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Resistance of highland barley seedlings to alkaline salt and freeze-thaw stress with the addition of potassium fulvic acid

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Abstract: Crops are commonly subjected to freeze-thaw and salt stress factors simultaneously in Qinghai-Tibet Plateau. In the agricultural field, potassium fulvic acid can not only promote plant growth and increase crop yield but also enhance plant resistance to stress. In this study, the changes of osmotic adjustment substances, antioxidant enzyme activities and photosynthetic characteristics of barley seedlings under alkaline salt and freeze-thaw stress were investigated by laboratory simulation. The results showed that under single alkaline salt stress, the soluble protein content increased significantly ($P < 0.05$), and the malondialdehyde (MDA) content of seedlings increased by 63.1%; however, antioxidant enzymes activities and photosynthetic rate of barley seedlings decreased. Under combined stresses of alkaline salt and freeze-thaw, the soluble protein content, antioxidant enzyme activities, and photosynthetic rate of barley seedlings decreased; in contrast, the MDA content of seedlings increased. With the addition of potassium fulvic acid, the soluble protein content of seedlings increased, MDA content decreased significantly ($P < 0.05$), and enzyme activities tended to be stable. This study revealed that the addition of a proper amount of potassium fulvic acid could mitigate the damage of alkali salt and freeze-thaw stress on barley seedlings.

Keywords: net photosynthesis rate; salinity; frost; *Hordeum vulgare* L.; tolerance

The Qinghai-Tibet plateau has a large area of freeze-thaw areas (Wang et al. 2016). It seriously affects the physiological and ecological process of crops in Tibetan areas, resulting in a lower germination rate and growth rate of plants in the freezing-thawing zone; ultimately, crop yield reduces. Meanwhile, salinisation is a major environmental problem in Tibet, and permafrost in Tibet is alkaline (Zhang et al. 2014). Salt stress is not conducive to plant growth, resulting in osmotic stress, ion toxicity, oxidative stress, etc. (Zhao et al. 2020). Besides, under alkaline salt stress, the increase of pH will cause further damage to plants. Therefore, in addition to coping with physiological drought and ion toxicity, plants must

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maintain intracellular ion balance (Zhang and Mu 2009). Potassium fulvic acid, an organic compound potassium fertiliser, provides nutrients for plant growth (Kumar et al. 2013). Many studies showed that appropriate application of potassium fulvic acid could improve crop yield (Priya et al. 2014), enhance plant stress resistance and improve crop quality (Xiao et al. 2020). *Hordeum vulgare* L., also known as naked barley, is a gramineous plant which is a typical representative of evolution in an extreme ecological environment. Its special habitat makes it an important material for people to study the tolerance mechanism of crops to drought, high altitude, low temperature and other special environments. In this paper, the highland barley seeds were used as experimental material; based on the addition of salinity and potassium fulvic acid, a simulation of the freeze-thaw was carried out to observe the growth of seedlings under a complex environment.

The objectives of this study were to (1) investigate the variation regularity of photosynthesis, osmotic regulation ability and antioxidant enzymes activities of highland barley seedlings under single and combined stress – freeze-thaw and salinity; (2) assess the alleviation mechanism of potassium fulvic acid to seedlings under combined stresses of freeze-thaw and salinity (Figure 1). And the result aimed to reveal the mechanisms by that highland barley seedlings respond to combined stress, and it was of theoretical value and practical significance to boost yields of highland barley in the Tibet area.

MATERIAL AND METHODS

Plant materials. The seeds in this experiment were Beqing-3 barley from Hainanzhou, Qinghai province, China. They were disinfected with 0.1% $KMnO_4$ for 2 h and washed with deionised water. The seeds were arranged neatly on a grid layer of trays (45 seeds per row × 30 rows), and the dimension of the trays with the lids was 28 cm × 22 cm × 10 cm (length × width × height). 500 mL solution with different treatments was added to trays, respectively, and the grid layer was immersed in the solution. Then, the trays were placed at 25/20 °C day/night temperatures in a light incubator (MGC-450BP), and the illumination intensity was 297 μmol/m^2/s. The trays were sufficiently watered with Hogland nutrient solution daily.

Design of potassium fulvic acid and alkaline conditions. Solution of 0.5 g/L potassium fulvic acid (purchased from Xinjiang Heise Eco-tech Co., Ltd, Changji, China) was prepared with Hogland nutrient medium (purchased from Qingdao Hope Bio-Technology Co., Ltd, Qingdao, China) (Tan et al. 2021). 60 mmol/L $NaHCO_3$ solution was obtained with half-strength Hoagland solution for alkaline salt stress. The $NaHCO_3$-potassium fulvic acid solution was obtained by adding potassium fulvic acid to the $NaHCO_3$ solution until the concentration of potassium fulvic acid was 0.5 g/L. This experiment was divided into 8 groups (Table 1), 4 freeze-thaw treatment groups: single freeze-thaw stress (F); combined stresses of potassium fulvic acid and freeze-thaw (FK); combined stresses of alkaline salt and freeze-thaw (FH); combined stresses of alkaline salt, potassium fulvic acid and freeze-thaw (FHK); 4 non-freeze-thaw groups: control group (CK); single potassium fulvic acid stress (K); single alkaline salt stress (H); combined stresses of alkaline salt and potassium fulvic acid (HK).

