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ARTICLE

Effects of Exogenous Calcium on Datura Seed Germination under Drought Stress

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ABSTRACT

With polyethylene glycol (PEG-6000), of 0% (CK), 5%, 10%, 15%, 25% used to simulate drought stress, and CaCl2 concentration 0 (CK), of 15, 20, 25 and 30mmol/L as ion gradient of exogenous calcium, the effects of drought, exogenous calcium and the interaction between the two on the Datura seed germination, so as to explore the optimal application amount of exogenous calcium to ease the suppression of drought stress on Datura seed germination. The results showed that the germination rate, germination potential and germination index of the Datura seeds were significantly lower than those of the control group. Under the normal moisture condition, exogenous calcium of moderate and low concentration had no significant effect on the Datura seed germination, while that of high concentration showed an inhibitory effect on the seed germination. Under drought stress, with the increasing concentration of exogenous calcium, the three indicators of Datura seeds showed a trend of increasing first and then decreasing. When the exogenous calcium had the concentration of 20 mmol/L, all the indicators of seed germination reached the maximum value, while showed a downward trend when exogenous calcium concentration was 25-30 mmol/L, and even increasingly sharp with drought intensifying. Therefore, in the production and utilization of Datura, 20 mmol/L of exogenous calcium can be used to soak seeds before sowing to improve the emergence rate under low and moderate drought conditions.

1. Introduction

Seeds are an important stage in the life history of a plant, and an important time for the study of drought resistance of the plant [1]. The germination rate, germination potential and germination index of the seeds mirror the germination speed, uniformity and the strength potential of seedlings, all of which declined dramatically with the increasing of drought stress intensity [2,3]. Under the action of severe drought stress (over 15%), the seed germination rate was extremely low, implying that even if the seeds were germinated, the growth of the seedlings was significantly inhibited, showing that it is feasible to study the drought resistance of Datura with PEG solution of different osmotic potential gradients to simulate drought stress [3].

33.6 percent of the land area on earth is arid or semi-arid. Years of research data show that among the meteorological disasters in China, drought influences as much as half the national territorial area, more seriously than flood(27.8%) [4]. In the blue book of China's Science and

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Technology, drought is listed as top of climate disasters in China. Since the beginning of the 21st century, drought occurs every year in China, different in impact scope and severity, but seasonal drought occurs almost yearly [5,6]. Compared with the second half of the 20th century, the frequency of drought has increased significantly, and the average area affected by drought remains almost the same, but the percentage of the disaster area has increased [7,8].

Since Shanxi Province is home to limestone resources [9], plus the requirements of national development, mountain quarrying has become an important economic source in parts of Shanxi Province. However, because of the fact that the quarrying technology is not advanced, the policy provisions are not strict enough, with excessive pursuit of economic benefits, over-exploitation and the like inappropriate actions, the regional ecological environment has been dramatically damaged. There lies a decline in biodiversity, serious soil erosion, environmental pollution, broad distribution of bare rock, etc [10,11]. The exposed quarries and quarrying wasteland will lead to intensified soil erosion, loss of species diversity, waste of resources, reduced vegetation cover, and the like problems [12,13,14]. However, with a further increase in the demand for limestone, the mining scale is expanding year by year, and problems like environmental damage becomes increasingly prominent, so is the difficulty of rectification. The recovery management of limestone after mining is urgent [15]. In the process of ecological restoration, the selected species need to show good adaptability to the soil environment with high calcium.

In recent years, the impact of the mining of such limestone mountains on the environment in Shanxi Province has been studied by many experts and scholars, who try to solve the environmental problems in limestone lands through a scientific method and approach [16,17]. Unfortunately, there has been no mature theoretical and technical standard for this technology. In the restoration project, imported plants like Bermudagrass, Chloris virgata, and alfalfa are adopted. These plants have various problems, such as their ability to adapt to the slope habitat and the distinct climate of the Loess Plateau, their shortcomings of single variety, poor resistance against diseases, population degradation, potential threat to the native species and so on [15,18,19,20,21,22]. However, there is less report on the restoration of limestone mountains with native species.

