EFFECTS OF DROUGHT STRESS SIMULATED BY PEG ON SEED GERMINATION OF TWO ECOTYPES OF *CERATOIDES ARBORESCENS*

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ABSTRACT

Ceratoides arborescens is widely distributed in arid area of Inner Mongolia. They are fine sand binding plants, and the main feed of livestock in arid desert area in the dry season. Under drought stress, seed germination and seedling root growth is very important to the survival and development of plant seedlings. Therefore our main objective of this study was to determine the effects of drought stress on seed germination and seedling growth of two ecotypes, Wu and Kerqin, of Ceratoides arborescens. The effects of drought stress on seed germination under PEG treatment and germination recovery under optimal conditions after presoaking with PEG solution were studied in a series of laboratory tests. The germination and radical growth of the two ecotype Wu and Kerqin of Ceratoides arborescens were studied to different degrees of inhibition with reduced osmotic potential of a series of PEG solutions. However, slight drought stress promoted the seed germination of the two ecotype Wu and Kerqin of Ceratoides arborescens and improved the germination index of the two ecotypes, simulating the radical growth. The lowest osmotic potential for germination was -2.1MPa for Kerqin and -1.8MPa for Wu. The result shows that the drought resistance of seed germination of Kerqin was stronger than that of Wu.
Under optimal conditions, the seeds presoaked with PEG solution recovered their germination after drought stress was relived. The seeds presoaked longer, suffered greater from drought stress and germination was suppressed completely. The radical length of the primary root of the two species had no significant difference between the CK after presoaking with PEG solution in 3d，and then decreased significantly with the increase of days of presoaking.

KEY WORDS： *Ceratoides arborescens*, drought stress, seed germination, germination recovery

INTRODUCTION

Desertification is one of the most serious environmental problems in the world. China is one of the countries with large desertification area, wide distribution and serious harm, specially the northwest of China is the most prominent area in the desertification problem. The precipitation is scarce in this area, evaporation is strong, more wind and sand, large temperature difference between day and night, and with soil salinization [1]. The main body of the vegetation was formed by the short perennial shrubs in the area. These drought tolerant plants can withstand long-term, severe water deficit, with obvious drought and physiological characteristics [2]. They survive in extreme desert conditions, forming a special adaptation mechanism [3]. The germination strategy of seed in desert environment is an important aspect of the survival strategy of desert plants. The special mechanism of seed germination of desert plants ensures the seed germination and seedling growth at the appropriate time and place. The seed structure and environmental factors play an important role in germination, and ultimately affect the formation and succession of desert vegetation [4]. Overall, seed germination is a product of the interaction of various ecological factors. And the germination response to ecological factors defers with the species and ecotypes.

The two ecotype Wu and Kerqin of *Ceratoides arborescens* are widely distributed in arid area of Inner Mongolia. They are fine sand binding plants, and the main feed of livestock in arid desert area in the dry season. In the previous research of the germination ecology of this two ecotypes, there are existing data involving salinity on effect of seed germination of *Ceratoides* [5,6], effects of drought stress simulated on seed germination of four desert plant species [7], temperature, light, storage and germination bed and other factors on the seed germination of *Ceratoides arborescens* [8,9].

Seed germination of desert plants has a complex process, each desert plants evolve into germination strategies adapted to the harsh habitats in the long-term evolution. Therefore objective of this study was to determine the effects of drought stress on seed germination and seedling growth of two ecotypes, Wu and Kerqin, of *Ceratoides arborescens*.

MATERIALS AND METHODS

Plant materials

The seeds of two ecotypes (Wu and Kerqin) of *Ceratoides arborescens* were collected from the desert grassland in Siziwangqi, Wulanchabu of Inner Mongolia in late October 2014. Approximately a plant contains at least 100 mixed seeds. Thousand seed weight was 2.6 and 2.1 g. After air dried, the seeds were placed in a low temperature storage at -5℃. The test was conducted in the forage laboratory of Grassland Research Institute, Inner Mongolia Academy of Agricultural & Animal Husbandry Sciences.

