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RESEARCH

Dynamics of physiological and biochemical effects of heat, drought and combined stress on potato seedlings

Xingxing Wang[1], Mingfu Shi[2], Ruyan Zhang[1], Yong Wang[1], Weina Zhang[1], Shuhao Qin[1*] and Yichen Kang[1*]

Abstract

Background Heat and drought stresses usually occur together in nature, and both are expected to increase in frequency and intensity as a result of climate change. The synergistic impacts of these compound climate extremes on potatoes are far from the effects of individual stresses. However, the dynamics of the effects of combined heat and drought stresses on potato physiology and biochemistry have yet to be thoroughly assessed. To elucidate this point, we set up a pot experiment using 'Atlantic' potato seedlings as test material. A total of six treatments were set up: CK (normal growth conditions: 21 ℃, 0 PEG), A1B1 (31 ℃, 20% PEG), A1B2 (31 ℃, 10% PEG), A1B3 (31 ℃, 0 PEG), A2B1 (21 ℃, 20% PEG), and A2B2 (21 ℃, 10% PEG), and 15 physiological indices were determined with the stress time of 0, 6, 12 and 18 days.

Results After 18 days of stress, the phenotype of potato seedlings was significantly different. Compared with CK, the thickness of potato leaves and palisade tissue increased under heat and drought stress, and the combined stress reduced the photosynthetic efficiency of potato leaves. In all treatments except CK, the chlorophyll content decreased significantly, the antioxidant enzyme activity increased first and then decreased, and the relative conductivity and malondialdehyde content increased significantly. The heat and combined treatment made the content of the osmotic regulator first increase and then decrease, while the treatment of 21 ℃ had no significant change. According to the correlation, principal component and interaction analysis, both heat and drought treatment had significant effects on each index, and the longer the stress time, the greater the effect, and the effect of combined stress was greater than that of single stress. However, after 6 days of stress, the activity of antioxidant enzymes and the content of transparent regulatory substances increased.

Conclusions In conclusion, potato can cope with heat, drought and combined stress by adjusting leaf tissue structure, antioxidant enzyme activity and osmotic regulatory substances in a short time.

Keywords Potato seedlings, Heat, Drought, Compound stresses, Physiological characteristics
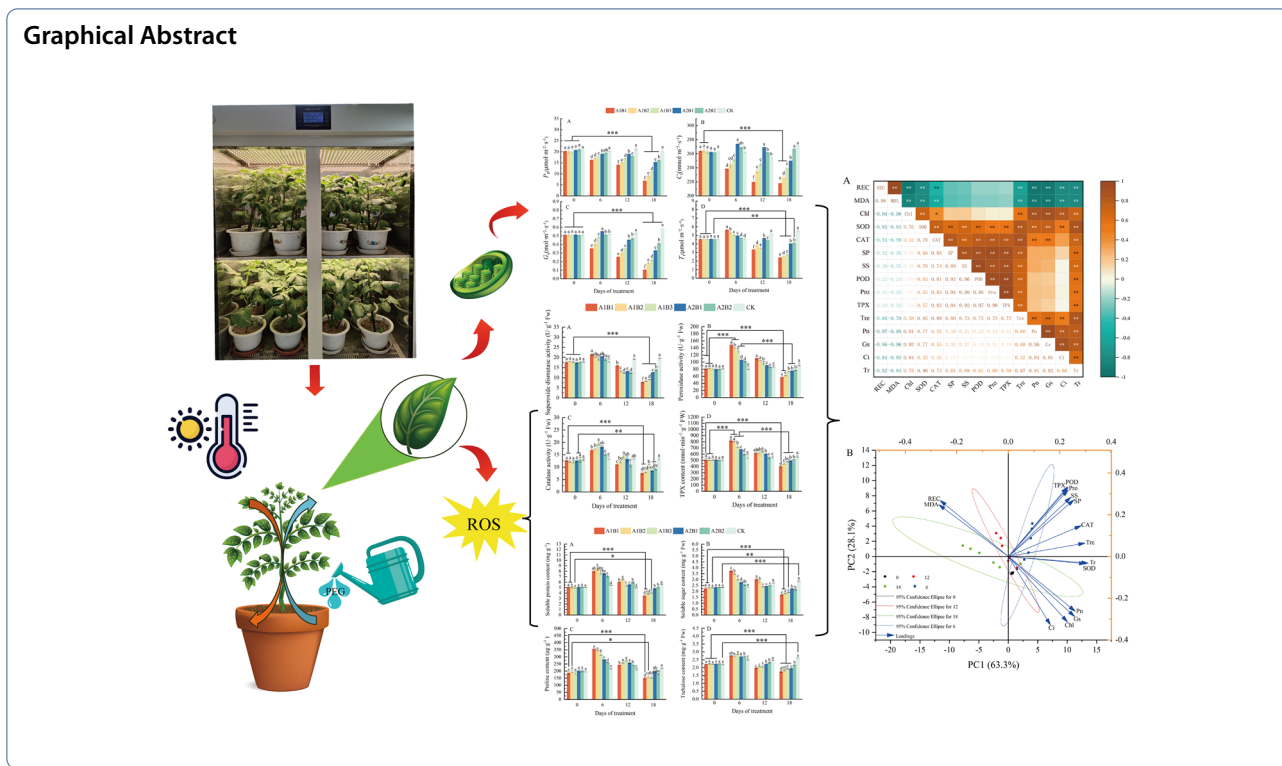
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Graphical Abstract

Introduction

Temperature and environmental patterns are changing around the world [1]. This change is expected to exacerbate regional and global water scarcity to a greater extent, which undoubtedly has a negative impact on multiple agricultural systems. Plants adapt to new conditions by sensing changes in temperature and water in their environment and adjusting their development patterns [2]. Studies have shown that plants can mitigate the negative effects of heat and drought through morphological and physiological changes. Leaf curling, stomata opening, transpiration rate reduction, leaf surface area reduction and leaf elevation, etc. (thermal morphogenesis) during seedling stage may enhance leaf cooling capacity and thus increase plant survival at higher temperatures [3]. When a plant is starved of water (drought morphogenesis), drought leads to a reduction in the number of leaves, closure of stomata, a decrease in stomatal conductance, an increase in the content of reactive oxygen species, and the establishment of an extensive root system, etc. [4]. The combined stress of heat and drought leads to contradictory stomatal movements, exposing plants to severe dehydration and higher leaf temperatures [5]. Temperature changes during the nutrient growth phase of a plant can alter water movement in the plant and affect water and nutrient uptake, ultimately leading to physiological wilting as well as a shorter life cycle [6].

In nature, heat and drought stresses often occur simultaneously, and the synergistic impacts of these climate extremes on agriculture are far greater than the impacts of individual stresses, each stress factor amplifies the effect of the other [7]. Heat and drought have significant adverse effects on photosynthesis mechanism. When plants are exposed to heat stress, the deactivation of Rubisco, along with a reduction in chlorophyll content and photosystem II (PSII) efficiency, leads to a severe decline in photosynthesis [8]. Studies have shown that drought stress reduces photosynthesis by reducing the rate of carbon dioxide diffusion caused by stomatal and non-stomatal constraints [9]. Piao et al. [10] pointed out that environmental conditions such as heat and drought inhibit plant photosynthesis and respiration, resulting in lower crop productivity. Among many environmental factors, water content, temperature and light had more significant effects on leaves, and the differences were mainly in leaf shape, leaf thickness and microstructure [11]. The thickness of plant leaves is determined by its own growth and water content. The greater the thickness of leaves, the stronger the water storage capacity. Meanwhile, the differentiation of palisade tissue and sponge tissue reflects the water status in the environment [12].

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Therefore, leaf thickness is often used as a measure of drought.

