Contribution of Mitochondrial Structure and Respiratory Metabolism to The Cold-Resistance of Alfalfa Seedling Root

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Contribution of mitochondrial structure and respiratory metabolism to the cold resistance of alfalfa seedling root

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Abstract:

Background: Fall dormancy of alfalfa is significantly associated with its cold tolerance, while root, the main body of alfalfa for overwintering, is critical for the cold resistance of alfalfa. The effect of low temperature on mitochondrial ultrastructure and respiratory metabolism of alfalfa seedling root with different fall dormancy was examined, to study the root cold resistance mechanism by which fall dormancy affects alfalfa cold tolerance. **Results:** Low temperature induced mitochondrial swelling, and the decline of ATP and accumulation of H_2O_2 in alfalfa seedling root. Both the Cytochrome pathway (CP) and Alternative pathway (AP) respiratory rate were restrained and mETC complex I, II, III and IV activities were inhibited directly by low temperature in both kinds of alfalfa seedling root, while the decline of mETC complex II and III activities were more serious in Gannong No. 5. These results indicated that the damage of mitochondrial structure and the inhibition of mETC complex I, II, III and IV activities directly by low temperature declined the ATP synthesis and aggravated the ROS

accumulation, which inhibit the growth of alfalfa seedling root. Moreover, the lower damage on mitochondrial structure and mETC complex II, III activities and higher the percent of AP to total respiratory rate lead to the lower ATP lack and H_2O_2 accumulation, which contributed to the root growth of Xinmu No.4 seedling. **Conclusions:** Low sensitivity of mitochondrial structural stability and mETC complex II, III and Alternative respiration to low temperature contributed to the root cold resistance of alfalfa with low fall dormancy grade.

Key words: low temperature; mitochondria; respiratory electron transport chain; fall dormancy; alfalfa seedling root

Background

Alfalfa (*Medicago sativa* L.), a perennial legume forage, is an important forage crop in many countries around the world，especially in China. However, in northern China, extreme weather, such as frost and cold tide in spring and low temperature in winter, often occur. The growth of alfalfa is threaten by cold and frost damage, and the alfalfa turning green would be significantly affected, which results in the drop production of alfalfa.

Fall dormancy is defined as reduced growth during the fall that comes with reducing day length and temperature (Malinowski et al., 2007), and is correlated with improved winter survival in alfalfa, It is reported that, fall dormancy was significantly associated with cold tolerance (Wu et al., 2011). It is classified into three groups: dormant (1-3), semi-fall dormant (4-6), and non-dormant cultivars (>6) (Malinowski et al., 2007), among them, dormant cultivars produce short and prostrate shoots in autumn, exhibit slow stem elongation after summer harvest, and possess high winter hardiness and cold tolerance. While, non-dormant cultivars grow vigorously in autumn, forming long erect shoots, and resume rapid shoot elongation after cutting in summer and autumn, but own low winter hardiness and cold tolerance (Dhont et al., 2002; Rina

et al., 2011).

The root is an important organ for absorbing, transporting, storing nutrients and water, directly affects plant regeneration. Its germination is important for alfalfa to overwinter and turn green. And the cold tolerance of alfalfa is closely related to its root (Zhang et al., 2003). At present, research about alfalfa root after low temperature mainly focuses on the development of root morphology (Nan et al., 2012), the relationship between root characteristics and environment factors, such as atmospheric CO_2 (Bertrand et al. 2007) and Rhizobium Symbiosis (Liu et al.,2019). Duke and Doehlert (1981) reported that root functions (respiration, the ability to nodulate, and levels of various enzyme activities) increase to a greater extent or are more pronounced in hardy cultivars compared to those with lesser hardiness during the hardening process. While, Walton(1974) showed that those cultivars which showed greater frost hardiness under field conditions gave higher tissue impedance values and greater cell survival in the presence of sucrose than did the frost-susceptible cultivars. These reports indicated that energy metabolism of alfalfa root is important for its hardiness in low temperature.

Respiration is the only source of energy in alfalfa root without chloroplasts. It is also one of the most important physiological processes for life, and an important indicator for measuring the strength of life activities. Mitochondria, the hub of cellular material metabolism and energy metabolism, plays an important role in the process of plant growth metabolism (Saks et al., 2007), and is also one of the main damage sites of stress (Jones, 2000; Lam et al., 2001). Mitochondria electron transport chain complexes are located in the mitochondrial inner membrane. Mitochondrial enzyme complexes interact and coordinate with each other to complete electron transfer and energy conversion. Only if all the structures and functions of enzyme complexes are intact and collaborative can mitochondria guarantee normal respiratory electron transport and ATP synthesis (Millar et al., 2001). So it is important to maintain the mitochondrial function for growth of alfalfa root under stress condition.

Here, the effects of low temperature stress on seedling root mitochondrial ultrastructure and respiratory metabolism of alfalfa with different fall dormancy were analysed by artificial temperature and light conditions. The changes of root vitality, ATP content and reactive oxygen species (ROS) in alfalfa seedling root were studied at low temperature, and the effect of low temperature on mitochondrial respiration, including different respiratory pathways and the mECT complexes activities of alfalfa seedling root were analysed. The effect of low temperature on the structure and function of root was explored to enrich the theoretical system between alfalfa's fall dormancy and cold resistance, and provide new ideas for genetic improvement of cold-resistant alfalfa varieties and breeding of cold-resistant varieties in the future.

Results

1 Effect of low temperature on growth, vitality of alfalfa seedling root

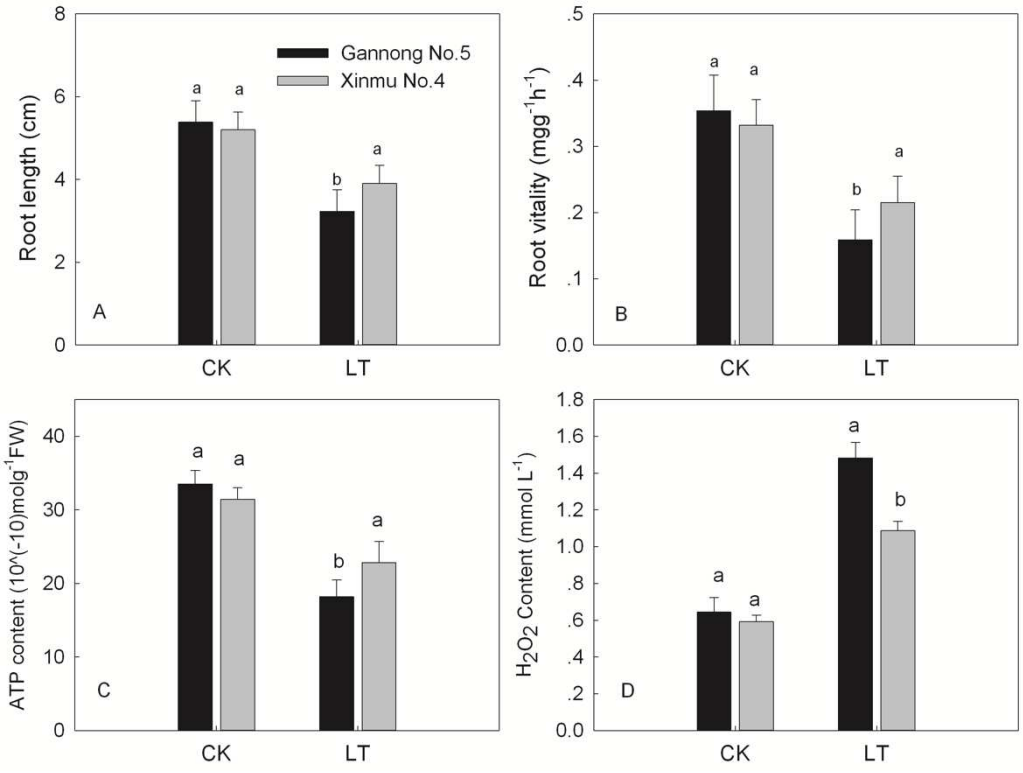


Fig. 1 Effect of low temperature on growth, vitality of alfalfa seedling root. The length (A), vitality (B), ATP(C) and H_2O_2 content (D)of alfalfa seedling root after low temperature treatment. Data are the means

± SE of 3-5 independent measurements.

