERIAL AND METHODS

Plant materials and treatments

The seeds of two barley cultivars, including alkali-tolerant Sichuan MS (named SCMS) and alkali-sensitive Qitai 9919 (named QT9919), were sterilized with 5 % sodium hypochlorite for 10 min and then washed with sterile distilled water. Seeds germination was carried out under two conditions: one group was treated with a mixed solution of $NaHCO_3$-Na_2CO_3 at a molar ratio of 9:1 (90 mmol.L^{-1}), while the other group served as the control and was treated with distilled water alone (0 mmol.L^{-1}). These seeds were cultured in Petri dishes containing sterile filter paper. Each Petri dishes represented a single replication with 100 seeds, and three replications were included in each group. The Petri dishes were placed in an incubator, maintaining temperature ranging from 23-27 ^{o}C during the daytime and 20-24 ^{o}C at night.

Measurement of plant height, root height, water uptake

Plant height and root height were measured using rulers after culturing for 1, 2, 3, 4, and 6 days. Different weights of samples were measured using a chemical balance. Water absorption of seed (A_t) was calculated after soaking for different time intervals, namely, after culturing for 2, 4, 6, 8, 10, 12, 16, 24, 36, and 48 hours by applying the following formula: A_t (%) = (M_t – M_0) / M_0 × 100; The rate of seed water absorption (R_t) was calculated using this formula: R_t (% · h^{-1}) = (A_t – A_{t-1}) / [t - (t-1)]; where M_0 (g) represents the initial weight of 100 seeds before soaking, t (hours) represents the duration that these seeds have been soaked, M_t (g) represents their turgor weight after soaking for t hours (h), A_t (%) is the water absorption of seed after soaking for t hours, and A_{t-1} (%) is the water absorption of seed after soaking for (t-1) hours. Each individual experiment represented a single replication with 100 seeds, and five replications were included in each group.

Determination of storage substances contents and the activity of related enzymes

The samples used to measure physiological parameters were selected as the material, and immediately frozen in liquid nitrogen for 30 min before stored at -80 °C. The total contents of starch and soluble sugar were determined using ELISA kits. Starch and soluble sugar in the sample were separated by 80% ethanol and analyzed through acid hydrolysis and anthrone colorimetriy (Solarbio Life Sciences, Beijing, China). The activity of ten enzymes involved in starch degradation and carbohydrate metabolism, including α-amylase, β-amylase, sucrose synthase (SuSy), hexokinase (HK), phosphofructokinase-1 (PFK1), pyruvate kinase (PK), isocitrate dehydrogenase (IDH), succinic dehydrogenase (SDH), malic dehydrogenase (MDH) and glucose-6-phosphate dehydrogenase (G6PDH) was measured using ELISA kits (Solarbio Life Sciences, Beijing, China). Five replications were included in each group.

RNA Isolation and Quantitative real-time PCR

Quantitative real-time PCR (qRT-PCR) was performed to measure the expression of seven genes related to carbohydrate metabolism. Total RNA from all samples was extracted using RNA extraction kit (Takara, Dalian, China) according to the manufacturer's instructions. RNA quality and quantity were evaluated by agarose gel electrophoresis as well as OD_{260}/OD_{280} values. First-strand cDNA synthesis was carried out using a PrimeScript™ RT reagent kit (Takara, Dalian, China). According to the manufacturer's instruction of SYBR Green PCR Master Mix (Takara, Dalian, China), qRT-PCR was conducted using iQ5 Real-Time PCR System (Bio-Rad, USA). *Actin* gene was selected as candidate reference gene (Walling et al., 2018), and primer pairs for all target genes were designed using Primer Software (http://www.Premierbiosoft.com) (Table 1). Three biological replications were performed for each sample. The relative expression levels of target genes including *α-amylase*, *β- amylase*, *SuSy*, *PFK1*, *IDH*, *MDH*, and *G6PDH* were calculated by $2^{-\Delta\Delta CT}$ method using Microsoft Excel program (Walling et al., 2018).

Statistical Analysis

Analysis of variance (ANOVA) was calculated with Software SPSS (IBM Corp, USA) based on multiple replications. The least significant difference (LSD) was used in conducting the means at a p values < 0.05 threshold. Figures were plotted using Software Microsoft Office Excel 2010 (Microsoft, USA).

