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Comparative Study of the Impact of Drought Stress on *P.centrasiaticum* at the Seedling Stage in Tibet

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Abstract: *Pennisetum centrasiaticum* is widely distributed in arid and semi-arid areas of Tibet. Its rhizome system is developed and has strong resistance to adversity. In this study, the physiological characteristics and drought resistance of *P.centrasiaticum* seedlings from 12 drought-stressed sites in Tibet were examined at the Lhasa Plateau Ecosystem Research Station of the Chinese Academy of Sciences. PEG-6000 solution with five levels of water potential (0, –0.7, –1.4, –2.1, and –2.8 MPa) was used to simulate drought stress, and malondialdehyde (MDA), proline (Pro) and chlorophyll contents were determined. The balance between production and elimination of reactive oxygen species in *P.centrasiaticum* was destroyed, leading to membrane lipid peroxidation and the production of MDA, and accelerating the decomposition of chlorophyll. *P.centrasiaticum* absorbed water from the outside to resist drought by secreting proline and other osmotic regulating substances. The Pro and chlorophyll contents in *P.centrasiaticum* showed a temporary rising trend, and then decreased with the decrease in water potential. MDA content increased with the decrease in water potential. By using the membership function method, the drought resistance of *P.centrasiaticum* seedlings from the 12 areas was evaluated, and the results showed that the drought resistance at the sites went from strong to weak in this order: Xietongmen > Linzhou > Sog > Damxung > Tingri > Namling > Gyirong > Linzhi > Purang > Dingjie > Longzi > Sa'gya. The drought resistance of *P.centrasiaticum* was strong in Xietongmen, Linzhou and Sog. Whether *P.centrasiaticum* from these three areas is suitable for cultivation in arid and semi-arid areas of Tibet needs further study.

Key words: Tibet; *P.centrasiaticum* Tzvel.; seedling stage; physiological characteristics; drought resistance

1 Introduction

Nearly half of the land in Tibet is arid or semi-arid. Due to its special topographical, geomorphic and climatic conditions, the natural conditions are harsh, the ecosystem is fragile, and the carrying capacity is very limited (Sun et al., 2012; Zhang et al., 2015). Global climate change and the impact of human activities have further aggravated the situation in recent years, and grassland degradation has become one of the most serious ecological problems facing Tibet (Liu et al., 2012; Fu et al., 2018). In order to relieve the pressure of natural grassland and protect the ecological environment in Tibet, the vigorous promotion of human-made grassland has become a key measure (Gao et al., 2014; Duan et al., 2019). *Pennisetum centrasiaticum* Tzvel is a perennial forage of *Pennisetum* in Gramineae. *P.centrasiaticum* is widely distributed in arid and semi-arid

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areas of Tibet. Its rhizome system is developed and has strong resistance to adversity. It has genes to resist disease, insects, cold and drought that wheat crops lack. The biomass and nutritional quality of *P.centrasiaticum* are high at jointing stage (Li, 1983; Gao, 2008). PEG-6000 can simulate drought stress by regulating the osmotic pressure of solutions to limit water entering seeds, so the use of PEG-6000 offers a fast and reliable method to simulate a drought environment for the purpose of screening drought-resistant varieties (Hegarty, 1977; Van den et al, 2006). At present, there are few studies of *P.centrasiaticum* in Tibet. There is no data about the use of PEG-6000 to simulate a drought environment to screen out the *P.centrasiaticum* from different areas. The meteorological data from 1971 to 2014 showed that drought conditions in Tibet have worsened over the years, and this may cause devastating, long-term damage to the agriculture, economy and ecosystem of Tibet (Li et al., 2019). Seedling stage is the key stage of forage growth, and is sensitive to water response. Therefore, identifying varieties with strong drought resistance at seedling stage can be of significant value as a guide for production. Drought resistant forage types are key to the development of animal husbandry in Tibet (Cui et al., 2015).