The growth of alfalfa root was seriously inhibited by LT in both kinds of alfalfa seedling, indicated by the decrease of root length after the LT treatment. And the suppression of LT on growth in Gannong No.5 was more serious than that in Xinmu No.4(Fig. 1A). Moreover, LT caused a significant decrease in root vitality in both kinds of alfalfa seedling, and the decline of root vitality in Gannong No.5 was more serious than that in Xinmu No.4 (Fig.1B), which indicated that low temperature not only slowed root growth but also inhibited the root vitality.

ATP is the only energy source that maintains root vitality and growth. Then the ATP was tested to explore the mechanism of low temperature slow the root growth.

2 Effect of low temperature on ATP and ROS content of alfalfa seedling root

LT caused the depletion of ATP in both kinds of alfalfa seedling root, and after the treatment for 72h, the ATP content declined to 27% of the control group in Xinmu No.4, while the decline of ATP in Gannong No.5 was 45%(Fig. 1C). Simultaneously, after LT treatment, intracellular H_2O_2 content significantly increased, approximately 1.6 times of the control group in Xinmu No.4, while about 2.3 times in Gannong No.5(Fig. 1D). These results showed that low temperature treatment induced ROS accumulation.

Both the ATP synthesis and ROS production depend on the respiration metabolism, so respiration metabolism was targeted to explored the reason of ATP depletion and the production site of ROS, by which clarify the mechanism that low temperature inhibited the root growth.

3 Effect of low temperature on mitochondrial ultrastructure in alfalfa seedling root

The biological function of mitochondria is inseparable from the specific internal structure. Mitochondrial swelling degree is a hallmark of mitochondrial dysfunction[21]. The results showed that, the root mitochondrial structure of alfalfa was complete, the inner mitochondrial membrane was folded into cristae that permeate the soluble, internal matrix, and the intervals of mitochondrial cristae was obvious, both in gannong

No.5 and Xinmu No.4 seedling root under RT (Fig. 2A,B). After the LT treatment, the mitochondrial volume became larger, transparent blank parts appeared in the matrix, the original ordered specific structure was destroyed, intervals of mitochondrial cristae expanded, part of them disappeared, and increase of mitochondrial swelling degree was observed in LT-treated mitochondria as showed in Fig. 2C,D. Moreover, the increase of mitochondrial swelling degree was more serious in Gannong No.5than that in Xinmu No.4 (Fig. 2). This suggested that low temperature destroyed the mitochondrial integrity in alfalfa seedling root.

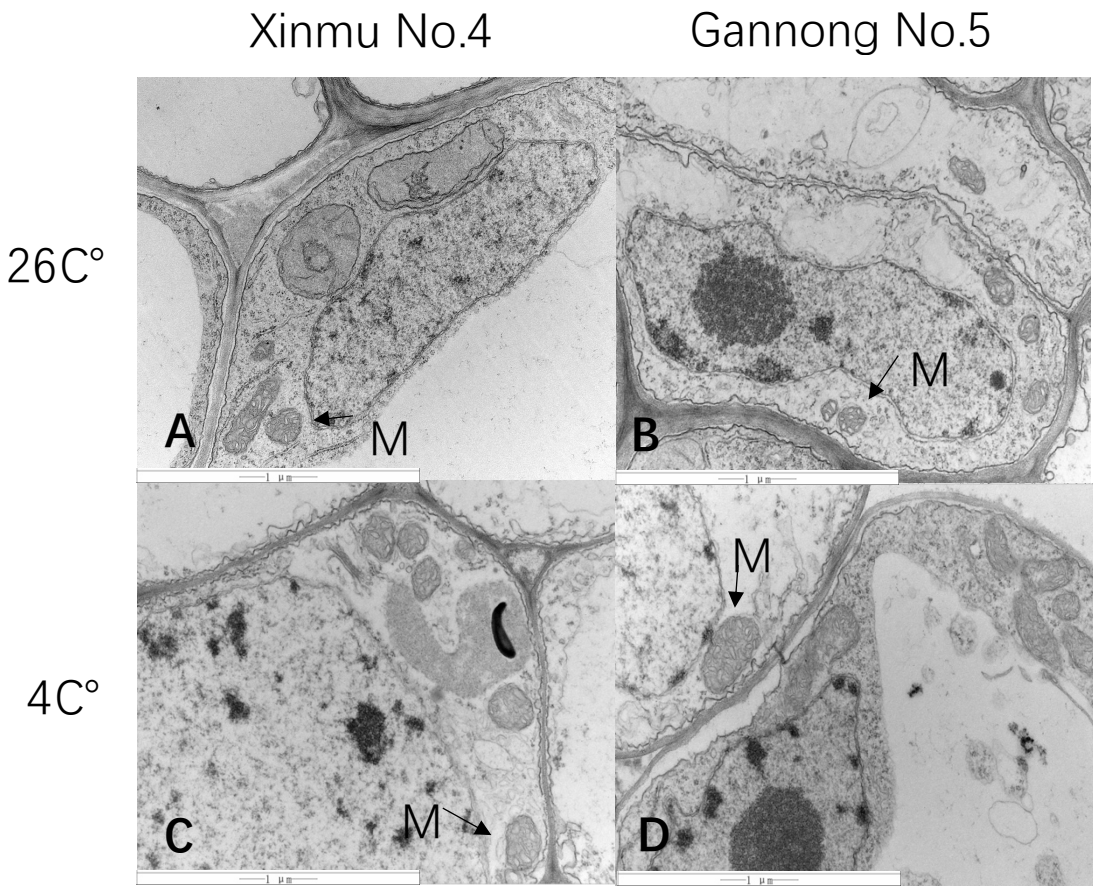


Fig. 2 Effect of low temperature on alfalfa seedling root mitochondrial ultrastructure(30000×). The mitochondrial structure of Xinmu No.4 under 26℃(A)and 4 ℃(C), of Gannong No.5 under 26℃(B)and 4 ℃(D) . M：Mitochondria

4 Effect of low temperature on total respiration via CP and AP capacity in alfalfa

seedling root

The effect of low temperature on total respiration, CP and AP capacity in alfalfa was studied. LT treatment decreased the total respiration and CP and AP capacity in alfalfa seedling root both in Gannong No. 5(Fig. 3A) and Xinmu No.4(Fig. 3B). The respiration inhibition was mainly resulted from the Cytochrome respiration decreased by LT in alfalfa seedling root, and the decline of respiration was more serious in Gannong No. 5 than in Xinmu No.4(Fig. 3C).