Heat and drought stress lead to excessive accumulation of reactive oxygen species (ROS) and superoxide anion radicals in plants, resulting in oxidative damage to proteins, nucleic acids and cell membranes [13]. Higher plants have a ROS-scavenging scavenging antioxidant defense mechanism system, in which many antioxidant enzymes, such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and thioredoxin peroxidase (TPX), play important roles in the antioxidant defense system of plants [14]. Cellular osmotic regulation is considered to be an important physiological characteristic related to heat and drought resistance [15]. As osmoregulatory substances, proline (Pro), soluble sugar (SS) [16] and trehalose (Tre) [17] play a key role in resistance to stress. At the same time, prevention of drought-induced osmotic stress depends on minimizing stomatal and cuticular water loss and maximizing water uptake (through root growth and osmotic adjustment) [18]. But these studies have rarely been reported on potato seedlings. Therefore, it is of great theoretical significance and practical application to study how potato activates a series of responses at the cellular and physiological levels to respond and adapt to heat and drought environments [19].

The potato (*Solanum tuberosum* L.) is an annual herb of the Solanaceae family and the genus Solanum. It is one of the major crops grown worldwide under different climate conditions, and it is a major cash crop [20–22]. According to the Food and Agriculture Organization of the United Nations (FAO), China was the largest potato producer from 1990 to 2022 [23]. Potatoes are widely distributed in China and make a significant contribution to China's food security and poverty alleviation [24–26]. However, potatoes prefer moist and cool growing conditions, and their seedling stage is sensitive to water deficit and heat [27]. It has been pointed out that a water deficit can slow down the growth of potato seedlings and reduce the transpiration rate of leaves, which can reduce the potato formation rate and yield [28]. Milan et al. [29] concluded that heat can lead to problems such as reduced stomatal conductance, smaller leaves, and deformed tuber growth in potatoes, resulting in severe yield loss. In addition, studies have shown that both water deficit and excess water can exacerbate the effects of temperature [30, 31]. This suggests the need to understand temperature and moisture interactions to develop more effective adaptation strategies [6].

At present, the effects of abiotic stresses on the physiology and ecology of potato seedlings are mainly focused on the response of a single stress factor to potatoes [32–34]. However, there are few studies on the effects of the combined stress of heat and lack of water on the internal mechanism of potato seedlings. Therefore, in this study, we used an artificial climate chamber (RDN type) and PEG-6000 to simulate the natural precipitation method. By controlling the temperature and creating a drought-stressed environment at different time segments, we studied the dynamic changes in plant phenotypic morphology, leaf microstructure, photosynthesis, chlorophyll content, antioxidant defense, cellular osmotic regulation and growth of potato seedlings under heat and drought stress at different treatments. To reveal the determining characteristics of the different temperatures and moisture environments on potato production and the compensatory growth effects, and to clarify the ecological adaptations of potato seedlings under certain temperatures and precipitation conditions. And to analyze their coping patterns under different temperatures and moisture.

Materials and methods
Test material and test site
The tested potato variety is –'Atlantic', with an upright plant shape, strong stalk, medium branch number and strong growth potential. The experiment was selected as a high-quality seed potato to be tested from May to October 2022–2023 at Gansu Agricultural University (52°12′59″N, 22°34′37″E) in Gansu Province, Northwest China. The plant was grown in pots with a size of 23.5 cm (diameter) * 15 cm (height) and holes at the bottom. The substrate was made from a mixture of vermiculite, perlite and seedling substrate at a volume ratio of 3:1:1. The substrate was sterilized with carbendazim, and the height of the substrate in the pot was 11 cm.

Experimental design and methodology
The experiment was set at heat (31 ℃) and normal temperature (21 ℃). Drought stress was simulated by PEG (PEG-6000) with 20% PEG and 10% PEG, respectively. CK was treated under normal growth conditions (21 ℃, 0 PEG) (Table 1). There were 3 replicates per treatment and 12 pots per replicate.

Table 1 Experiment design

Treatment	Temperature(Day/Night) ℃	PEG moisture treatment (%)
A1B1	31/23	20
A1B2	31/23	10
A1B3	31/23	0
A2B1	21/13	20
A2B2	21/13	10
CK	21/13	0

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The seed potatoes with full sprouts were screened and dried. Fill the substrate with water 2 days before sowing, and apply basal fertilizer one day before sowing. Sowed in May 2022 and 2023, 1 seed potato per pot, bud side up. Seedlings were watered with 150 ml of water every 3 days after emergence and soil water content of potted plants was determined by weighing and irrigated from 17:00 to 18:30 pm. The plants were exposed to uniform light of 10,000 lx in RDN-type artificial climate chamber for 16 h/day and darkness of 8/h. The humidity of the culture area is 60%. Heat and drought treatment were carried out 20 days after emergence, and PEG-6000 was watered at different concentrations on day 0, day 6 and day 12.

Determination items and methods
On the 0th, 6th, 12th and 18th d of treatment, the functional leaves of the apical 3rd-5th nodes of the plants were taken to determine the indexes, and three repetition mixed leaf samples were taken from each repeat.

Observation on the microstructure of potato leaves
The functional leaf tissue at 3–5 nodes at the top of the plant was put into 4% paraformaldehyde fixing solution, removed in the fume hood and trimmed with scalpel, then dehydrated, paraffin embedded and sliced. Tissue sections were stained with saffron solid green (plant). The leaf microstructure was observed and compared under different treatments using inverted integrated fluorescence microscope (Revolve RVL-100-G). The thickness of leaves, palisade tissue and spongy tissue were measured and analyzed by Image J (1.52p) software.

Determination of photosynthetic index
Photosynthetic parameters were measured using a Li-6400XT photosynthesis, and net photosynthetic rate (P_n), intercellular CO_2 concentration (C_i), stomatal conductance (G_s), and transpiration rate (T_r) were determined. The third and fourth leaves of the apical part of the plant were selected for the determination of potatoes, and plants with representative growth were selected for each treatment, including three replications. Photosynthetic parameters were measured from 8:50 to 11:00 am., and the LED light intensity was controlled at 800 μmol m^{-2} s^{-1}.

Determination of chlorophyll content
Chlorophyll content was determined by spectrophotometry, and 0.5 g of potato leaves were taken from each sample. For specific methods, refer to Lichtenthaler and Wellburn [35]. The absorbance was measured at 665 nm, 649 nm and 470 nm, respectively, and the chlorophyll a (chl a), chlorophyll b (chl b) and chlorophyll a+b (chl a+b) contents were calculated according to the formula.

Determination of antioxidant and osmoregulatory substances
Superoxide dismutase (SOD) enzyme activity was determined by the nitro blue tetrazolium method [36]; Determination of Peroxidase (POD) enzyme activity using guaiacol method [37]; The activity of Catalase (CAT) enzyme was determined by ultraviolet absorption method [38]; Determination of leaf enzyme activity: Thioredoxin peroxidase (TPX) enzyme activity was determined using a spectrophotometric method (kit provided by Suzhou Keming Biotechnology Co); The content of Malondialdehyde (MDA) was determined by Thiobarbituric acid method [39]; Relative conductance (REC) was measured with a conductometer [40];

The Soluble protein (SP) content was determined by the Coomassie brilliant Blue G-250 method [41]; Soluble sugar (SS) content was determined by the kit provided by Beijing Suoshen Technology Co., LTD. (Beijing, China); Proline (Pro) content was determined by reference to Wani et al. [42]; Trehalose (Tre) content was determined by anthrone colorimetry, and Suzhou Keming Biotechnology Co provided the kit. The sample was 0.1 g of potato leaves, and the specific method was described in the kit. The absorbance value at 620 nm was detected, and the alginate content in the sample was calculated according to the formula.

Data processing
Microsoft Excel 2019 was used for experimental data statistics. ANOVA analyzed the experimental data through SPSS 26.0 software using Duncan's new complex polar deviation method ($P \le 0.05$), plotted using Origin Pro 2021, and microscopic measurements were performed using Image J (1.52p) software.

