Effects of combined extreme cold and drought stress on growth, photosynthesis, and physiological characteristics of cool-season grasses

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Abstract

Abiotic stress is an important factor affecting turf establishment and limiting the sustainability of the turf industry. To alleviate the combined effects of cold and drought prevalent in cold- and drought-prone regions, the selection and introduction of turfgrass germplasm suitable for these conditions are essential for successful turf establishment. Thus, we evaluated the effects of combined extreme cold and drought stress on morphological, photosynthetic, and physiological and biochemical traits in 16 wild annual bluegrass (*Poa annua*) seedlings. We found that there were significant differences ($P < 0.05$) among the provenance, combined cold and drought stress, and main interaction factors. The combined cold and drought stress altered the morphological characteristics of the 16 germplasm to varying degrees. Furthermore, The combined cold and drought stress significantly also reduced the net photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate (Tr), instantaneous water use efficiency (WUE), Chlorophyll content, chlorophyll fluorescence parameters, however, ac-cumulated Intercellular CO_2 concentration (Ci), relative electrical conductivity (REC), the contents of malondialdehyde (MDA), proline (Pro), soluble protein (SP), soluble sugar (SS), superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), hydroxyl radical (·OH), and increased the activities of superoxide dismutase activity (SOD), peroxidase activity (POD), catalase activity (CAT), ascorbate peroxidase activity (APX) and glutathione reductase (GR). Comprehensive evaluation using PCA (Principal Component Analysis), affiliation function analysis, and clustered heat maps indicated that ‘HZ’ germplasm had better combined cold and drought tolerance, whereas ‘ZQ’ germplasm was more sensitive to combined cold and drought, which was roughly consistent with the order of morphological damage symptoms. It is recommended that ‘HZ’ seeds be used in planting projects under cold-and drought-prone areas, while ‘ZQ’ seeds are more suitable for use under non-cold and water-deficit conditions.

Introduction

Abiotic stresses, such as extreme temperature, drought, salinity and oxidative stress pose serious threats to agriculture and lead to ecological environment degradation. Abiotic stresses result in a series of morphological, physiological, biochemical, and molecular changes that adversely affect plant growth and productivity[1]. It is believed that among all the abiotic stressors globally, cold and drought are the major factors limiting plant growth and production[2]. Due to rapid and dynamic changes in the global environment, plants are more and more frequently exposed to the combination of various abiotic stresses[3], such as cold and drought, which can cause more severe damage to plants than a single stress and are not conducive to agricultural production and the improvement of the ecological environment[4]. Moreover, responses to combined stresses are unique and cannot be directly extrapolated from single-stress responses[5,6]. Whereas studies involving cold and drought stresses usually address the stresses individually, only a few studies combine cold and drought[4,7].

The combination of cold and drought stress can induce significant changes in plant morphology, such as leaf wilting and senescence, stem and root growth inhibition, and fruit damage, severely reducing crop

productivity[8,9]. Plants can develop a variety of defense mechanisms by method of self-regulation to counteract the damage caused by stress[10]. In general, the factors of cold (usually below 10˚C) and drought can affect photosynthesis and nutrient uptake of plants[11,12]. Both stresses of cold and drought normally lead to the disruption of chloroplast ultrastructure, blocked PSII electron transfer in chloroplast membranes, reduced chloroplast pigment synthase activity, insufficient raw material for chlorophyll synthesis, slowed plant organism metabolism and reduced chlorophyll content in leaves[4]. The response patterns of plants to single cold or drought stress are similar[5], but the effects of the combined cold and drought stress are more remarkable than each stress applied individually.

Combined cold and drought stress can accumulate large amounts of reactive oxygen species (ROS) such as superoxide anion radicals ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (·OH), impairing plant function by causing protein denaturation, lipid peroxidation, DNA damage, and enzyme inhibition[6]. Additionally, numerous studies have shown that the mitigation of oxidative damage induced by environmental stress and the enhancement of plant resistance may be related to the activation of their antioxidant defense systems[13,14]. Under adversity stress, plants will activate the mechanisms of the antioxidant enzyme system and non-enzymatic system to provide protection from reactive oxygen species and consequently sustain the normal growth and metabolism of plants. Among them, the activity of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) can effectively regulate the metabolic homeostasis in plants[15]. SOD not only plays the role of the first line of defense for plants to scavenge ROS but also is the main scavenger of $O_2^{\cdot-}$. The function of SOD is mainly to convert excess $O_2^{\cdot-}$ into H_2O_2 and O_2; the H_2O_2 is further converted into H_2O that is harmless to cells through POD[16]. In the AsA-GSH cycle, APX plays a key role in catalyzing H_2O_2, while GR provides substrates for APX mainly through the formation of AsA and GSH, thereby scavenging the overproduction of ROS[17]. Indeed, the level of malondialdehyde (MDA) and relative electrical conductivity (REC) content can indicate the degree of cellular membrane lipid peroxidation and the strength of plant response to adversity conditions [18,19]. Proline (Pro), soluble sugar (SS), and soluble protein (SP) are essential osmoregulatory substances involved in response to various environmental stresses, which in turn come to prevent cellular dehydration[20].

Northwest China is located in an arid and semi-arid climate with scarce rainfall, water scarcity, cold and dry winters, and plants suffering from prolonged cold and drought stresses[21,22]. Given these climatic conditions, the improvement of the ecological environment is facing significant difficulties, this situation requires the use of effective and unavoidable solutions that must rely on the redevelopment of these ecosystems through the introduction of cold- and drought-resistant species to reduce the impacts of extreme temperature and water scarcity and to promote the sustainability of ecological agriculture. Annual bluegrass (*Poa annua*) is one of the most widely distributed plant species in the world, with short life history, strong reproductive ability, resistance to trampling, pruning, stress resistance, and easy formation. It can be used as an excellent lawn grass and ecological grass. Due to its comprehensive

survival strategy, *Poa annua* plays an important role in urban green space ecosystems' productivity, species diversity, and ecological function[23,24]. This makes the introduction and selection of this turfgrass the perfect solution for restoring vegetation cover on ecologically degraded lands. However, a comprehensive study should be considered to evaluate the response of plant materials following their geographical origin before planting to better mitigate and without any fault with the problems of urban greening, sports field turf establishment and road berm construction in cold-and drought-prone areas. In this work, we propose to study the response of *Poa annua* seedlings from 16 provenances under extreme combined cold and drought stress to determine the introduction of this species in cold-and drought-prone areas. This study determined the morphological, photosynthetic and physiological and biochemical responses of different *Poa annua* germplasms to combined extreme cold and drought stress, and aims to observe the effects of combined extreme cold and drought stress on different germplasms of *Poa annua* for assessing the degree of tolerance of 16 *Poa annua* germplasms to combined cold and drought stress. In addition, using comparisons of the resistance of different *Poa annua* germplasms to combined temperature and water stress, we are looking to screen the provenance that provides the best growth results to select it as planting material in grasses construction projects in cold-and drought-prone regions of the world.

Results

Effects of combined cold and drought stress on plant morphology

The morphological changes of different *Poa annua* seedlings under combined cold and drought stress were observed in this experiment (Fig 1). The results showed that the growth morphology of *Poa annua* seedlings changed obviously under the combined stress. Among them, ‘ZQ’ changed more; the seedling showed the most severe symptoms of leaf yellowing, wilting, and curling after 24 h of the combined stress. The ‘HZ’ had strong characteristics of resistance, especially, the seedling leaves still maintained good vigor under 24 h of stress; it was found that those samples appeared only the morphological changes of yellowing of lower leaves and curling of upper leaf edges. Therefore, according to the phenotypic symptoms, it can be tentatively evaluated that ‘HZ’ had a high performance of resistance and ‘ZQ’ had a low resistance performance.

Combined analysis of provenance, combined stress and provenance x combined stress

According to the results of ANOVA, it can be seen that the effects of provenance, combined stress, and provenance x combined stress on 30 traits were prominently different (Table 1). The results revealed that the resistance of *Poa annua* was influenced by the provenance, combined stress, and interaction, and the effect of combined stress on these traits was more obvious. The genetic variation coefficients of 30 traits for 16 *Poa annua* germplasms confirmed the genetic differences under combined stress; the coefficient of variation for 30 traits ranged from 8.26% (qN) to 74.97% (Gs), with a mean value of 32.09%.

Table 1. Main interactions and coefficients of variation of growth and photosynthetic and physiological traits of different *Poa annua* seedlings under the stress treatments.

Trait	Provenance(P)	Temperature(T)	PxT Interaction	CV(CK)(%)	CV(T)(%)
LDMC	F=4.84(*p* 0.001)	F=296.39(*p* 0.001)	F=3.41(*p* 0.001)	12.27%	12.28%
RWC	F=17.12(*p* 0.001)	F=1695.90(*p* 0.001)	F=9.26(*p* 0.001)	13.74%	14.70%
Chla	F=36.04(*p* 0.001)	F=596.84(*p* 0.001)	F=22.76(*p* 0.001)	50.26%	30.48%
Chlb	F=14.62(*p* 0.001)	F=430.10(*p* 0.001)	F=10.59(*p* 0.001)	26.65%	13.74%
Chla/b	F=35.51(*p* 0.001)	F=105.08(*p* 0.001)	F=24.34(*p* 0.001)	32.01%	29.18%
Chla+b	F=15.26(*p* 0.001)	F=797.43(*p* 0.001)	F=5.01 (*p* 0.001)	37.61%	16.22%
Pn	F=21.10(*p* 0.001)	F=2837.23(*p* 0.001)	F=28.48 (*p* 0.001)	29.27%	44.36%
Ci	F=32.78(*p* 0.001)	F=448.38(*p* 0.001)	F=8.58 (*p* 0.001)	15.03%	14.35%
Gs	F=85.19(*p* 0.001)	F=1249.83(*p* 0.001)	F=15.06(*p* 0.001)	25.35%	74.97%
Tr	F=47.43(*p* 0.001)	F=1016.97(*p* 0.001)	F=9.69(*p* 0.001)	21.89%	64.48%
WUE	F=20.87 (*p* 0.001)	F=343.73 (*p* 0.001)	F=4.83 (*p* 0.001)	33.92%	46.98%
Fv/Fo	F=21.33(*p* 0.001)	F=1820.21(*p* 0.001)	F=26.28(*p* 0.001)	17.32%	46.52%
Fv/Fm	F=11.10(*p* 0.001)	F=875.81(*p* 0.001)	F=8.56(*p* 0.001)	6.60%	22.82%
ΦPSII	F=31.82(*p* 0.001)	F=315.97(*p* 0.001)	F=2.89 (*p* 0.05)	35.27%	69.27%
qN	F=4.81 (*p* 0.001)	F=398.03(*p* 0.001)	F=2.27(*p* 0.05)	5.30%	8.26%
qP	F=164.00(*p* 0.001)	F=237.95(*p* 0.001)	F=2.19(*p* 0.05)	46.91%	64.35%
ETR	F=50.89 (*p* 0.001)	F=1353.86(*p* 0.001)	F=7.74(*p* 0.001)	24.72%	47.76%
REC	F=4.63(*p* 0.001)	F=2729.41(*p* 0.001)	F=5.21(*p* 0.001)	25.92%	11.34%
MDA	F=51.57(*p* 0.001)	F=3332.63(*p* 0.001)	F=26.58(*p* 0.001)	9.37%	16.54%
$O_2^{\cdot-}$	F=30.78(*p* 0.001)	F=1501.05 (*p* 0.001)	F=38.59 (*p* 0.001)	11.66%	30.22%
H_2O_2	F=9.85 (*p* 0.001)	F=18.78 (*p* 0.001)	F=2.45(*p* 0.05)	21.70%	19.17%
·OH	F=98.32(*p* 0.001)	F=909.95(*p* 0.001)	F=8.81(*p* 0.001)	28.12%	22.47%
SOD	F=39.76(*p* 0.001)	F=39.76(*p* 0.001)	F=10.92 (*p* 0.001)	46.38%	22.42%
POD	F=34.60 (*p* 0.001)	F=34.60 (*p* 0.001)	F=22.71(*p* 0.001)	31.39%	36.37%
CAT	F=19.23 (*p* 0.001)	F=19.23 (*p* 0.001)	F=7.42(*p* 0.001)	43.99%	31.40%
APX	F=30.24(*p* 0.001)	F=30.24(*p* 0.001)	F=10.52(*p* 0.001)	50.54%	40.17%
GR	F=105.80(*p* 0.001)	F=105.80(*p* 0.001)	F=25.47(*p* 0.001)	43.63%	35.21%
Pro	F=90.16(*p* 0.001)	F=2010.95(*p* 0.001)	F=35.02(*p* 0.001)	66.66%	46.63%