RESULTS AND DISCUSSION

To clarify the impact of alkali stress on plant germination and growth, two barley cultivars with varying levels of alkali tolerance were selected as the experimental materials. Phenotypic measurements were taken during the germination stage for both alkali-tolerant SCMS and alkali-sensitive QT9919 cultivars. Generally, both barley cultivars showed

Table 1. Primers used to analyze the expression of seven genes related to carbohydrate metabolism through qRT-PCR.

Target gene	Forward primer (5'→3')	Reverse primer (5'→3')
Actin	GACTCTGGTCATGGTGTCAGC	GGCTGGAAGAGGACCTCAGG
α-amylase	GCAAGAGAGAGCTGAAGAACA	CCAGTTGAAGCCCTGAAAGA
β- amylase	TCATGGAGATGGCCAAGAAG	GGAAGTGGTATGGTGACTGAG
SuSy	GTATGTGAGGGTCAACGTGAG	AGTCCTTGTTGCTTCCTTCC
PFK1	CTGGCCCGATAAATGGGAAT	GCTGGTCTGTGACTGATCTAAC
IDH	CTCCCAGGCTACAAGACTAGAT	CTGATGCTCAAGGCCACTATAC
MDH	GCTCTCTCTGCTGGTCTTATC	GTCGCATCCATCTTCTTCCT
G6PDH	CATGTCTGCGGTGATCCTAATC	CGTGCCCAGATTCCTTCATATC

significant germination and growth under different treatments as the germination time increased. There was no notable difference in the germination between the two cultivars under normal condition or early alkali treatment (1d). Obviously, the alkali-tolerant SCMS exhibited superior germination and growth than alkali-sensitive QT9919 when subjected to prolonged exposure to alkaline conditions (2d, 3d, 4d, and 6d) (Figure 1A), indicating a stronger resistance to alkalinity in SCMS. Furthermore, both plant height and root height of SCMS were significantly higher than those of QT9919 under alkali treatment (Figures 1B and C), suggesting that seed germination ability of QT9919 was greatly inhibited by alkali conditions. Similar results have been reported in wheat studies where shoot and root growth were hindered by alkalinity (Guo et al., 2010). The germination stage is crucial for plant lifecycle initiation but also highly sensitive to osmotic stress, ion injury, and high pH resulting from an alkali environment which can reduce seed viability and overall vitality.

The availability of water during seed imbibition is a critical step for successful plant germination under alkaline conditions. Therefore, monitoring water uptake during early stages is essential. The water absorption capacity gradually increased over time for both SCMS and QT9919 under different conditions (Figure 2A). Specifically, during the early germination phase (0-24 h), two barley cultivars exhibited similar water absorption under alkali or normal condition, but significant differences were observed at later period (36 h and 48 h). Among them, SCMS demonstrated a stronger water absorbing capacity than QT9919 in all circumstances. After calculating the rate of seed water absorption, results showed that both cultivars reached peak absorption rates within two hours without any difference, and subsequent treatments resulted in fluctuating rates with small range (Figure 2B). Generally, the rate of seed water absorption for SCMS was slightly greater than that of QT9919 under alkali stress, suggesting that better water uptake by SCMS may contribute to its alkali resistance.

Water absorption is the crucial step in seed germination, and we found that the inhibitory effects of alkali treatments in barley were more apparent during later period of water uptake (Figure 2). However, no significant changes were