The basic conditions of germination

Optimum germination temperature of two ecotypes seeds was 25℃, under the condition of no light in incubator, refer to《International Rules for Seed Testing》[10], using the method of petri dish paper, and literature report [9,11,12]. All treatments were repeated 4 times, the germination period was 7 d with 50 seeds per replicate.

Treatment of drought stress simulated by PEG

The PEG solution (Polyethylene glycol, molecular weight of 6000) that simulates drought stress was used by the method of Michel and Kaufmannu [13]. The osmotic potential of PEG solution for 8 treatments was 0(CK), -0.3, -0.6, -0.9, -1.2, -1.5,

-1.8 and -2.1MPa. A filter paper soaked with 7 ml PEG solution was placed in the each Petri dish, and then put the seeds. Supplementing distilled water was done daily by weighing method to maintain constant osmotic potential of solution, and counted the germination number daily. The germination rate and germination index were calculated, and seedling bud embryos (including hypocotyl and terminal bud) and primary root length determined at 3d.

The seed germination after presoaking with PEG solution

Germination of two ecotypes (Kerqin: -2.1 MPa and Wu: -1.8MPa) soaking seeds was done under the threshold of the lowest osmotic potential on 1, 3, 5, 8, 10 and 15 d. Then re-sprouting experiment was done using different soaking seeds in appropriate conditions, and without soaking seeds as control.

Data analysis

Data analysis on the germination rate, germination index, primary root length and embryo length of different treatments were done using SPSS22.0-General linear Model-Univariate program.

RESULTS

Effects of drought stress on seed germination rate of two ecotypes

Generally, drought stress simulated by PEG had inhibitory effect on the germination rate of the seeds of two ecotypes and the degree of inhibition is generally increased with the decrease of the osmotic potential (Fig. 1). Seed germination rate of two ecotypes increased in the range of 0~0.3MPa, and then it was decreased along with the decrease of PEG osmotic potential. But the response of different ecotypes to drought stress was different. Under the conditions of 0~0.3 MPa, the germination rate of Kerqin was significantly higher than that of Wu. The lowest threshold of osmotic potential for germination were -2.1MPa for Kerqin and -1.8MPa for Wu at P<0.05.

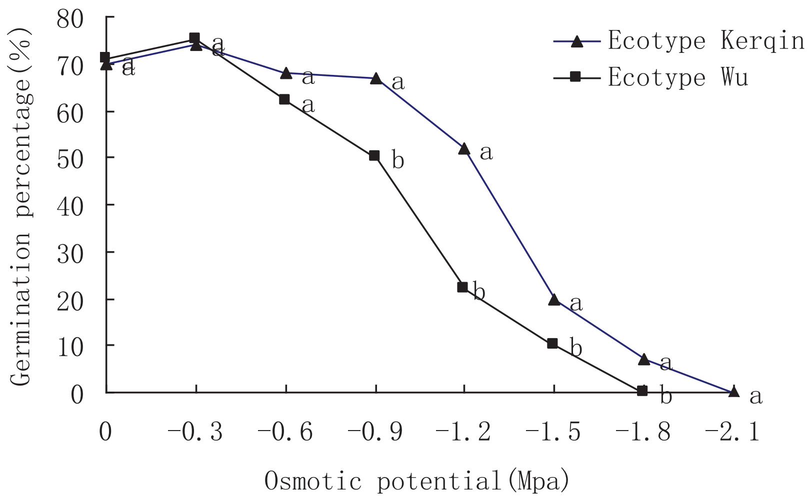

Figure 1 Germination rate of two ecotypes seeds under different osmotic potential. The different letters indicate significant differences at P<0.05 level between different species under the same osmotic potential.

Effects of drought stress on seed germination index of two ecotypes

The seed germination index of two ecotypes had no significant differences between 0 to 0.6MPa, and it decreased along with the decrease of PEG osmotic potential. The seed germination index of Kerqin was always significantly higher than that Wu at P<0.05.