SS	F=26.02(p 0.001)	F=26.02 (p 0.001)	F=7.53(p 0.001)	28.56%	19.71%
SP	F=12.93(p 0.001)	F=12.93(p 0.001)	F=7.39(p 0.001)	6.40%	10.36%

LDMC, leaf dry matter content; RWC, relative leaf water content; Chla, chlorophyll a content; Chlb, chlorophyll b content; Chla/b, chlorophyll a:b ratio; Chla+b, chlorophyll a+b content; Pn, net photosynthetic rate; Ci, intercellular CO_2 concentration; Gs, stomatal conductance; Tr, transpiration rate; WUE, instantaneous water use efficiency; Fv/Fo, potential photochemical efficiency of PSII; Fv/Fm, maximum photochemical efficiency of PSII; ΦPSII, the actual photochemical quantum efficiency of PSII; qN, non-photochemical quenching coefficient; qP, photochemical quenching; ETR, the relative rate of electron transfer; REC, relative electrical conductivity; MDA, malondialdehyde content, $O_2^{\cdot-}$, superoxide anion; H_2O_2, hydrogen peroxide; ·OH, hydroxyl radical; SOD, superoxide dismutase activity; POD, peroxidase activity; CAT, catalase activity; APX, ascorbate peroxidase activity; GR, glutathione reductase activity; Pro, proline content; SS, soluble sugar content; SP, soluble protein content. Same as below.

Effects of combined cold and drought stress on the morphological characteristics of 16 *Poa annua* seedlings

As shown in Fig. 2, the RWC of 16 *Poa annua* seedlings under combined cold and drought stress was significantly decreased, while the LDMC was significantly increased ($P < 0.05$). The RWC of 'ZQ' germplasm reduced the most under stress, which was significantly reduced by 58.15% compared with non-stressed germplasm ($P < 0.05$), and the RWC of 'ZQ' germplasm was not significant compared to other germplasms except 'HZ' 'JC' ' AN' 'LZ' and 'TZ' germplasms. The LDMC of 'ZQ' germplasm indicated the greatest increase under stress, which was significantly increased by 78.72% compared with non-stressed germplasm ($P < 0.05$). The LDMC of 'ZQ' germplasm showed significant differences compared to other germplasms under stress ($P <0.05$), except that it was not significantly different from 'HZ', 'QC', 'KT', 'LZ', and 'XH' germplasms.

Effect of combined cold and drought stress on photosynthetic characteristics of 16 *Poa annua* seedlings

Effect of combined cold and drought stress on photosynthetic gas exchange parameters of 16 seedlings

The Pn of 'JC' germplasm was significantly higher than that of other germplasms under non-stressed conditions ($P<0.05$) (Fig. 3). The Pn, Gs, Tr and WUE of 16 *Poa annua* seedlings under combined cold and drought stress decreased significantly, while Ci increased significantly ($P < 0.05$). The greatest decrease in Pn was observed in 'ZQ' germplasm under combined cold and drought stress, whereas the smallest decreases in Pn, Gs, Tr and WUE were observed in 'HZ' germplasm, which was significantly reduced by 40.36%, 19.24%, 18.99%, and 26.46%, respectively, compared with non-stressed germplasms ($P < 0.05$). The smallest increase in Ci was observed in 'HZ' germplasm and the largest increase was observed in 'ZQ' germplasm under combined cold and drought stress, which significantly increased by 69.65% ($P < 0.05$) compared to non-stressed germplasms.

Effect of combined cold and drought stress on chlorophyll content in 16 seedlings

The contents of Chla, Chla+b and Chla/b were significantly higher in ‘ZQ’ germplasm under non-stressed conditions ($P < 0.05$) (Fig. 4). In contrast, the Chla, Chlb, Chla+b contents and Chla/b of 16 *Poa annua* seedlings were significantly decreased ($P < 0.05$) under the combined cold and drought stress. Among them, ‘TZ’ germplasm showed the smallest decrease in Chla, Chlb and Chla+b contents, which were reduced by 24.93%, 13.23% and 18.54%, respectively, compared with non-stressed germplasms. The Chla, Chlb and Chla+b contents of ZQ germplasm showed the greatest decrease, with a significant reduction of 80.35%, 61.70% and 73.92%, respectively, compared with non-stressed germplasms ($P < 0.05$). The Chla/b of ‘XH’ germplasm showed the greatest decrease under combined cold and drought stress, with a significant reduction of 60.39% compared with non-stressed germplasms ($P < 0.05$), and the Chla/b of XH germplasm was not significant with all other germplasms except with ‘SD’, ‘HZ’, ‘JC’ and ‘MJ’ germplasms.

Effect of combined cold and drought stress on chlorophyll fluorescence parameters in 16 seedlings

The Fv/Fo of ‘ZQ’ germplasm was significantly higher ($P < 0.05$) than that of other germplasms under non-stress conditions (Fig. 5). In contrast, Fv/Fo, Fv/Fm, ΦPSII, qN, qP and ETR of 16 *Poa annua* seedlings were reduced significantly under combined cold and drought stress ($P < 0.05$). Among them, Fv/Fo of the 'ZQ’ germplasm showed the greatest decrease under stress, which was significantly reduced by 84.55% ($P < 0.05$) compared with non-stressed germplasm. While the 'HZ’ germplasm showed the smallest decrease and was significantly different from all other germplasms except for ‘LX’ seedlings ($P < 0.05$). Both ΦPSII and ETR of ‘HZ’ germplasm showed the smallest decrease under combined cold and drought stress, while ‘ZQ’ germplasm showed the largest decrease, which was significantly reduced by 53.54% and 74.70%, respectively, compared with non-stressed germplasm ($P < 0.05$). Both Fv/Fm and qN decreased the least in ‘LX’ germplasm under stress, whereas ‘ZQ’ germplasm showed the greatest decrease, with significant reductions of 61.92% and 72.60% compared with non-stressed germplasm, respectively ($P < 0.05$). In addition, ‘LX’ germplasm showed the smallest decrease in qP under stress, whereas ‘JC’ germplasm showed the largest decrease, which was significantly reduced by 30.67% compared with non-stressed germplasm ($P < 0.05$), and was significantly different from all other germplasms ($P < 0.05$).

Effect of combined cold and drought stress on relative conductivity and malondialdehyde content of 16 *Poa annua* seedlings

The REC of 16 seedlings increased significantly under the combined cold and drought stress ($P < 0.05$) (Fig. 6). The smallest increase in REC was observed in HZ germplasm, while the largest increase was observed in ‘ZQ’ germplasm, which significantly increased by 345.13% compared to non-stressed germplasm ($P < 0.05$), and the REC of ‘ZQ’ germplasm was significantly different from all other germplasms, except for non-significant differences with ‘SD’, ‘JC’, ‘QC’, ‘YZ’ and ‘LZ’ germplasms. Similarly, the MDA content of 16 *Poa annua* seedlings increased significantly under stress ($P < 0.05$). Among them, ‘LX’ and ‘HZ’ germplasms showed a smaller increase in MDA content, which significantly

increased by 36.17% and 31.67% compared to non-stressed germplasms, respectively ($P < 0.05$). While ‘ZQ’ germplasm showed the largest increase, which was significantly increased by 111.36% compared to non-stressed germplasm ($P < 0.05$), and the MDA content of ‘ZQ’ germplasm was significantly different from all other germplasms except for ‘CX’ germplasm.

Effect of combined cold and drought stress on reactive oxygen species levels in 16 *Poa annua* seedlings

As shown in Fig. 7, $O_2^{\cdot-}$ increased significantly in all 16 *Poa annua* seedlings under combined cold and drought stress ($P < 0.05$). Among them, the $O_2^{\cdot-}$ increase of ‘HZ’ germplasm was the smallest, and the $O_2^{\cdot-}$ increase of ‘ZQ’ germplasm was the largest, which was significantly increased by 262.47% compared to non-stressed seedlings ($P < 0.05$), and there was a significant difference between the $O_2^{\cdot-}$ of ‘ZQ’ germplasm and that of all other germplasms ($P < 0.05$). The H_2O_2 and ·OH contents of 16 *Poa annua* seedlings were increased under combined cold and drought stress. Among them, the 'HZ’ germplasm showed the smallest increase in both H_2O_2 and ·OH contents, which increased by 2.79% and 13.04% compared with non-stressed germplasms, respectively ($P < 0.05$). In addition, the largest increase in H_2O_2 content was observed in ‘KT’ germplasm, while the largest increase in ·OH content was observed in ‘JC’ germplasm under stress conditions, with significant increases of 23.50% and 152.25%, respectively ($P < 0.05$), and the H_2O_2 content of ‘KT’ germplasm was significantly different from all other germplasms except for ‘XH’ germplasm ($P < 0.05$).

Effect of combined cold and drought stress on antioxidant enzyme activities of 16 *Poa annua* seedlings

As shown in Fig. 8, the activities of SOD, POD, CAT, APX and GR in 16 *Poa annua* seedlings significantly increased under combined cold and drought stress ($P<0.05$). Among them, the POD and CAT activities of ‘HZ’ germplasm increased the most, significantly increasing by 261.20% and 230.40% compared to non-stressed seedlings ($P<0.05$), and there were significant differences between ‘HZ’ germplasm and other germplasms. The POD and CAT activities of the 'ZQ’ germplasm showed the smallest increase, significantly increasing by 42.23% and 88.36% compared to non-stressed germplasm ($P<0.05$), respectively. Moreover, the CAT activity of the 'ZQ’ germplasm showed a significant difference compared to other germplasms, except for the ‘XH’ germplasm which was not significantly different from each other. In addition, the SOD and GR activities of the 'ZQ’ germplasm showed the smallest increase under combined cold and drought stress, increasing by 1.15 and 1.58 times compared to non-stressed germplasm, respectively. The APX activity of the 'ZQ’ germplasm showed the smallest increase under stress, while the 'LX’ germplasm showed the largest increase, significantly increasing by 329.65% compared to non-stressed germplasm ($P<0.05$). Moreover, the APX activity of ‘LX’ germplasm showed significant differences compared to other germplasms, except for no significant differences with ‘HZ’ and ‘QC’ seedlings

Effect of combined cold and drought stress on osmoregulatory substances in 16 *Poa annua* seedlings

The Pro, SS, and SP contents of 16 *Poa annua* seedlings were significantly increased under combined cold and drought stress ($P < 0.05$) (Fig. 9). Among them, 'ZQ' germplasm showed the smallest increase in both Pro and SP contents, while 'HZ' germplasm showed the largest increase in both contents, which increased 6.18 and 1.53 times compared with non-stressed germplasm, respectively, and the Pro contents of 'HZ' germplasm differed significantly from those of all other germplasms, whereas the SP contents of 'HZ' germplasm differed significantly from those of all other germplasms, except for non-significant differences with 'TZ' germplasm. In addition, the SS content of 'HZ' germplasm increased the most under combined cold and drought stress, while the increase in 'XH' germplasm was the smallest, and the SS content in 'XH' germplasm was significantly different from other germplasms except for 'SD', 'ZQ', 'AN' and 'CX' germplasms, which were not significantly different.

