PHYSIOLOGICAL EFFECTS OF FREEZE-THAW, CADMIUM, AND AMBROSIN COMBINED STRESS ON *SECALE CEREALE* L. SEEDLINGS

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Abstract. Dongmu 70th, a rye (*Secale cereale* L.) cultivar known for its frost resistance, may potentially be impacted by Ragweed (*Ambrosia trifida* L.) ambrosin. This study explored the individual and combined effects of freeze-thaw cycles, cadmium (Cd), and ambrosin on Dongmu 70th's physiological responses. Parameters measured included soluble sugars, proteins, malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD) activities, and net photosynthetic rate. Our results provide a preliminary understanding of Dongmu 70's resilience to these environmental stressors and guide the development of integrated stress management strategies.
Keywords: *ambrosin-based allelopathic substances, cadmium stress, freeze-thaw stress, Secale cereale L.*

Abbreviations: FT: freeze-thaw; MDA: malondialdehyde; CAT: catalase; SP: soluble protein; SOD: superoxide dismutase TBA: thiobarbituric acid; TCA: tricarboxylic acid; NFT: no freeze-thaw; A: *Ambrosia trifida* L. (ragweed).

Introduction

Rye (*Secale cereale* L.) is an important crop in the Triticeae tribe. In Europe, large quantities of rye are used annually for rations and fodder, while in northern China, rye is also widely grown. Rye is a highly tolerant crop to a variety of environmental stresses and is particularly resistant to drought and frost. Rye also has the advantage of rapid growth and high yields. Freeze-thaw is a process by which soils freeze and thaw due to seasonal or diurnal temperature changes. Freeze-thaw always occurs in spring and winter in northern Asia and damages not only the soil but also plant tissue cells (Kreyling et al., 2008).

Heavy metals have a long latent time in the soil, are poorly migrated, and are also difficult to be degraded by microorganisms. Their accumulation and migration not only lead to soil degradation, but also pollute the water environment. In addition, they may accumulate step by step through the food chain and eventually threaten human health. Northeast China has a large proportion of land contaminated by heavy metals, and the potential ecological hazard level is high. Among a variety of heavy metal pollution, cadmium (Cd) pollution is particularly serious. Due to the high solubility of Cd in soil (about 35%), it has a higher absorption coefficient than other heavy metals, such as Zn, Cu and Pb, and is more easily absorbed. Under the influence of Cd, changes in membrane lipid peroxidation occur, leading to an increase in membrane lipid peroxidation and K + leakage rate and a decrease in chlorophyll content. Physiological and biochemical processes that control photosynthesis, water consumption efficiency,

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mineral nutrition and sugar metabolism are disrupted by Cd. As a result, the yield of plant biomass decreases (Atabayeva et al., 2020).

Ambrosia trifida L. (ragweed) is a world-recognized public nuisance weed. It competes with crops for light and nutrients, and it also releases ambrosin to having an allelopathy effect on other plants, ultimately leading to crop yield reduction. Ragweed has seriously invaded northeastern China, and in terms of population trends in recent years, and its populations will continue to increase steadily in the coming years (Liu et al., 2021).

Studies have been conducted to investigate the effects of cadmium, freeze-thaw or ragweed on Secale cereale but there is a gap in studies on the compound stress caused by the above three factors. We used the control variable method to set True or False as three dimensions of freeze-thaw, cadmium and ragweed stresses, and thus arranged and combined eight compound stresses. In this paper, the following parameters of Secale cereale L were studied under the above eight compound stresses in a controlled manner: osmotic substance content, catalase (CAT) activity, superoxide dismutase (SOD) activity, and changes in net photosynthetic rate. By analyzing these data, we could determine the physiological status of Secale cereale L in general.

Materials and methods

Plant materials

Experiments were conducted using Dongmu-70th rye seeds supplied by Ditong Seed Company, China. About 1000 plump and uniformly sized seeds were selected and soaked in 0.1% mass fraction of acidic $KMnO_4$ solution for 2 h, and then rinsed with distilled water. The seeds were placed on a 26 × 18 cm rectangular tray, lined with two layers of filter paper, moistened with Hoagland, and covered with cling film.

Experimental apparatus and drugs

Ambrosin is an extract from ragweed. The collection site of ragweed (*Ambrosia trifida* L.) was Changchun City, Jilin Province, with geographic coordinates 125.28° 43.78° and 223 m. The sampling area was a mono-dominant community of ragweed, and the sampling time of the adult planting period was in the first half of September. After collection, ragweed was cleaned with distilled water, dried naturally, and the roots, stems, and leaves were ground together into powder and mixed well. Weighing 100 g of the powder, put into a beaker containing 1000 mL of distilled water, soaked for 48 h, intermittent shaking, filtered with sterile gauze, out of the filtration with 0.25 μm microporous filter membrane filtration decontamination, to get the concentration of 10% (W/V) of the ragweed extract, 4°C refrigerated preservation.