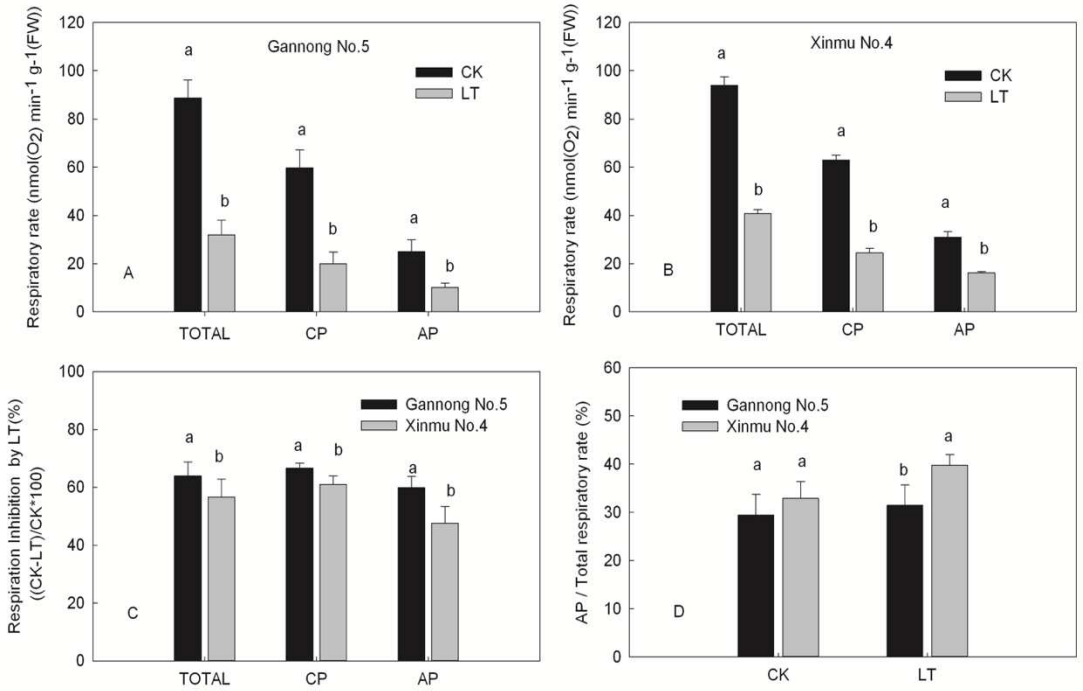


Fig. 3 Effect of low temperature on respiration in alfalfa seedling root. The respiratory rate of total respiration, CP and AP pathway capacity in Ganong No.5 (A) and Xinmu No.4(B) seedling root, the respiration inhibition by low temperature analysed by (CK-LT)/CK*100(C), and the percent of AP pathway to the total respiration (D) in alfalfa treated with low temperature. Data are the means ± SE of 15-20 independent measurements. Letters represent values that differed significantly between different alfalfa in the LSD range test ($P < 0.05$).

Alternative oxidase oxidizes ubiquinone directly and reduces oxygen to water, bypassing the two coupling sites of complexes III and IV of the cytochrome electron transport chain. The percent of AP to total respiration shows the electron flow through the AP, by which, alternative respiration could relieve the over-reduction of respiratory

electron transport chain and reduce the production of reactive oxygen species (ROS) efficiently. After the LT treatment, the percent of AP to total respiration increased, especially in Xinmu No.4, though the AP respiratory rate decreased (Fig. 3D). Thisresult indicated that the respiration was inhibited by low temperature, which was more serious in Gannong No. 5 seedling root, while the increased percent of AP to total respiration may contribute to the less ROS accumulation in Xinmu No.4 seedling root.

5 Effect of low temperature on the activities of complexes I, II, III, IV and ATP synthase of alfalfa seedling root mitochondria

To further study the attack sites of low temperature in the electron transport chain, the activities of the five complexes in the mECT were measured. There was obvious inhibition in the activities of complexes I, II, III, IV, except ATP synthase in the mitochondria of alfalfa with LT treatment both in Gannong No.5(Fig. 4A) and Xinmu No.4(Fig. 4B). Furthermore, the decrease of complexes II and III activities were more serious in Gannong No.5 than that in Xinmu No.4 (Fig. 4C). These results indicated that low temperature affected the activities of complexes, especially complexes I, II, III, IV of alfalfa seedling root mitochondria, which subsequently inhibited alfalfa mitochondrial respiration. Moreover, the activities complexes in the mECT, especially complexes II and III of Gannong No.5 seedling root was more sensitive to low temperature than that in Xinmu No.4.

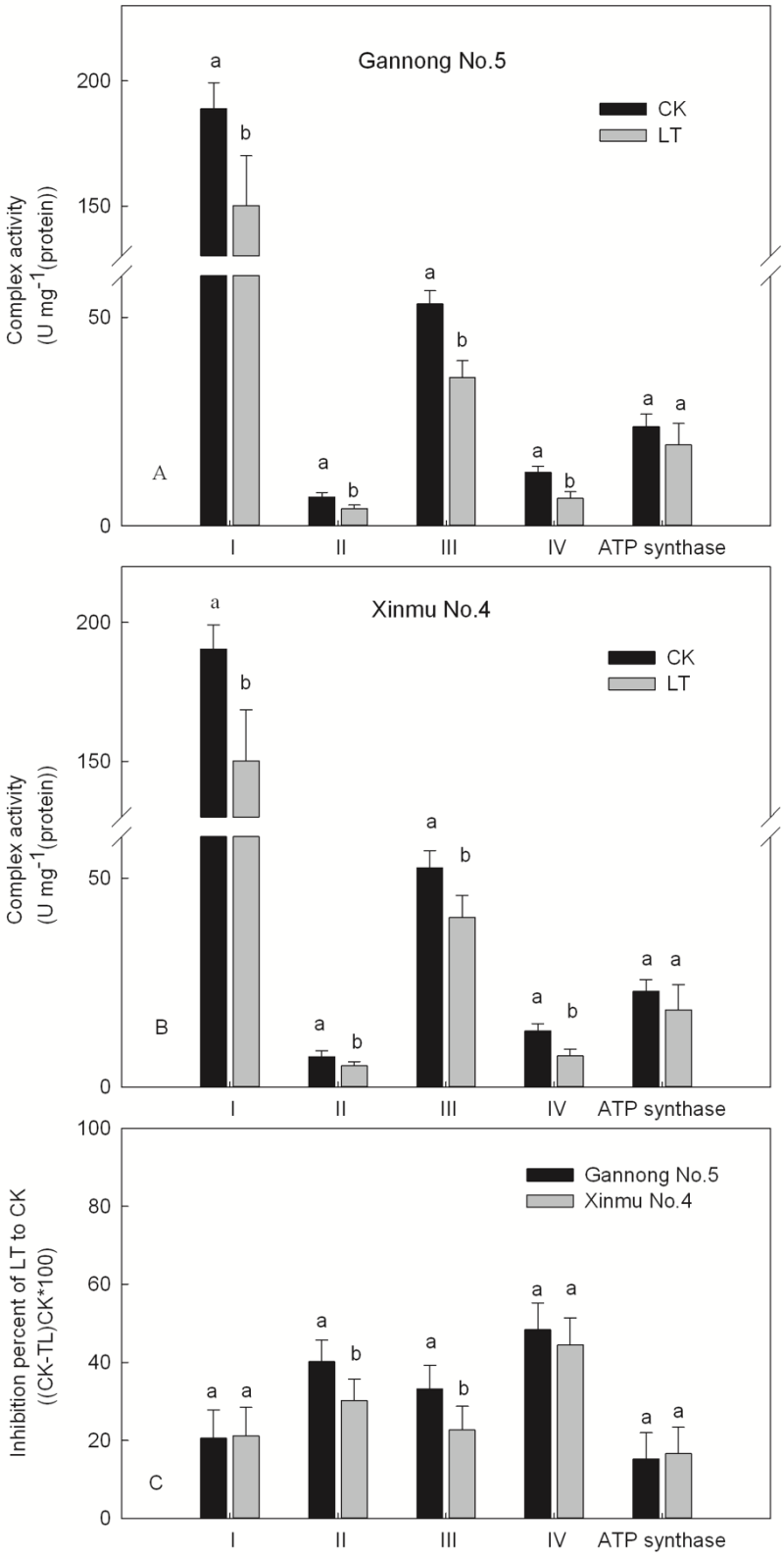
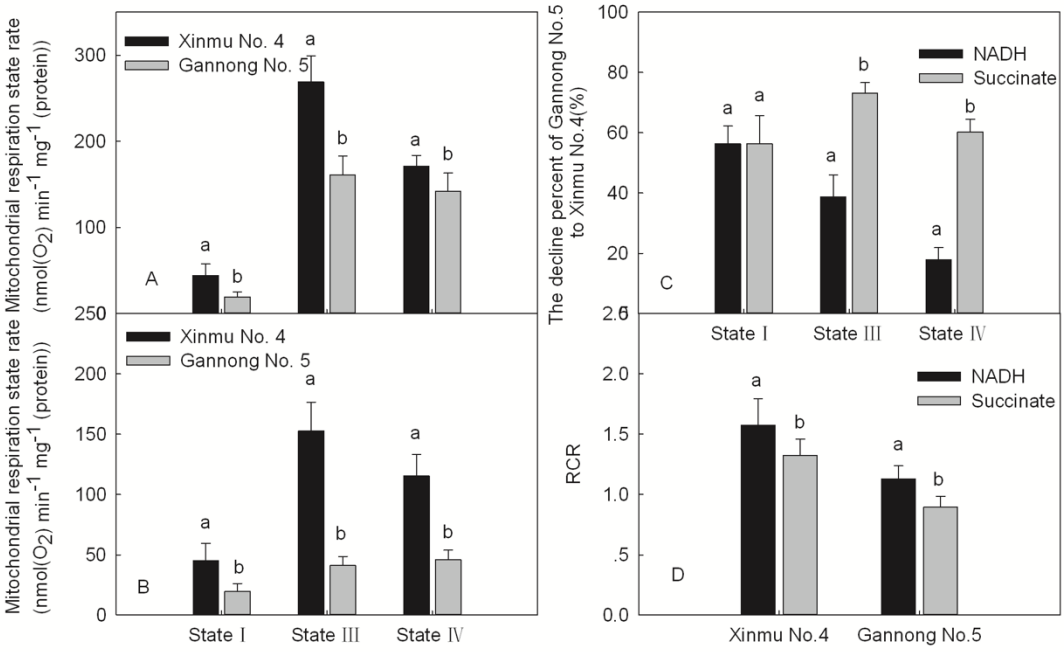