P.centrasiaticum from 12 sites in Tibet were selected as the experimental materials for this paper. Physiological indexes were measured at seedling stage, and the drought resistance of the *P.centrasiaticum* from the 12 sites was evaluated using the membership function method. The main objectives of this study were to identify *P.centrasiaticum* with strong drought resistance ability in the seedling stage, to provide raw materials and a material basis for the development of local grassland animal husbandry, to alleviate the contradiction between grass and livestock, and to protect the deteriorating natural grassland ecological environment in Tibet.

2 Materials and methods

2.1 Plant materials

The study materials used in this study were *P.centrasiaticum* seeds taken from 12 sites in Tibet in 2017 (Table 1).

Table 1 Locations where *P.centrasiaticum* seedlings were collected in 12 sites in Tibet

Source of seedlings	Geographical coordinates	Elevation (m)	Compact form
Linzhi	93° 94′ N, 29° 79′ E	3117	LZS
Linzhou	91° 08′ N, 29° 88′ E	3889	LZX
Xietongmen	88° 21′ N, 29° 42′ E	3891	XTM
Sog	93° 79′ N, 31° 83′ E	3940	SX
Longzi	92° 32′ N, 28° 42′ E	3948	LZ
Namling	89° 08′ N, 29° 64′ E	3949	NML
Purang	81° 16′ N, 30° 34′ E	4061	PL
Dingjie	87° 77′ N, 28° 37′ E	4163	DJ
Gyirong	85° 32′ N, 28° 89′ E	4198	JL
Sa’gya	87° 90′ N, 29° 00′ E	4233	SJ
Damxung	91° 05′ N, 30° 51′ E	4333	DX
Tingri	87° 04′ N, 28° 47′ E	4413	DR

2.2 Experiment design

Seedling cultivation: According to the germination rate test, the *P.centrasiaticum* seeds from the 12 sites were sown in seedling trays on May 24, 2018. After sowing, the seedling tray was placed in a 25 °C incubator for cultivation, with 14 hours of level 5 light and 10 hours of darkness per day. An appropriate amount of water was sprayed every day into the seedling trays to ensure normal growth of the *P.centrasiaticum* seeds. When the seedlings had grown to a stage with three to four leaves, seedlings at the same growth level were selected for stress treatment. Seedlings with consistent growth were transferred to PEG-6000 solutions with different water potentials (0, –0.7, –1.4, –2.1 and –2.8 MPa) that has 4 replicates for each water potentials. The quantity was weighed and hydrated every day to maintain the water potential. Samples were taken after 5 days of drought stress. The seedling samples obtained were quick-frozen at –80 ℃ for assessment.

2.3 Index measurement and methods

The formula for calculation of PEG-6000 for different water potentials (Michel and Kaufmann, 1973) is as follows:

$$\Psi S = -(1.18\times10^{-2})\times C-(1.18\times10^{-4})\times C^2+(2.67\times10^{-4})\times C\times T+(8.39\times10^{-7})\times C^2\times T \quad (1)$$

where ΨS represents the solution of water potential (MPa); C is the concentration of the PEG-6000 solution (g kg^{-1}); T is the temperature (℃).

The methods used for determination of physiological indexes: The content of malondialdehyde was determined by a kit method. The content of proline was determined by Ninhydrin colorimetry. The content of total chlorophyll, chlorophyll a, chlorophyll b and carotenoid were determined by Colorimetry (Li, 2000).

The calculation formula for the membership function method: The parameters of proline and chlorophyll content that correlated positively with drought resistance were expressed in the following formula (Yan et al., 2018):

$$u(X_{ijk}) = (X_{ijk}-X_{\min})/(X_{\max}-X_{\min}) \quad (2)$$

The parameters of malondialdehyde content that correlated negatively with drought resistance were expressed in the following formula:

$$u(X_{ijk}) = 1-(X_{ijk}-X_{\min})/(X_{\max}-X_{\min}) \quad (3)$$

where $u(X_{ijk})$ is the membership degree of k index in j water potential stage of i grass species, $X_{\min}$ and $X_{\max}$ represent the minimum and maximum values of the k comprehensive index; the average value of each index membership degree was used as the comprehensive evaluation standard of the drought resistance ability of germplasm.