PCA analysis of physiological and photosynthetic characteristics of *Poa annua* seedlings under different stress treatments

To eliminate the differences caused by the basic traits of the provenances, relative values were used to describe the responses of plants to combined cold and drought. Thirty-two relevant growth, photosynthetic and physiological indicators of sixteen germplasms under regular watering (the soil moisture content was 80% of the maximum field capacity) at room temperature (25℃) and combined drought (the soil moisture content was 30% of the maximum field capacity) and cold (0℃) were transformed into different principal components. The contribution rates of the variance of the first four principal components of the *Poa annua* seedlings related to stress treatments were 54.27%, 9.69%, 6.43%, and 5.29%, respectively, with a cumulative contribution rate of 75.68%, which represented 75.68% of the comprehensive information of the related indexes(Fig. 10). Therefore, only the first three principal components that can comprehensively reflect the main information of the raw data of each test index were selected as effective principal components for the analysis.

Thirty-two indicators divided different stress treatments and *Poa annua* germplasm into four quadrants (Q) of PCA. Fig. 11A showed a clear separation of the two treatments, with the stress group located on the right side of PC1 (Q3 and Q4) and the control group located on the left side of PC1 (Q1 and Q3). In addition, we also found significant segregation among sixteen *Poa annua* germplasms: 'HZ', 'YZ', and 'AN' germplasms in Q1, QC, 'KT', 'XH', and 'MJ' germplasms in Q2, 'TZ', 'LX', and 'LZ' germplasms in Q3, while 'SD', 'LX', 'JC', 'GZ', 'QZ', and 'ZQ' germplasms were distributed in Q4 (Fig.11A). The double-label plot showed that there was a strong positive correlation between chlorophyll fluorescence parameters and chlorophyll content in *Poa annua*. However, in the opposite region, it exhibited physiological and biochemical characteristics, indicating that combined drought and cold stress enhanced osmotic regulation ability, ROS accumulation, and antioxidant enzyme activity (Fig. 11B). Moreover, By conducting PCA analysis on thirty-two indicators of *Poa annua* germplasm under different stress treatments, four naturally separated populations were formed. The first group was mainly characterized by osmoregulation substances and antioxidant enzyme activity, including the contents of SP, Pro, and the activities of POD, CAT, and GR. The second group was mainly characterized by lipid peroxidation and reactive oxygen species levels, namely REC, the contents of MDA, $O_2^{\cdot-}$, H_2O_2, and ·OH. Photosynthetic gas

parameters, including Gs and Tr mainly characterized the third group. The fourth group was mainly characterized by chlorophyll fluorescence parameters and chlorophyll content, including the contents of Chla, Chlb, Chla+b, Chla/b, Fv/Fm, Fv/Fo, ETR, and qN.

Comprehensive evaluation and cluster analysis of stress resistance of *Poa annua* seedlings

To eliminate the influence of different orders of magnitude on the evaluation results, and based on the results of PCA screening, the relative values of each trait were standardized by using the affiliation function method. Meanwhile, 16 germplasm were clustered and analyzed according to the average affiliation function values (Fig. 12). All germplasms were divided into three categories. Cluster I contained five combined cold and drought tolerant germplasms, namely, 'HZ' 'LX' 'TZ' 'YZ' and 'AN' germplasms. Cluster II contained nine moderately tolerant to combined cold and drought tolerant. Two germplasms in Cluster III were sensitive to combined cold and drought: 'CX' and 'ZQ' germplasms.

Discussion

Temperature and water are the main abiotic stresses limiting plant growth and distribution[25,26], especially in arid and semi-arid regions. The damage symptoms of plant external morphology can directly reflect the impact of stress on plants and therefore can be used as an indicator of plant stress resistance[27,28]. In this study, by observing the external morphology of 16 *Poa annua* germplasms under combined cold and drought stress for 24 h, we found that the 'HZ' germplasm showed less severe damage symptoms than the other germplasm, and the 'ZQ' germplasm showed the most severe damage symptoms. In contrast, changes in biomass are an integrated expression of plant response to adversity stress and a visual indicator of plant resilience[27]. In this study, the LDMC of 16 germplasms increased significantly under stress conditions, indicating that 16 seedlings were tolerant. Among them, 'ZQ' germplasm showed the highest increase in LDMC and 'HZ' germplasm showed the least increase, which indicated that different germplasms had different growth strategies in response to stress, which was the main characteristic of plant adaptive growth and stress tolerance. Numerous studies have shown that plants maintaining higher RWC during stress indicate stronger stress resistance[29–32]. In this study, the RWC of 16 germplasms decreased under combined temperature and water stress, among which 'HZ' germplasm maintained higher RWC, indicating that 'HZ' germplasm had higher combined cold and drought resistance.

Photosynthesis is the first damaged physiological process inhibited by abiotic stress. Consequently, photosynthesis is very sensitive to changes in environmental conditions[33,34]. According to relevant studies, abiotic environmental stress severely disrupts the photosynthetic system of plants[35,36], resulting in reduced photosynthetic rate and weakened photosynthesis in plants. More importantly, in this study, the Pn, Gs, Tr, and WUE of 16 *Poan annua* germplasms were significantly inhibited under combined cold and drought stress, which is consistent with previous research results[37]. Moreover, the decrease in Pn, Gs, Tr, and WUE of 'HZ' germplasm was the smallest under stress conditions, indicating that 'HZ' germplasm reduced Tr and WUE by self-regulation and reducing Gs, and maintaining relative stability of the internal microenvironment to cope with combined cold and drought stress, and thus maintaining stronger

photosynthetic capacity under stress. Interestingly, Ci was significantly increased in 16 seedlings under combined cold and drought stress, and it can be assumed that the impaired photosynthetic system of *Poan annua* seedlings is due to the limitation of non-stomatal factors[38]. Meanwhile, non-stomatal processes such as Rubisco activity and electron transfer efficiency are closely related to photosynthetic mechanisms[36]. Thus, It can be seen that combined cold and drought stress is the main factor leading to a decrease in leaf photosynthetic activity. To a certain extent, the changes in chlorophyll content can reflect the strength of plant stress tolerance[38–40]. In this study, we found that the Chla, Chlb, Chla + b contents and Chla/b of 16 seedlings were significantly inhibited after being subjected to combined cold and drought stress, a phenomenon that suggested that chloroplasts were damaged and chlorophylls were decomposed to varying degrees when plants suffered from combined stress. In addition, the Chla, Chlb and Chla + b contents of 'TZ' germplasm were significantly higher than those of the other germplasms after being subjected to the combined cold and drought stress, suggesting that the 'TZ' germplasm seems to be better adapted to the combined cold and drought stress by protecting chlorophylls to reduce decomposition. Variations in chlorophyll fluorescence parameters can reflect the effects of abiotic stress on processes in plant photosynthesis[41]. The damage to the PSII reaction center caused by adversity stress inhibits photosynthetic electron transport and the photosynthetic activity of PSII, which is one of the main reasons for the decrease in the pho-tosynthetic rate[40]. Fv/Fo, Fv/Fm, ΦPSII, qP, qN, and ETR of 16 *Poan annua* seedlings showed a decreasing trend under combined stress, and the 'ZQ' germplasm showed the largest decreases in Fv/Fo, Fv/Fm, ΦPSII and ETR. It can be seen that the effective electron transfer efficiency of PSII in the center of action of the 'ZQ' germplasm was greatly impeded under stress conditions, and the photosynthetic system was damaged, which greatly reduced the ability to utilize light energy.

What is noteworthy is that the production of ROS in plants exposed to combined cold and drought stress severely disrupts the dynamic balance between ROS production and clearance in plant cells, leading to a series of cell signaling events, such as cellular senescence and apoptosis, which in turn inhibit plant growth and development[42,43]. In this study, 16 *Poan annua* germplasms accumulated large amounts of $O_2^{\cdot-}$ H_2O_2 and ·OH contents when subjected to the combined cold and drought stress, which is consistent with previous findings[44,45]. Besides, the excessive ROS produced by plant cells may be associated with membrane lipid peroxidation in the leaves of *Poa annua* seedlings. Excessive ROS accumulation causes oxidative damage to cell membrane lipids and tissue senescence, producing toxic oxidation products such as the polyunsaturated fatty acid oxidation product MDA[46]. MDA is a marker of lipid peroxidation associated with oxidative stress and redox signaling, and accumulated MDA could predispose to oxidative damage to the cell membrane system, especially when adversity stress negatively affects plants[47]. In this study, the combined stress of cold and drought increased the MDA levels of 16 seedlings, and the MDA content of 'ZQ' germplasm increased significantly compared to other germplasms, which indicated that the cell membrane lipid peroxidation damage and severe damage to cell membrane structure and function of 'ZQ' germplasm. The size of REC is closely related to the plant's ability to resist adversity. Our study showed that 16 *Poa annua* germplasms increased significantly after being subjected

to combined cold and drought stress, with the smallest increase in ‘HZ’ germplasm and the largest increase in ‘ZQ’ germplasm, suggesting that the combined cold and drought stress resulted in damage to the cell membrane system of 16 seedlings and that ‘ZQ’ germplasm was weakly combined cold and drought resistant. The balance between antioxidant enzyme activities plays a crucial role in stabilizing ROS levels[48]. Hence, the antioxidant system is considered to be a good indicator to characterize plant tolerance under adversity stress. In this study, we found that the SOD, POD, CAT, APX, and GR activities were significantly elevated in 16 *Poa annua* seedlings under combined cold and drought stress, which is consistent with the results of previous studies[49,50]; from this, it can be inferred that plants activate a self-stress mechanism to activate antioxidant enzyme activities in adversity through stress-induced membrane lipid peroxidation, and then adapt to the adversity stress and re-establish the balance between ROS generation and scavenging[51]. It is well known that Pro, SS and SP are important osmoregulatory substances in plants, which can improve water uptake by increasing cytosol concentration and decreasing osmotic potential. At the same time, osmoregulators also enhance the stability of cell membranes, thereby playing an important role when plants are subjected to adversity stress[52,53]. The results of this study showed that the Pro, SS, and SP contents of 16 *Poa annua* seedlings increased to different degrees under combined cold and drought stress, among which, the ‘ZQ’ germplasm showed the smallest increase in both Pro and SP contents, and the SS content of ‘XH’ seedlings also showed the smallest increase, while the ‘HZ’ germplasm showed the largest increase in all of them, which indicated that the ‘HZ’ germplasm had stronger osmoregulatory ability. In addition, The difference in osmoregulation ability of different germplasm under the same stress condition may be related to their genetic background (soil, rainfall, temperature, etc.), which needs to be further investigated.

Based on the previous research findings with PCA analysis in this study[14,44,50,54,55], we found that the onset and accumulation of stress injury in *Poa annua* seedlings involved different physiological processes. In the meantime, it also alleviates the stress effects of plants to stress by inducing various mechanisms within the cell. For this purpose, we constructed a map of the response mechanisms for combined cold and drought resistance in *Poa annua* (Fig. 13). Briefly, *Poa annua* seedlings activate antioxidant systems under combined cold and drought stress, thereby altering a variety of physiological processes such as biomass, chlorophyll biosynthesis, photosynthetic properties, chlorophyll fluorescence parameters, lipid peroxidation levels, osmoregulatory capacity, ROS levels and antioxidant enzyme systems. Moreover, the interaction between these physiological response mechanisms provides strategies for improving the performance of *Poa annua* under combined cold and drought stress.

Conclusions

By determining the morphological, photosynthetic and physiological biochemical traits of 16 *Poa annua* germplasms in response to combined cold and drought stress at the seedling stage, we found that combined cold and drought stress caused the photosynthetic system of *Poa annua* germplasms to be significantly impaired, and the cell membranes showed varying degrees of lipid peroxidation. However, the adverse effects of combined cold and drought stress can be mitigated by a variety of intracellular

mechanisms. Meanwhile, we also obtained ‘HZ’ and ‘ZQ’ germplasm, which are tolerant and sensitive to combined cold and drought stress. This is due to the adaptation of the ‘HZ’ germplasm to its hostile environment. Thus, in the context of planting actions in cold and dry regions where temperature and water conditions are often unfavorable, it is recommended to use seeds from provenances providing seedlings with the best characteristics of resistance to environmental stress.