Freeze-thaw treatment. When the seedlings grew for 8 days, and the seedlings in the control group grew to 15 cm, 4 groups of freeze-thaw stress were placed in a high-low temperature alternating test chamber (BPHJ-120A) for freeze-thaw treatment with a cycle of 14 h, and the 4 non-freeze-thaw groups were placed in the light incubator. The freeze-thaw temperature was set and controlled by the machine program. The initial tem-

Table 1. Experimental design of groups of different treatment

	CK	K	H	HK	F	FK	FH	FHK
0.5 g/L potassium fulvic acid		+		+		+		+
60 mmol/L $NaHCO_3$			+	+			+	+
Freeze-thaw					+	+	+	+

+ is for stress treatment; CK – control group; K – single potassium fulvic acid stress; H – single alkaline salt stress; HK – combined stresses of alkaline salt and potassium fulvic acid; F – single freeze-thaw stress; FK – combined stresses of potassium fulvic acid and freeze-thaw; FH – combined stresses of alkaline salt and freeze-thaw; FHK – combined stresses of alkaline salt, potassium fulvic acid and freeze-thaw

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perature of the machine was set at 14.5 °C, and the rate of temperature change was 0.5 °C/12 min with a freezing and thawing cycle from 14.5 °C to –5 °C and then to 10 °C. The corresponding freeze-thaw sampling temperatures are 10, 2.5, –5, 2.5 and 10 °C (denoted as T1–T5, respectively). At each sampling time point, barley seedlings leaves of 8 treatment groups were randomly sampled. After the leaves were wrapped in tin foil and fixed for 50 s in liquid nitrogen, they were stored in an ultra-low temperature refrigerator at –80 °C for the following assays.

Determinations

Measurement of osmotic regulation substances. The soluble protein content was assayed according to the method of Bao et al. (2020). 0.1 g leaves were weighed, 5 mL distilled water was added, and they were ground into homogenate; the homogenate was then centrifuged at 3 000 rpm for 10 min. 1.0 mL supernatant was taken into the test tube and 4 mL distilled water was added, then 1 mL diluted solution was absorbed, and 5 mL Coomassie Bright Blue G-250 solution was added. The mixture was shaken well and let stand for 2 min. The absorbance of the supernatant was recorded at 595 nm with distilled water as blank set to zero, and the soluble protein content was obtained by standard curve.

Measurement of lipid peroxidation. The malondialdehyde (MDA) content was assayed according to the method of Gong et al. (2013). 0.3 g leaves were weighed, 3 mL 10% trichloroacetic acid (TCA) solution was added, and they were ground into homogenate. The homogenate was then centrifuged at 4 000 rpm for 10 min, 2.0 mL supernatant was taken into the test tube, 2.0 mL 0.6% thiobarbituric acid (TBA) solution was added, and the mixture was shaken well. Then, the test tube was boiled in water for 15 min, then placed in a refrigerator at 4 °C for rapid cooling, and centrifuged at 4 000 rpm for 10 min subsequently. The absorbance of the supernatant was recorded at 532, 600 and 450 nm with distilled water as blank set to zero.