Fig. 4 Effect of low temperature on the complexes activities of alfalfa seedling root mitochondria. The activities of complexes in Gannong No.5(A) and in XInmu No.4(B). The inhibition percent of low temperature to CK(C). Data are the means ± SE of 15-20 independent measurements. Letters represent

183 values that differed significantly in the LSD range test ($P < 0.05$).

184 **6 Effect of low temperature on the respiration state of alfalfa seedling root**
185 **mitochondria**



186

187 **Fig. 5** Effect of low temperature on the respiration state of alfalfa seedling root mitochondria.
188 Respiration states of root mitochondria when NADH (A) and succinate (B), respectively,
189 are used as substrate after LT treatment, and the decline percent of Gannong No.5
190 respiration state compared to Xinmu No.4 respiration state analysed by (Xinmu No.4 -
191 Gannong No.5)/ Xinmu No.4 *100 with different substrates after LT treatment (C). The
192 respiration control rate (RCR) with different respiration substrates after LT treatment (D).
193 The data are the means ± SE of 15-20 independent measurements. Letters represent
194 values that differed significantly in the LSD range test ($P < 0.05$).

195 Mitochondrial respiration states were measured to assess the effect of LT
196 treatment on respiratory electron transport. The results showed that the rates of
197 respiration state I, state III, and state IV of Gannong No.5 seedling root mitochondria
198 were more significantly inhibited than that in Xinmu No.4, whether NADH (Fig. 5A) or
199 succinate (Fig.5B) was used as a respiratory substrate. Compared to NADH, when

succinate was the respiratory substrate, the rates of respiration state I, state III and state IV were declined more seriously in Gannong No.5 after LT treatment (Fig. 5C), which indicated that mitochondrial electron transport based on complex II was more sensitive to low temperature in Gannong No.5 than that in Xinmu No.4.

RCR is used to assess the coupling degree of mitochondrial electron transport and oxidative phosphorylation. The results showed that after LT treatment, the RCR of Gannong No.5 were decreased more seriously than that in Xinmu No.4, both with the NADH and succinate as substrates, among them, the decline of RCR was greater when succinate was used as a substrate compared to NADH ((Fig. 5D). These results indicated that mitochondrial electron transport based on complex II was decoupled oxidative phosphorylation, and limited ATP synthesis in Gannong No.5 after LT treatment.

The activities of complexes I, II, III, IV were declined both in Gannong No.5 and Xinmu No.4 after LT treatment, but the less damaged complex I still contributed to the transmembrane proton gradient that is used to synthesize ATP to support cell alive.

Discussion

This study demonstrated that the damage of mitochondrial structure and the inhibition of complex I, II, III and IV activities in the mETC by low temperature restrained respiratory metabolism, declined the ATP synthesis and aggravated the ROS accumulation, which inhibited the growth of alfalfa seedling root.

Low temperature induced mitochondrial swelling (Fig. 3A), which led to the inner and outer mitochondrial membranes into closer proximity, increased the contact sites, and negatively affected mitochondrial electron transport and oxidative phosphorylation (Halestrap et al., 2000). Saeki et al. (2008) reported that lipid bilayers and contents mixed, and resonance energy transferred between aggregated mitochondria (Françoise et al., 1995). Low temperature might increase the surface charge density of the mitochondria, and result in repulsion between mitochondria (Saeki et al., 2008), which then reduces the aggregation degree of the mitochondrial membrane. The

destroyed structure leads to mitochondrial dysfunction.

Low temperature not only damaged the mitochondrial structure, but also inhibited the activities of mitochondrial complexes I, II, III and IV(Fig. 5), which inhibited proton transport to the intermembrane space (Leeuwen et al., 2011); or induced proton electrochemical gradient across the mitochondrial membrane to drive proton leak and not involve ATP synthesis (Porter et al., 1999), so that proton electrochemical gradient across the mitochondrial membrane was not enough to drive the ATP synthase to complete ATP synthesis, eventually led to the ATP depletion (Fig. 2A), while the ATP synthase had not been damaged (Fig. 5). ATP is the direct energy for cell metabolism and is also an important signalling molecule. The shortage of energy in the cells inhibited cell growth.

Moreover, mitochondria are key place of reactive oxygen species (ROS) production. Low temperature blocked the respiration mETC and then increased the leak of electrons from the mETC. In addition, the AP is efficiently to reduce the production of ROS (Kornfeld et al., 2013; Zhang et al., 2012). Therefore, the increase of ROS was aggravated by the inhibition of low temperature to AP activity or capacity, which indicated by the result that the higher decline of alternative respiration in Gannong No.5 showed higher H_2O_2 content. All of these mechanisms were involved in the accumulation of ROS in alfalfa treated by low temperature. The accumulation of ROS would damage cell membrane lipids, proteins and nucleic acids, eventually leading to cell death (Pospíšil, 2012). The excessive accumulation of ROS caused by the inhibition of the mitochondrial electron transport by low temperature may be another important mechanism by which low temperature inhibits alfalfa seedling root growth.