Cd solution, produced by Guobiao (Beijing) Testing & Certification Co., Ltd. (GBTC); BPHJ-120A; Enzyme preparation, produced by the Institute of Nanjing Jiancheng Biological Engineering; CIRAS-3, DC CO2/H2O gas analyzer, produced by Portable Photosynthesis System; UV-6100, ultraviolet spectrophotometer produced by Shanghai Metash Instruments Co., Ltd.; TDL-40 centrifuge produced by ShangHai Anting Scientific Instrument Factory.

Plant growth and stress treatment

Rye seeds, after a 36-h soaking period, were evenly distributed among eight groups [Cadmium (Cd) t or f × Ambrosin (A) t or f × Freeze Thaw (FT) t or f] on the lattice

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layers of culture trays (34×25×12 cm). Each tray was supplemented with respectively conditional Hogeland, that ambrosin, Cd solution were added as group condition design. The trays were then covered with transparent plastic lids and placed in an incubator set to a 12-h light/dark cycle at day/night temperatures of 25°C/20°C. Subsequently, 500 ml of the respective conditional Hogeland was added to each tray bi-daily. Once the majority of the seedlings reached a height of 3 cm, the lids were removed. After 6 days, seedlings from the FT group were transferred to a BPHJ-120A alternating chiller, where the temperature started at 10° cycled through 10° -5° and back to 10°C over 12-h periods. Samples were taken every 6 h, when temperature in 10° -5° and back to 10°C. For sampling purposes, leaves from about 600 plants were excised every group, immediately wrapped in aluminum foil, and flash-frozen in liquid nitrogen for 50 s to preserve the biochemical integrity.

Biochemical characterization

The soluble protein content in leaf samples was quantified using the Coomassie Brilliant Blue staining method. Initially, 0.1 g of fresh leaf tissue was weighed and homogenized in 5 mL of distilled water. The homogenate was then centrifuged at 3000 rpm for 10 min. Subsequently, 1.0 mL of the supernatant was extracted and diluted to 5.0 mL. From this dilution, 1.0 mL was taken and 5 mL of Coomassie Brilliant Blue G-250 solution was added. After thorough mixing, the mixture was allowed to stand for 2 min to ensure complete reaction. The absorbance was measured at a wavelength of 595 nm. Protein concentration was then determined by comparing the absorbance values to a pre-established standard curve. The Coomassie brilliant blue stain is used in the Bradford assay, a colorimetric protein assay, to quantify the proteins separated by gel. This assay is performed by determining the absorbance shift of the Coomassie Brilliant Blue G-250 (Ku, 2013).

$$\text{Soluble protein content} = C * V_T / (V_S * W_F * 1000)$$

where C: Value in pre-established standard curve; V_T: Total volume of the extract (5 mL); V_S: Sample volume for measurement (1 mL from V_T); W_F: Fresh weight of sample leaf tissue.

The contents of malondialdehyde and soluble sugar were determined by the thiobarbituric acid (TBA) method (Heath and Packer, 1968); 0.5 g of chopped leaves were weighed, 1 ml of 10%TCA was added, ground to homogenate, then 4 mL TCA was added for further grinding, and centrifuged for 10 min at 4000 rpm. 2 ml supernatant was taken and 2 ml 0.6%TBA solution was added. The mixture was reacted in a boiling water bath for 15 min and was cooled quickly before centrifugation. The extinction of the supernatant at 532 nm, 600 nm, and 450 nm was determined.

$$\text{Soluble sugar content } (\mu\text{mol/L}) = 11.71 * D450$$

$$\text{MDA } (\mu\text{mol/L}) = 6.45 * (D532 - D600) - 0.56 * D450$$

CAT and SOD activities were measured by enzyme preparation.

Put the sample in the incubator to recover for 30 min, and measure it with the CIRAS-3 portable photosynthesis system.

Measurements of all the above biochemical indicators were repeated at least 3 times.

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Data processing

Statistical analysis is performed with Original statistical software (R Foundation for Statistical Computing, Vienna, Austria). ANOVA is applied to inspect treatment differences at each time point. The mean value was obtained by de-averaging the data from multiple sets of experiments.

Results

Changes in soluble protein content

Following freeze-thaw cycles (T1 to T3), the soluble protein content in the FT group exhibited a general increase slightly. In comparison, the soluble protein levels in the Cd + FT and A + Cd + FT groups showed a more modest increase throughout the freeze-thaw process relative to the CK + FT and A + FT groups. Cross-sectional analysis over the same period revealed significantly higher soluble protein content in groups subjected to stress compared to the CK group. These alterations were particularly accentuated in the group exposed to ambrosin stress. Under the influence of the Cd + FT stress factor, there was a large increase in soluble protein content at sampling time T2, but relatively decreased at sampling time T3 (*Fig. 1*).