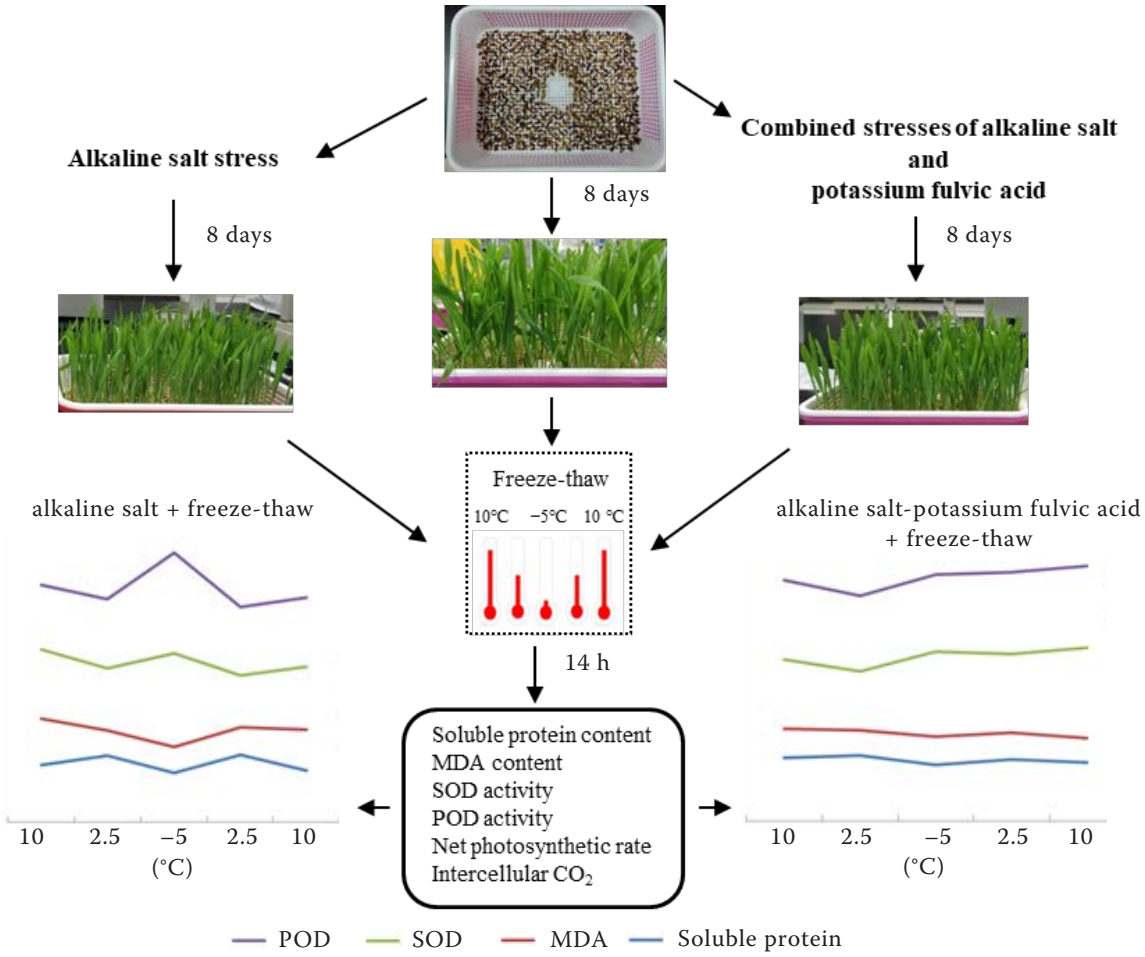

Figure 1. Graphical schema. POD – peroxidase; SOD – superoxide dismutase; MDA – malondialdehyde

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Determination of antioxidant enzymes. 0.1 g leaves were weighed, 5 mL phosphate-buffered saline (pH = 7.56) solution was added, and they were ground into homogenate. The homogenate was then centrifuged at 3 500 rpm for 10 min, and the supernatant was used for the following assays. The superoxide dismutase (SOD) activity was assayed using a reagent kit produced by Nanjing Jiancheng Biological Engineering Institute.

0.1 g leaves were weighed, 10 mL phosphate-buffered saline (pH = 7.32) solution was added, and they were ground into homogenate. The homogenate was then centrifuged at 3 500 rpm for 10 min, and the supernatant was used for the following assays. The peroxidase (POD) activity was assayed using a reagent kit produced by Nanjing Jiancheng Biological Engineering Institute. In this experiment, the results presented were means of three replicates (n = 3).

Measurement of photosynthesis indices. The photosynthetic parameters were measured by a CIRAS-3 portable photosynthetic instrument (Jin et al. 2017). The CO_2 concentration was set to 390 ppm, the H_2O concentration was set to 100%, and the internal light intensity (PARi) was set to 1 200 μmol/m^2/s. The cuvette flow was 200 mL/min, and the leaf room temperature was controlled to 25 ± 2 °C. In sunny and windless weather, the highland barley seedlings that have undergone a freeze-thaw cycle were placed under natural light for 1 h. The photosynthetic instrument (CIRAS-3, produced by PP Systems, Amesbury, USA) was used to measure the net photosynthetic rate (A, μmol/m^2/s), intercellular CO_2 concentration (C_i, ppm).

Statistical data analysis

Statistical analysis was performed using R 3.3.1 statistical software (R Foundation for Statistical Computing, Vienna, Austria). All data were analysed using one-way analysis of variance (ANOVA) followed by the least significant difference test at P = 0.05 level and Pearson correlation analysis. The results were expressed as means ± standard error (SE).

RESULTS AND DISCUSSION

Osmotic regulation substances

Under the stress of potassium fulvic acid and alkaline salt, the soluble protein content in seedlings leaves of single potassium fulvic acid stress, single alkaline salt stress and combined stresses of alkaline salt and potassium fulvic acid was higher than that of the control group and increased by 11.5, 20.9 and 40.5% compared with CK (P < 0.05), respectively. During the freezing-thawing period, with the decrease of temperature (T1–T3), the protein content in seedling leaves of all freeze-thaw groups (F, FK, FH, FHK) increased first and then decreased, and that of single freeze-thaw stress reached the lowest at T3 (Figure 2). When the temperature increased (T3–T4), the protein contents of single freeze-thaw stress, combined stresses of alkaline salt and freeze-thaw and combined stresses of alkaline salt, potassium fulvic acid and freeze-thaw increased by 17.8, 38.1, 8.8%, respectively. When the temperature rose to T5, the protein content of each freeze-thaw group decreased, and the protein content of FHK was significantly higher than that of the other three freeze-thaw groups (P < 0.05).