Result
Effect of heat and drought on phenotype and leaf microstructure of potato seedlings
In order to evaluate the transient effects of heat and drought stress on potato seedlings, leaf phenotype and leaf microstructure changes were recorded on day 6 and day 18. The results showed that potato plants and leaves morphology changed significantly under different temperature and moisture treatments. On the 6 days of treatment (Fig. 1), the potato seedlings under 21 ℃ and normal water treatments have better overall growth, thicker stems and better leaf elongation than those under 31 ℃ treatment, and the control (CK) leaves are green and abundant; After 18 days of treatment (Fig. 2), 31 ℃ treatment of potato seedlings under the overall status of

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the poorer, and there is the phenomenon of futile growth, leaf wilting obviously, leaf yellowing, curled edges, weak stalks, which was an adaptive response to the damages caused by water loss, extreme temperatures, or combined stresses. There were no significant changes in the leaves under the 21 ℃ treatment, and the thick and strong growth of the stalks was better.

As observed by the leaf microstructure, the thickness of potato leaves and their palisade tissue significantly changed under different temperature and moisture treatments. On the 6 days of treatment, the leaf thickness of A1B2 and A1B3 potato seedlings at 31 ℃ was thinner than that at 21 ℃, and the leaf thickness and palisade tissue thickness of A2B2 and CK seedlings at 21 ℃ had little changes, and their chloroplast content was higher. On the 18 days of treatment, the thickness of leaves increased, and the number of chloroplasts in palisade tissues
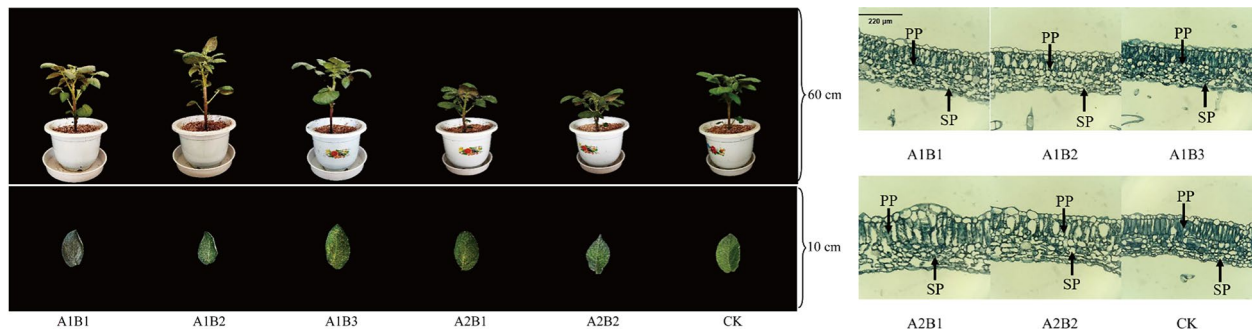

Fig. 1 Effects of heat and drought treatment for 6 days on morphology and leaf microstructure of potato seedlings. *PP* palisade parenchyma, *SP* spongy parenchyma, Scale bars = 220 μm
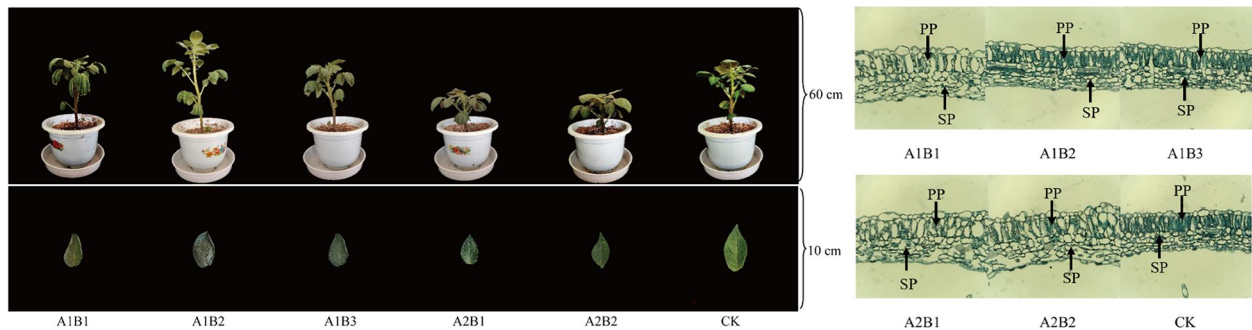

Fig. 2 Effects of heat and drought treatment for 18 days on morphology and leaf microstructure of potato seedlings. *PP* palisade parenchyma, *SP* spongy parenchyma, Scale bars = 220 μm

Table 2 Measurement of microstructure and thickness of leaves

Treatment	6 days		18 days	
	Blade thickness(μm)	Palisade tissue thickness (μm)	Blade thickness(μm)	Palisade tissue thickness (μm)
A1B1	352±2.20bc	135±6.91c	393±3.25b	137±7.48bc
A1B2	317±8.11d	122±2.30 cd	343±2.26c	134±2.21bc
A1B3	332±10.27 cd	114±3.55d	332±9.53c	142±3.98abc
A2B1	468±21.04a	181±7.14a	416±18.64ab	151±8.31ab
A2B2	381±1.37b	151±0.93b	444±24.88a	166±14.27a
CK	319±2.64 cd	124±3.14 cd	312±5.31c	119±3.94c

Data are means of scoring ± SE from three replications. Different lowercase letters indicate significant difference between different treatments in the same stress time (*P* ≤ 0.05)

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Fig. 3 Photosynthetic Parameters of potato seedlings under heat and drought treatment: P_n(**A**)、C_i(**B**)、G_s(**C**)、T_r(**D**). Data are means of scoring ± SE from three replications. Different lowercase letters indicate significant difference between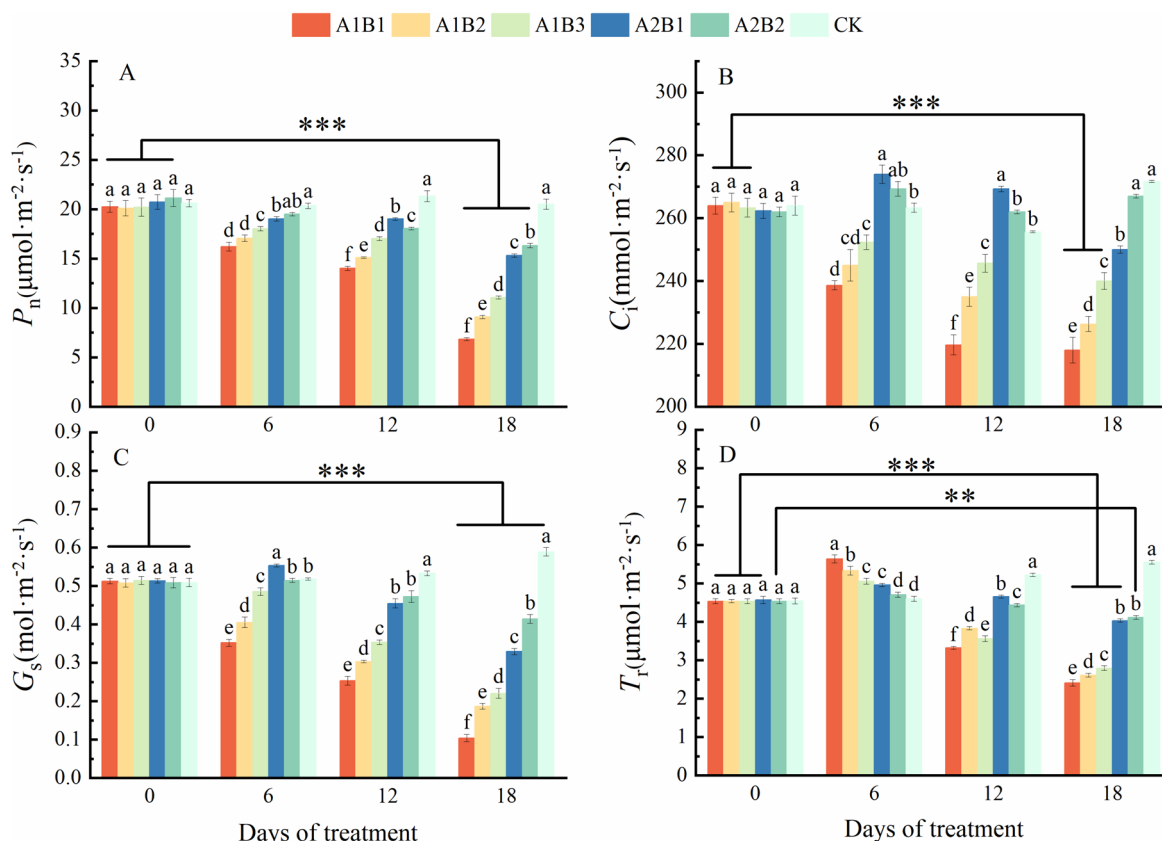 different treatments in the same stress time ($P \le 0.05$). * indicates a significant correlation at $P \le 0.05$ level; ** indicates a very significant correlation at the $P \le 0.01$ level. *** indicates a very significant correlation at the $P \le 0.001$ level