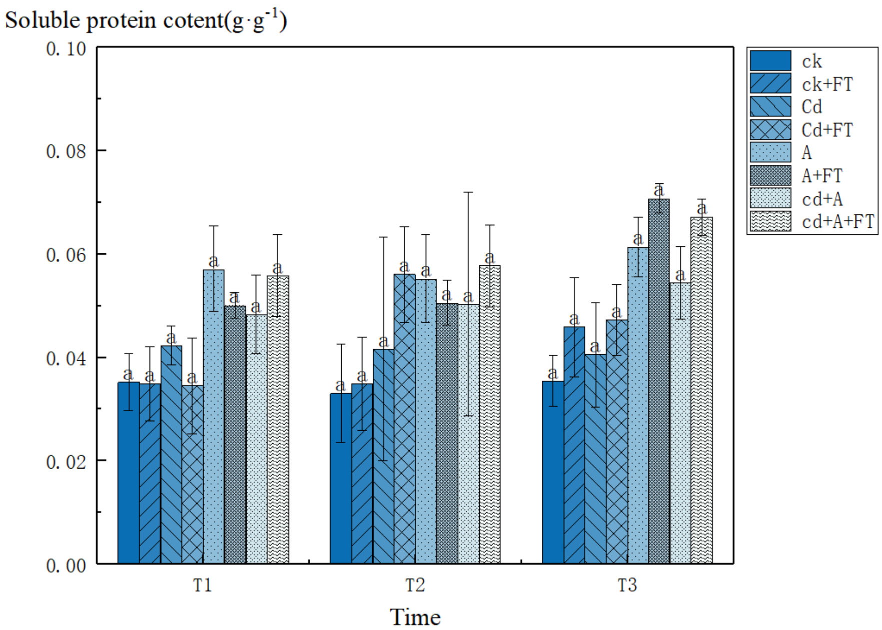

Figure 1. *Effects of combined stress of freezing and thawing, ambrosin and cadmium on soluble protein in seedlings. Rye was subjected to freeze-thaw stress after 6 days of growth. In the figure, T1 is when the temperature drops to 10℃ after freeze-thaw for 2h; T2 is when the temperature drops to −5℃ after freeze-thaw for 8h; T3 was frozen and thawed for 14h, and the temperature rose to 10℃. Different letters above the error bars indicate statistically significant differences between the groups*

Changes in soluble sugar

During the freeze-thaw (T2), the soluble sugars in the CK + FT group decreased, whereas there was no significant change observed in the A + FT group, and the Cd + FT

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group experienced a significant increase in soluble sugar content. Among the control groups, only the CK group showed an increase in soluble sugars, with no significant changes noted in the other groups.

Transitioning into the thawing phase (T3), the soluble sugar content in the CK + FT group within the FT treatment remained relatively stable compared to the freeze-thaw period. However, the soluble sugar levels in the Cd + FT group continued to rise, while those in the A + FT and Cd + A + FT groups exhibited a significant decline. In the control group, the soluble sugar levels in the CK group fell below the initial levels, whereas in the A group, there was a significant increase compared to the T1 and T2 periods. The remaining control groups did not show significant changes in their soluble sugar content.

Cross-sectional analysis of the same time frame revealed that soluble sugar content was significantly higher in groups subjected to stress compared to the CK group, with the Cd + FT group showing the most pronounced increase (*Fig. 2*).

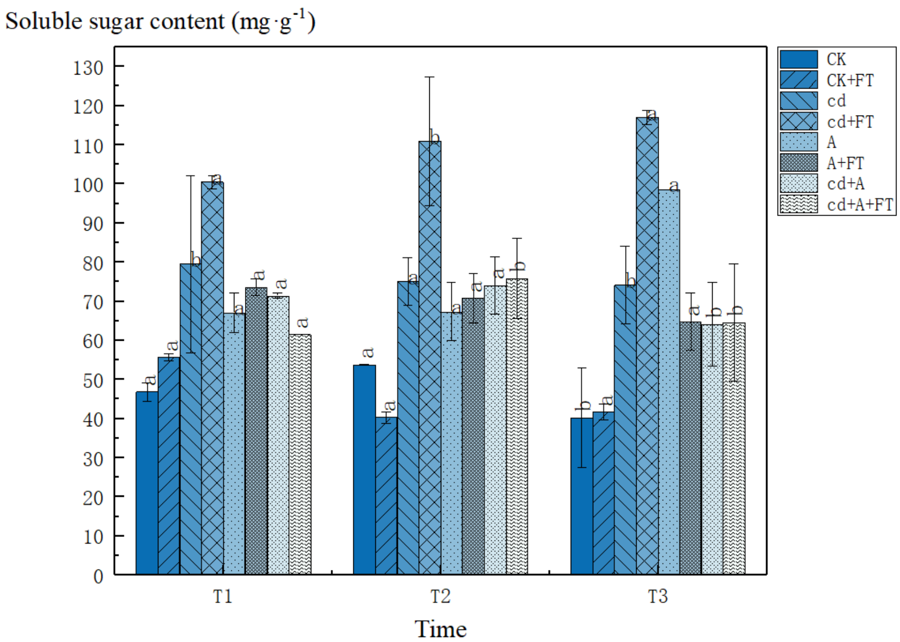

Figure 2. *Effects of combined stress of freezing and thawing, ambrosin and cadmium on soluble sugar in seedlings. Rye was subjected to freeze-thaw stress after 6 days of growth. In the figure, T1 is when the temperature drops to 10℃ after freeze-thaw for 2 h; T2 is when the temperature drops to −5℃ after freeze-thaw for 8 h; T3 was frozen and thawed for 14 h, and the temperature rose to 10℃. Different letters above the error bars indicate statistically significant differences between the groups*