Under adverse conditions, plants will accumulate a large number of osmotic adjustment substances to reduce the damage caused by stress and enhance their stress resistance of plants (Dong et al. 2013). In this study, the protein content of group H and group HK was higher than that of group CK, and the increase of protein content of group HK was more obvious than that of group H (Figure 2). The reason may be that the content of Na^+ in the alkaline salt stress group increased, leading to water loss and the accumulation of abundant osmotic adjustment substances in barley seedlings; meanwhile, plenty of Na^+ showed antagonistic action against K^+ (Zhao et al. 2020), which inhibited the uptake of K^+ by barley seedlings, thus limiting the growth of seedlings. However, potassium fulvic acid slowed down this process and accelerated the synthesis of protein in barley seedlings. Under low-temperature stress, in order to resist cold, cold resistance genes in plants were expressed, and antifreeze proteins were synthesised (Du et al. 2011). In this study, as highland barley was one of the main crops in high-altitude freeze-thaw intensive areas, it had certain resistance to low temperature (Yuan et al. 2017). The soluble protein content in combined stresses of alkaline salt, potassium fulvic acid and freeze-thaw was higher than that in other freeze-thaw treatments, indicating that potassium fulvic acid could alleviate the adverse effects of alkaline salt and freeze-thaw on barley seedlings and enhance the osmotic adjustment ability of seedlings.

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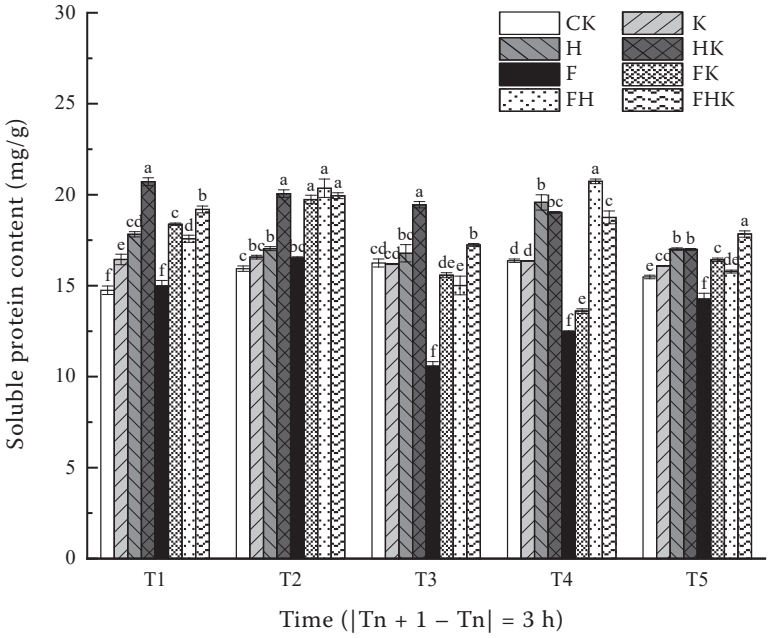

Figure 2. Effects of potassium fulvic acid (K), alkaline salt (H) and freeze-thaw (F) stress on soluble protein content of highland barley seedlings. CK – control group; FK – combined stresses of potassium fulvic acid and freeze-thaw; FH – combined stresses of alkaline salt and freeze-thaw; FHK – combined stresses of alkaline salt, potassium fulvic acid and freeze-thaw. Different letters indicated significant differences between different treatment groups at the same temperature ($P < 0.05$). Data represent means ± standard error of three replicates ($n = 3$). T1 – 10, T2 – 2.5, T3 – –5, T4 – 2.5, T5 – 10 °C

Lipid peroxidation

Under non-freeze-thaw treatment, MDA content in leaves of single potassium fulvic acid stresses significantly decreased, while MDA content in leaves of single alkaline salt stress and combined stresses of alkaline salt and potassium fulvic acid significantly increased, which increased by 63.1% and 12.7% compared with CK ($P < 0.05$) (Figure 3). After freeze-thaw treatment, with the decrease of temperature (T1–T3), MDA content in single freeze-thaw stress and combined stresses of potassium fulvic acid and freeze-thaw groups showed a trend of first increasing and then decreasing, while that in combined stress of alkaline salt and freeze-thaw and combined stress of alkaline salt, potassium fulvic acid and freeze-thaw showed a trend of first decreasing and then increasing, compared with T1, MDA content in F and FH groups decreased by 25.0% and 43.1%, respectively. With the increase of temperature (T3–T5), MDA content in F, FK and FH increased, while MDA content in FHK decreased. When the temperature increased to T5, the MDA content increased in the order: FHK < FK < F < FH.