decreased significantly under the A1B1 and A2B1 treatments (Table 2).

Effect of heat and drought on photosynthetic characteristics of potato seedlings

As can be seen from Fig. 3A, P_n of potato seedling leaves showed a decreasing trend with the prolongation of treatment time. P_n decreased sharply in the A1B1 and A1B2 treatments, followed by the A1B3 treatment. Compared with day 0, P_n was higher than that of all treatments in CK; P_n decreased by 66.23%, 54.80%, 45.30%, 26.15% and 22.80% under A1B1, A1B2, A1B3 treatments and ambient A2B1 and A2B2 treatments at day 18, respectively.

The C_i of potato seedling leaves showed different trends depending on temperature and moisture (Fig. 3B). C_i decreased sharply in the A1B1 and A1B2 treatments, followed by the A1B3 treatment. C_i of A2B1 and A2B2 treatments increased slowly and higher than all treatments. Compared with day 0, at day 18, C_i decreased by 17.42%, 14.59%, and 8.86% in the A1B1, A1B2, and A1B3 treatments, respectively.

The G_s of potato seedling leaves showed a decreasing trend with increasing treatment time (Fig. 3C). The G_s of A1B1, A1B2 and A1B3 treatments decreased significantly, and G_s under A2B1 and A2B2 treatments decreased at day 12 and day 18, respectively. Compared with day 0, on day 18, G_s treated with A1B1, A1B2, A1B3, A2B1 and A2B2 decreased by 79.68%, 63.23%, 57.13%, 35.86% and 18.56%, respectively.

The leaf T_r of potato seedlings increased first and then decreased with the treatment time (Fig. 3D). T_r changed significantly under heat, and T_r of A1B1, A1B2 and A1B3 treatments increased first and then decreased. T_r did not change significantly under 21℃. From day 6 to day 18, the T_r treated by A1B1, A1B2 and A1B3 treatments decreased by 46.77%, 42.46% and 38.42%, respectively.

Effect of heat and drought on total chlorophyll content of potato seedlings

From Fig. 4, the chlorophyll a, b, and a+b contents showed an overall decreasing trend with treatment time. The changes in chlorophyll a+b content were obvious
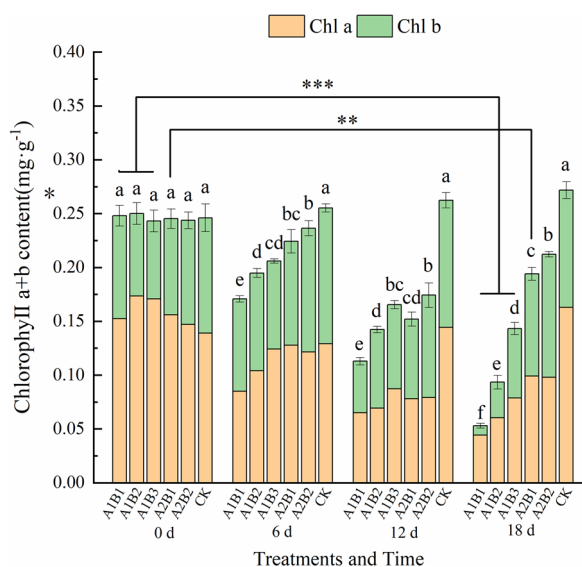
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Fig. 4 Effect of heat and drought treatments on chlorophyll a+b content of potato seedlings Data are means of scoring ± SE from three replications. Different lowercase letters indicate significant difference between different treatments in the same stress time ($P \leq 0.05$). * indicates a significant correlation at $P \leq 0.05$ level; ** indicates a very significant correlation at the $P \leq 0.01$ level. *** indicates a very significant correlation at the $P \leq 0.001$ level

under heat; the chlorophyll a+b content of A1B1 and A1B2 treatments decreased sharply, and the chlorophyll a and chlorophyll b content followed, followed by A1B3 treatment; the chlorophyll a+b content of CK was higher than that of all the treatments and showed a rising trend. Processed for 18 days, the chlorophyll a+b content of A1B1, A1B2, A1B3, A2B1 and A2B2 treatments decreased by 78.63%, 62.60%, 41.05%, 20.88% and 12.88%, respectively. Among them, A1B1 and A1B2 treatments decreased chlorophyll b content by 90.88% and 56.82%. On day 18, with reference to A2B3, the decrease in chlorophyll content was more significant in A1B3 vs. A2B1, indicating that heat was the main effect.

Effect of heat and drought on antioxidant and osmoregulatory systems in potato seedlings

Effect of heat and drought on antioxidant enzyme activities in potato seedlings

In Fig. 5, the antioxidant enzyme activities showed varying degrees of first increase and then decrease with time. As seen in Fig. 5A, at day 6, the SOD activities of A1B1 and A2B1 treatments increased by 18.34% and 14.93%, respectively. However, from day 6 to day 18, SOD activity decreased sharply in all treatments except for CK. From day 0 to day 18, SOD activity under A2B1 and A2B2 treatments showed a tendency first to increase and then decrease. The POD activity of each treatment increased

at day 6 (Fig. 5B), with the A1B1 treatment having the highest POD activity of 147.4 U g^{-1}. Between day 0 and day 18, the POD activity of the A1B1 and A1B2 treatments decreased by 28.74% and 23.79%, respectively. The change in POD activity was not significant under the 21 ℃ treatment. The CAT activity of the treatments also increased at day 6 (Fig. 5C), with the A1B3 treatment having the highest CAT activity, followed by A2B1. From day 0 to day 18, the CAT activity of A1B1 and A1B2 treatments decreased by 39.47% and 34.01%, respectively. The CAT activity of A2B1 and A2B2 treatments decreased by 31.52% and 28.21%, respectively. In the same trend as the first three antioxidant enzyme activities, the TPX activity of each treatment increased at day 6 (Fig. 5D), with the A1B1 treatment having the highest TPX activity of 818.63 nmol·min^{-1} g^{-1} FW. Between day 0 and day 18, the TPX activity of the A1B1 and A1B2 treatments was reduced by 26.87% and 11.49%, respectively. There was no significant change in TPX activity under 21 ℃.

Effect of heat and drought on malondialdehyde content and relative conductivity of potato seedlings

As can be seen from Fig. 6A, the MDA content showed an overall increasing trend with treatment time. From 0 to 18 days, A1B1, A1B2, and A1B3 treatments led to a sharp increase in the MDA content, which increased by 66.30%, 64.31%, and 61.55%, respectively. The highest MDA content was observed in the A2B1 and A2B2 treatments, while the CK maintained the MDA content at a low value. With the extension of the treatment time, the relative electrical conductivity of the blade showed an overall increasing trend (Fig. 6B). The relative conductivity increased greatly under heat and slowly at 21 ℃ treatments. From 0 to 18 day, the relative conductivities of A1B1, A1B2, A1B3 and A2B1 treatments increased by 59.86%, 54.61%, 49.73%, and 40.49%, respectively. After 18 days of stress, A1B3 decreased significantly compared to CK.