Changes in SOD activity

During the freeze-thaw period (T1 to T2), superoxide dismutase (SOD) activity in rye seedlings within the CK + FT and Cd + FT groups exhibited a decline. Within the control group, SOD activity decreased in the CK group, increased in the Cd + A group, and remained stable in the Cd group. Transitioning into the thawing stage (T2 to T3), SOD activity showed an upward trend in both the CK + FT and Cd + FT groups. Among the controls, there was an increase in SOD activity observed in the CK and Cd groups. Over the entire period from T1 to T3, the SOD activities in the A + FT and Cd + A + FT groups demonstrated a continuous decline.

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Comparative analysis of SOD activities across the same period revealed a significant decrease under cadmium (Cd) stress, an increase during T1 and T2 under ambrosin (A) stress, and intermediate levels under combined Cd and A stress, lower than the control but higher than the Cd-only group. This suggests an antagonistic interaction between Cd and A stresses, influencing the SOD activity dynamics (*Fig. 3*).

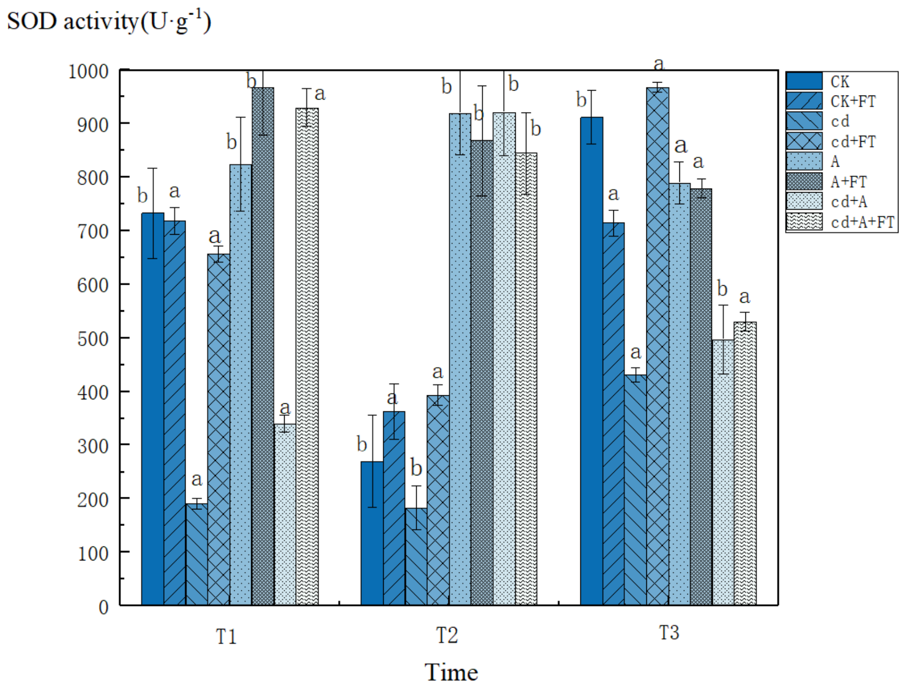

Figure 3. *Effects of combined stress of freezing and thawing, ambrosin and cadmium on SOD activity in seedlings. Rye was subjected to freeze-thaw stress after 6 days of growth. In the figure, T1 is when the temperature drops to 10℃ after freeze-thaw for 2 h; T2 is when the temperature drops to −5℃ after freeze-thaw for 8 h; T3 was frozen and thawed for 14 h, and the temperature rose to 10℃. Different letters above the error bars indicate statistically significant differences between the groups*

Changes in CAT activity

Under individual stress conditions from cadmium (Cd), ambrosin (A), and freeze-thaw (FT), catalase (CAT) content exhibited an increase relative to the control group (CK). However, under combined stress conditions such as Cd + Cd + F and A + F although CAT content significantly rose compared to the CK group, the increment was less pronounced than that observed in the groups subjected to single stress factors. Notably, in the CK + F Cd + F and A + FT groups, CAT content increased when the temperature was lowered to -5°C and subsequently decreased when the temperature returned to 10°C. This dynamic response suggests a temperature-dependent modulation of CAT activity in response to stress conditions. The Cd + A + FT group showed a greater increase in CAT values at T2 compared to the Cd + A group. The relationship between Ambrosin, FT and sampling time: CAT of both group A and A + FT showed a large increase at sampling time T2, but at sampling time T3, the CAT value of group A + FT dropped back, while group A basically remained the same compared to T2 (*Fig. 4*).