Alfalfa fall dormancy is an adaptive response of alfalfa to changes in autumn environment. Studies have shown that the cold resistance of alfalfa is related to the fall dormancy level. The higher the fall dormancy level, the lower the cold resistance (Wu et al., 2011). In this study, the seedling root mitochondrial structure and function of the

Gannong No. 5 alfalfa with high fall dormancy grade were highly sensitive to low temperature. This may be one of the reasons why alfalfa's cold resistance is lower due to the damage caused by low temperature to alfalfa seedling root. In addition, the lower decline of alternative respiration may contribute to the resistance of low temperature in Xinmu No.4. with low fall dormancy grade.

Conclusions

In a conclusion, by damaging the mitochondrial structure and inhibiting mECT complex I, II, III and IV activities directly, low temperature inhibited the respiration, reduced ATP synthesis, this is the main cause for low temperature to inhibit alfalfa seedling root growth. The higher sensitivity of root mitochondrial structure and mECT complex II, III activities to low temperature, which aggravated by higher decline of alternative respiration, led to the lower cold-resistance of alfalfa with higher fall dormancy grade.

Methods

1 The alfalfa growth and low temperature treatment

1.1 Growth of alfalfa

The different fall dormancy alfalfa were tested in this experiment. *Medicago sativa* L. Xinmu No. 4 with fall dormancy 2 was acquired from Department of Grassland Science, Xinjiang Agricultural University, and *Medicago sativa* L. Gannong No. 5 with fall dormancy 8 was acquired from College of Grassland Science, Gansu Agricultural University. The culture was carried out at a constant temperature of 26 °C in the culture chamber, and the nutrient solution was poured 3 times a week to promote its growth.

1.2 Low temperature treatment of alfalfa

When the plants grow to the seedling stage (after 2 week growth period), the whole plants with the same growth are transferred to the artificial climate incubator, and treated at room temperature 26°C (RT) and low temperature 4°C (LT), respectively. After 72h of treatment, the root samples are taken and each sample is determined.

2 Experimental methods

2.1 Measurement of root vitality

Take 0.2g the root tip of the plants with different treatment, use the filter paper to dry the surface water, and put them into a petri dish to test the root vitality by TTC method (Dorota, 2010). The dehydrogenase activity in root is represented by the reduction of TTC, which would be used to reflect the root activity.

2.2 Measurement of intracellular H_2O_2 content

Intracellular H_2O_2 was extracted and analysed according to Patterson et al. (1984). Fresh weight (0.3 g) of alfalfa root were ground and extracted with 5 mL of 5% (w/v) trichloroacetic acid (TCA) and centrifuged at 12 000 g for 10 min. The supernatant was used for the H_2O_2 assay. The results presented were the means of 3-5 independent measurements.

2.3 Measurement of root respiratory rate

The effects of low temperature on root respiration were examined by measuring the oxygen consumption in alfalfa root with an Oxytherm oxygen electrode (Hansatech, UK). Fresh weight (0.5 g) of alfalfa root in 1 mL of assay buffer and the required amount of low temperature mother liquor added to Oxytherm oxygen electrode incubation chamber (final concentration was 0.2 mmol·L-1) were used to examine oxygen consumption at 25 ºC. In this experiment, AP capacity and CP capacity were measured in the presence of KCN and salicylhydroxamic acid (SHAM), respectively (Yip and Vanlerberghe, 2001; Robson and Vanlerberghe, 2002).

2.4 Measurement of mitochondrial ultrastructure

The samples were fixed in 2.5% glutaraldehyde diluted in phosphate buffer (0.1 M, pH 7.3) and the washed in phosphate buffer and post-fixed in 1% osmium tetroxide also diluted in phosphate buffer. The samples were washed in distilled water and placed on uranyl acetate 0.5% for 2h, after which, the material was dehydrated in an increscent series of acetone (50-100%), transferred to a mixture of acetone and 100% resin Araldite^{TM} (1:1) and left overnight. After incubation (37°C for 1h), the samples were embedded in the appropriate Araldite^{TM} resin molds and incubated at 60°C for

48h. The cuttings were made with an ultrafine diamond knife microtome Ultracut (Leica™) and sections were examined and photographed in a Philips CM-100TM electron microscope(Borgo et al., 2015).

2.5 Measurement of ATP content

ATP was quantified according to Liu et al.(2012) and Chen et al.(2013). A total of 0.5g of alfalfa seedling root was homogenized in 5ml boiling water, the homogenate incubated at 37 °C for 30 min, followed by centrifugation at 4000 rpm for 10 min to obtain the supernatant. An ATP analysis kit (A095) was purchased from the Chengjian Bioengineering Institute (Nanjing, China) and the ATP content was measured at 636nm according to the manufacturers.

2.6 Measurement of mECT complexes activities and respiration states

The root mitochondria from alfalfa seedling was isolated according to Martin et al.(2011). Alfalfa seedling roots were harvested in the buffer (0.4M Mannitol, 1mM EGTA, 0.1% BSA, 50mM Tricin, 20mM β-mercaptoethanol, 1% w/v Polyvinylpyrrolidone, NaOH pH 7.8) and homogenized using a Polytron blender (9500 min-1). The homogenate was filtered through cheesecloth and nylon net. The filtrate was centrifuged twice and the resulting pellet was resuspended in wash media [0.4M Mannitol, 10mM MOPS (KOH), 1mM EGTA, 0.1% BSA, pH 7.8 with NaOH] and loaded on top of Percoll step gradient (40, 28, and 20% (v/v) Percoll). After centrifugation, the enriched mitochondria diluted in 3 volumes of mannitol wash media and centrifuged at 11,500 rpm. The pellet was resuspended in sucrose wash media [0.3M Sucrose, 0.1% (w/v) BSA and 10mM TES- NaOH, pH 7.5 and loaded on top of a 28% Percoll gradient (Millar et al., 2001). After centrifugation mitochondrial band near the top of the gradient was collected and concentrated by two successive centrifugations. The final pellet was suspended in mannitol wash media (Teodoro et al., 2015).

The mECT complexes activities were measured according to the methods of the instructions of the activities test kit of complexes I, II, III, IV and V in the mitochondrial respiratory electron transport chain (Cominbio, Chain). The respiration states were

340 measured according to Liu et al.(2014).

341 3 Statistical analyses

342 Least significant difference (LSD) was used to analyse differences between the
343 different treatments by using SPSS 11.5.

344 **Abbreviations**

345 Cytochrome pathway : CP; Alternative pathway :AP; Low temperature : LT

346 **Declarations**

347 **Ethics approval and consent to participate:** Not applicable

348 **Consent for publication:** Not applicable

349 **Availability of data and materials:** All data generated or analysed during this study are
350 included in the manuscript.

351 **Competing interests:** The authors declare that they have no conflicts of interest with the
352 contents of this article.

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357 analyses, and interpretation of data; in the writing of the manuscript, and in the decision to
358 publish the results.

359 **Authors' contributions:** Meijun Liu conducted most of the experiments, analyzed the
360 results, and wrote most of the paper. Wenjing zhao and Haoyang conducted experiments
361 of mitochondrial ultrastructure and respiration rate. Zhang Xiaoqing Sui and Yuxiang Wang
362 conducted experiments searching for mitochondrial respiratory state rate and ATP assay.