Effect of heat and drought on the osmoregulatory system of potato seedlings

According to Fig. 7A, the SP content showed a general trend of increasing and then decreasing from day 0 to day 18. On day 6, the SP content increased in all treatments except CK, which increased by 36.61%, 40.30%, 36.60%, 33.60% and 28.13%, respectively. For 18 days of stress, the composite treatments of heat and drought treatments reduced the SP content. The lowest SP content was found in the A1B1 treatments, followed by the A1B2 treatment.

There was a general trend of increasing and then decreasing SS content with increasing treatment time (Fig. 7B). On day 6, SS content increased in A1B1, A1B2 and A1B3 treatments by 40.35%, 33.45% and 25.04%,
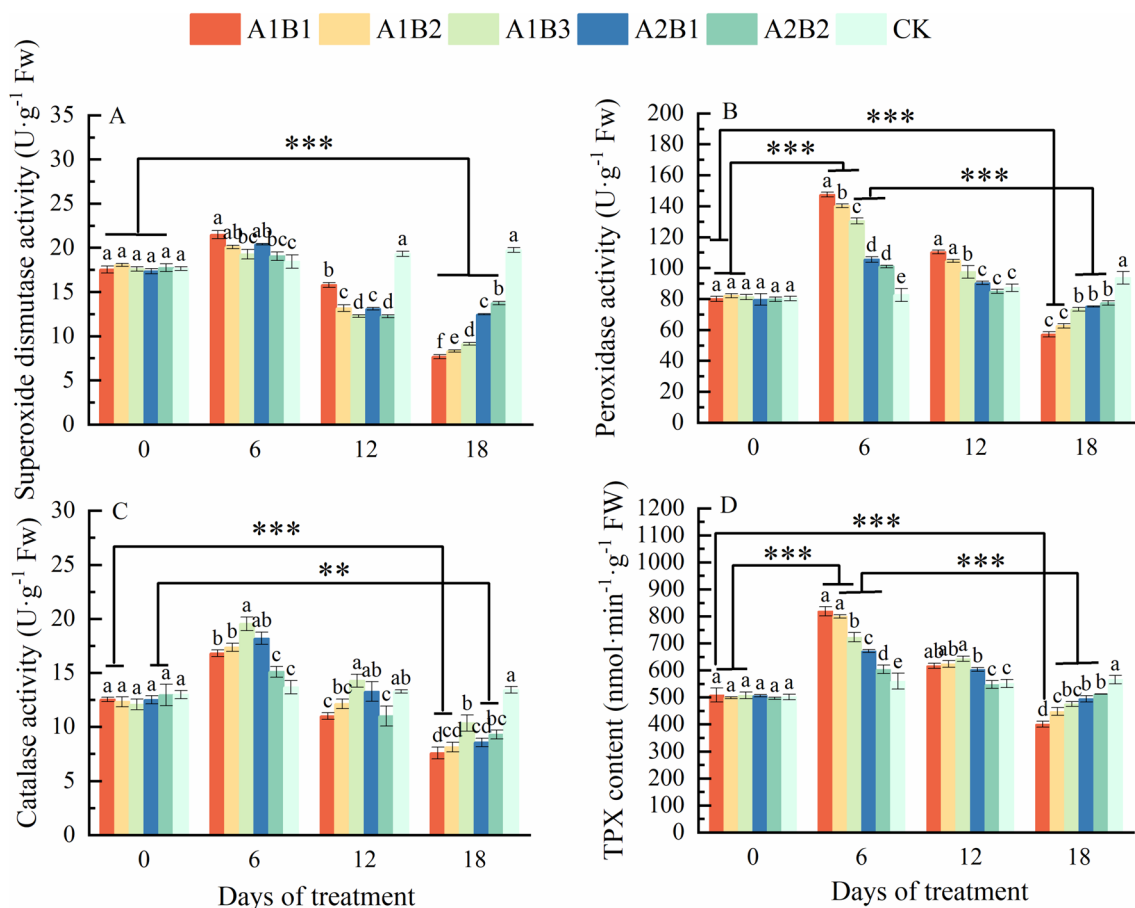
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Fig. 5 Effect of heat and drought treatment on antioxidant enzyme activities of potato seedlings: SOD (**A**) 、POD (**B**) 、CAT (**C**) 、TPX (**D**). Data are means of scoring ± SE from three replications. Different lowercase letters indicate significant difference between different treatments in the same stress time ($P \leq 0.05$). * indicates a significant correlation at $P \leq 0.05$ level; ** indicates a very significant correlation at the $P \leq 0.01$ level. *** indicates a very significant correlation at the $P \leq 0.001$ level

respectively, but then SS content gradually decreased from day 6 to day 18. After 18 days of stress, both temperature and moisture and combined treatments decreased SS content. Among them, the lowest SS content was found in A1B1, A1B2 and A1B3 treatments. The CK showed insignificant changes in SS content.

As shown in Fig. 7C, the Pro content showed a general trend of increasing and then decreasing due to the prolongation of stress time. On day 6, the Pro content increased in A1B1, A1B2 and A1B3 treatments by 45.94%, 42.50% and 39.72%, respectively. The Pro content gradually decreased again from 6 to 18 days. After 18 days of stress, both temperature and moisture and combined treatments decreased Pro content. Among them, A1B1, A1B2 and A1B3 treatments had the lowest Pro content. The A2B2 treatment had the lowest Pro content under 21 ℃ treatment.

Tre content did not change significantly with increasing treatment time, with a general trend of increasing and then decreasing (Fig. 7 D). After 6 days of stress, Tre content of A1B1, A1B2 and A1B3 treatments increased by 19.88%, 16.36% and 22.35%, respectively, but then gradually decreased from 6 to 18 days. Tre content was lowest in A1B1, A1B2 and A1B3 treatments after 18 days of stress. The Tre content of the A2B1 treatment was significantly reduced by 15.48%.

Difference analysis between heat and drought on various indexes of potato seedlings

Correlation analysis between physiological and biochemical indicators of potato seedlings under heat and drought treatments

To investigate the correlation between physiological and biochemical characteristics of potato seedlings after heat and drought treatments, we performed Pearson
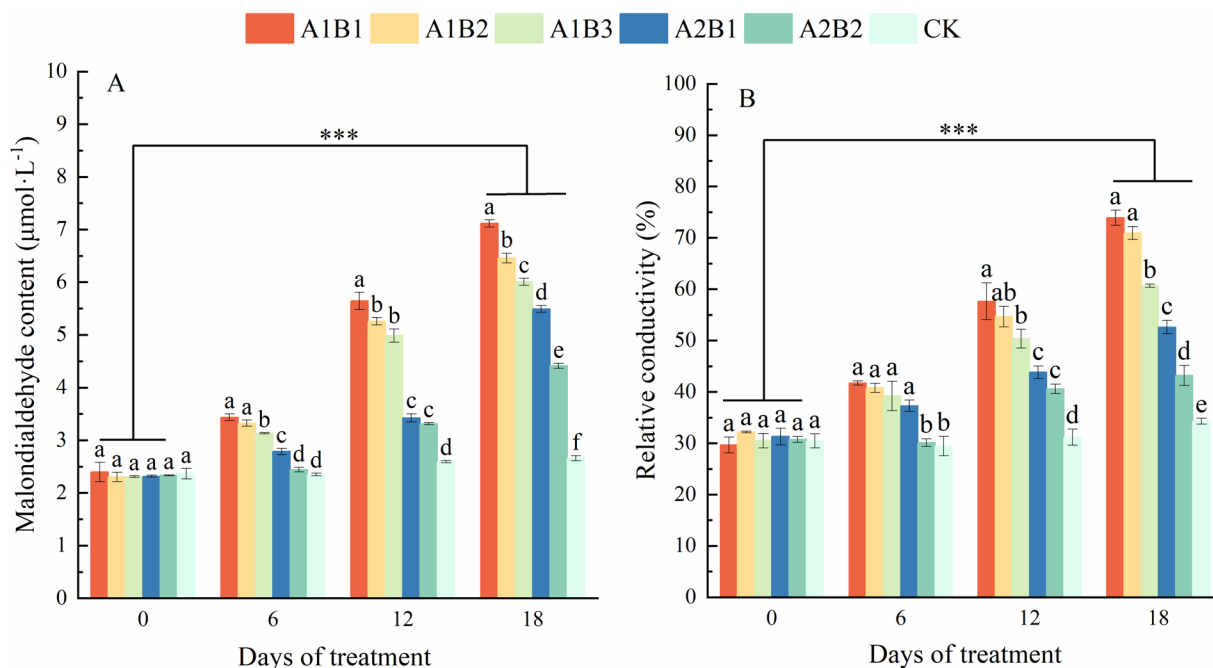
Fig. 6 Effect of heat and drought treatment on malondialdehyde content and relative conductivity of potato seedlings: MDA (**A**) and REC (**B**). Data are means of scoring ± SE from three replications. Different lowercase letters indicate significant difference between different treatments in the same stress time ($P \leq 0.05$). * indicates a significant correlation at $P \leq 0.05$ level; ** indicates a very significant correlation at the $P \leq 0.01$ level. *** indicates a very significant correlation at the $P \leq 0.001$ level