The massive production of free radicals in plants will lead to membrane lipid peroxidation, resulting in the destruction of the cell membrane system and even the death of plant cells in serious cases. The accumulation of MDA was an important indicator reflecting the degree of lipid peroxidation in plant tissues (Tsikas 2017). Alkaline salt stress could aggravate lipid peroxidation of the plant cell membrane (Liu et al. 2015). In this study, MDA content significantly increased in single alkaline salt stress and combined stresses of alkaline salt and potassium fulvic acid groups. The research found that fulvic acid significantly reduced MDA content (Lotfi et al. 2015). With the addition of potassium fulvic acid, MDA content in single potassium fulvic acid stress decreased (Figure 3). After freeze-thaw treatment, MDA content in combined stresses of alkaline salt, potassium fulvic acid and freeze-thaw was lower than that in combined stress of alkaline salt and freeze-thaw, indicating that potassium fulvic acid could enhance the resistance of barley seedlings to alkaline salts and freeze-thaw stress, and weak the lipid peroxidation of the cell membrane to a certain extent.

Antioxidant enzymes

Under potassium fulvic acid and alkaline salt stress, SOD activity in single alkaline salt stress and combined stress of alkaline salt and potassium fulvic acid was significantly lower than that of CK ($P < 0.05$) and decreased by 24.7% and 22.2%, respectively (Figure 4). Under freeze-thaw treatment, with the decrease in temperature (T1–T3), SOD activity in all freeze-thaw groups (F, FK, FH, FHK) decreased first and then increased rapidly. The SOD activity in F, FH and FHK groups increased by 21.5, 35.5 and 21.7%, respectively, while that in combined stresses

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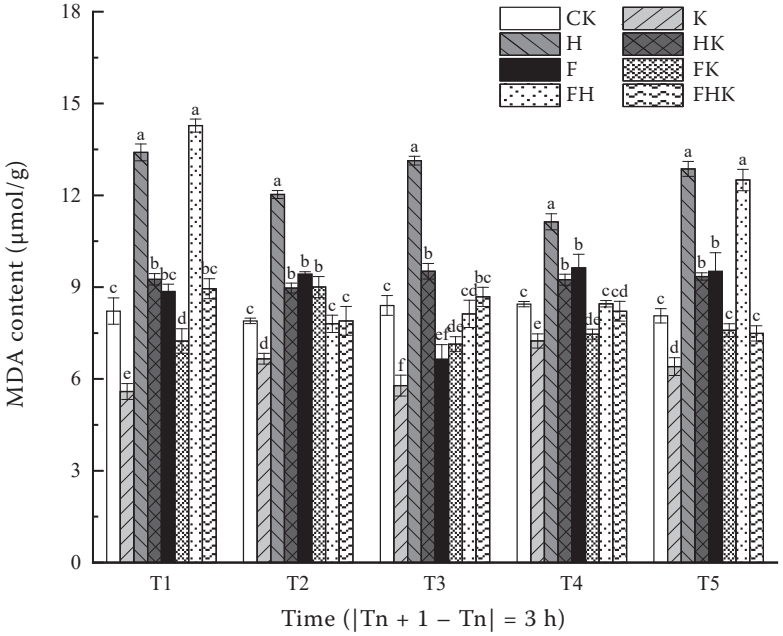

Figure 3. Effects of potassium fulvic acid (K), alkaline salt (H) and freeze-thaw (F) stress on malondialdehyde (MDA) content of highland barley seedlings. CK – control group; FK – combined stresses of potassium fulvic acid and freeze-thaw; FH – combined stresses of alkaline salt and freeze-thaw; FHK – combined stresses of alkaline salt, potassium fulvic acid and freeze-thaw. Different letters indicated significant differences between different treatment groups at the same temperature ($P < 0.05$). Data represent means ± standard error of three replicates ($n = 3$). T1 – 10, T2 – 2.5, T3 – –5, T4 – 2.5, T5 – 10 °C

of potassium fulvic acid and freeze-thaw changed little. At T3, SOD activity in single freeze-thaw stress reached the maximum; SOD activity was 145.50 ± 4.53 U/mgprot, which was significantly higher than that in the non-freeze-thaw treatment group ($P < 0.05$). With the increase in temperature (T3–T5), the SOD activity in group F showed a decreasing trend; on the contrary, the SOD activity in combined stresses of alkaline salt and freeze-thaw and combined stresses of alkaline salt, potassium fulvic acid and freeze-thaw showed a decreasing trend first and then increasing trend. When the temperature rose to T5, SOD activity in group FH was the lowest, which was significantly lower than that in other treatment groups ($P < 0.05$).