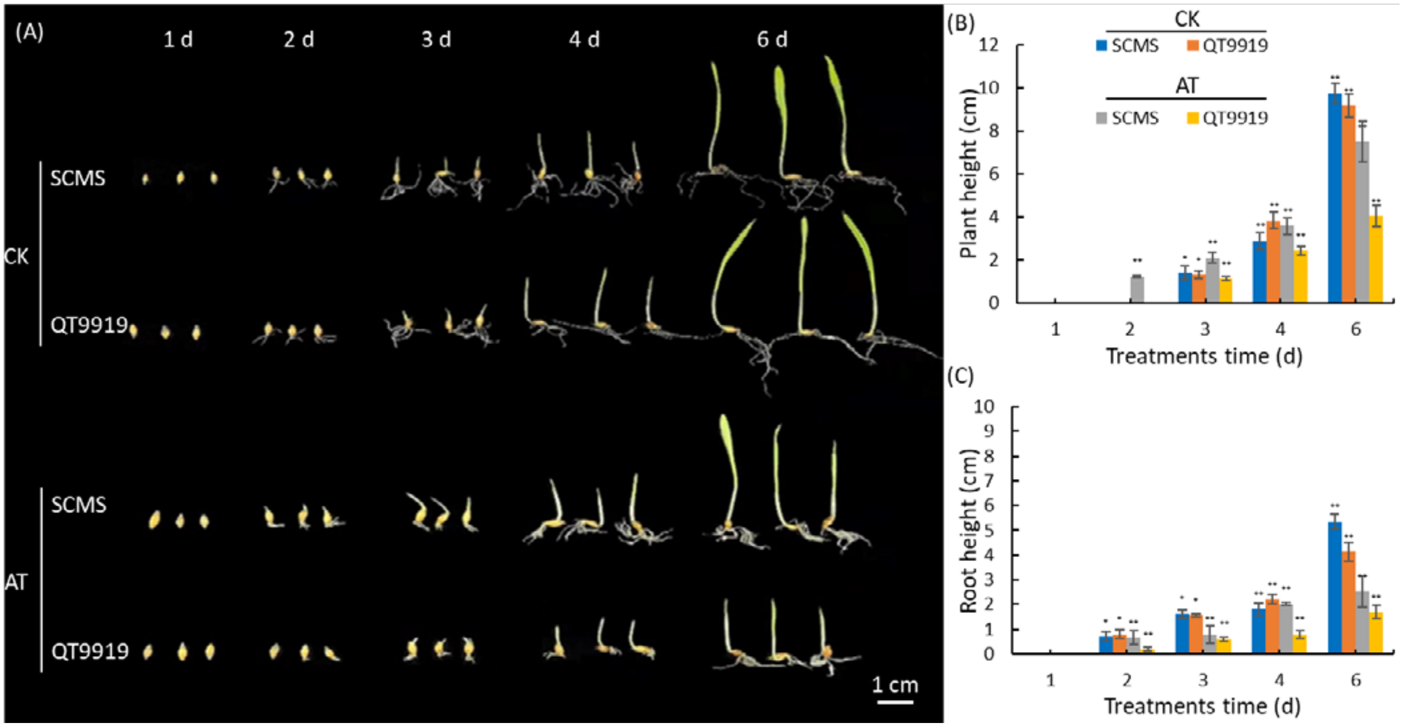

Figure 1. Morphology of seeds germination (A), plant height (B) and root height (C) of two barley cultivars (SCMS and QT9919) under alkaline conditions. Barley seeds were cultured for 1, 2, 3, 4, and 6 days under two conditions, including distilled water alone (0 mmol.L^{-1}) as control (CK) and NaHCO$_3$-Na$_2$CO$_3$ mixed solution (90 mmol.L^{-1}) as alkali treatment (AT).