Materials and Methods

Plant and culture

Seeds of *Poa annua* from sixteen provenances were obtained from May to July 2021 in Gansu and Qinghai Provinces, China, and named after the provenances. We have permission to collect *Poa annua*. The voucher specimen, YZ, PE 01850519, was identified by Guoliang Zhang and its sheet was deposited in the herbarium PE (https://sweetgum.nybg.org/science/ih/herbarium-list). AN, PE 00925127, was identified by Guoliang Zhang and its sheet was deposited in the herbarium PE (https://sweetgum.nybg.org/science/ih/herbarium-list). LX, KUN 1403520, was identified by Wei Qi and its sheet was deposited in the herbarium KUN (https://www.cvh.ac.cn/spms/detail.php). SD, QYTC QYTC0005973, was identified by Shirong Ma and its sheet was deposited in the herbarium QYTC (https://www.cvh.ac.cn/spms/detail.php). HZ, IBSC 0125425, was identified by Shaoqing Chen and its sheet was deposited in the herbarium IBSC (https://www.cvh.ac.cn/spms/detail.php). XH, HNWP 0285609, was identified by Kun Liu and its sheet was deposited in the herbarium HNWP (https://www.cvh.ac.cn/spms/detail.php). Simultaneously, The voucher specimen of MJ and QZ, PE 00925122, was identified by Marina Olonova and its sheet was deposited in the herbarium PE (https://sweetgum.nybg.org/science/ih/herbarium-list). The voucher specimen of CX, JC and KT, BNU 0020964, was identified by Yi He and its sheet was deposited in the herbarium BUN (https://www.cvh.ac.cn/spms/detail.php). The voucher specimen of TZ, GZ and LZ, PE 02078373, was identified by Shuliang Hu and Xuezhong Liu and its sheet was deposited in the herbarium PE (https://www.cvh.ac.cn/spms/detail.php). They can be searched in the Chinese Virtual Herbarium (https://www.cvh.ac.cn/index.php). The geographical locations and climatic conditions of provenances are shown in Table 2. The test was conducted at Gansu Agricultural University, Gansu Province, China (36°5'N, 103°34'E), with an average annual temperature of 10.3°C and average yearly precipitation of 350 mm. The seeds were sown in plastic pots (18 cm inner diameter, 12 cm height, with holes in the bottom) in June 2022. The cultivated substrate was farmland soil, sand, sheep manure, and organic nutrient soil (7:1:1:1; v/v). Each pot was filled with 1.8 kg of mixed substrate, with a sowing rate of 8 $g \cdot m^{-2}$, and cultivated under natural conditions. Furthermore, the position of the pots was moved periodically to reduce differences in the microenvironment.

Table 2
Geography of the main distribution areas of the 16 wild *Poa annua* germplasms for testing

Number	Collection Place	Longitude	Latitude	Elevation /m	Average annual rainfall /mm	Average annual temperature /℃	Habitat
SD	Shandan County, Zhangye City, Gansu Province	101°36'	38°72'	1781	328	8	In front of the house and behind the house
HZ	Huanzhong District, Xining City, Qinghai Province	101°78'	36°51'	2677	560	3	In front of the house and behind the house
JC	Jingchuan County, Pingliang City, Gansu Province	107°37'	33°47'	1193	555	10	In front of the house and behind the house
ZQ	Zhouqu County, Gannan Tibetan Autonomous Prefecture, Gansu Province	104°22'	33°46'	1420	689	12	River Valley
QC	Qingcheng County, Qingyang City, Gansu Province	107°60'	35°89'	1316	572	10	In front of the house and behind the house
KT	Kongdong District, Pingliang City, Gansu Province	106°53'	35°59'	1540	477	9	In front of the house and behind the house

Number	Collection Place	Longitude	Latitude	Elevation /m	Average annual rainfall /mm	Average annual temperature /℃	Habitat
LX	Linxia County, Linxia Hui Autonomous Prefecture, Gansu Province	103°08'	35°49'	2012	573	6	Green Belt
QZ	Qinzhou District, Tianshui City, Gansu Province	105°44'	34°30'	1350	536	10	In front of the house and behind the house
MJ	Maiji District, Tianshui City, Gansu Province	105°58'	34°21'	1603	560	11	Roadside
AN	Anning District, Lanzhou City, Gansu Province	103°42'	36°52'	1543	325	10	Green Belt
YZ	Yuzhong County, Lanzhou City, Gansu Province	104°11'	36°40'	1940	390	7	Green Belt
LZ	Liangzhou District, Wuwei City, Gansu Province	102°63'	37°30'	1523	144	9	In front of the house and behind the house
CX	Chongxin County, Pingliang City, Gansu Province	107°02'	35°31'	1149	647	10	In front of the house and behind the house

Number	Collection Place	Longitude	Latitude	Elevation /m	Average annual rainfall /mm	Average annual temperature /℃	Habitat
GZ	Ganzhou District, Zhangye City, Gansu Province	103°15'	16°99'	1478	128	7	Green Belt
XH	Xihe County, Longnan City, Gansu Province	104°20'	33°47'	1736	533	8	In front of the house and behind the house
TZ	Tianzhu County, Wuwei City, Gansu Province	101°4'	38°96'	2783	397	4	Green Belt

Stress treatment

The seedlings were subjected to combined cold and drought stress when they grew to 6 ~ 7 true leaves. The experiment was conducted in a completely randomized design. The seedlings of *Poa annua* from each provenance were set up in a control group (regular watering at room temperature) and a stress group (combined cold and drought stress), with 3 replicates of each treatment and a total of 96 pots. One week before the start of the stress experiment, both groups of plants were weighed and watered daily to maintain the same soil moisture content (the soil moisture content was 80% of the maximum field capacity). After that, the control group plants continued to be grown under regular watering (the soil moisture content was 80% of the maximum field capacity) at room temperature (25°C), whereas plants in the stress group were subjected to natural drought to gradually reduce the soil moisture content to 30% of the maximum field capacity, and then seedlings were subjected to combined cold stress while maintaining the soil moisture content unchanged. Firstly, seedlings were pre-cooled in an incubator at 15°C for 24 h. Then, the program was reduced to 0°C at a rate of 5°C/2 h, followed by cold stress for 24 h. A stratified soil coring procedure was used to determine the maximum field capacity of the soil, and the soil water content was determined by the weighing method[56]. After the end of the stress, a portion of seedlings in each group was randomly selected for the determination of growth indexes, and another portion of middle and upper functional leaves was sampled for the determination of relevant physiological and biochemical indexes.

Measurement items and methods

Biomass

Before the stress treatment, 9 seedlings (3 per pot) were randomly selected from each group, weighed for fresh weight (FW) and saturated fresh weight (SFW), then placed in an oven to kill at 105℃ for 0.5 h and baked at 75℃ for 48 h, and weighed for dry weight (DW), Finally, leaf dry matter content (LDMC) and relative leaf water content (RWC) were calculated, the morphological changes of leaves before and after stress were photographed and recorded.

Chlorophyll content

The leaf pigments were extracted using 95% (v/v) ethanol in accordance with the method of Zhu et al.[57]. The chlorophyll a (Chl a) and chlorophyll b (Chl b) contents of the extracts were measured at an absorbance of 665 nm and 649 nm, and the contents of Chl a/b and Chl a + b were calculated.

Photosynthetic parameters

Photosynthetic parameters were measured in September 2022 on leaves of *Poa annua* seedlings in the non-stress and stress groups. The net photosynthetic rate(Pn), intercellular CO_2 concentration(Ci), stomatal conductance (Gs), and transpiration rate (Tr) of seedling leaves were measured every morning (9:00–11:00 on sunny days) using a portable photosynthesis meter (Ciras-2, MA01913) on selected functional leaves with similar growth conditions at the upper edge of the plant and healthy. Besides, Water use efficiency (WUE) was expressed as WUE = Pn/Tr.

Chlorophyll fluorescence

Chlorophyll fluorescence parameters were measured using a portable chlorophyll fluorescence meter IMAGING-PAM (WALZ, Nuremberg, Germany) from 9:00 to 11:00.

REC and MDA Content

Relative electrical conductivity (REC) was determined using the electrical conductivity method[58]. 0.1 g of fresh leaves were placed in a test tube containing 10 mL of ultrapure water and then incubated at 40℃for 0.5 h to confirm the conductivity of the solution (R1), followed by heating in boiling water for 15 min to make sure the conductivity of the solution (R2). REC was calculated as REC (%) = R1/R2*100%.

A thiobarbituric acid (TBA) colorimetric method was utilized to determine malondialdehyde (MDA) content[59].

Reactive oxygen levels

The superoxide anion radical ($O_2^{\bullet-}$) was determined by the hydroxylamine oxidation method[60]. The H_2O_2 content was employed by the acetone method[61]. The scavenging rate of hydroxyl radicals (·OH) was determined by the salicylic acid method[62].

Antioxidant enzyme activity

The antioxidant enzymes were confirmed by the method of Khalid et al.[63] with some improvements. Actually, 0.2 g of leaves were homogenized in 5 mL of pre-cooled phosphate buffer (50 mM, pH 7.8) and then centrifuged at 5000 rpm/min for 10 min under the temperatures of 4°C, and the resulting supernatant was the crude enzyme extract. The activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) were determined.

Osmoregulatory substances

The content of proline (Pro) was determined using the ninhydrin method[64]. Briefly, 0.1 g of leaves were added to 1 mL of sulfosalicylic acid (3%) extract and shaken in a boiling water bath for 10 min, then centrifuged at 1000 rpm/min for 10 min at 25°C, 1 mL of supernatant was mixed with 1 mL of acidic ninhydrin and 1 mL of glacial acetic acid, and after 30 min of the boiling water bath, 2 mL of toluene was added, and the toluene layer was collected, the absorbance was measured at 520 nm by a UV − Vis spectrophotometer (Cary 60 UV − Vis, Agilent Technologies Inc., Santa Clara, CA, USA).

The soluble sugar (SS) content was determined by the anthrone colorimetric method[60]. 0.2 g of leaves were placed in 10 mL of distilled water, then the sample was heated in boiling water for 30 min, 1 mL of the extract was mixed thoroughly with 1.5 mL of distilled water, 0.5 mL of ethyl anthrone acetate, and 5 mL of concentrated sulfuric acid, held in boiling water for 1 min, after cooling, the absorbance at 630 nm was recorded on a UV − Vis spectrophotometer.

The soluble protein (SP) content was tested by use of the coomassie staining method[60]. Furthermore, 0.2 g of leaves were homogenized in phosphate buffer (50 mM, pH 7.8), centrifuged at 12000 rpm/min for 20 min at 4°C, 0.1 mL of enzyme solution was mixed thoroughly with 2.9 mL of Thomas Brilliant Blue solution. after cooling, the absorbance at 595 nm was recorded on a UV − Vis spectrophotometer.

Statistical Analysis

Using the SPSS 20.0 software, the data were subjected to ANOVA and Duncan multiple comparisons. Two-way analyses of variance (ANOVA) were conducted to detect the effects of provenance and combined drought and cold stress and their interactions. All statistical effects were considered significant at $P < 0.05$, and Origin 2021.0 software was used for plotting figures, rates of change of indicators, principal component analysis (PCA) and cluster analysis heat maps. The data were comprehensively analyzed using the fuzzy mathematical membership function method to evaluate the combined drought and cold tolerance of the 16 *Poa annua* germplasms. The value of the affiliation function was calculated as follows: μ(Xj)=(Xj-Xmin)/(Xmax-Xmin), and in-verse membership function value: μ(Xj) = 1-(Xj-Xmin)/(Xmax-Xmin), where μ(Xj) de-notes the value of the affiliation function of the jth index; Xj denotes the value of the jth indicator value; Xmin indicates the jth indicator minimum value; Xmax indicates the jth indicator maximum value. In addition, the coefficient of genetic variation (CV%) for single traits of *Poa annua* under each treatment was calculated:(CV%) = SD/ $\bar{X}$, where SD represents the standard deviation, and $\bar{X}$ represents the mean value of single traits among the germplasm under each treatment.