2.4 Statistical analysis

Data were collected in Excel 2016, variance analysis was conducted using SPSS 19 software, the LSD method was used to carry out multiple comparisons of *P.centrasiaticum* in different areas under the same osmotic pressure were carried out by, and plots were completed with SigmaPlot 12.5. The membership function method was used to evaluate the drought resistance of *P.centrasiaticum* from the 12 sites.

3 Results

3.1 Differences of MDA among the sites

For the control, the MDA content of seedlings from Linzhi was the highest, and the MDA content of seedlings from Tingri was the lowest (Fig. 1). MDA content decreased gradually as elevation increased. When the water potential was –0.7 MPa, the MDA content of seedlings from Namling and Longzi was low, and the MDA content of seedlings from Linzhi was the highest, which indicated that drought resistance of seedlings from Namling and Longzi was high, and that of seedlings from Linzhi was low. When the water potential was –1.4 MPa, the MDA of seedlings from Gyirong was the lowest, significantly lower than that of seedlings from other sites ($P < 0.05$); While, the MDA contents of seedlings from Xietongmen, Sog, Linzhou and Linzhi were higher, significantly higher than that of seedlings from other sites. When the water potential was –2.1 MPa, the MDA of seedlings from Gyirong, Damxung and Tingri were lower than that of other sites, and that of seedlings from Purang and Xietongmen were higher than that of other sites. When the water potential was –2.8 MPa, the MDA contents of seedlings from Linzhi and Xietongmen were higher, while the MDA contents of seedlings from Longzi, Dingjie and Tingri were lower. With the decrease of osmotic type, the MDA of *P.centrasiaticum* in the same site showed a tendency of increasing first, then decreasing and then increasing. As a whole, with the increase in drought stress, the MDA content generally increased.

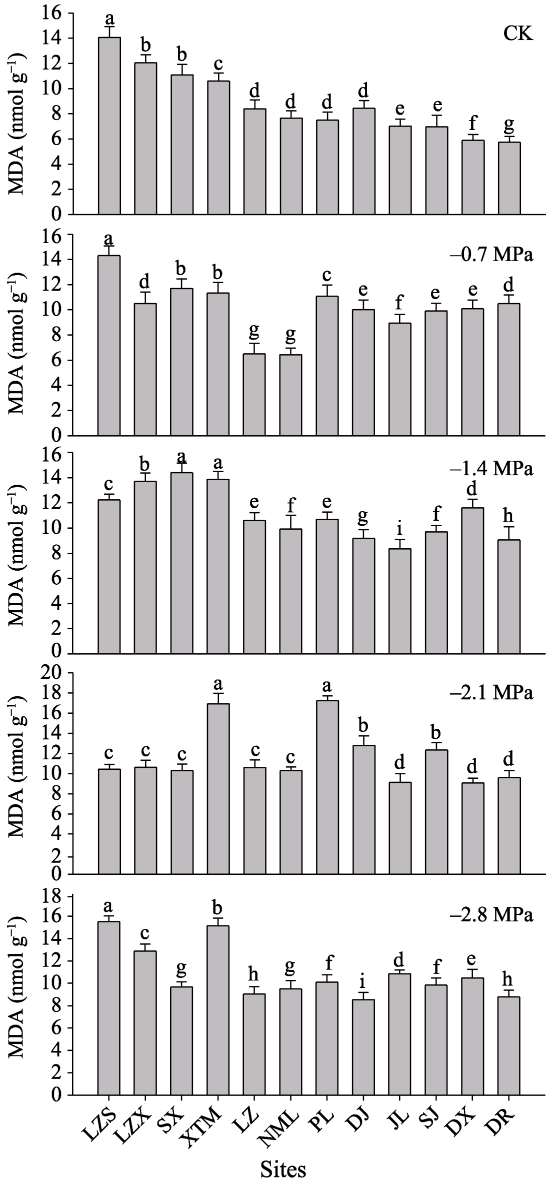

Fig. 1 Comparison of MDA content in *P.centrasiaticum* seedlings from different sites

Note: Different lowercase letters indicate significant differences for Differences of MDA among the sites ($P < 0.05$).