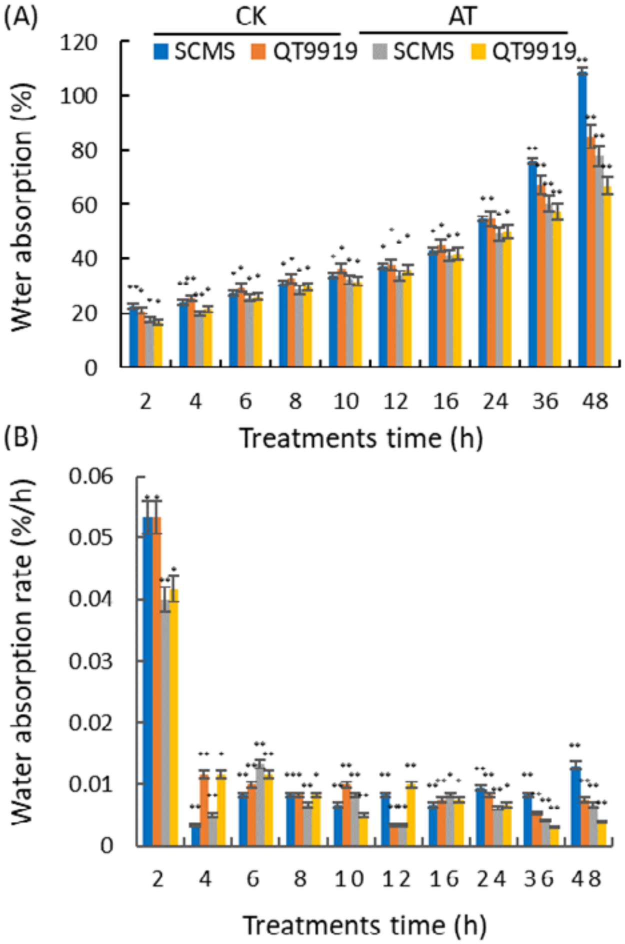

Figure 2. Water absorption (A) and rate of water absorption (B) of seeds in two barley cultivars (SCMS and QT9919) under alkaline conditions. Barley seeds were cultured for 2, 4, 6, 8, 10, 12, 16, 24, 36, and 48 hours under two conditions, including distilled water alone (0 mmol.L^{-1}) as control (CK) and $NaHCO_3$-Na_2CO_3 mixed solution (90 mmol.L^{-1}) as alkali treatment (AT).

observed in the rate of water absorption during this period. This phenomenon may be attributed to alkalinity-induced plant physiological dehydration rather than the rate of water absorption.

The main storage substance of barley seed is starch, which contributes to germination by providing essential nutrients. Storage substances, including starch and soluble sugars, as well as the enzymes involved in these processes, play a crucial role in protecting from damages induced by abiotic stress (Couée et al., 2006). To systematically evaluate the relationship between storage substance metabolism and alkali tolerance during barley germination, we examined changes in storage substance content and related enzymes activity in two barley cultivars under alkali stress. Results showed that the total starch content decreased significantly with prolonged germination time for all four types of seeds tested. Moreover, the reduction in starch content was greater under normal conditions compared to alkali stress (Figure 3). Specifically, compared to the total starch contents after germinating for 1d, that of SCMS under normal conditions were respectively degraded by 15.02%, 45.70%, 66.18% after germinating for 2d, 4d, 6d, and that of SCMS under alkali stress were respectively degraded by 8.14%, 41.80%, 64.89%. Similarly, QT9919 respectively exhibited reductions by 17.23%, 41.80%, 64.89% under normal conditions, and 3.00%, 13.32%, 41.80% under alkali stress. The sugars produced from the starch degradation during germination processes revealed that the soluble sugar contents initially increased and then decreased in all samples, reaching their highest level after germinating for 2d. The soluble sugar contents of SCMS and QT9919 were lower than control after responding to