Effects of drought stress on the radical length of the primary roots of two ecotypes

Different osmotic potential of PEG had inhibitory effect on the radical growth of the primary root of two ecotypes, and the degree of inhibition is generally increased with the decrease of the osmotic potential (Fig. 2). The radical length of primary root of two ecotypes were increased (0 ~ -0.6MPa) and then decreased. Under the control condition, the radical length of primary root of Kerqin was significantly higher than that of Wu at P<0.05.

Under -1.8 MPa, the primary root of Kerqin still could grown, but the root growth of the other had been completely inhibited (Fig. 2).

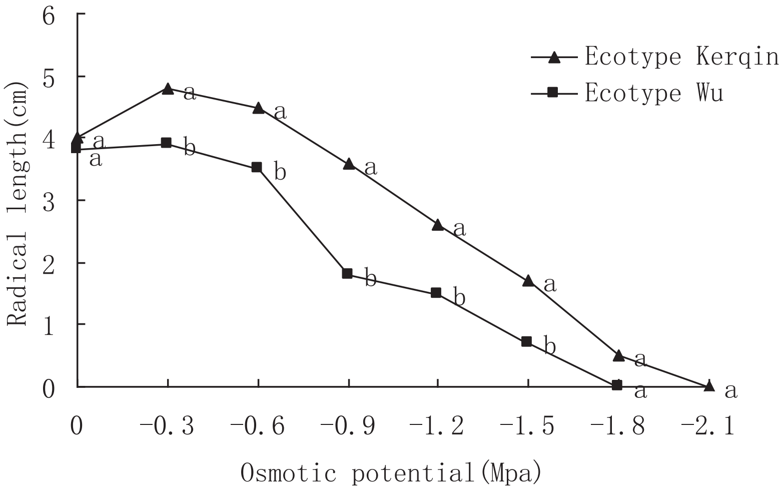

Figure 2 Radical length of the primary roots of two ecotypes seeds under different osmotic potential. The different letters indicate significant differences at P<0.05 level between different species under the same osmotic potential.

The seed germination of two ecotypes after presoaking with PEG solution

The results of seed germination of two ecotypes after presoaking with PEG solution showed that the response of seed germination rate and germination index of two species to PEG drought stress showed a declined tendency, and the longer the stress time caused the greater the effect of drought stress (Table 1, 2). At 15 days, seed germination of two ecotypes was completely inhibited.

Table 1

Percentage of germination recovery of two ecotypes seeds after presoaking with PEG solution

Presoaked days	Ecotypes	
	Kerqin (－2.1 Mpa)	Wu (－1.8 Mpa)
0	90±0.3 a	92±0.7 a
1	68±1.2 b	55±0.3 b
3	52±2.3 c	33±0.5 c
5	46±0.9 d	26±1.5 d
8	30±0.7 e	13±2.3 e
10	7±0.8 f	3±0.5 f
15	0±0.0 g	0±0.0 g

Note：The different letters indicate significant different at P＜0.05 level within species，the numbers in brackets behind the species are the osmotic potential of presoaking PEG solution.

Table 2

Germination index of two ecotypes seeds after presoaking with PEG solution

Presoaked days	Ecotypes	
	Kerqin (－2.1 Mpa)	Wu (－1.8 Mpa)
0	37±0.3 a	40±0.7 a
1	31±0.8 b	37±0.5 b
3	22±0.6 c	24±0.6 c
5	16±0.9 d	13±0.8 d
8	11±0.8 e	7±0.2e
10	7±0.4 f	2±0.2 f
15	0±0.0 g	0±0.0 g

Note：The different letters indicate significant different at $P<0.05$ level within species，the numbers in brackets behind the species are the osmotic potential of presoaking PEG solution.

The radical length of primary roots of the two ecotypes after presoaking with PEG solution

After relieving PEG treatment, the radical length of the primary roots of the two ecotypes both increased first and then decreased with the increasing of the days of drought stress in PEG (Table 3). Compared with CK, the primary root length of the two ecotypes increased significantly with 1d PEG soaking, reached to 5.3 and 5.1 cm, and 1.3 and 1.2 times of CK respectively. The radical length of the primary root of the two species had no significant difference between the CK after presoaking with PEG solution in 3d，and then decreased significantly with the increase of days of presoaking.