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466 ## Figure Legends

467 **Fig. 1 Effect of low temperature on growth, vitality of alfalfa seedling root.**

468 The length (A), vitality (B), ATP(C) and H_2O_2 content (D)of alfalfa seedling root after low temperature
469 treatment. Data are the means ± SE of 3-5 independent measurements.

470 **Fig. 2 Effect of low temperature on alfalfa seedling root mitochondrial ultrastructure(30000×).**

471 The mitochondrial structure of Xinmu No.4 under 26℃(A)and 4 ℃(C), of Gannong No.5 under 26℃(B)and
472 4 ℃(D) . M：Mitochondria

473 **Fig. 3 Effect of low temperature on respiration in alfalfa seedling root.**

474 The respiratory rate of total respiration, CP and AP pathway capacity in Ganong No.5 (A) and Xinmu
475 No.4(B) seedling root, the respiration inhibition by low temperature analysed by (CK-LT)/CK*100(C), and
476 the percent of AP pathway to the total respiration (D) in alfalfa treated with low temperature. Data are the
477 means ± SE of 15-20 independent measurements. Letters represent values that differed significantly
478 between different alfalfa in the LSD range test ($P < 0.05$).

479 **Fig. 4 Effect of low temperature on the complexes activities of alfalfa seedling root mitochondria.**

480 The activities of complexes in Gannong No.5(A) and in XInmu No.4(B). The inhibition percent of low
481 temperature to CK(C). Data are the means ± SE of 15-20 independent measurements. Letters represent

values that differed significantly in the LSD range test ($P < 0.05$).

Fig. 5 Effect of low temperature on the respiration state of alfalfa seedling root mitochondria.

Respiration states of root mitochondria when NADH (A) and succinate (B), respectively, are used as substrate after LT treatment, and the decline percent of Gannong No.5 respiration state compared to Xinmu No.4 respiration state analysed by (Xinmu No.4 - Gannong No.5)/ Xinmu No.4 *100 with different substrates after LT treatment (C). The respiration control rate (RCR) with different respiration substrates after LT treatment (D). The data are the means ± SE of 15-20 independent measurements. Letters represent values that differed significantly in the LSD range test ($P < 0.05$).

Figures

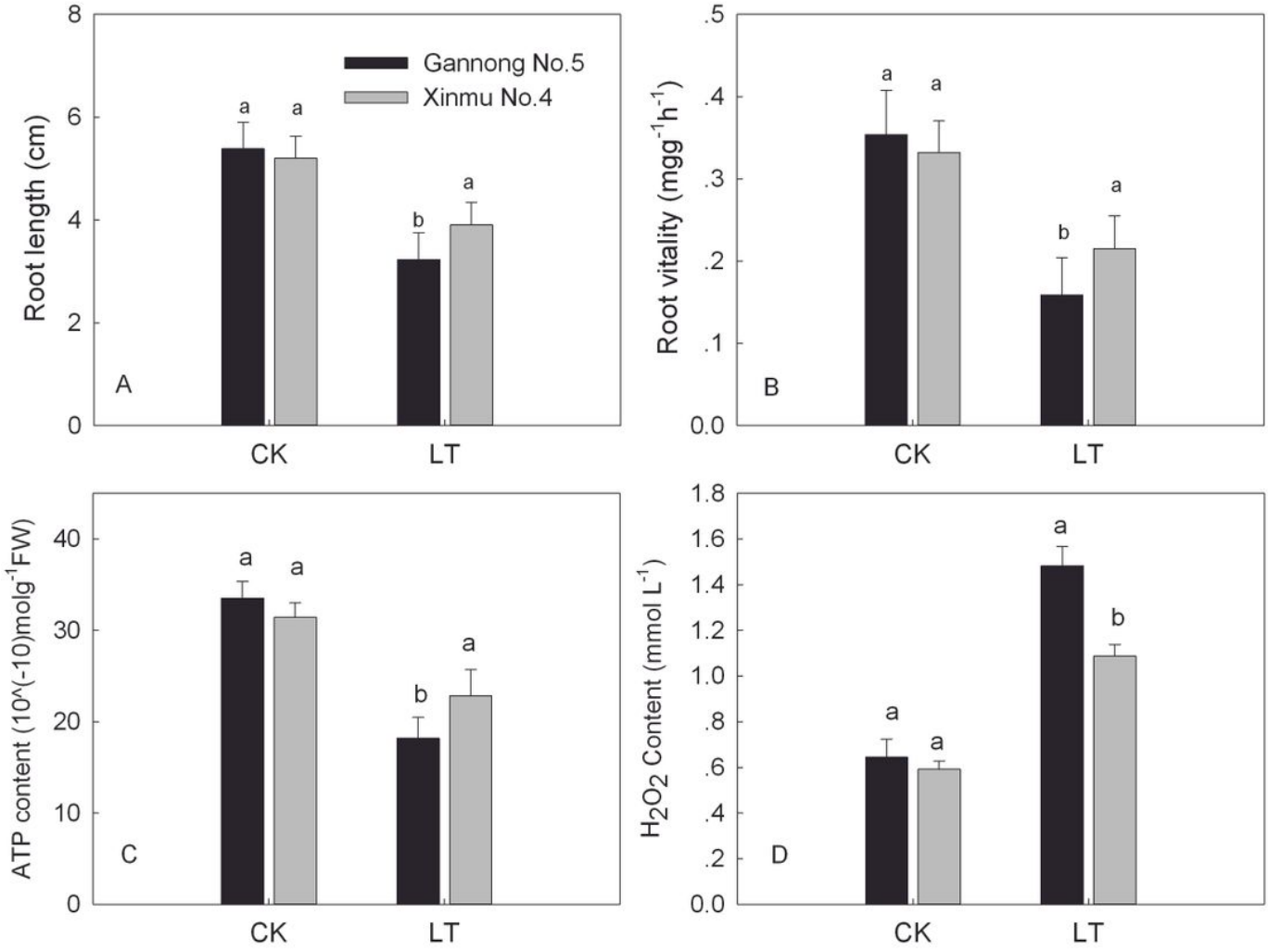

Figure 1

Effect of low temperature on growth, vitality of alfalfa seedling root. The length (A), vitality (B), ATP(C) and H2O2 content (D)of alfalfa seedling root after low temperature treatment. Data are the means ± SE of 3-5 independent measurements.