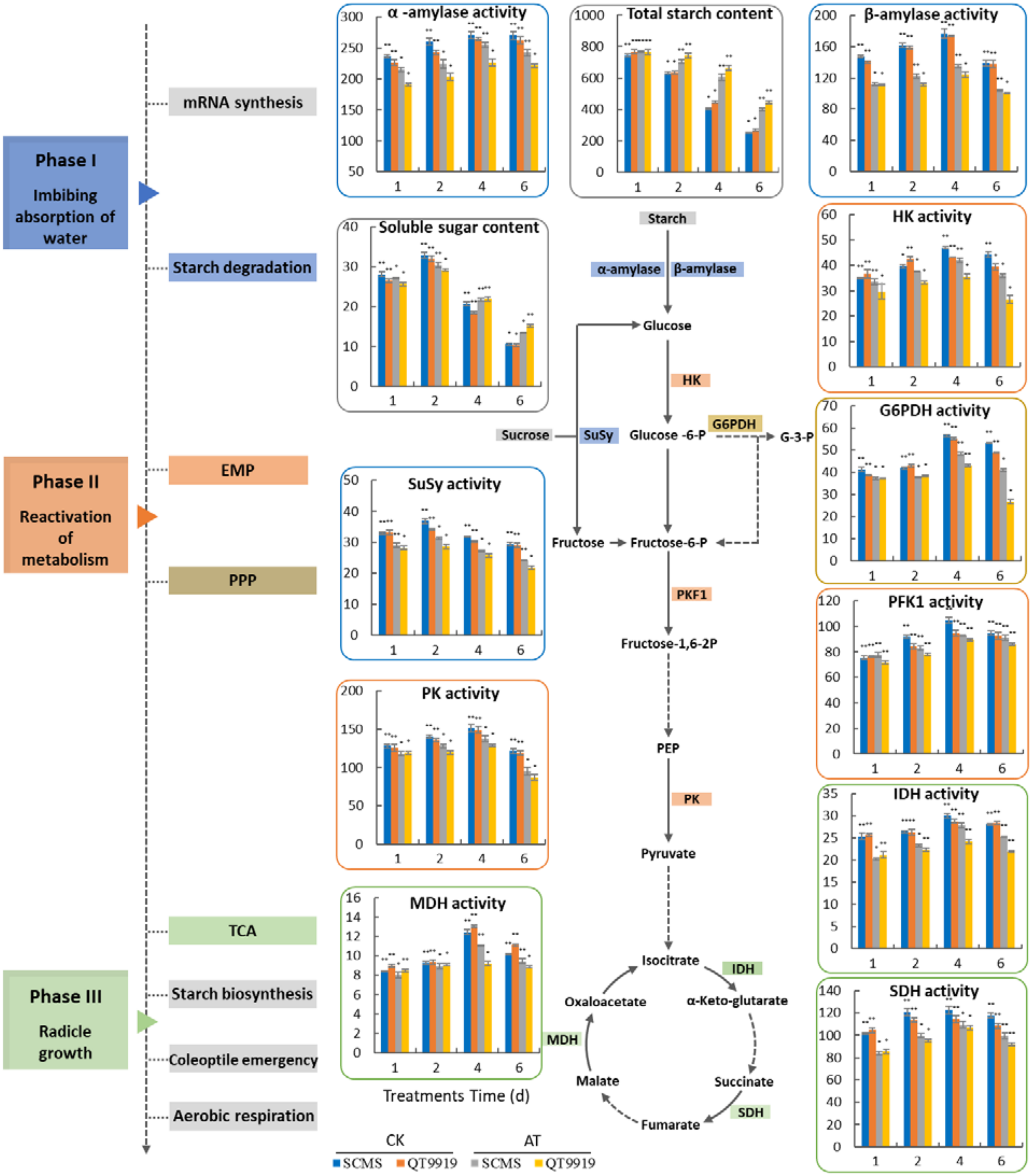

Figure 3. Changes in storage substances contents and related enzymes activity of two barley cultivars (SCMS and QT9919) during three germination phases under alkaline conditions, including total starch content, soluble sugar content, α-amylase activity, β-amylase activity, sucrose synthase (SuSy) activity, hexokinase (HK) activity, phosphofructokinase-1 (PFK1) activity, pyruvate kinase (PK) activity, isocitrate dehydrogenase (IDH) activity, succinic dehydrogenase (SDH) activity, malic dehydrogenase (MDH) activity and glucose-6-phosphate dehydrogenase (G6PDH) activity. Barley seeds were cultured for 1, 2, 3, 4, and 6 days under two conditions, including distilled water alone (0 mmol.L^{-1}) as control (CK) and $NaHCO_3$-Na_2CO_3 mixed solution (90 mmol.L^{-1}) as alkali treatment (AT).

alkali stress for 1d and 2d, but were significantly higher than controls after germinating for 4d and 6d. Furthermore, the soluble sugar content of QT9919 was lower than that of SCMS under alkali stress after germinating for 1d and 2d, but was higher for 4d and 6d.

The activities of enzymes involved in starch degradation and soluble sugar production were measured, including the α-amylase, β-amylase and sucrose synthetase (SuSy). The results displayed that the activities of these three enzymes were initially increased and then decreased in all samples (Figure 3). Specifically, the activities of α-amylase and β-amylase reached their highest levels after germinating for 4d, while SuSy achieved its peak after 2d. Overall, the enzymatic activities of two barley cultivars under control conditions were significantly higher than those under alkali treatment. Additionally, the enzymatic activities of SCMS were significantly higher than those of QT9919 under both conditions. These findings suggested that alkali stress inhibited starch degradation and storage substance transformation during germination stage, with a more pronounced effect on alkali-sensitive QT9919.