Table 3

Radical length of two ecotypes seeds after presoaking with PEG solution

Presoaked days	Ecotypes	
	Kerqin (－2.1 Mpa)	Wu (－1.8 Mpa)
0	4.1±0.2 b	4.0±0.7 a
1	5.3±0.1 a	5.1±0.3 b
3	4.0±0.1 b	3.3±0.5 c
5	3.6±0.2 c	2.6±0.5 d
8	2.2±0.0 d	2.3±0.3 d
10	0.6±0.2 e	0.5±0.0 e
15	0±0.0 f	0±0.0 f

Note：The different letters indicate significant different at $P<0.05$ level within species，the numbers in brackets behind the species are the osmotic potential of presoaking PEG solution.

DISCUSSION

Moisture is the key factor, affecting on seed germination [4]. It is also an important factor which restricts the growth of plants and the distribution of vegetation, which is particularly important to the effects of the arid desert plant [13, 14]. Different osmotic potential of PEG had inhibitory effect on the germination of the two ecotypes, and the germination rate and germination index were generally decreased with the Increasing of intensity of drought stress (Fig. 1, 2). This is consistent with the reports in the literature [16-20]. It can be seen from the results of the study that the seed germination rate of the two ecotypes both had a trend of increasing in the slight drought stress, which showed that the slight drought stress can promote the seed germination of the two ecotypes. This is consistent with the report of Yang Jing-ning and Wang Yan-rong on the four desert plants including *Ceratoides latens* [7].

Under drought stress, seed germination and seedling root growth is very important to the survival and development of plant seedlings [21, 22]. In general, the seed of the wild plants and the seeds of the super dry plants have the unique characteristics of germination. Under the same environmental conditions, deeply rooted seedlings have strong drought resistance, in order to adapt to the conditions of precipitation in arid desert area [11]. The study found that slight drought stress can promote the growth of the two ecotype of *Ceratoides arborescens*, it stimulated the growth and development of the primary root. This is consistent with the study of Zeng Yanjun on the report of *Caragana korshinskii*, *Hedysarum scoparium* and *Artemisia sphaerocephala* [23].

Each kind of plant has different threshold of tolerance of PEG drought stress [23]. The lowest threshold of osmotic potential for germination were -2.1 MPa for Kerqin and -1.8 MPa for Wu, the result shows that the drought resistance of seed germination of Kerqin was stronger than Wu. It may be the different forms of their long-term adaptation to the environment.

In the arid environment, it is essential for the survival of plants to keep the vigor of the seed and to keep the growth of the seedlings [21]. When the stress environment was weakened, the most of the desert plants showed a significant complex sprouting state, that they may be more resistant to drought than plants normally grown. With the extension of soaking time, after the relieving of PEG stress, the germination percentage and germination index were increased first and then decreased (Table 1, 2). It showed that short time presoaking with PEG solution can promote the seed germination of two ecotypes, and if the soaking time is longer, the seeds were more affected by drought stress. When presoaking with PEG solution in 15 d, after relieving drought stress, the seed germination of the two ecotypes were completely inhibited. It showed that there was a certain threshold value for drought tolerance of cold resistant plants, and once the drought extent exceeded this threshold, it would cause the damage on plants to recover.

CONCLUSION

The germination and radical growth of the two ecotype Wu and Kerqin of *Ceratoides arborescens* were studied to different degrees of inhibition with reduced osmotic potential of a series of PEG solutions. However slight drought stress promoted the seed germination of the two ecotype Wu and Kerqin of *Ceratoides arborescens*, improved the germination index of the two ecotypes， and simulated the radical growth of the two ecotypes.

The lowest threshold of osmotic potential for germination were -2.1MPa for Kerqin and -1.8MPa for Wu, the result shows that the drought resistance of seed germination of Kerqin was stronger than that of Wu. Under optimal conditions, the seeds presoaked with PEG solution recovered their germination after drought stress was relived. The seeds presoaked for longer suffered greater damage from drought stress， and at the longest times germination was suppressed completely.

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