Under potassium fulvic acid and alkaline salt stress, POD activity in single alkaline salt stress and combined stresses of alkaline salt and potassium fulvic acid was significantly lower than that in CK ($P < 0.05$) and decreased by 20.1% and 10.7%, respectively. POD activity in single potassium fulvic acid stress increased by 10.2% compared with CK. Under freeze-thaw

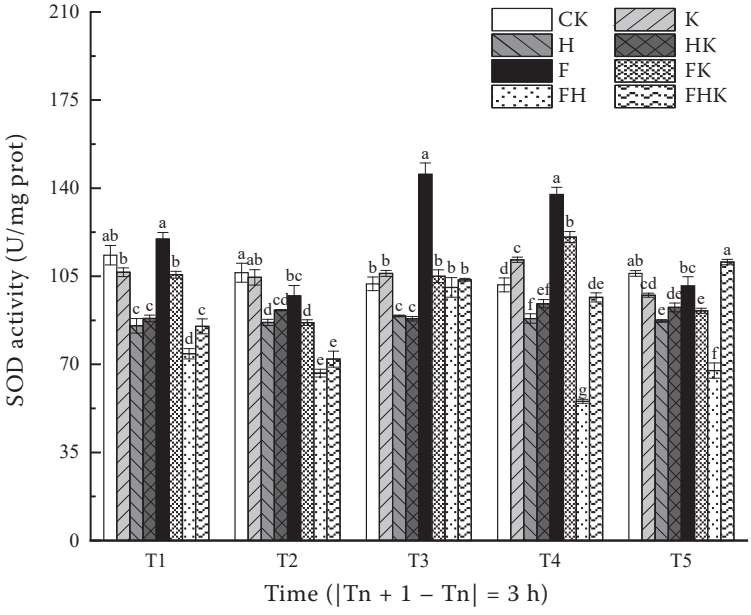

Figure 4. Effects of potassium fulvic acid (K), alkaline salt (H) and freeze-thaw (F) stress on superoxide dismutase (SOD) activity of highland barley seedlings. CK – control group; FK – combined stresses of potassium fulvic acid and freeze-thaw; FH – combined stresses of alkaline salt and freeze-thaw; FHK – combined stresses of alkaline salt, potassium fulvic acid and freeze-thaw. Different letters indicated significant differences between different treatment groups at the same temperature ($P < 0.05$). Data represent means ± standard error of three replicates ($n = 3$). T1 – 10, T2 – 2.5, T3 – –5, T4 – 2.5, T5 – 10 °C

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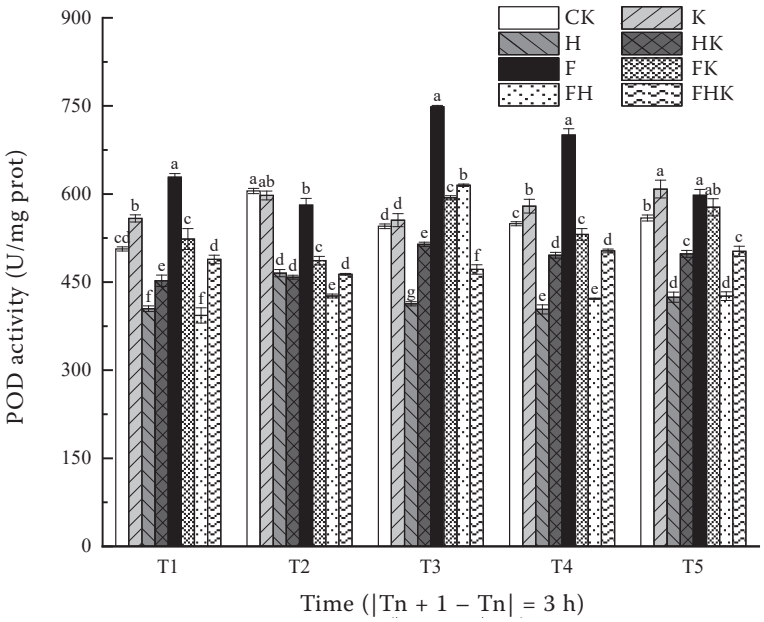

Figure 5. Effects of potassium fulvic acid (K), alkaline salt (H) and freeze-thaw (F) stress on peroxidase activity (POD) activity of highland barley seedlings. CK – control group; FK – combined stresses of potassium fulvic acid and freeze-thaw; FH – combined stresses of alkaline salt and freeze-thaw; FHK – combined stresses of alkaline salt, potassium fulvic acid and freeze-thaw. Different letters indicated significant differences between different treatment groups at the same temperature ($P < 0.05$). Data represent means ± standard error of three replicates ($n = 3$). T1 – 10, T2 – 2.5, T3 – –5, T4 – 2.5, T5 – 10 °C

treatment, POD activity in F, FK and FHK groups decreased first and then increased rapidly with the decrease of temperature (T1–T3), while POD activity in leaves of FHK increased rapidly. The POD activity in F, FK and FH groups increased by 19.1, 11.8, and 56.3%, respectively, compared with T1 (Figure 5). With the increase in temperature (T3–T5), POD activity in group F decreased gradually. When the temperature rose to T5, POD activity in group FH was the lowest and significantly lower than in other treatment groups ($P < 0.05$).