To further understand the physiological mechanisms underlying barley's responses to alkali stress, key enzymes involved in carbohydrate metabolism were determined. This included HK, PFK1, and PK associated with EMP pathway; IDH, SDH, and MDH related to TCA cycle; as well as G6PDH involved in PPP pathway. The changes observed in all enzymes across four samples followed a similar trend - initially increasing and then decreasing (Figure 3), with peak activity observed on the fourth day. Under alkali stress conditions, every enzyme's activity was significantly reduced for both SCMS and QT9919 cultivars. Among them, HK exhibited the greatest difference between two cultivars under alkali stress condition, followed by MDH. The activity level of HK was 17.67% higher in SCMS compared to QT9919, and those of MDH was 16.8% higher in SCMS compared to QT9919. Meanwhile, the impact on SDH was not significant (2.4%). Therefore, the combination treatment using $NaHCO_3$ and Na_2CO_3 significantly suppressed carbohydrate metabolism enzyme activity, and intensified negative effects caused by alkali stress.

After sufficient water intake, breaking seed dormancy activate the storage substances in seeds (Pandey and Suprasanna 2017; Shelke et al., 2017). Respiration, which includes EMP, TCA cycle and PPP, is accompanied by the decomposition, metabolism and energy supply of storage substances. This can directly affect seed vitality, germination and growth. The activities of related enzymes can reflect seed germination (Couée et al., 2006), and researchers have suggested that respiration was activated during both the seedling and early germination stages under salt treatments (Shelke et al., 2017). We found that alkali stress inhibited the starch degradation and sugar metabolism in both SCMS and QT9919 by reducing the enzymes activities involved in EMP, TCA cycle and PPP (Figure 3).

Systematic transformation of storage substance analysis was widely used as an effective strategy to reveal physiological mechanisms responding to abiotic stresses. As the dominant component of barley seeds, the accumulation, composition and structure of starch have central effects on the yield and quality. We speculated that differences in starch composition between two barley seeds might lead to difference in germination through affecting the mobilization efficiency. Previous reports have shown that amylase activities decreased during seed germination under salt stress inhibiting decomposition and transformation of stored substances (Mallik et al., 2011). This was supported by lower soluble sugar as well as weaker α-amylase, β-amylase and SuSy in QT9919 compared to SCMS (Figure 3), but further experiments are need to prove this difference on starch composition. Additionally, it was observed that enzyme activity for HK and MDH showed significant changes under alkali stress for both barley genotypes (Figure 3). This result is consistent with previous reports indicating high-efficient carbohydrate metabolism contributes to plant tolerance induction against environmental stress through improving osmotic potential regulation and energy supply (Li et al., 2014; Li et al., 2021).

In order to investigate the expression pattern of genes involved in starch degradation and carbohydrate metabolism under alkali stress, qRT-PCR analyses were conducted on seven representative genes. Two different barley cultivars under varying germination conditions showed similar trends in the expressions of these seven genes, initially increasing then declining. Furthermore, the expressions of these seven genes were significantly down-regulated in response to alkali stress (Figure 4 and Figure 5). Notably, a sharp increase in *α-amylase* transcripts was observed after 4d of germination. The relative expression level of α-amylase in QT9919 was found to be 56.87% lower than that in SCMS under alkali stress with significant difference (Figure 4A). Similarly, *β-amylase* reached its highest expression level after 4d of germination, with QT9919 showing a decrease by 40.15% compared to SCMS under alkali stress (Figure 4B). In contrast to α-amylase and β-amylase, SuSy displayed peak expression levels after 4d and 6d for germination followed by a drastic reduction (Figure 4C). As for four genes involved in carbohydrate metabolism including *PFK-1*, *IDH*, *MDH* and *G6PDH*, their expression peaked after treatment for 4d (Figure 5). At this point, there were diverse differences observed between control and stressed SCMS as well as QT9919 regarding the expression levels of target genes.