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Changes in malondialdehyde (MDA) content

During the freezing phase, malondialdehyde (MDA) content in the CK + FT group increased compared to the CK group, whereas it decreased during the thawing phase. In comparison, MDA levels were elevated in both the Cd and A groups relative to the CK group. Notably, MDA content was highest under dual stress conditions, but decreased under triple stress, exhibiting lower levels than observed in the double stress groups. This pattern suggests complex interactions between the stress factors affecting lipid peroxidation and cellular damage in rye seedlings (*Fig. 5*).

Changes in net photosynthetic rate

During the freeze-thaw cycle (T1 to T2), the net photosynthetic rate of rye seedlings in both CK + FT groups exhibited a decrease, while in the Cd + FT group, it increased, with no significant changes observed in other groups. As the temperature rose, the net photosynthetic rate in the Cd + FT and Cd + A + FT groups declined, whereas rates in other groups remained stable. Comparative analysis of the photosynthetic rates across the same period revealed that both cadmium (Cd) and ambrosin (A) stresses individually reduced the net photosynthetic rates of rye seedlings, yet no significant interaction between these stresses was evident. This suggests independent effects of Cd and A on the photosynthetic capacity of the seedlings (*Fig. 6*).

Discussion

Soluble protein is an essential osmotic regulator and nutrient, and the increase in soluble protein content will induce cells to maintain a low osmotic potential and enhance water absorption and water-holding capacity. Plant proteins participate in processes such as cell osmosis stabilization, cell compartmentalization, lipid alteration and fatty acid membrane composition, increased antioxidant activity, energy metabolism, and activation of primary and secondary metabolites in response to cold stress. When under stress, plants can increase their resistance by changing protein content (Kazemi-Shahandashti and Maali-Amiri, 2018). The data showed that the soluble protein content of the CK + FT and A + FT groups gradually increased as the freeze-thawing progressed, indicating that freeze-resistant varieties can effectively improve the freezing tolerance of plants by increasing protein content (Takahashi et al., 2013). The soluble protein content increased relative to the CK group under Cd stress, demonstrating that rye seedlings can maintain osmotic balance by increasing the soluble protein content and have a certain resistance to Cd stress. In addition, it is also possible that Cd causes damage to the protein of the seedlings, inhibits the normal metabolism of rye, and increases the mass fraction of the original protein (Atabayeva et al., 2020). The soluble protein content of the Ragweed Group was significantly higher than in the CK Group due to the allelopathic effect of the allelopathic substances in ragweed on rye (Bruckner et al., 2003), and the microorganisms bred in Group A also promoted the production of various enzymes in the rye to cope with external stresses, resulting in increased protein content. The soluble protein content of the Cd + A + FT group in the T3 state was lower than that in the A + FT group, which may be due to the presence of Cd interfering with the allelopathic effect of ragweed on the growth of rye seedlings (Wei et al., 2020).

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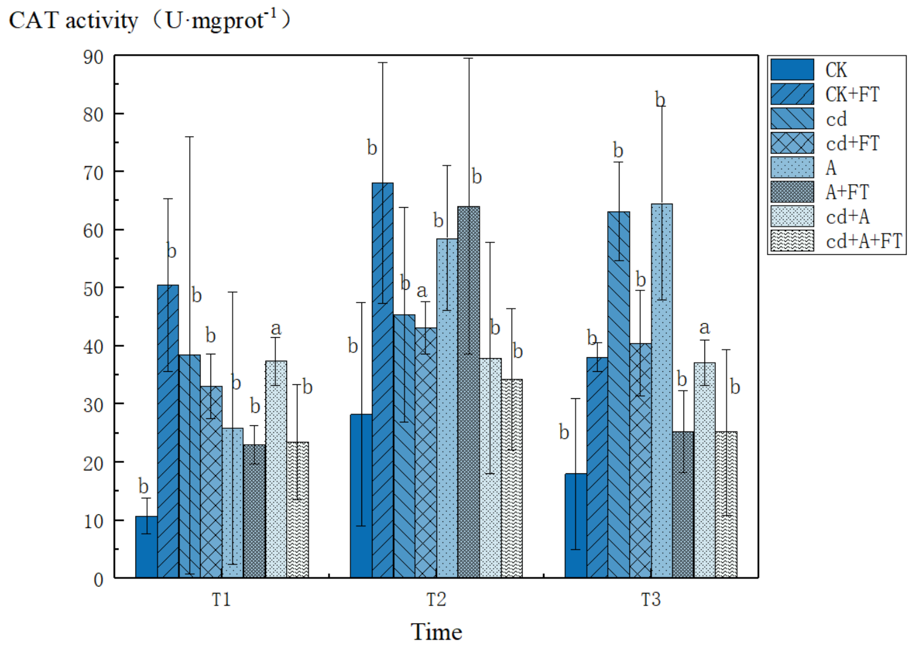