3.2 Differences of Pro in seedlings from different sites

For the control, the Pro content of seedlings from Linzhi, Linzhou and Xietongmen was higher, significantly higher than that in other areas ($P < 0.05$) (Fig. 2), while the Pro content of seedlings from Damxung was the lowest. When the water potential was –0.7 MPa, the Pro content of seedlings from Xietongmen was the highest, and compared with the control, the Pro content of seedlings from Tingri increased the fastest. When the water potential was –1.4 MPa, the Pro content of seedlings from Linzhou and Xietongmen was significantly higher than that of seedlings from other areas ($P < 0.05$). The Pro content of seedlings from Namling, Gyirong and Sa'gya was relatively low. Compared with the control, the Pro content of seedlings from Damxung increased the fastest. When the water potential was –2.1 MPa, the Pro content of seedlings from Xietongmen was the highest; it was significantly higher than of seedlings from other areas ($P < 0.05$). Compared with the control, the Pro of content seedlings from Damxung increased the fastest, and the Pro content of seedlings from Purang and Tingri increased faster than others. When the water potential was –2.8 MPa, the Pro content of seedlings from Linzhou, Xietongmen, Namling, Dingjie, Gyirong and Tingri and Sa'gya continued to increase, among which the Pro content of seedlings from Longzi, Sa'gya and Tingri increased rapidly, while the content of seedlings from Linzhou and Xietongmen was the highest. Overall, as drought stress

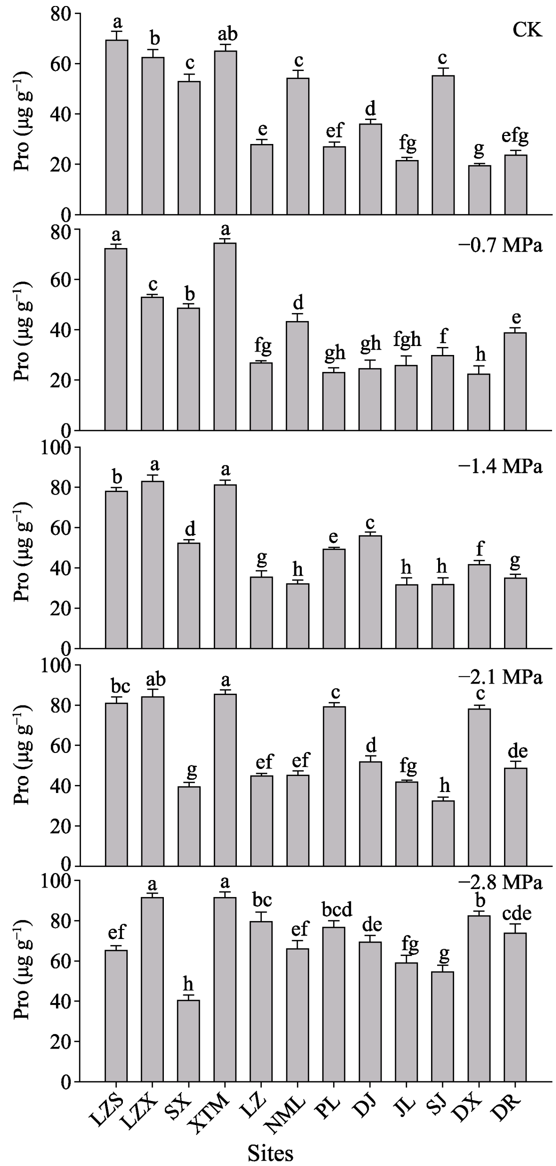

Fig. 2 Comparison of Pro content in *P.centrasiaticum* seedlings from different sites

Note: Different lowercase letters indicate significant differences for Differences of Pro among the sites ($P < 0.05$).