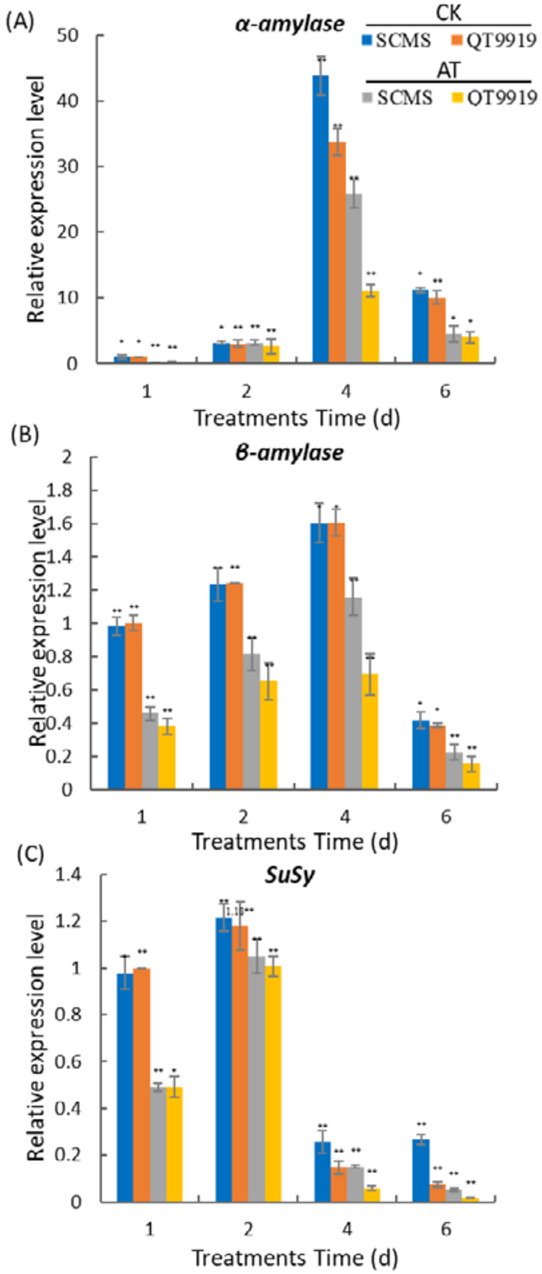

Figure 4. Expression of three genes related to starch degradation by qRT-PCR. Three genes included *α-amylase*, *β-amylase*, and *SuSy*. Barley seeds were cultured for 1, 2, 3, 4, and 6 days under two conditions, including distilled water alone (0 mmol.L^{-1}) as control (CK) and $NaHCO_3$-Na_2CO_3 mixed solution (90 mmol.L^{-1}) as alkali treatment (AT). Samples of QT9919 grown in distilled water were taken as the control, and three biological replications were used. Different labels indicate significant differences at *P* value < 0.05.