Declarations

Data availability

Data available on reasonable request from corresponding author.

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Author contributions

X. B initiated and designed the experiment, J. L. F. R. C. Z. Y. Y. and P. L. performed the experiments and collected the data, J. L analyzed the data and wrote the manuscript, X. B., F. R. and H. C. revised the manuscript. All authors reviewed the manuscript.

The use of this material complies with the laws and guidelines of relevant institutions or countries.

Competing interests

The authors declare no competing interests.

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Figures

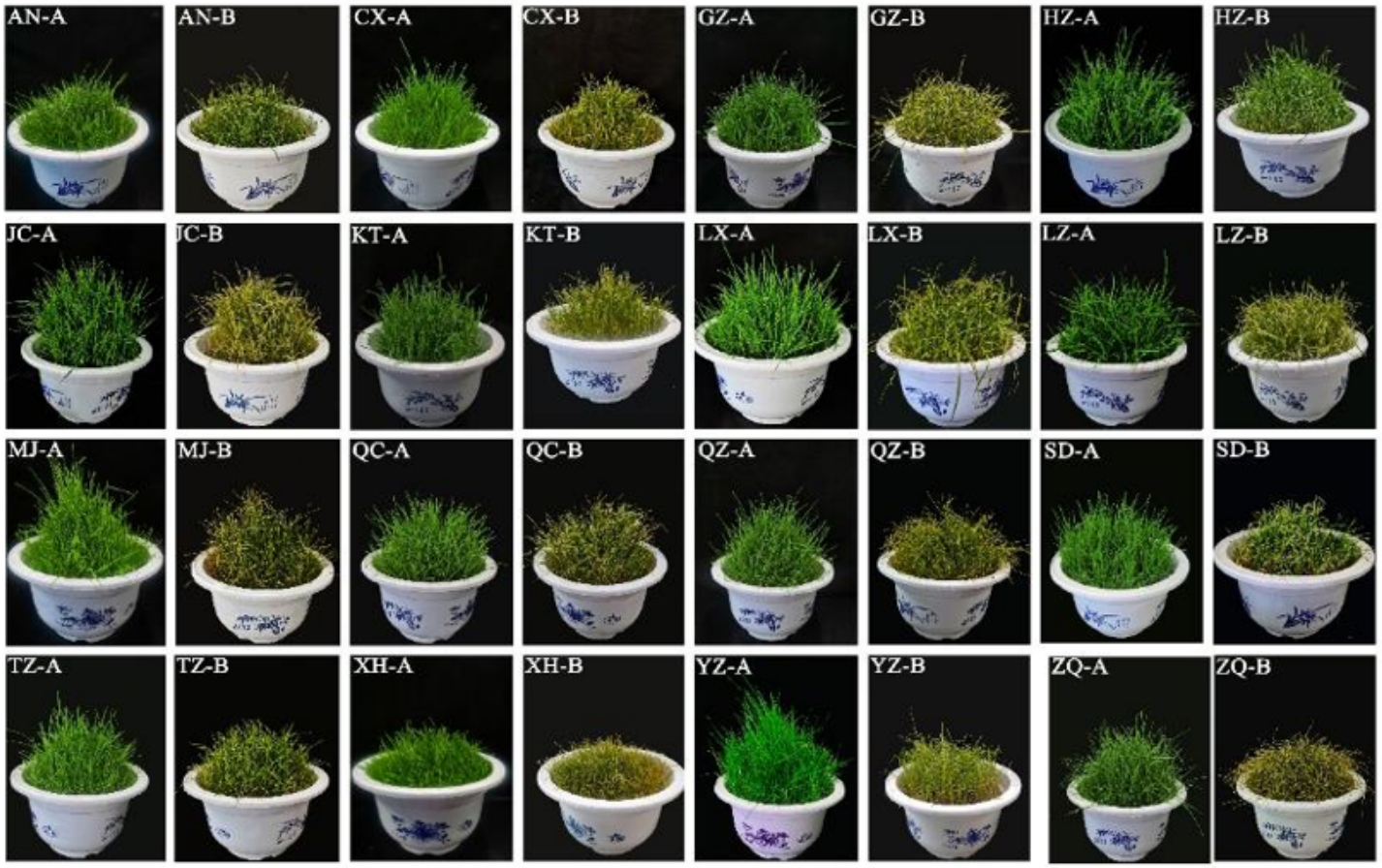

Figure 1

Comparison of morphological changes of each *Poa annua* seedling in the non-stress

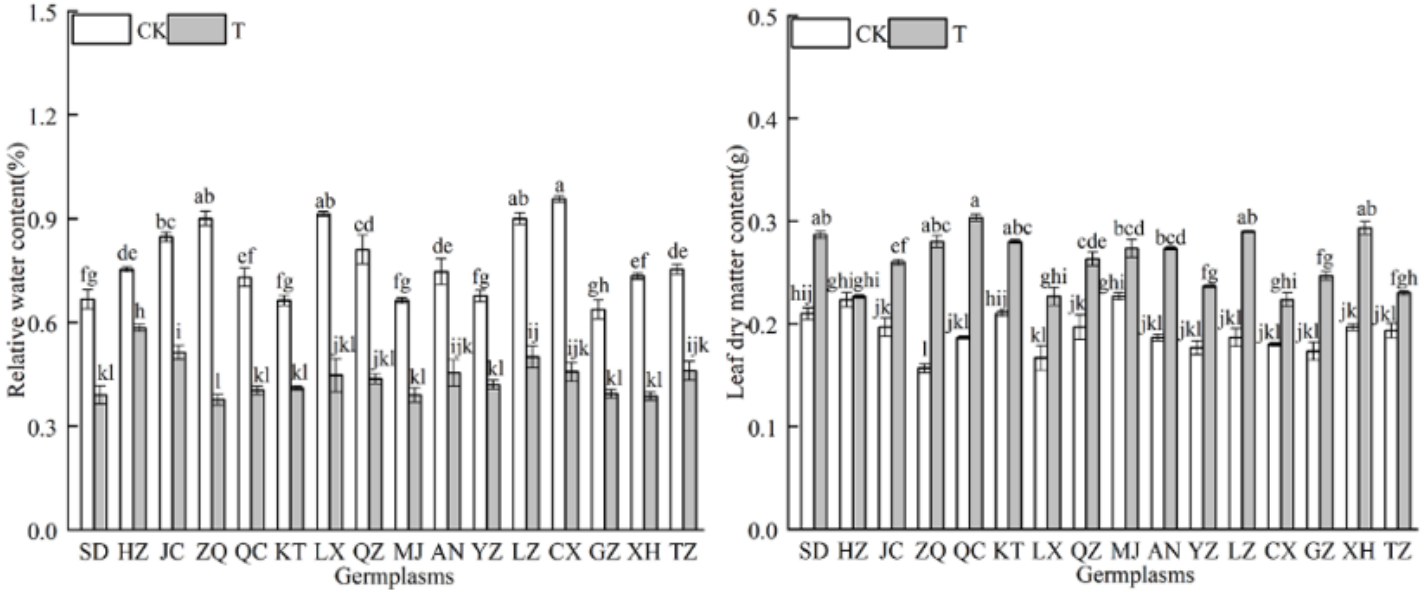

Figure 2

Effect of combined cold and drought stress on morphological characteristics of different germplasms. Values are means ± standard deviation (n=3). Different letters indicate significant differences among treatments and germplasms ($P < 0.05$).

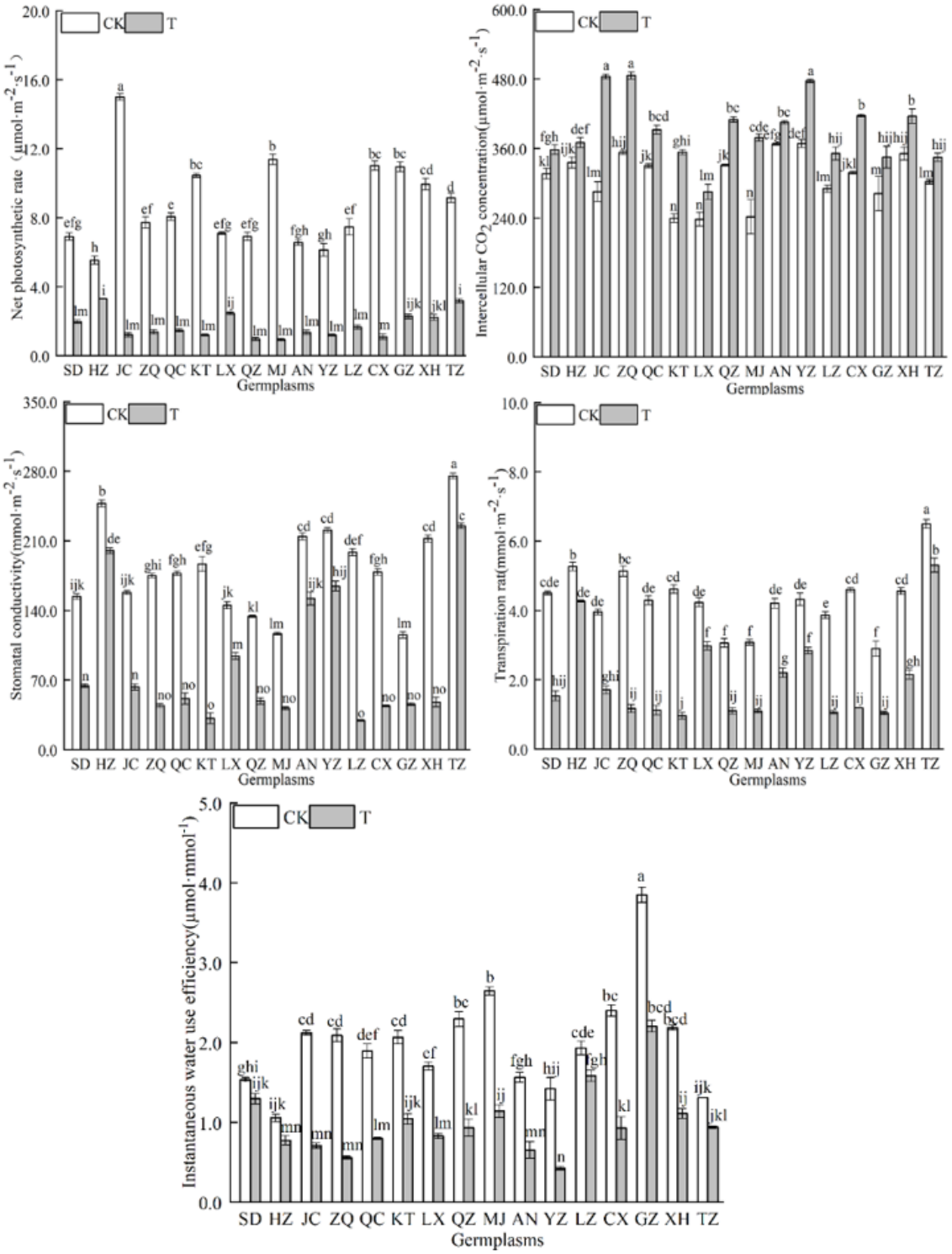

Figure 3

Effect of combined cold and drought stress on photosynthetic gas exchange parameters of different seedlings