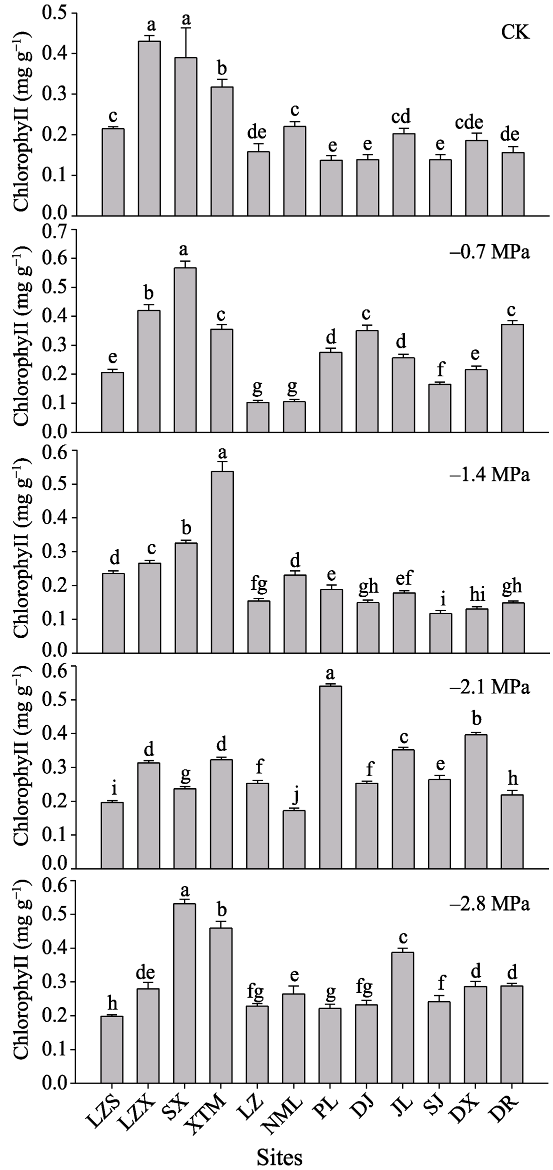

Fig. 3 Comparison of Chlorophyll content in *P.centrasiaticum* seedlings from different sites

Note: Different lowercase letters indicate significant differences for Differences of chlorophyll among the sites ($P < 0.05$).

increased, the Pro content of seedlings generally increased first and then decreased.

3.3 Differences of chlorophyll in seedlings from different sites

In the control, the chlorophyll content in *P.centrasiaticum* seedlings from Linzhou and Sog was the highest (Fig. 3), while the content in seedlings from Dingjie, Gyirong and Sa'gya was lower. When the water potential was –0.7 MPa, the chlorophyll content of seedlings from Sog was the highest, and it was significantly higher than that in other sites ($P < 0.05$). When the water potential was –1.4 MPa, the chlorophyll content of seedlings from Xietongmen was the highest, and it was significantly higher than that in other sites ($P < 0.05$). In addition, the chlorophyll content of seedlings from Sog was significantly higher than that in all sites except Xietongmen ($P < 0.05$). When the water potential was –2.1 MPa, the chlorophyll content of *P.centrasiaticum* seedlings from Purang was the highest, the content of seedlings from Linzhi and Damxung was also higher, and the content of seedlings from Namling was the lowest. When the water potential was –2.8 MPa, the chlorophyll content of *P.centrasiaticum* seedlings from Sog and Xietongmen was higher, while that of Linzhi was the lowest. As a whole, with the increase in drought stress, the chlorophyll content of seedlings generally increased firstly and then decreased.