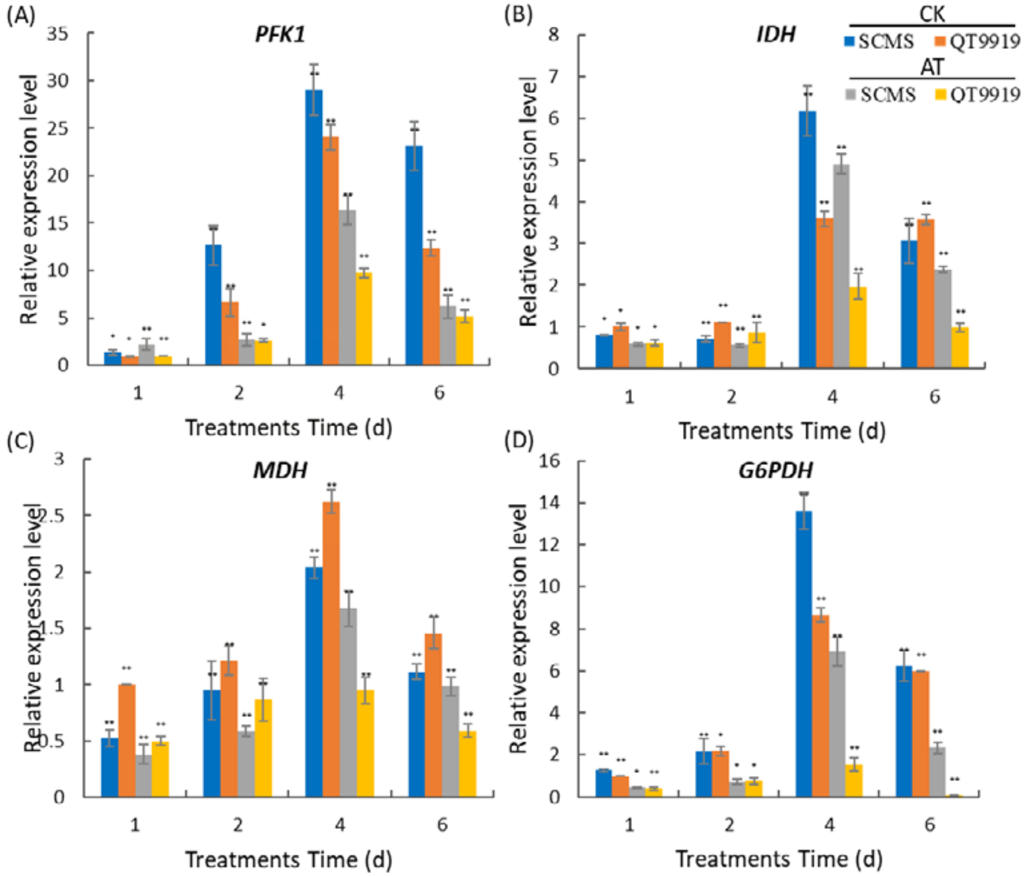

Figure 5. Expression of four genes related to carbohydrate metabolism by qRT-PCR. Four genes included *PFK1*, *IDH*, *MDH*, and *G6PDH*. Barley seeds were cultured for 1, 2, 3, 4, and 6 days under two conditions, including distilled water alone (0 mmol.L^{-1}) as control (CK) and $NaHCO_3$-Na_2CO_3 mixed solution (90 mmol.L^{-1}) as alkali treatment (AT). Samples of QT9919 grown in distilled water were taken as the control, and three biological replications were used. Different labels indicate significant differences at *P* value < 0.05.

Notably, the accumulation of *G6PDH* transcripts in SCMS was found to be approximately three times higher than that in QT9919. Overall, the accumulation patterns of these gene transcripts were associated with the respective barley cultivar's level of alkali tolerance.

This result is consistent with previous research indicating Putrescine soaking significantly enhances salt-tolerance in white clover seed through activation of α-amylase and β-amylase activities promoting starch metabolism, strengthening TCA cycle for better osmotic adjustment and energy supply, up-regulating the *AsSOS1*, *NHX6*, *SKOR*, *HKT1*, and *HKT8* expression levels to maintain higher K^+/Na^+ (Cheng et al., 2022).

In 2050, approximately half of the arable lands worldwide will be affected by salt-alkalization, posing a significant threat to sustainable agricultural development and food security (Butcher et al., 2016; Ganapati et al., 2022). As the management and improvement strategies for salt-alkaline land shift towards cultivating tolerant salt-alkali crops, there is a growing trend in exploiting and utilizing these lands. Barley has been recognized as a pioneering crop in the field of plant salt-alkali stress due to its tolerance properties. Therefore, developing barley varieties with high salt-alkaline tolerance is considered the most effective approach to address soil salt-alkalization. Barley varieties SCMS, as the alkali-tolerant crop, was a promising choice for modern approaches aimed at enhancing saline-alkali tolerance in barley. Future studies should focus on exploring molecular mechanisms underlying contrasting barley cultivars using high-throughput sequencing and investigating gene function, particularly those related to alkali-tolerant barley relatives like SCMS. This will enable identification and utilization of genetic resources that confer saline-alkali tolerance for accurate breeding purposes.

CONCLUSIONS

Alkali stress inhibited germination in the alkali-sensitive barley variety QT9919, resulting in significant decreases in germination vigor, plant height, and root length. In contrast, alkali-tolerant barley variety SCMS exhibited stronger resistance against alkali stress conditions.

Under alkali stress during seed germination stage, starch degradation and sugar metabolism were suppressed in both QT9919 and SCMS through down-regulation of genes expression levels along with reduced enzymes activities involved in EMP, TCA cycle and PPP processes. However, enzyme activity levels of HK and MDH in SCMS was significantly higher than those in QT9919.

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