Under stress, plants produce excessive reactive oxygen radicals, POD and SOD, scavengers of reactive oxygen species (ROS) in plants, which can convert the excess ROS in plants into harmless substances such as water, thus improving the tolerance of plants to various adverse stresses (Sharma et al. 2012). The results of single alkaline salt stress were consistent with the research results of Tang et al. (2021); this may be owing to that the accumulation of ROS exceeded the regulatory threshold, and the excess ROS cannot be scavenged, thus leading to a decline of antioxidant enzymes activities (Song et al. 2006). The addition of potassium fulvic acid could increase POD and SOD activities in leaves under salt stress. The activities of SOD and POD in combined stresses of alkaline salt, potassium fulvic acid and freeze-thaw showed a fluctuant rising and tended to be stable, indicating that potassium fulvic acid was beneficial to regulating the antioxidant system of barley seedlings; moreover, the decrease of MDA content reflected ROS was in normal physiological level, and the antioxidant substances in seedlings kept a stable level.

Pearson correlation analysis showed that soluble protein was significantly negatively correlated with SOD and POD, with correlation coefficients of –0.806 and –0.816 ($P < 0.01$), respectively (Table 2). At the beginning of freeze-thaw treatment, barley seedlings synthesised antifreeze proteins; the soluble protein decreased, and the antioxidant defence system was activated, which induced an increase in SOD and POD activities. As the temperature rose, the soluble protein increased, and antioxidant enzyme activities decreased. SOD activity showed a significantly positive correlation with POD activity, with a correlation coefficient of 0.856 ($P < 0.01$). This may be ascribed to the adversity stress caused by the excess production of ROS, which induced the increase of antioxidant enzyme activities, O_2^- was transformed to H_2O_2 by SOD, H_2O_2 was reduced by POD, and transformed to H_2O (Pandhair and Sekhon 2006).

Photosynthesis

Under non-freeze-thaw treatment, the net photosynthetic rate of CK, K, H and HK groups decreased gradually (Figure 6A). With the decrease in temperature (T1–T3), the net photosynthetic rate of F, FK, FH and FHK groups decreased by 16.6, 9.3, 21.5 and 24.0%, respectively. With the increase in temperature (T3–T5), the net photosynthetic rate of each group continued to decrease, indicating that the damage caused by freezing and thawing stress to the net

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Table 2. Correlation analysis of indexes in all freeze-thaw treatment groups

	Soluble protein	MDA	SOD	POD
Soluble protein	1	0.045	−0.805**	−0.816**
MDA		1	−0.382	−0.408
SOD			1	0.856**
POD				1

*represents a significant correlation at the level of 0.05 (bilateral); **indicates a significant correlation at the level of 0.01 (bilateral); MDA – malondialdehyde; SOD – superoxide dismutase; POD – peroxidase

photosynthetic rate of the barley seedlings could not be recovered by heating up. The net photosynthetic rate in each group decreased continuously and reached the minimum value at T5, which was 1.75 ± 0.12, 1.05 ± 0.086, 1.7 ± 0.063, 1.67 ± 0.061 μmol/m^2/s, respectively. From the overall view of Figure 6A, after freeze-thaw treatment, the variation of net photosynthetic rate decreased in the order: FK > FHK > FH > F.

Under non-freeze-thaw treatment, the concentration of intercellular CO_2 in CK, K, H and HK groups increased gradually. The intercellular CO_2 concentration of group H and HK was lower than that of group CK (Figure 6B). With the decrease in temperature (T1–T3), intercellular CO_2 concentration in each freeze-thaw treatment group increased, and F, FK, FH and FHK increased by 13.8, 14.0, 37.6 and 34.3%, respectively. At T3, each group showed a trend of FH < FHK < F < FK (P < 0.05). With the increase in temperature (T3–T5), the intercellular CO_2 concentration of each freeze-thaw treatment group continued to increase and reached the maxi-