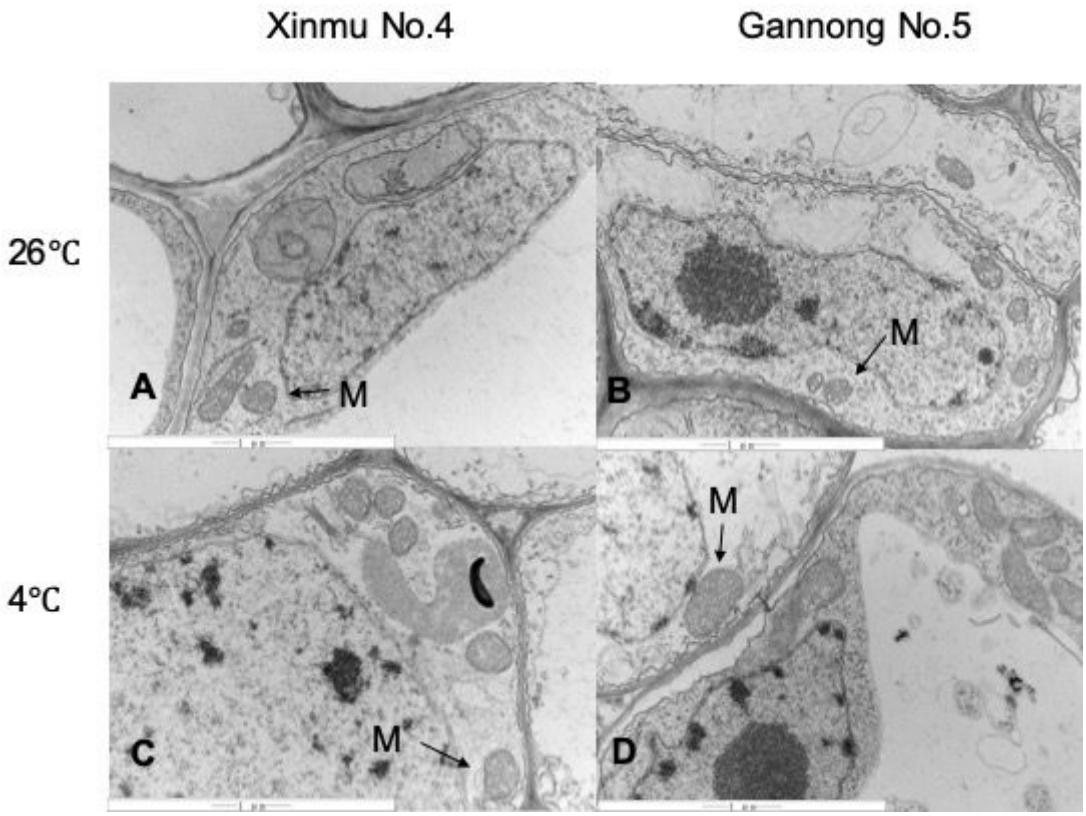

Figure 2

Effect of low temperature on alfalfa seedling root mitochondrial ultrastructure(30000×). The mitochondrial structure of Xinmu No.4 under 26℃(A)and 4 ℃(C), of Gannong No.5 under 26℃(B)and 4 ℃(D) . M：Mitochondria

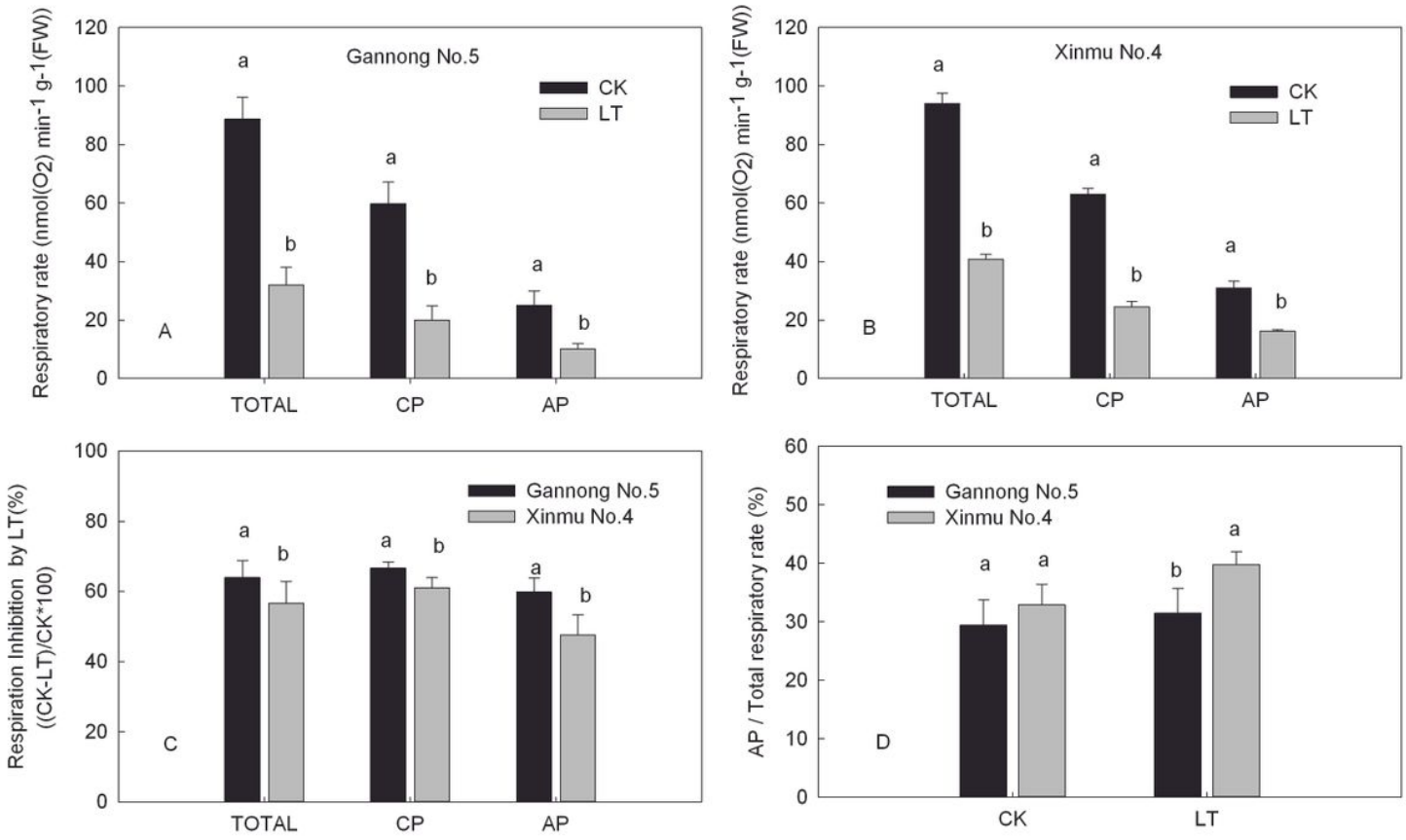

Figure 3

Effect of low temperature on respiration in alfalfa seedling root. The respiratory rate of total respiration, CP and AP pathway capacity in Ganong No.5 (A) and Xinmu No.4(B) seedling root, the respiration inhibition by low temperature analysed by (CK-LT)/CK*100(C), and the percent of AP pathway to the total respiration (D) in alfalfa treated with low temperature. Data are the means ± SE of 15-20 independent measurements. Letters represent values that differed significantly between different alfalfa in the LSD range test ($P < 0.05$).