3.4 Comprehensive evaluation

The results of the above indexes can only indicate the total physiological response of a single index under drought stress, and it is difficult to judge the drought resistance capacity of specific regions. Using the membership function analysis method, the membership function values of the 12 *P.centrasiaticum* materials were calculated by using the mean values of the three drought resistance indexes measured in this experiment under different water potentials (Table 2). Membership function values were used to calculate the averages, which ranked from high to low were: Xietongmen > Linzhou > Sog > Damxung > Tingri > Namling > Gyirong > Linzhi > Purang > Dingjie > Longzi > Sa'gya.

4 Discussion

4.1 Relationship between membrane lipid peroxidation and drought resistance

Plant cell membranes play an important role in maintaining the stability of cells. Drought stress subjects plants to peroxidation and change in the content of MDA in the plants (Zhang et al., 1994; Jiang et al., 2001). With the decrease of water potential, the balance between the production and elimination of reactive oxygen species in *P.centrasiaticum* was destroyed, and this resulted in oxidative damage, membrane lipid peroxidation, and malondialdehyde production. Finally the growth and development of *P.centrasiaticum* were affected (Liu et al., 2009; Pan et al., 2014). The increase of plasma membrane permeability was positively correlated with the accumulation of MDA, while the accumulation of MDA was negatively correlated with growth (Wong et al., 1997). Many studies of the photosynthetic parameters and physiological indexes of plants have shown that the content of MDA can be used to effectively evaluate the drought resistance of plants (Wu et al., 2014; Wang et al., 2015). The higher the content, the more drought resistance a plant has, and the slower the increase, the stronger the drought resistance. In drought stress situations, the relative content of MDA in the leaves of herbaceous plants increases gradually as a result of the aggravation caused by drought stress (Ding et al., 2013; Zhang et al., 2018). The increase of MDA content in drought resistant plant is smaller (Zhou et al., 2009). As the time of drought stress lengthens and the degree of aggravation from drought stress increases, the content of MDA in Brassica napus seedlings increased (Xie et al., 2013). In this study, with the decrease of water potential, the balance between the production and elimination of reactive oxygen species in *P.centrasiaticum* was destroyed, which resulted in oxidative damage, membrane lipid peroxidation, malondialdehyde production. Finally the growth and development of *P.centrasiaticum* were affected. With the decrease of water potential, the MDA in seedlings from the 12 sites showed an overall upward trend, and this was consistent with the results of previous studies (Liu et al., 2009; Pan et al., 2014). Therefore, the content of MDA can be used as a physiological index to evaluate the drought resistance of *P.centrasiaticum* germplasm. The MDA content of *P.centrasiaticum* in Linzhi was higher under 5 osmotic patterns, which indicated that the drought resistance ability of these seedlings was weaker than those from other areas.

Table 2 Membership function values of drought resistance indexes and comprehensive drought resistance ranking of *P.centrasiaticum* from 12 sites

Sites	MDA	Pro	Chlorophyll	Average membership function value	Ranking
Linzhi	0.341	0.746	0.232	0.440	8
Linzhou	0.460	0.768	0.515	0.581	2
Sog	0.505	0.379	0.662	0.515	3
Xietongmen	0.318	0.834	0.637	0.596	1
Longzi	0.714	0.326	0.165	0.402	11
Namling	0.737	0.398	0.207	0.448	6
Purang	0.515	0.438	0.366	0.440	9
Dingjie	0.647	0.390	0.263	0.433	10
Gyirong	0.729	0.229	0.372	0.443	7
Sa'gya	0.651	0.296	0.178	0.375	12
Damxung	0.680	0.407	0.302	0.463	4
Tingri	0.740	0.340	0.289	0.456	5