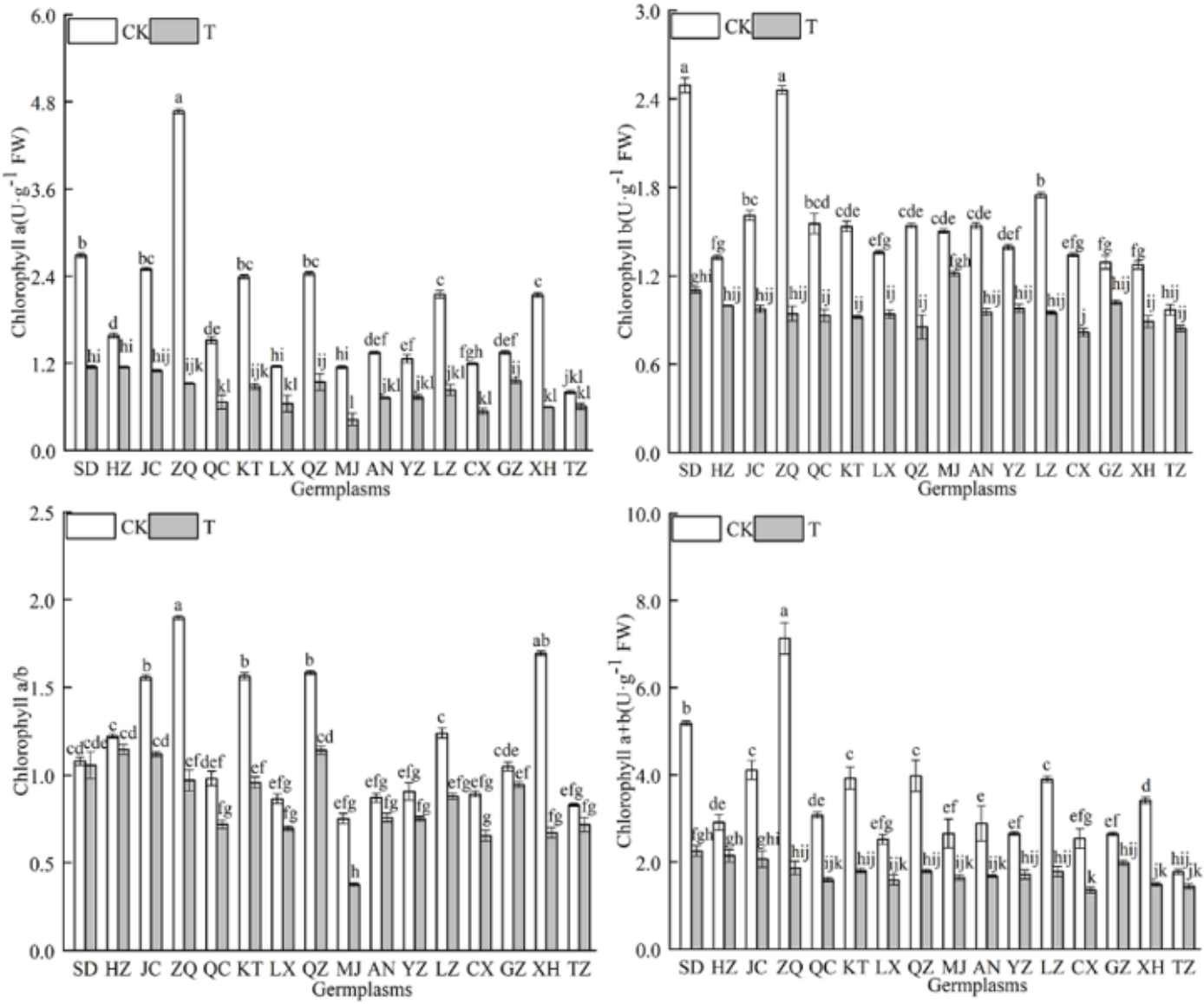

Figure 4

Effect of combined cold and drought stress on chlorophyll content of different seedlings

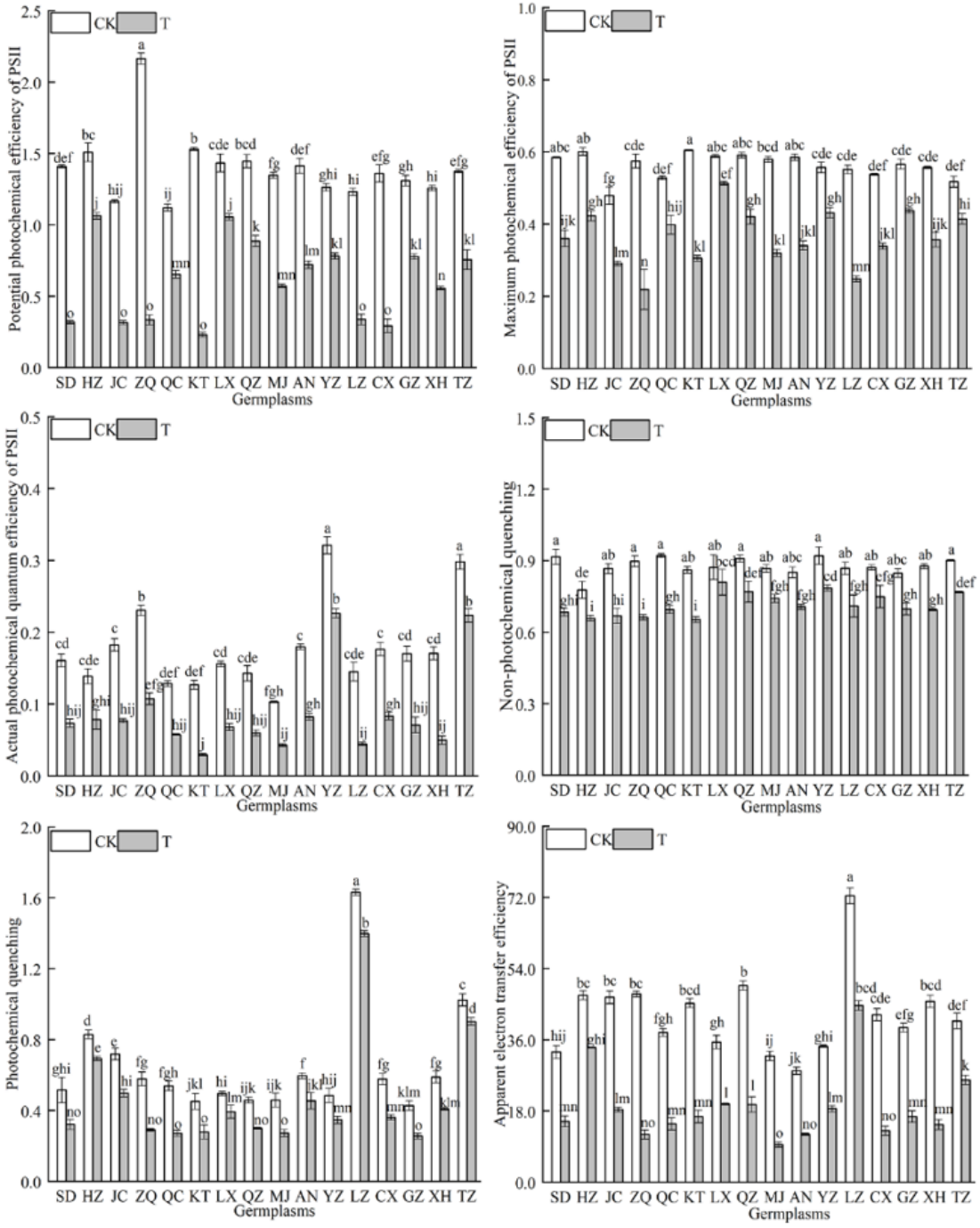

Figure 5

Effect of combined cold and drought stress on chlorophyll fluorescence parameters of different seedlings

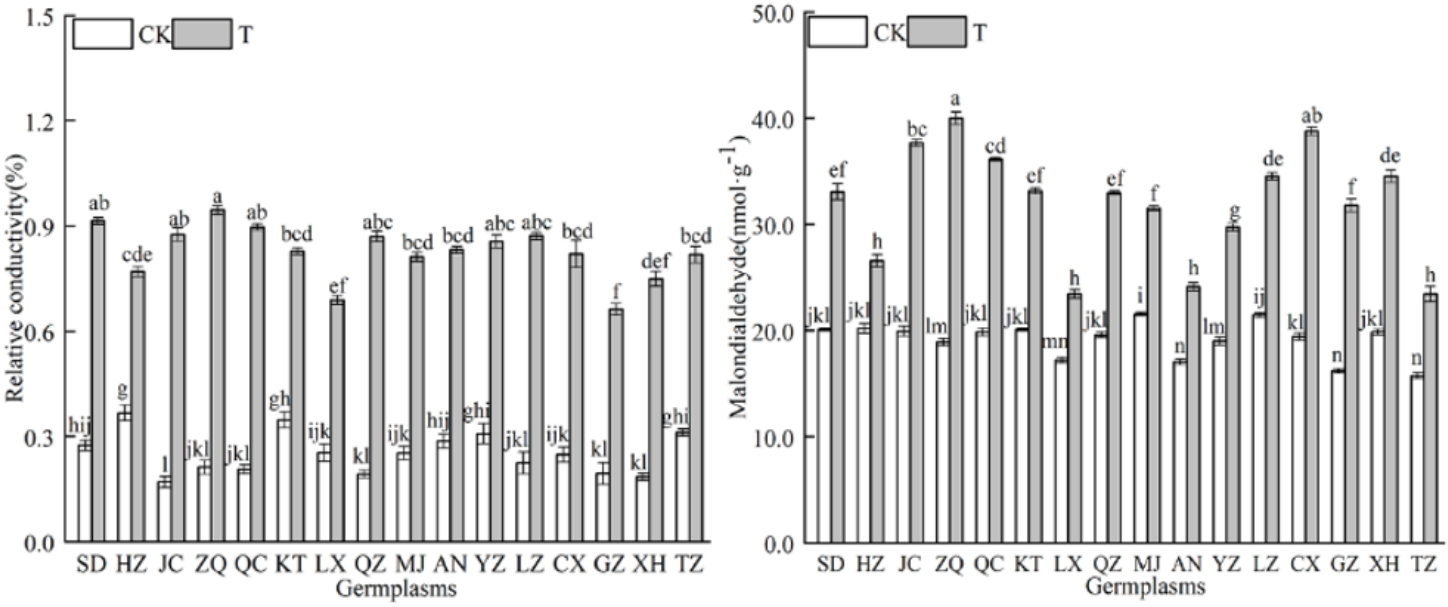

Figure 6

Effect of combined cold and drought stress on relative conductivity and malondialdehyde content of different seedlings

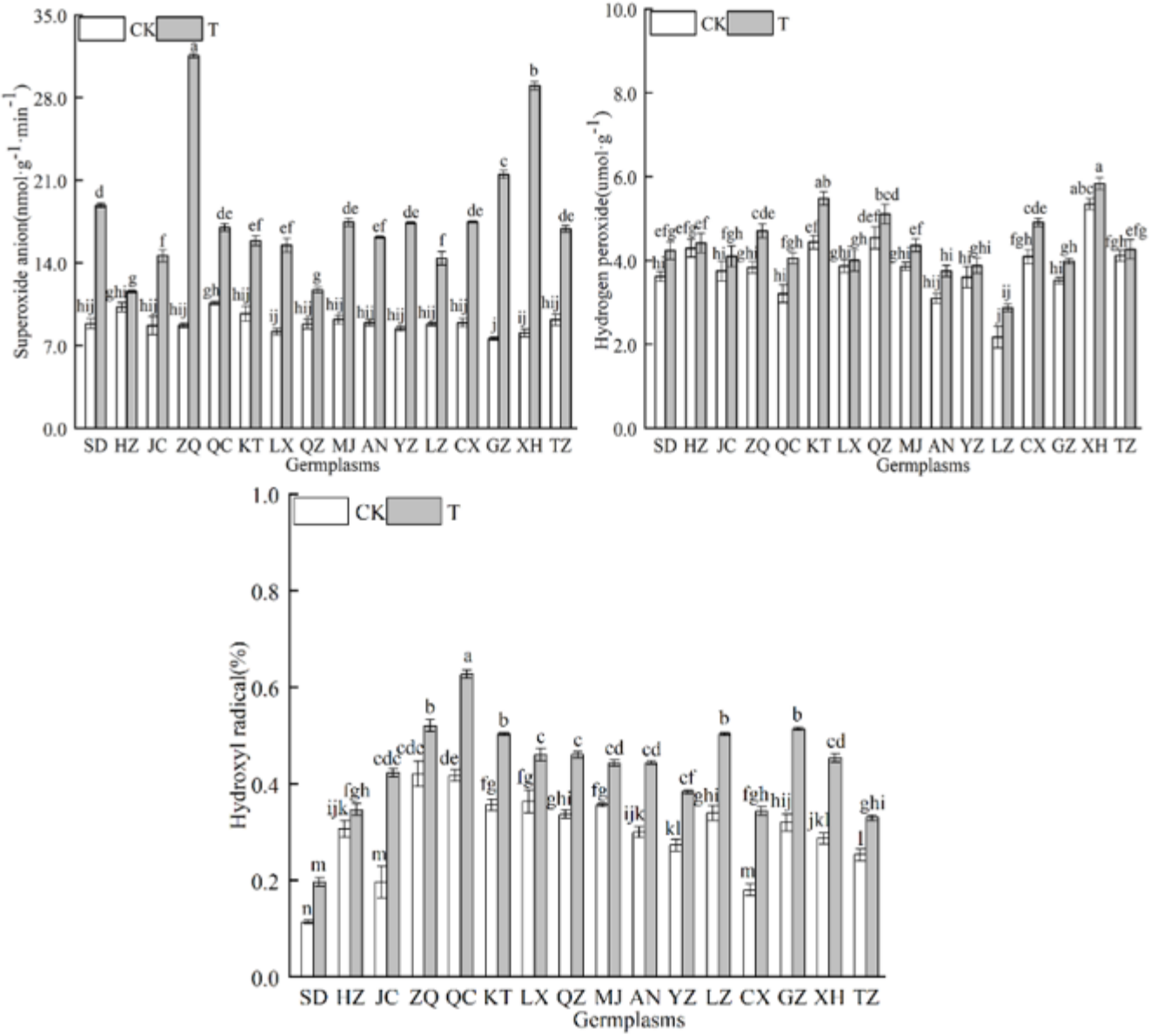

Figure 7

Effect of combined low temperature and drought stress on reactive oxygen levels in different seedlings