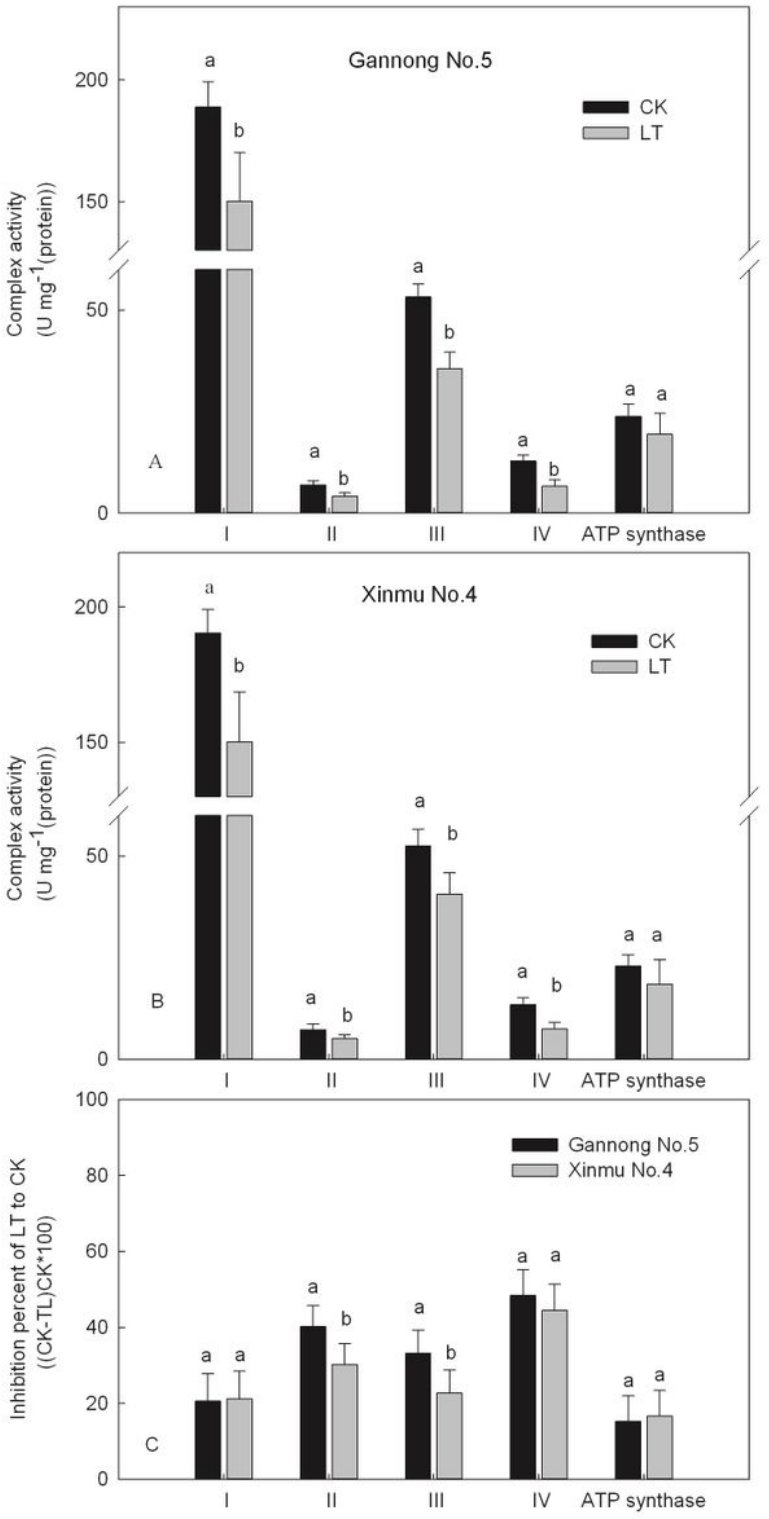

Figure 4

Effect of low temperature on the complexes activities of alfalfa seedling root mitochondria. The activities of complexes in Gannong No.5(A) and in XInmu No.4(B). The inhibition percent of low temperature to CK(C). Data are the means ± SE of 15-20 independent measurements. Letters represent values that differed significantly in the LSD range test ($P < 0.05$).

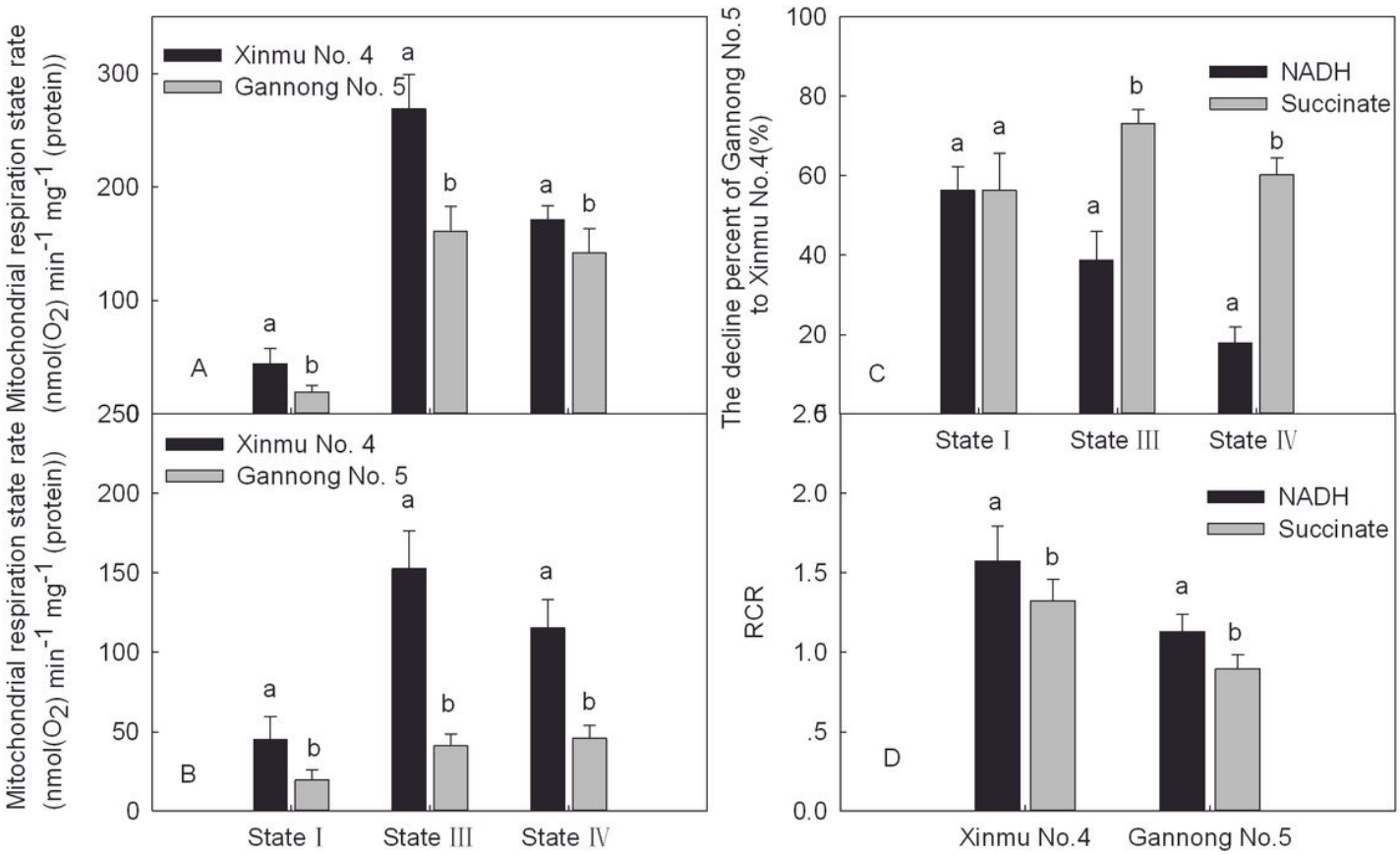

Figure 5

Effect of low temperature on the respiration state of alfalfa seedling root mitochondria. Respiration states of root mitochondria when NADH (A) and succinate (B), respectively, are used as substrate after LT treatment, and the decline percent of Gannong No.5 respiration state compared to Xinmu No.4 respiration state analysed by (Xinmu No.4 - Gannong No.5)/ Xinmu No.4 *100 with different substrates after LT treatment (C). The respiration control rate (RCR) with different respiration substrates after LT treatment (D). The data are the means ± SE of 15-20 independent measurements. Letters represent values that differed significantly in the LSD range test ($P < 0.05$).