Figure 4. *Effects of combined stress of freezing and thawing, ambrosin and cadmium on catalase activity in seedlings. Rye was subjected to freeze-thaw stress after 6 days of growth. In the figure, T1 is when the temperature drops to 10℃ after freeze-thaw for 2 h; T2 is when the temperature drops to −5℃ after freeze-thaw for 8 h; T3 was frozen and thawed for 14 h, and the temperature rose to 10℃. Different letters above the error bars indicate statistically significant differences between the groups*

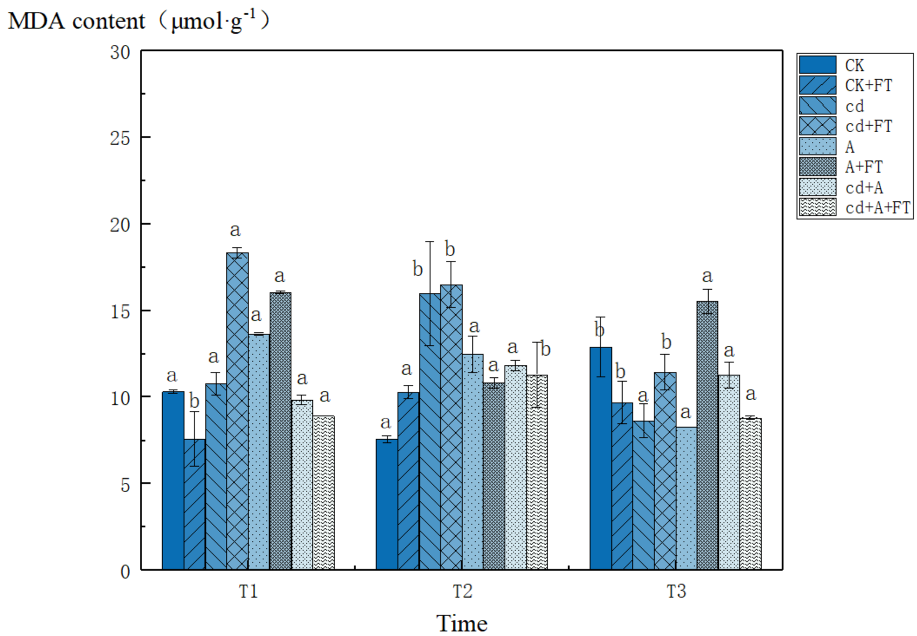

Figure 5. *Effects of combined stress of freezing and thawing, ambrosin and cadmium on Malondialdehyde (MDA) content in seedlings. Rye was subjected to freeze-thaw stress after 6 days of growth. In the figure, T1 is when the temperature drops to 10℃ after freeze-thaw for 2 h; T2 is when the temperature drops to −5℃ after freeze-thaw for 8 h; T3 was frozen and thawed for 14 h, and the temperature rose to 10℃. Different letters above the error bars indicate statistically significant differences between the groups*

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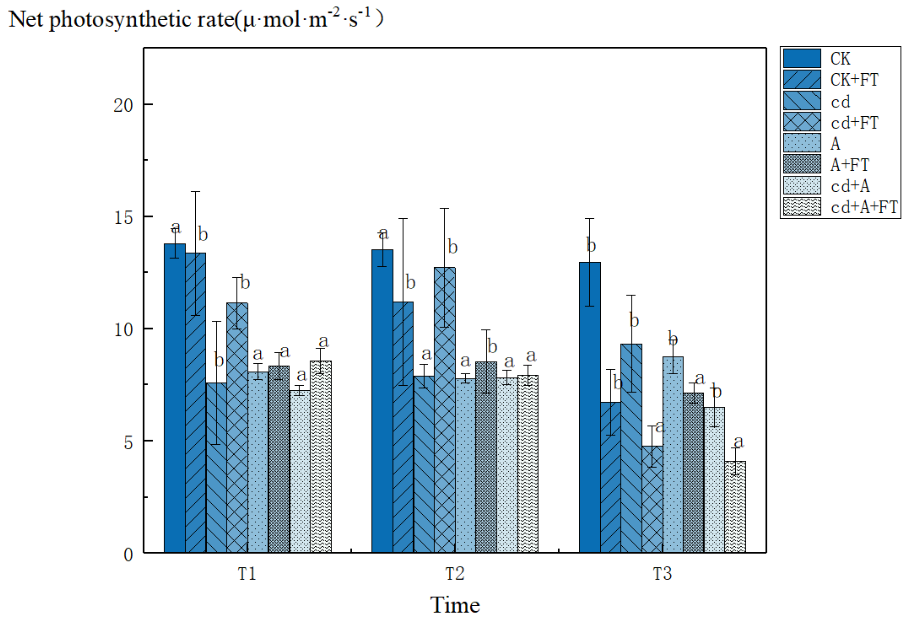