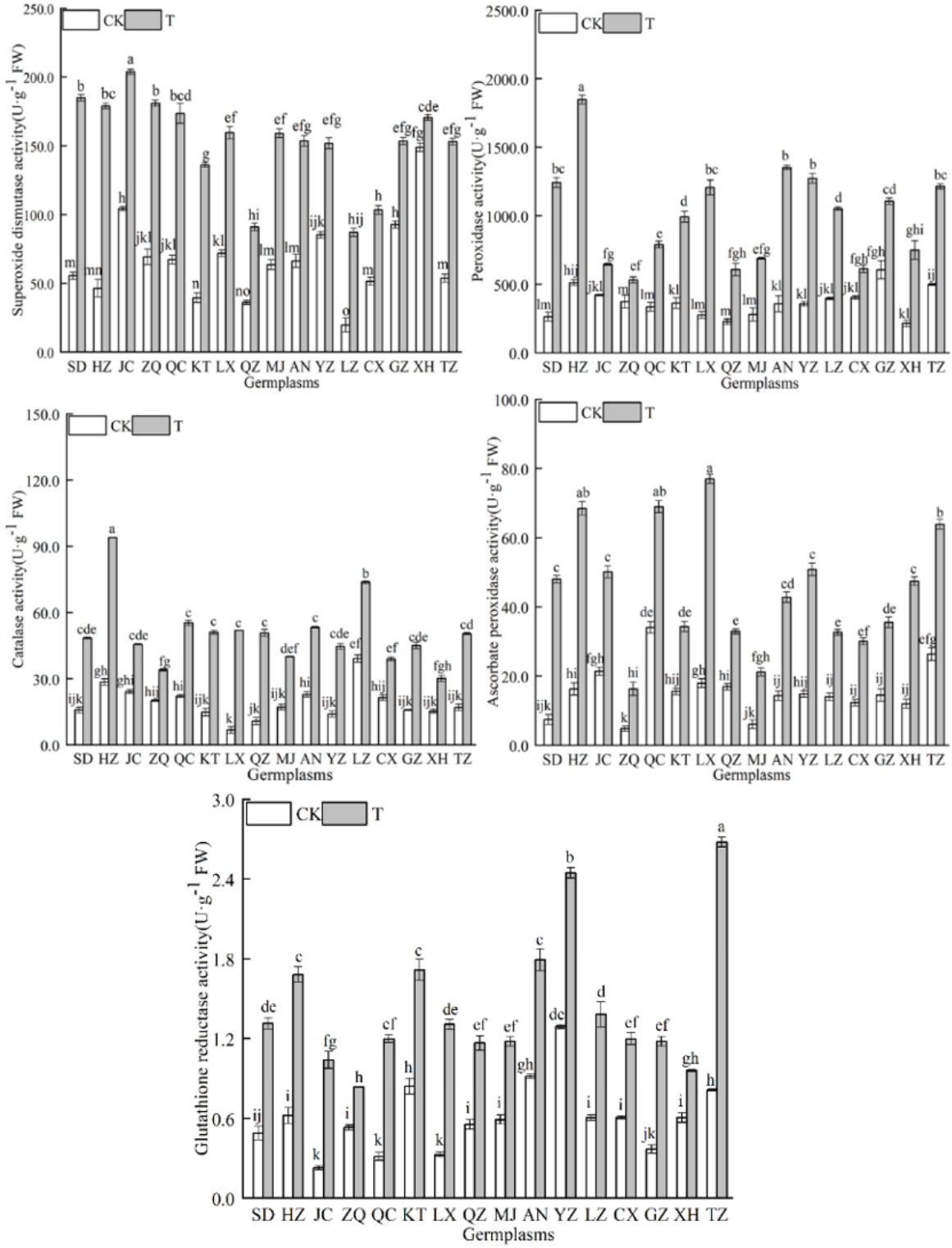

Figure 8

Effect of combined cold and drought stress on antioxidant enzyme activities of different seedlings

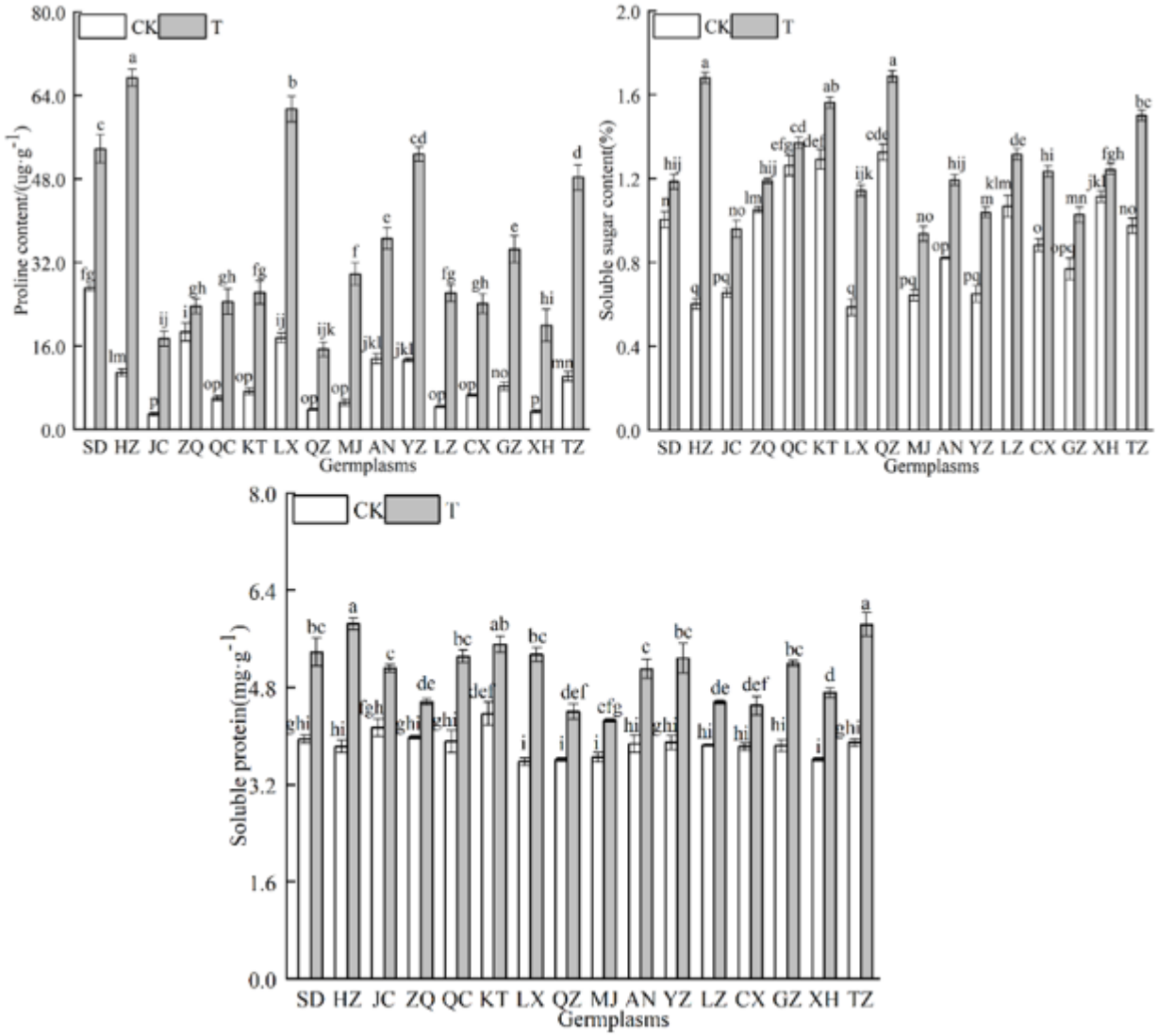

Figure 9

Effect of combined cold and drought stress on osmoregulatory substances in different seedlings