4.2 Relationship between osmoregulation substances and drought resistance

Osmoregulation is an important physiological mechanism for plants to adapt to drought stress, and as a result, has become the most active research field in drought resistance physiology (Lutts et al., 1999; Singh et al., 1972). Many studies have shown that Pro is accumulated in plants under conditions of water loss, and that plants absorb water from the outside to resist drought stress, using substances such as Pro to make osmotic adjustments. The higher the content of Pro, the stronger a plant's drought resistance ability (Munns et al., 1979; Abrams et al., 1990; Medrano et al., 2002; Ni et al., 2004). The relative content of soluble sugar and proline increased with the increase of drought stress and the extension of stress time, and the increased content was positively correlated with drought resistance (Xie et al., 2013). It was found that the relative value of Pro content could be used as a physiological index to evaluate the drought resistance ability of potatoes in the early stage of growth (Ding et al., 2013). Furthermore, osmoregulation substances are related to stomatal opening and closing. In order to avoid too much transpiration, plants need to close stomata to reduce transpiration, but plants also need to absorb carbon dioxide for photosynthesis, so stomatal opening and closing plays a key role in the balance between photosynthesis and transpiration, while osmoregulation matter can make plants maintain a moderate transpiration rate (Wright, 1969). In this study, Pro content first decreased and then increased with the decrease of water potential. This may have been because mild drought did not cause a rapid reaction of proline. Therefore, the content of Pro can be used as a physiological index to evaluate drought resistance of *P.centrasiaticum* germplasm.

4.3 Relationship between photosynthetic physiology and drought resistance

When a plant is under drought stress, the stomata on the leaves close, and the amount of carbon dioxide absorbed by the plant is reduced, resulting in a slowdown of photosynthesis. The decrease of chlorophyll content in plants with strong drought resistance was small, while the decrease in plants with weak drought resistance was large (Mou et al., 2016). The chlorophyll content of grass with strong drought resistance was higher than that of the grass with weak drought resistance. On the one hand, this is a result of the sharp decrease of water content caused by the weakening of chlorophyll biosynthesis; on the other hand, it is a result of the accumulation of active oxygen in plants caused by drought. The accumulation of oxygen free radicals directly or indirectly activate membrane lipid peroxidation, resulting in membrane permeability damage and accelerating chlorophyll decomposition. Under drought stress conditions, the contents of chlorophyll a, chlorophyll b and total chlorophyll, and the chlorophyll a/b ratio decreased compared with the control. This result was consistent with previous results (Zhang et al., 2003; Liao et al., 2018).

5 Conclusions

In summary, the balance between production and elimination of reactive oxygen species in *P.centrasiaticum* was destroyed, leading to membrane lipid peroxidation, the production of MDA, and the accelerated decomposition of chlorophyll. *P.centrasiaticum* absorbed water from the outside by secreting proline and other osmotic regulating substances to resist drought. The drought resistance of *P.centrasiaticum* was strong in Xietongmen, Linzhou and Sog.

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西藏地区白草幼苗期抗旱性比较研究

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摘　要：白草广泛分布在西藏干旱、半干旱地区，其根茎系统发达，抗旱能力较强。本文以西藏 12 个地区的野生白草为材料，在中科院拉萨高原农业生态综合试验站研究野生白草幼苗在干旱胁迫下的生理特性，并用隶属函数法对抗旱性进行综合评价，拟筛选出抗旱能力较强的白草种类。实验采用 5 种不同水势（0，–0.7，–1.4，–2.1，–2.8 MPa）的 PEG-6000 溶液模拟干旱胁迫，测定 12 份白草幼苗体内丙二醛（MDA）、脯氨酸（Pro）和叶绿素等生理指标。结果表明，随着水势的下降，白草体内活性氧的产生和消除平衡受到破坏，导致膜脂过氧化，产生丙二醛，加速叶绿素的分解；通过脯氨酸等渗透调节物质从外界吸水来抵御干旱。随着干旱胁迫的加剧，丙二醛的含量总体呈上升趋势，脯氨酸和叶绿素的含量总体呈先升后降趋势。利用隶属函数法对 12 个地区白草幼苗的抗旱性进行综合性评价，研究得出抗旱性由强到弱依次为：谢通门>林周>索县>当雄>定日>南木林>吉隆>林芝>普兰>定结>隆子>萨迦，表明谢通门、林周和索县白草的抗旱性较强，其是否适宜在西藏干旱、半干旱地区种植尚需进一步研究。

关键词：西藏；白草；幼苗期；生理特性；抗旱性