Figure 6. *Effects of combined stress of freezing and thawing, ambrosin and cadmium on net photosynthetic rate in seedlings. Rye was subjected to freeze-thaw stress after 6 days of growth. In the figure, T1 is when the temperature drops to 10℃ after freeze-thaw for 2 h; T2 is when the temperature drops to −5℃ after freeze-thaw for 8 h; T3 was frozen and thawed for 14 h, and the temperature rose to 10℃. Different letters above the error bars indicate statistically significant differences between the groups*

Chilling stress with oxidative facet is associated with ROS accumulation and triggers membranes' unsaturated fatty acids peroxidation, which can be assayed by MDA content (Aghdam et al., 2016). MDA is an end product of lipid peroxidation used as an indicator of membrane oxidative damage. In this experiment, the MDA content of Cd, CK + F Cd + F and A + FT was increased, indicating that the heavy metal cadmium stress, freeze-thaw stress, ambrosin stress can increase the content of MDA in rye seedlings, causing damage to cell membranes. In the results of three separate samplings of the CK + FT group at T1-T3, the MDA showed a trend of first up and then down, indicating that under long-term freeze-thaw stress, the tolerance of rye seedlings to low temperature increased to reduce the damage of the plant tissue (Guo et al., 2006). The MDA content of the Cd + FT group at T1 and T2 moments is higher than that of C single stress, and other compound stresses groups, which shows that under freeze-thaw and cadmium composite stress, rye seedlings have the highest level of lipid peroxidation in cell membranes and the highest levels of cell damage, but at the T3 time, the MDA content of Cd + FT is reduced. Except for the reason that the tolerance of rye seedlings to low temperatures is enhanced with the prolongation of freeze-thaw stress time, it may also be the adaptation function of rye seedlings to low doses of heavy metal cadmium increases at this time, damaging to plants (Sandbichler and Hockner, 2016, Maresca et al., 2020). The MDA content of Cd + A + FT stress was lower than that of bifactorial stress and did not continue to rise due to the increase in the types of compound stress, which is deduced to the change of the enzyme system dominating the free radical scavenging system in seedlings under combined stress, which improved the activity of the free radical scavenging system and could effectively reduce membrane lipid peroxidation damage MDA content no longer increased based on combine stress (Chen et al., 2020).

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Soluble sugars are a common component of most organisms and a fundamental part of biosynthesis. Therefore, the response of plants to stress is often accompanied by changes in carbohydrate content (Maness, 2010). Low temperatures generally increase the concentration of soluble sugars (Rosa et al., 2009). At different temperatures, the phenomenon of first increasing and then decreasing soluble sugars in the CK + FT group is inconsistent with Rosa's findings, which may be due to the damage of cell structure in this group of rye under freeze-thaw conditions, the osmotic pressure is unbalanced, and the soluble sugars are not stored typically. However, the increase in the Cd + FT group indicates that the Cd content of rye leaves has decreased under freeze-thaw conditions, and the soluble sugar content of rye is more affected by temperature conditions (Wang et al., 2021). The A + F and Cd + FT groups tended to be roughly the same, indicating that the osmotic pressure stress caused by ragweed on rye culture dominated.

Abiotic stresses usually cause H2O2 accumulation, with harmful effects, in plants. Catalase may play a key protective role in plant cells by detoxifying this excess H2O2 (Nie et al., 2015). Wheat CAT expressed in transgenic rice improved its tolerance to low-temperature stress (Matsumura et al., 2002), indicating that CAT is an essential gene related to abiotic stresses and can be used to improve plant stress tolerance. The increase in T1-T2 CAT activity may be due to the gene regulation of rye under low-temperature stress, increasing the release of CAT to reduce the accumulation of H2O2 in leaves, thereby enhancing the ability of plants to resist cold stress. The downward trend of CAT expression in T2-T3 out of line can be deduced to the gradual increase of T2~T3 temperature, the stress intensity of plants has diminished, the mRNA level and enzyme activity have been adjusted under cold stress, and the expression mode varies with the type of stress, which corresponds with the Nie's research. Cd stress will cause the metabolism maladjustment of reactive oxygen species in ryes, accumulating free radicals and damaging the cell membrane structure. The rye will enhance the release of CAT to mitigate the damage of reactive oxygen species such as H2O2 to plant cells. Cd and Cd + FT groups increased compared to the CK and CK + FT groups in releasing CA which is consistent with Iranpour's results. However, the CAT content of the Cd + FT group is lower than that in the Cd group, indicating that the CAT release capacity in plant cells was weakened under the combined stress of Cd and low temperature (Wu et al., 2012). Compared with the CK group, the CAT content of group A was higher, and with the extension of the experimental time, the CAT content continued to increase, and the CAT content of group A reached the highest at T3 time. CAT is a critical enzyme that clears H2O2 and can reduce H2O2 to H2 and the increase in CAT content indicates that rye seedlings quickly adapt to the stress of reducing ROS damage to plant cells by increasing CAT (Gong et al., 2013). Moreover, the CAT content in the Cd + A + FT group is lower than that in other stress groups, demonstrating that the CAT production capacity in plant cells is worn down under the influence of Cd-containing compound stress.