correlation analysis for each index. As can be seen in Fig. 8A, REC was highly significant ($P \leq 0.01$) and positively correlated with MDA content with an r-value of 0.98. REC was highly significant ($P \leq 0.01$) and negatively correlated with P_n and G_s, respectively; the r values were -0.97, -0.96. MDA was highly significant ($P \leq 0.01$) and negatively correlated with P_n and G_s with r values of -0.95 and -0.96, respectively. SS content was highly significant ($P \leq 0.01$) and positively correlated with POD activity with an r-value of 0.96. POD activity was highly significant ($P \leq 0.01$) and positively correlated with Pro content, TPX activity; the r values were 0.95 and 0.97, respectively. Pro content showed a highly significant ($P \leq 0.01$) positive correlation with TPX activity with an r-value of 0.98. P_n and G_s were highly significant ($P \leq 0.01$) and positively correlated with an r-value of 0.96.

Multicollinearity among numerous factors was addressed by exploring the contribution of different principal components to the variance of the data and the relationship between the principal components and the original variables through principal component analysis (PCA). Eigenvalues should be used to determine the number of principal components (PCs) to retain. The results of the study show that the sample points corresponding to the samples from the 13 treatments in

Fig. 8B are clustered with each other, indicating a high degree of similarity between the treatments. The consistency of the arrow direction of the response variable with the axis direction (negative to positive) can be used to determine whether there is a positive or negative correlation between the response variable and the PC. As indicators of variance, the indicators of photosynthetic system, antioxidant enzymes, and osmoregulatory system loaded significantly on the first and second quadrants, respectively, and were positively correlated with PC 1. PC 1 and PC 2 add up to 91.4%, with PC 1 and PC 2 accounting for 63.3% and 28.1% of the total variance, respectively.

Testing of interactive intersubjective effects

According to Table 3, there were highly significant ($P \leq 0.01$) effects of heat, drought, stress time and interaction test heat×drought, heat×stress time, drought×stress time and heat×drought×stress time treatments on SOD, MDA, C_i、T_r and Pro. There were highly significant ($P \leq 0.01$) effects of stress time, heat×stress time and drought×stress time treatments on the measured indices. The effect of heat on CAT was non-significant ($P \geq 0.05$), while the effect of drought and other combined treatments was highly significant ($P \leq 0.01$). Heat×drought×stress time treatments did not significantly ($P \geq 0.05$) affect POD, TPX, REC, P_n and
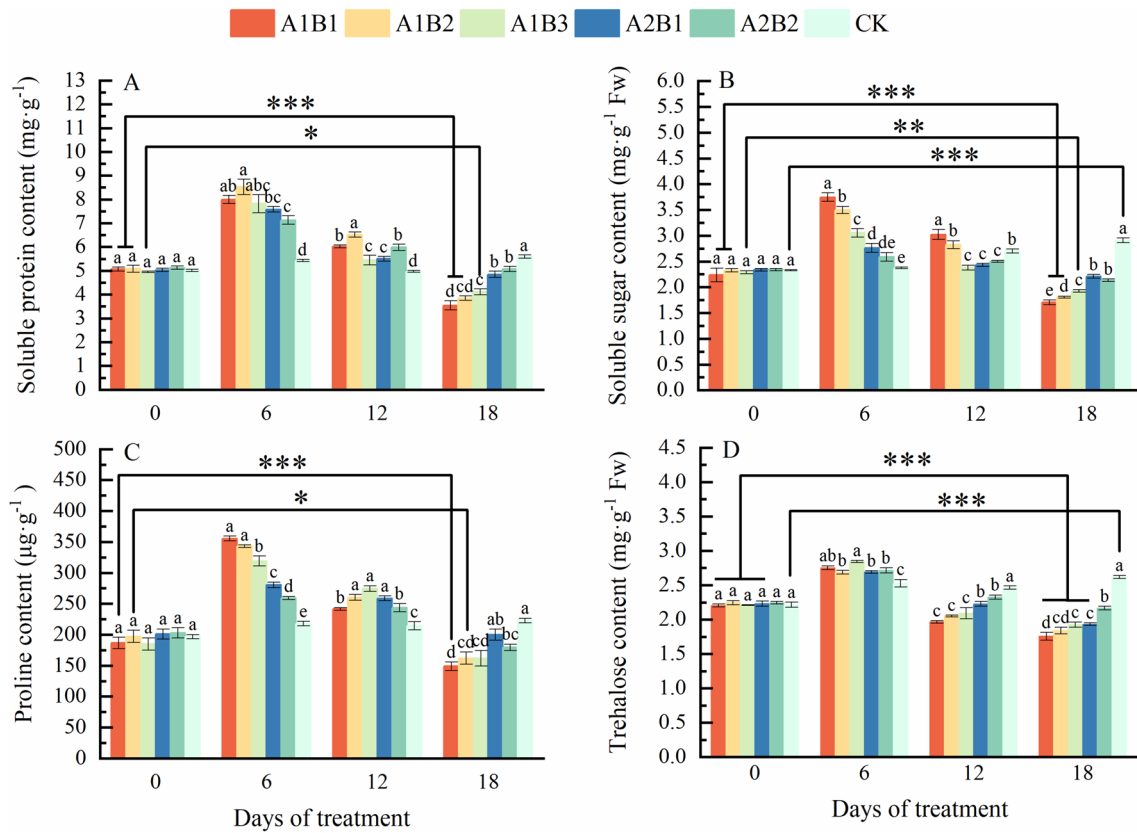
Fig. 7 Effect of heat and drought treatment on osmotic regulation system of potato seedlings: SP (**A**)、SS (**B**)、Pro (**C**)、Tre (**D**). Data are means of scoring ± SE from three replications. Different lowercase letters indicate significant difference between different treatments in the same stress time ($P \leq 0.05$). * indicates a significant correlation at $P \leq 0.05$ level; ** indicates a very significant correlation at the $P \leq 0.01$ level. *** indicates a very significant correlation at the $P \leq 0.001$ level
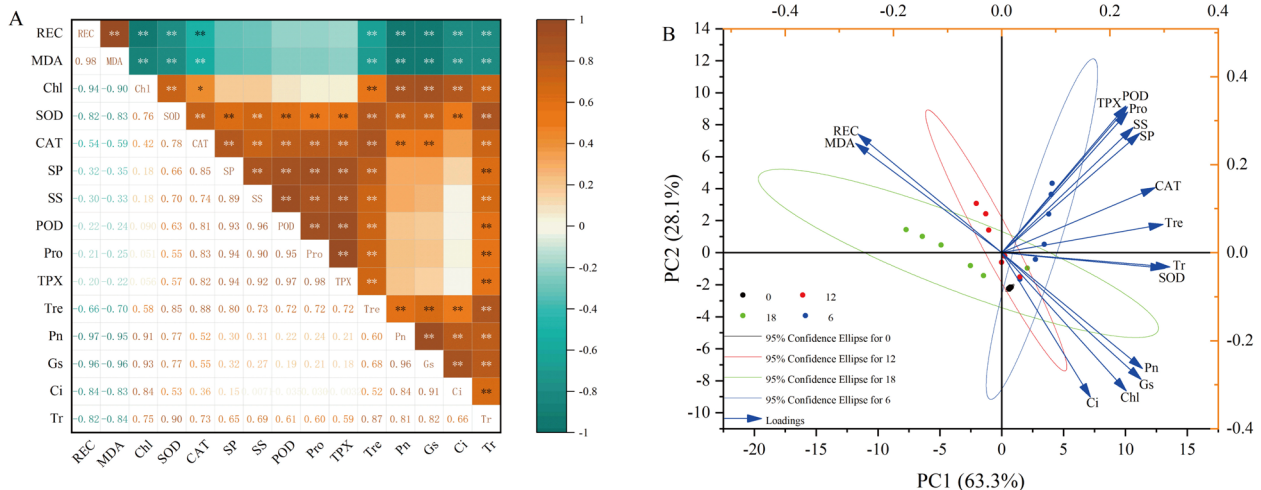