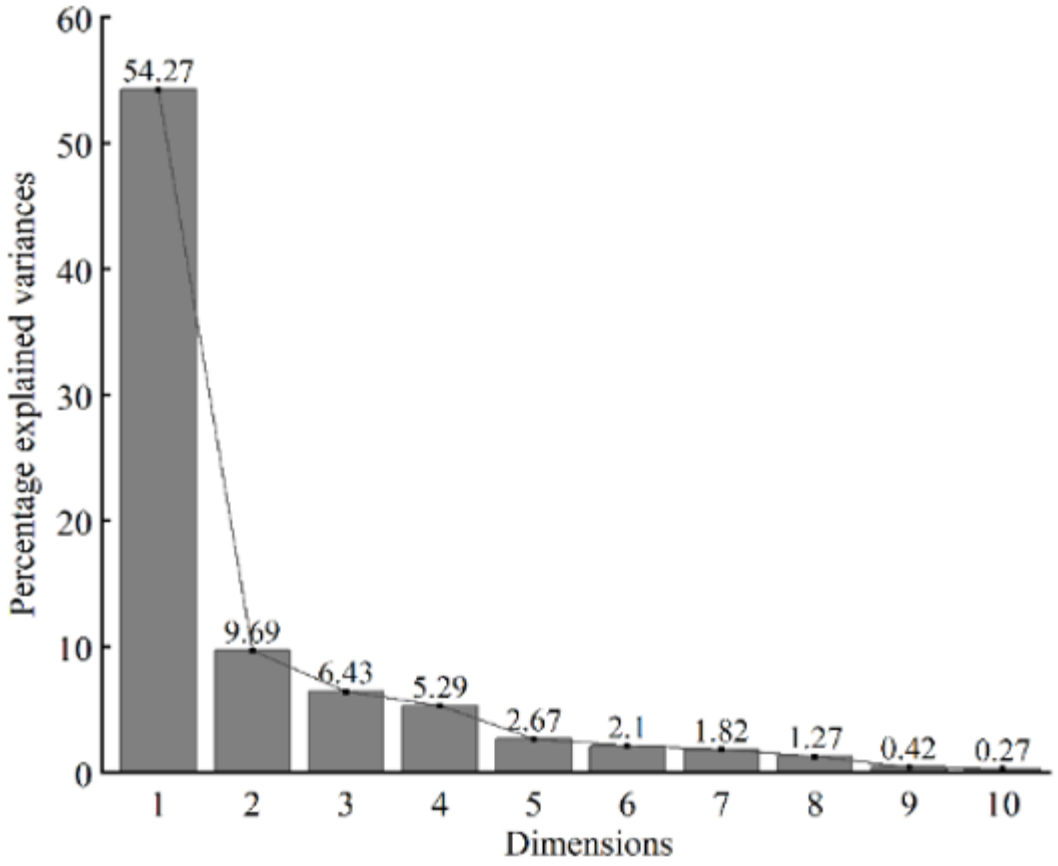

Figure 10

The analysis of the contribution rate of the principal components

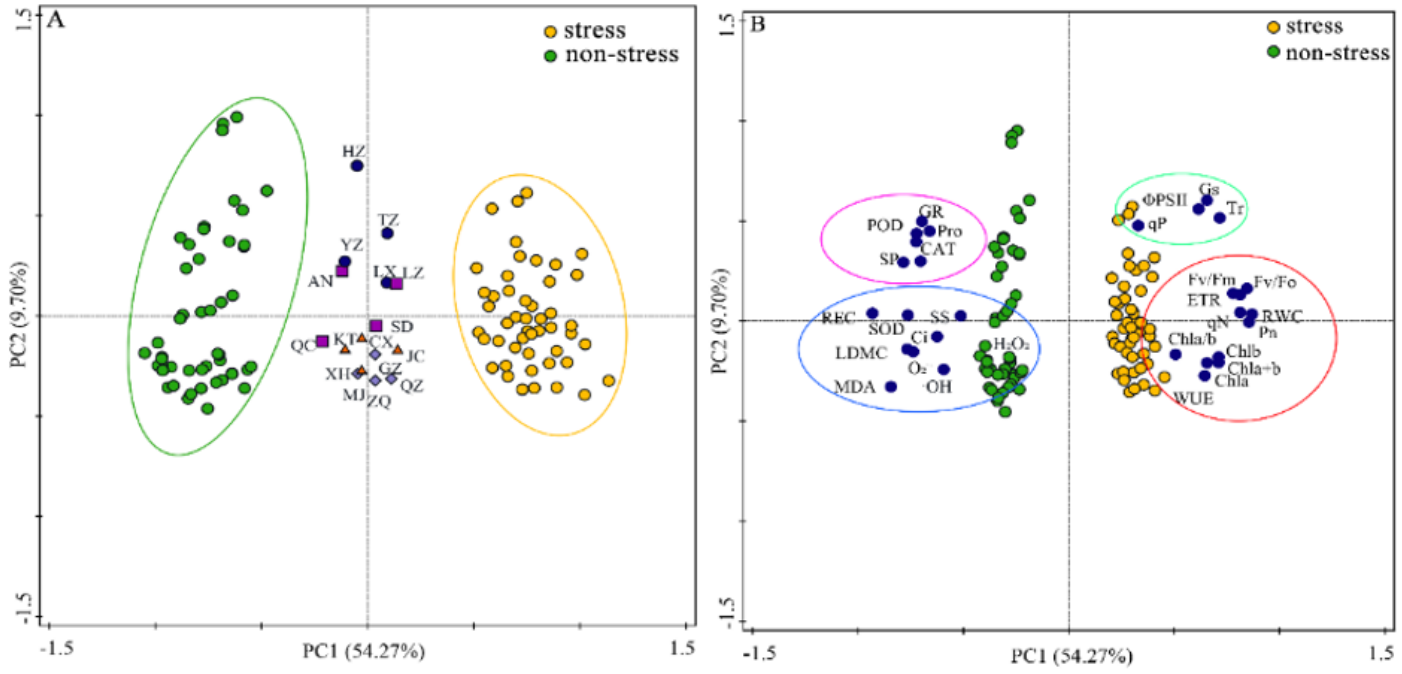

Figure 11

Observation plot showing separation of different treatments and *Poa annua*germplasms in different quadrants (A) PCA biplot showing the correlations between growth, photosynthetic, and physiological and biochemical characteristics of *Poa annua*seedlings under different combined water and temperature stress (B). The abbreviations of the corresponding indicators are shown in Table 1.

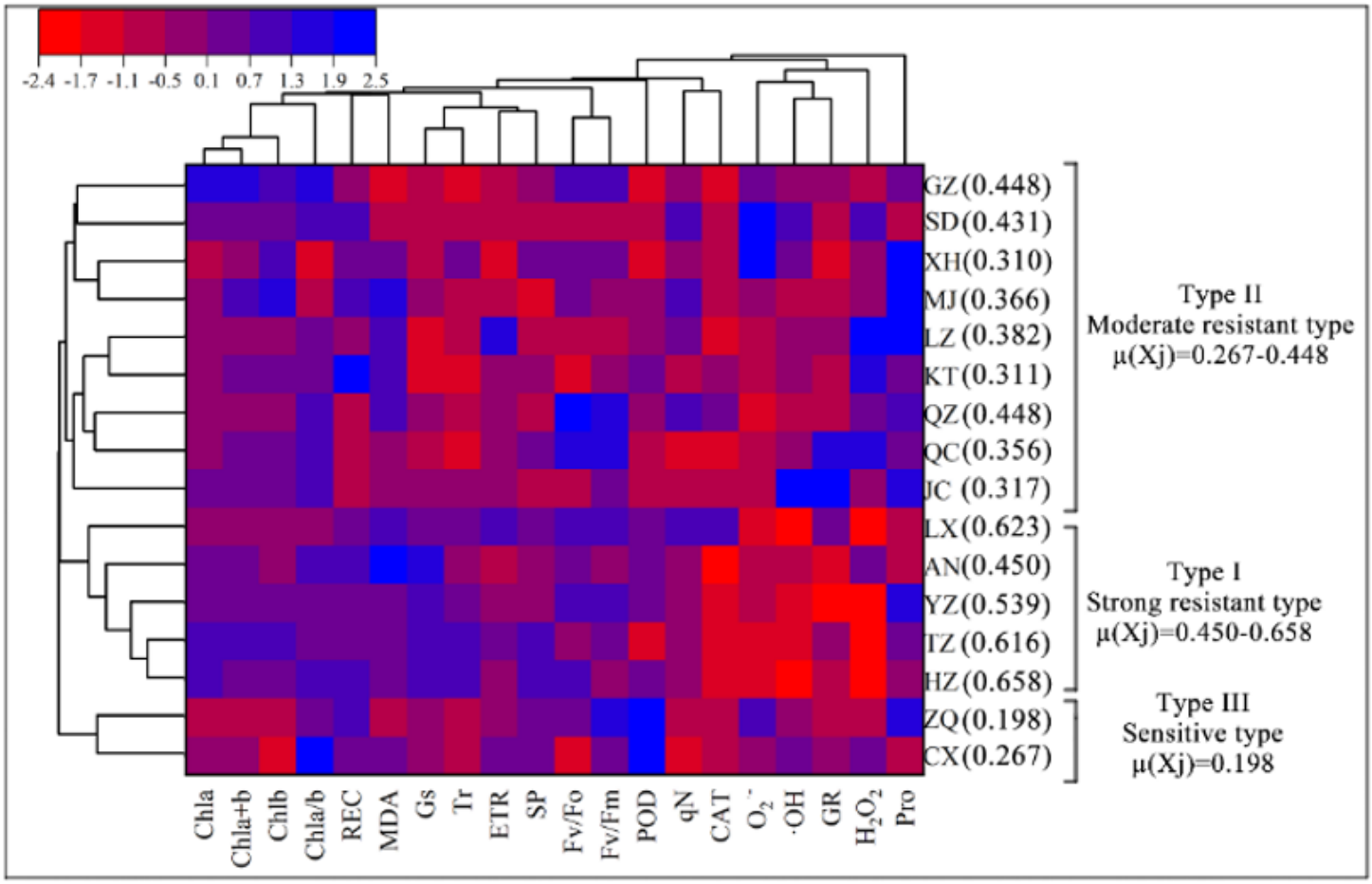

Figure 12

Heat map of cluster analysis for stress resistance among 16 *Poa annua*germplasms.

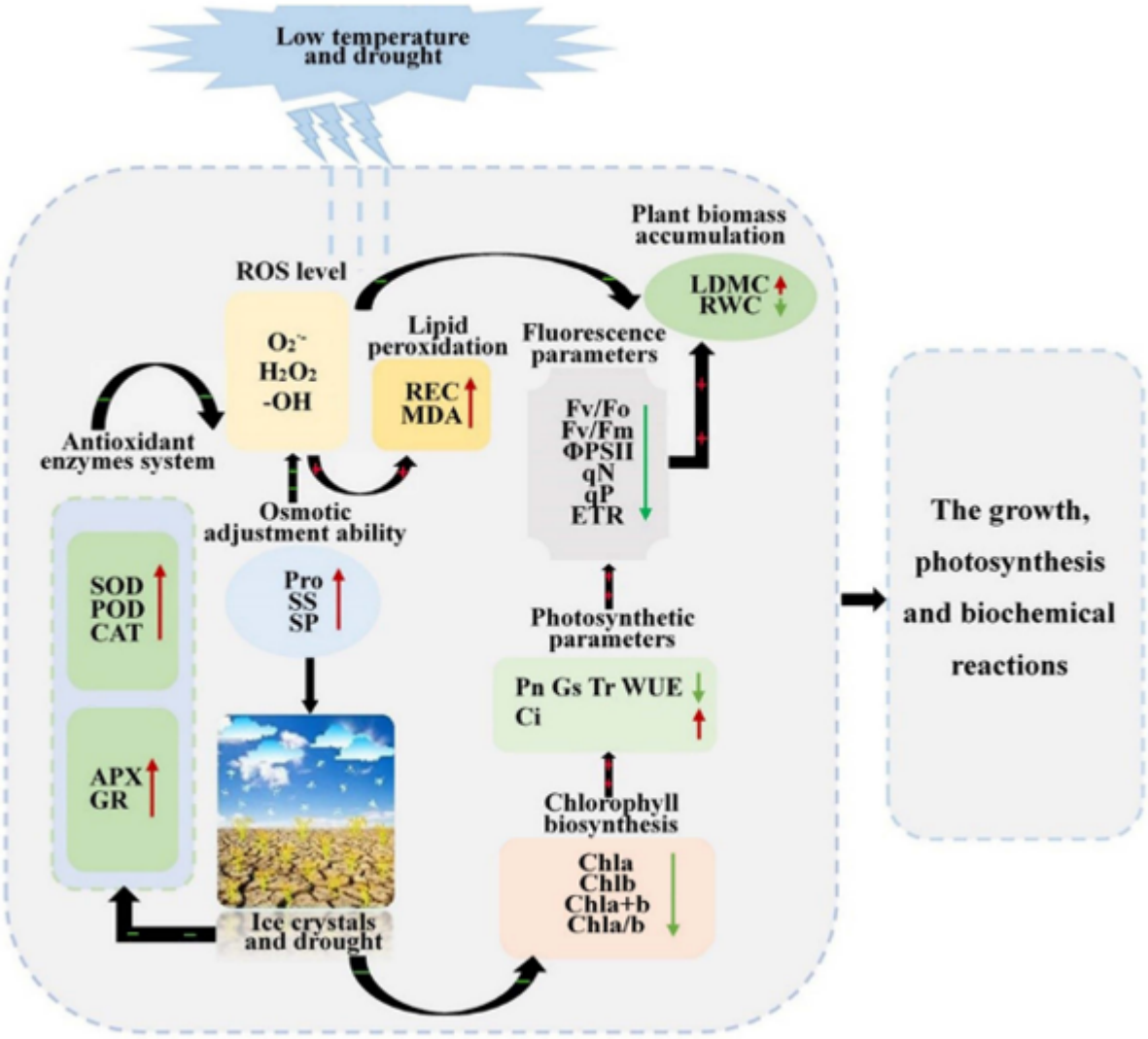

Figure 13

Response mechanism underlying resistance to combined cold and drought stress in *Poa annua* seedlings. The red and green arrows indicated that the corresponding traits were increased and decreased under stress treatment, respectively. "+ +" and "- -" indicated that the corresponding pathways acted positively and negatively, respectively.