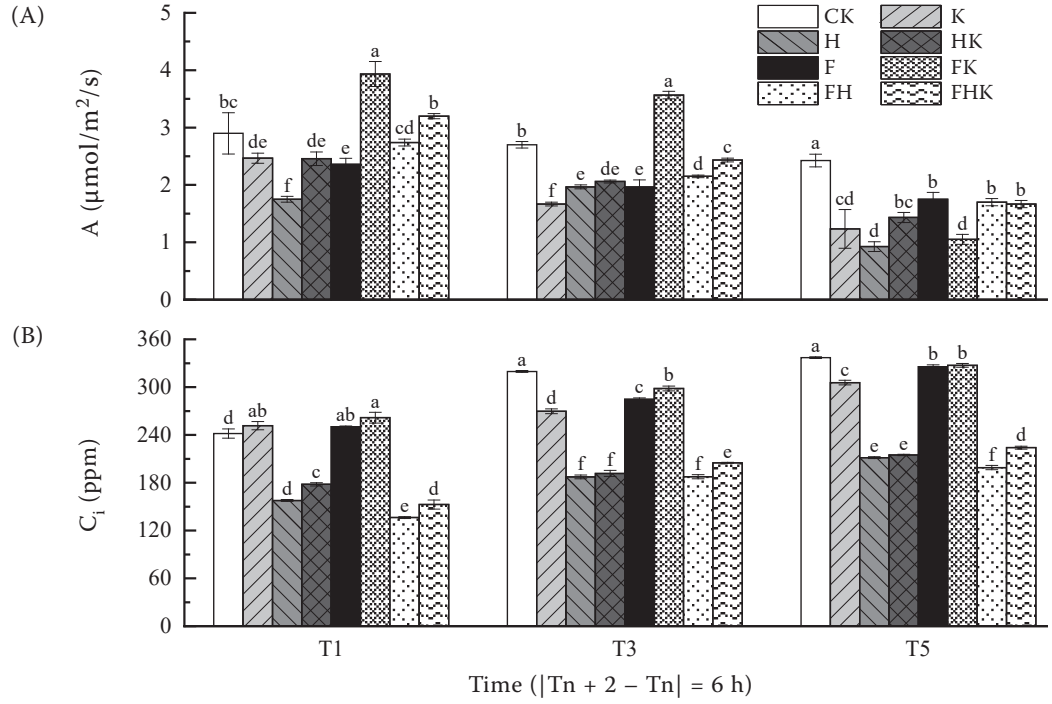

Figure 6. Effects of potassium fulvic acid (K), alkaline salt (H) and freeze-thaw (F) stress on (A)A (net photosynthetic rate) and (B) C_i (intercellular CO_2 concentration) of highland barley seedlings. CK – control group; FK – combined stresses of potassium fulvic acid and freeze-thaw; FH – combined stresses of alkaline salt and freeze-thaw; FHK – combined stresses of alkaline salt, potassium fulvic acid and freeze-thaw. The A and Ci in the vertical ordinate represent net photosynthetic rate, intercellular CO_2 concentration, respectively. Different letters indicated significant differences between different treatment groups at the same temperature (P < 0.05). Data represent means ± standard error of six replicates (n = 6). T1 – 10, T3 – −5, T5 – 10 °C

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mum value at T5. There was no significant difference between single freeze-thaw stress and combined stresses of potassium fulvic acid and freeze-thaw ($P > 0.05$), and the intercellular CO_2 concentration in combined stresses of alkaline salt and freeze-thaw was significantly lower than those in FHK, F and FK groups. As can be seen from Figure 6B, the variation of intercellular CO_2 concentration decreased in the order: FHK > FH > F > FK.

Photosynthesis was the physiological basis of various eco-physiological processes in plants (Taiz and Zeiger 2002). Plant photosynthesis will be damaged under low-temperature and alkaline salt stress (Liu et al. 2016). In this study, the net photosynthetic rate of single freeze-thaw stress continued to decrease during the whole freezing-thawing cycle, which might be because freeze-thaw stress would reduce the activity of photosynthetic enzymes in barley seedlings and damage the chloroplast of seedlings, thus reducing the net photosynthetic rate (Gan et al. 2019). In addition, the net photosynthetic rate of all freeze-thaw treatment groups decreased. The change rate of combined stresses of potassium fulvic acid and freeze-thaw was higher than that of the other three groups (F, FH, FHK), indicating that potassium fulvic acid could not effectively reduce the effect of stress on the net photosynthetic rate of barley seedlings.

Intercellular CO_2 concentration in plants was affected by stomatal size in leaves (Tominaga et al. 2018), while low-temperature stress caused water in plants form ice crystals and blocked leaf pores, resulting in reduced CO_2 uptake and concentration (Jurczyk et al. 2019). Under salt stress, stomatal guard cells can reduce water transpiration loss by controlling the stomatal aperture. Stomatal conductance decreased, and carboxylation reaction was limited, which was not conducive to CO_2 uptake in plants (Safdar et al. 2019). In this study, the intercellular CO_2 concentrations of single alkaline salt stress and combined stresses of alkaline salt and potassium fulvic acid were both lower than those of the CK (Figure 6B). However, the intercellular CO_2 concentration of HK was close to that of H, and that in combined stresses of alkaline salt, potassium fulvic acid, and freeze-thaw was close to that in combined stresses of alkaline salt and freeze-thaw. The result showed potassium fulvic acid could not effectively reduce the adversity stress effect of alkaline salt on the intercellular CO_2 concentration of barley seedlings.

Under short-term freeze-thaw and alkaline salt stress, the soluble protein content decreased, the MDA content increased, and the antioxidant enzyme activities and the photosynthetic rate decreased. With the addition of the appropriate amount of potassium fulvic acid, the damage of alkaline salt and freeze-thaw stress on enzyme activity and osmotic regulation ability of barley seedlings can be alleviated; specifically, the soluble protein content increased, MDA content decreased, antioxidant enzyme activities increased. Nevertheless, the effects on the photosynthetic rate were irreversible.

In conclusion, the results of this study showed that alkaline salt and freeze-thaw stress had a superposition effect on plants, and this effect could be effectively mitigated by the addition of potassium fulvic acid. In a word, potassium fulvic acid could improve the freezing resistance and salt tolerance of highland barley under short-term adversity. However, the effect of potassium fulvic acid on the photosynthesis of seedlings under stress was not obvious. In the future, the frequency of freezing and thawing could be increased to explore further the mechanism of potassium fulvic acid in alleviating the damage of freeze-thaw and saline-alkali stress to the photosynthesis of barley seedlings.

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