Fig. 8 Correlation Analysis and Principal Component Analysis (PCA) of Physiological and Biochemical Characters of potato seedlings: Correlation Analysis (**A**)、Principal Component Analysis (**B**). * indicates a significant correlation at $P \leq 0.05$ level; ** indicates a very significant correlation at the $P \leq 0.01$ level. The number represents the correlation coefficient

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Table 3 Test of inter-subjectivity effect

T I	A	B	t	A×B	A×t	B×t	A×B×t
SOD	176.63**	38.36**	726.60**	90.98**	188.40**	44.51**	29.91**
POD	158.08**	2.80	527.36**	0.46	223.33**	30.03**	2.05
CAT	0.00	15.07**	177.52**	6.65**	14.00**	8.18**	8.09**
TPX	64.64**	2.73	438.62**	4.97*	127.55**	20.64**	1.66
REC	372.92**	50.02**	304.39**	4.85*	71.23**	10.06**	0.77
MDA	1629.08**	187.73**	1697.77**	20.50**	296.76**	56.68**	15.93**
P_n	488.42**	55.88**	303.46**	2.00	87.37**	12.24**	1.58
C_i	441.63**	18.17**	66.23**	19.32**	60.38**	7.84**	8.83**
G_s	1284.18**	142.79**	512.09**	4.92*	213.02**	34.13**	23.16**
T_r	584.22**	29.71**	538.96**	46.79**	459.76**	45.82**	14.95**
Pro	38.98**	6.09**	518.63**	10.61**	138.93**	14.14**	7.87**
SS	18.22**	2.76	276.88**	31.95**	166.56**	29.81**	7.59**
SP	4.85*	21.02**	416.16**	3.82*	85.28**	17.63**	5.92**
Tre	118.09**	34.60**	450.73**	3.01	84.79**	23.11**	16.82**
Chl	384.58**	94.32**	184.48**	5.00*	84.31**	17.37**	2.95

T indicates treatment; I indicates index; A indicates heat; B indicates drought; t indicates time; The number represents the F value. The number represents the F value. * indicates a significant influence at $P \leq 0.05$ level; ** indicates a very significant influence at the $P \leq 0.01$ level

Chl. Heat×drought treatment and heat×drought×stress time treatment did not significantly ($P \geq 0.05$) affect P_n drought treatments did not significantly ($P \geq 0.05$) affect POD, TPX and SS.

Discussion

Temperature and moisture are key factors affecting the growth of above-ground parts and tubers of potatoes [43]. In order to cope with deleterious heat and drought stress, plants undergo a variety of morphological, physiological and biochemical changes. Therefore, plant stress response is a complex dynamic process, and the changes in leaf morphology and physiological characteristics can more accurately indicate the degree of stress [44]. The results of this study showed that potato seedlings had the best indicators when the average air temperature was 21 ℃; this is consistent with the findings of Rykaczewska et al. [34]. Heat and drought had significant effects on potato phenotypes, such as small and few leaves, wilted leaves, curled edges, and plants that were prone to collapse (Fig. 1). In contrast, potato plants in the normal water treatment (A2B3) at 21 ℃ grew well and had bright green, bushy leaves [45]. Studies have shown that when the temperature increases, the vapor pressure in the intercellular space in the lower stomatal cavity increases more than the atmospheric vapor pressure, so the vapor pressure difference between the inside and outside of the leaves increases, resulting in water escaping from the leaves, enhanced transpiration, and increased leaf thickness [46]. This is similar to what we observe in the microstructure (Fig. 2). On the 18th day of stress, compared with CK, A1B1 and A2B1 treatments increased the thickness of leaves and significantly reduced the number of chloroplasts in palisade tissue, which explained the reason for the change of plant phenotype [47]. The thickness of palisade tissue and spongy tissue of potato leaves increased with the increase of temperature. Demonstrating that when stress intensity increases, plants change their structure to adapt to the arid environment [48].

Both the duration and intensity of heat and droughts have an influence on the growth and development of plants [49]. In this study, the P_n, C_i, G_s and T_r of potato seedlings showed a decreasing trend under the continuous heat and drought treatment for 18 days. Stomatal closure helps plants maintain leaf water potential, but it also reduces CO_2 uptake, which affects photosynthesis [50]. Stomatal opening is a mechanism for blade cooling, which means that an increase in stomatal opening leads to a decrease in blade temperature [51]. However, Wani et al. [52] pointed out that plants increase their adversity adaptation capacity due to compound stresses of different adversities. Therefore, the opening and closing time and speed of plant leaf stomata during this period need to be further studied. Furthermore, researchers have demonstrated that one strategy to improve plant tolerance to heat and drought stress is to increase transpiration by improving stomatal size, which reduces leaf temperature and thus improves the cooling and water retention capacity of leaves [53]. In this study, with the prolongation of stress time, T_r showed a trend of increasing and then

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decreasing under heat and drought co-treatment, and the change was not significant under 21 ℃ treatment on 6 days; instead, it also increased C_i; The increase in temperature leads to the development of palisade tissue and the reduction of sponge tissue, which is also a response to water shortage. This facilitates the transport of CO_2 from stomata to photosynthesis sites and also counteracts the low CO_2 transport rate due to stomatal closure and altered leaf structure, thereby increasing water utilization by the plant, which also reflects the plant's ability to adapt to heat and drought stresses [54]. Chlorophyll is the main pigment for photosynthesis [55]. Studies have shown that a certain degree of drought or salt stress can lead to the destruction of chloroplast structure in plant leaves [56]. Similar results were also observed in the present study, where Chl a+b content decreased under temperature, drought, and their combined treatments as the duration of stress increased. After 18 days of stress, the leaves of potato seedlings under heat and drought treatments were light green with yellow spots (Fig. 1). Moreover, because the environmental stimulation of heat and drought interferes with the main sites in photosynthesis and cannot normally work when the duration of stress is longer, the content of each Chl is lower, which in severe cases can lead to the death of the plant [57]. But under combined stress, the main limiting factor of photosynthesis shifts to mesophyll conductance [58]. These findings emphasize the importance of mitigating the effects of compound climate extremes on crop productivity by targeting mesophyll conductivity and improving dynamic photosynthesis. In this study, the photosynthetic system was damaged under the heat compound drought treatment, which may be attributed to the closure of stomata at heat, which prevented CO_2 from entering the mesophyll cells and impaired photosynthesis, as well as affecting the energy balance of redox [59]. Overall, the effects of the combined stress of heat and drought on photosynthesis are thought to be synergistic, as stomatal closure impairs carbon fixation and leads to energy excess, which enhances photodamage to photosystems [5].