In the cytoprotective enzyme system, SO which can counteract reactive oxygen species (ROS) produced under stressful conditions, is the most effective antioxidant enzyme to prevent cellular damage and can resist adverse conditions by increasing SOD content when the external environment is unfavorable (Sanchez-Parra et al., 2015). Compared with the CK group, the SOD content of the CK + FT group was reduced. In addition, under the pressure of F the SOD content shows a trend of a decrease in T2 and an increase in T3, which corresponds to the essential characteristic of enzymes. The

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SOD content of the Cd group is significantly lower than the CK group, possibly because the Cd concentration is too high, causing irreversible damage to rye seedlings, and the alleviating effect of SOD on Cd stress is restricted (Lu et al., 2006). Ragweed produces allelopathic substances, which can contribute to the production of reactive oxygen species (ROS), inhibit antioxidant enzyme activity, and induce oxidative stress in plants. At the T1, and T2 stages, compared with the CK group, the SOD content of group A is higher. It is deduced that rye has developed an adaptive mechanism to clear ROS to alleviate oxidative stress response (Gong et al., 2020). At the T3 moment, the SOD content of group A diminished compared to the CK group, proving that the large number of reactive oxygen species produced exceeded the ability of antioxidant defense enzymes, leading to oxidative stress and plant cell death (Sucur et al., 2021).

Net photosynthetic rate reflects the ability of plants to accumulate organic matter through photosynthesis and is an important physiological indicator of plant growth under stress. In the experiment, when F and Cd treatments were present alone, they did result in a decrease in net photosynthetic rate. However, during T2, FT + Cd resulted in an increase in net photosynthetic rate. The freeze-thaw cycle caused more significant damage to carbon flux than continuous low temperature. It was not difficult to find that photosynthesis tended to decrease from T1 to T3 stages in the freeze-thaw group. Plant chloroplasts play an important role in light transmission, absorption and conversion, and are important organs for photosynthesis, which are very sensitive to low temperature environment. When the temperature of the external environment is shallow, the structure and function of chloroplasts will be seriously damaged (Lu et al., 2020). Secondly, sufficient soil moisture can provide ample material basis and energy transport guarantee for plant photosynthesis. Freezing soil-free water will inhibit the smooth progress of plant photosynthesis to a certain extent, and plant cells exposed to low-temperature stress will induce freezing and dehydration, blocking leaf stomata. Gases cannot enter the cells through stomata, resulting in reduced CO2 assimilation, thereby limiting the photosynthetic rate of plants and affecting plant photosynthesis (Du et al., 2014). Cadmium stress can lead to the reduction of chlorophyll in plants. The decrease in the net photosynthetic rate of Cd groups shows a corresponding result with universal rules in heavy metals' affection on photosynthesis. Compared with the CK group, the net photosynthetic rate decreased under the stress of indicating that the allelopathy of ragweed inhibited the photosynthesis of rye seedlings. It is deduced that rye seedlings produce ROS (reactive oxygen species) under stress, and rye seedlings avoid ROS production by increasing the non-radiative energy dissipation of pigment beds (Aroca et al., 2001). Thereby photosynthesis is restricted. The net photosynthetic rate of rye seedlings decreased significantly under triple stress. That is, the three stress factors synergistically affected the net photosynthetic rate of rye seedlings.

Conclusions

In this experiment, the physiological responses of DongMu-70 rye under the combined stress characteristics of freeze-thaw, Cd and ragweed were studied by measuring the physiological characteristics of non-photosynthesis and photosynthesis, and the following conclusions were obtained: under stress, rye seedlings underwent a series of physiological metabolic regulation, mainly manifested by an increase in MDA content, indicating cell membrane damage; soluble protein and soluble sugar content increased, which enabled The increase of soluble protein and soluble sugar content

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made the cells maintain low osmotic potential and enhanced water absorption and water holding capacity; the increase of CAT content alleviated the effect of ROS (reactive oxygen species) damage to the plant body; the decrease of SOD content under freeze-thaw and Cd stresses was an irreversible damage to the plant body in adversity. In addition, SOD content increased under A stress, indicating that rye has developed an adaptive mechanism to scavenge RO but its adaptability is limited. The net photosynthetic rate of rye seedlings was significantly affected by stress, showing that a single stress forced a reduction in net photosynthetic rate, and the reduction in compound stress was greater than that of a single stress. In the freeze-thaw cycle, low temperature had a negative effect on rye seedlings. The responses of SOD and MDA were evident during the temperature decrease. The highest degree of cell membrane damage was observed in rye under freeze-thaw and Cd stresses, and SOD content increased first under freeze-thaw stress, which is consistent with the essential characteristics of the enzyme. The above results indicate that the regulatory role of protective enzyme systems in rye seedlings is most pronounced under low temperature conditions. Cell membrane damage occurs in rye seedlings under low temperature, and plants show specific adaptations by reducing the damage through a series of mechanisms.

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