It is well known that harsh environments often lead to an increase in ROS in plant leaves, in which SOD is considered to have an important role in the plant's adversity tolerance and is the first line of defense against ROS toxicity [60]. APX is also thought to play an important role in protecting cells of higher plants, algae and other organisms from ROS damage [61]. In the present study, it was found that the SOD and APX activities of potato leaves showed an increasing and then decreasing trend with the prolongation of stress time. Increased leaf APX activity has been reported in mustard [62] and wheat [63] under Cd stress. CAT is an enzyme that contains tetramer heme and can break down hydrogen peroxide directly into water and oxygen, which is indispensable for ROS detoxification under stress conditions [64]. Simova-Stoilova et al. [65] reported that CAT activity was elevated in wheat under drought stress, but was higher in sensitive varieties. It was found that salt stress decreased the CAT activity of glycyrrhiza seedlings under the combined stress of salt and drought [66]. In this study, Under the stress of heat and drought for a short period of time (at the 6th day), the cells entered the state of oxidative stress, and the activities of SOD, POD, CAT and APX showed an increasing trend, which was consistent with the research results of Askim et al. [67]. Sharma and Dubey [68] found that chloroplast APX activity was higher in plants than in control plants under mild drought conditions, but decreased under severe drought stress. It suggests that the combined stress of heat and drought increases ROS scavenging capacity and antioxidant enzyme activity, which in turn improves resistance to adversity [61], However, with prolonged stress, plant survival depends on cellular adaptation, resistance to stress, and the ability to repair or replace damaged molecules [69].

MDA is one of the products of membrane lipid peroxidation, and its content can represent the degree of membrane lipid peroxidation and indirectly reflect the antioxidant capacity of plant tissues [70]. In this study, MDA content gradually increased with the stress time (except for CK), indicating that under prolonged heat and drought stress, the degree of lipid peroxidation of cell membranes exceeded the ability of seedlings to tolerate, resulting in damage to the structure and function of cell membranes and altering in membrane permeability, thus affecting a series of physiological and biochemical reactions. This is in agreement with previous findings on ginger lotus [71]. At the same time, the REC increased under the combined stress in this study, indicating that membrane lipid peroxidation caused a large amount of electrolyte extravasation from plant cells, and plant tissue was seriously damaged [72].

Slow plant growth is not only a passive consequence of an unfavorable environment but also an active slowing of growth to adapt to stressful conditions, and this "active" growth inhibition is achieved through stress-triggered cellular signaling. SP, SS, Pro and Tre are important osmoregulatory substances in plants and play an important role in plant stress [73]. Total carbohydrates are SS that act as osmotic pressure, and higher production of soluble carbohydrates is thought to signal metabolic regulation under drought stress [74]. In addition to its role in osmoregulation, the increase in Pro and total carbohydrate accumulation under drought stress is also intended to protect corn plant tissues from oxidative damage by scavenging free radicals [75]. In this study, the SP and

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SS contents of potato seedlings under heat treatment were first increased and then decreased. The short-term external stress treatment promoted the accumulation of SP and SS contents in the leaves of potato seedlings under heat and improved the osmotic adjustment ability of seedlings, thus alleviating the damage caused by heat, while the prolonged stress still reduced the osmotically regulated substances and caused irreversible effects [76]. In the present study, after 6 days of stress, the Pro content was significantly increased in all heat treatments, with an average increase of 45.26%. This phenomenon is similar to the performance of maize leaves under adverse circumstances [77]. However, after 6 to 18 days of stress, the Pro content gradually decreased; this is consistent with the study of Xue Xingning et al. [78]. Pro and Tre biosynthetic pathways are activated and inhibit their catabolism during dehydration, whereas sustained hyperthermia and drought are modulated in the opposite direction [79, 80].

According to the statistical analysis of each physiological index, heat, drought, stress time, and their interactive tests have extremely significant effects on SOD, MDA, Pro, C_i、G_s and T_r. Depending on further analysis of the correlation coefficients, REC was highly significantly negatively correlated with SOD activity, Same results as Anna et al. [81] study, antioxidants counteract the accumulation of ROS, thereby reducing electron loss from the leaves. In this study, the antioxidant enzyme activity increased after 6 days of stress, and the content of osmoregulatory substances increased, which to some extent scavenged the ROS produced by potato seedlings under stress, thus transiently adapting to the heat and drought stress. However, with the prolongation of the stress time, the antioxidant enzyme activity and the content of osmoregulatory substances decreased to different degrees. It has also been shown that adversity exacerbates the increase in MDA content in plants and that enzymatic and non-enzymatic antioxidant defense systems regulate overall ROS levels to maintain physiological homeostasis. Elevated ROS levels may also constitute stress signals with specific redox-sensitive signaling pathways that, once activated, may have destructive or potentially protective functions, resulting in a sharp decrease in osmoregulatory substances (SP, SS, Pro, Tre) levels [69, 82].

Conclusions

In this study, 15 physiological indexes of potato seedlings' Atlantic 'varieties were compared, analyzed, and comprehensively evaluated through heat, drought and combined heat and drought treatment, which deepened the understanding of the physiological and biochemical mechanisms of potato seedlings' resistance to heat and drought. Compared with single heat or drought stress, Under the combined stress of heat and drought for 18 consecutive days, the phenotype of potato seedlings changed significantly, the leaf edges curled and wilted, the leaf thickness increased, and the number of chloroplasts in the palisade tissue decreased significantly. The photosynthetic efficiency of potato seedlings decreased; The chlorophyll content showed a decreasing trend with the combined stress, which further indicated that the photosynthetic system of potato seedlings was damaged under the combined stress of heat and drought, thus reducing their production efficiency. In the compound stress, the antioxidant enzyme activity and osmoregulatory substance content had a rising trend after 6 days of stress, indicating that potato seedlings, on the one hand, obtained higher antioxidant capacity by decreasing the content of reactive oxygen species, thus reducing oxidative stress and protecting biomolecules from oxidative damage. On the other hand, by promoting the production of plant regulatory factors Pro and Tre, activating the signaling pathways related to heat and drought, improving the osmoregulatory capacity, and protecting the cellular structure and cell membranes from damage, the adaptive ability of potato seedlings to heat and drought stress was improved.

In summary, we believe that potatoes mainly respond to heat, drought and combined stress by regulating the activity of antioxidant enzymes and osmotic regulatory substances. These insights can help improve the modeling of sessile plants' response to climate change and expand our understanding of changes in plant dynamics observed in hot, arid environments. It is a well-known goal to improve crop stress resistance and reduce the negative effects of abiotic stress. If the combination of heat and drought priming at the seedling stage for a short time can alleviate the impact of sudden adverse environmental factors on potato growth at the later stage of growth, it will be the research direction for us to continue to explore.

Abbreviations

P_n	Net photosynthetic rate
C_i	Intercellular CO_2 concentration
G_s	Stomatal conductance
T_r	Transpiration rate
Chl a	Chlorophyll a
Chl b	Chlorophyll b
Chl a+b	Chlorophyll a+b
SOD	Superoxide dismutase
POD	Peroxidase
CAT	Catalase
TPX	Thioredoxin peroxidase
MDA	Malondialdehyde
REC	Relative conductance
SP	Soluble protein
SS	Soluble sugar
Pro	Proline
Tre	Trehalose

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Availability of data and materials
All data obtained from the current study are available from the corresponding author on a reasonable request. No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All co-authors have seen and agreed on the contents of the manuscript, and there is no financial interest to report.

Competing interests
The authors declare there are no conflicts of interests.

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