Datura stramonium Linn. can be seen throughout the country. As a herb or suffruticose therophyte, Datura grows well in places with abundant light. Since it is a heliophyte, it has a strong adaptability to the environment, with low requirements for soil conditions [23,24,25,26]. If there is a piece of soil rich in organic matter and calcareous, Datura can live here quite well. The Chinese herbal medicine field has not only developed a long history, but also has won a good reputation at home and abroad. As one of the traditional Chinese herbal medicines in China, Datura has been recognized and applied for a long time. Its seeds can be used to treat diseases like insomnia and headache, its leaves can be used to treat asthma, its flowers can be used for cough relieving, and furthermore, its flowers also have a positive effect in drugs cessation, especially for the elimination of heroin, which can effectively reduce the relapse of drug addicts [27,28,29,30].The Datura flower is relatively large, with a high ornamental value for a brightly beautiful color, tubular flower buds and a funnel-shaped corolla, so it plays an irreplaceable role in gardening, urban greening construction, environment beautifying, soil conditions improvement, etc. What's more, Datura has volatile oil with complex components, which can be used to make green pesticides, so it is of great value in agriculture and forestry [23,31,32,33,34].

The materials collected in this experiment are located in the limestone area of Huoshan, Linfen City, Shanxi Province, where it is dry, with low precipitation and many calcium ions. Therefore, the effects of different PEG and calcium chloride concentrations as well as the interaction of PEG and calcium chloride on seed germination can be explored[35,36], thereby providing references and a theoretical basis for the reproduction of native Datura and a scientific basis for the germination conditions of Datura seeds. In addition, it can also provide references for the improvement of the ecological adaptability and ecological restoration of Datura.

2. Materials and Method

2.1 Experimental Materials

Collection time of Datura seeds for the test: October 2017; collection location: Huoshan, Shanxi Province.

2.2 Experimental Method

The $CaCl_2$ concentration was designed to be the five gradients of 0, 15, 20, 25 and 30mmol/l, respectively, to simulate exogenous calcium. The PEG — 6000 solution was used to simulate the drought stress, and it was set a total of six osmotic pressure gradients0%, 5%, 10%, 15%, 25%at room temperature (25±2℃). The two factors have a total of 30 treatments, each of which was repeated 4 times.

The seed germination test was carried out on the petri dish paper. Full seeds were selected and placed on the petri dishes which has two layers of filter paper at the bottom. On each petri dish lay 25 seeds, with 2mL of

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pre-configured $CaCl_2$ and PEG solution added into it, and then the petri dishes were placed in a constant temperature incubator of 25 ℃ for germination. Every day, the number of germinated seeds was observed and recorded, and the fact of germ breaking through the seed coat by 2mm was taken as the standard of germination, and the evaporated moisture was supplemented every day.

Seed germination rate (Gr) = the number of germinated seeds in 15 days/ the number of seeds tested × 100%.

Germination potential (Gp) = the number of normally germinated seeds in the first 5 days / the total number of seeds × 100%.

Germination index (Gi) = MDG • PV. Seed vigor index (Vi) = seedling growth potential (seedling fresh quality) × Gi.

In the equations, MDG is the average number of seed germination per day, i.e. the number of germinated seeds at the end of the germination test / the number of days of the germination test; PV is the maximum germination rate of the seed, i.e. the largest germination number on any day during the test /the number of days the maximum value needed.

2.3 Data Processing

All data were processed with SPSS 16 software. The two-way analysis of variance and the least significant difference (LSD) were used to compare the differences between different data sets. The significance level was set as α=5.

3. Results and Analysis

3.1 The Length, Short Diameter, Thickness and Weight of Datura Seed

A mature Datura seed appears to be of a slight brownish or blackish color. The seed is morphologically large, kidney-shaped, with a leathery seed coat of a waxy structure. The umbilicus is triangular and inwardly recessed. The length diameter, short diameter, thickness and TKW of the seed are shown in Table 1.

3.2 Effects of Drought on the Datura Seed Germination

The germination rate (GR), germination potential (GP) and germination index (GI) of the Datura seeds decreased with the increase of drought stress intensity, and the difference between the drought treatments reached a significant level (P< 0.05). Based on table 2, it can be known that under normal moisture conditions, the germination rate and germination potential were 64% and 37%, respectively, both of the two indicators significantly reduced when drought stress was 15%, and reached the minimum when drought stress intensity was 25%, 21.8% and 21.6% of the control respectively. The GI of the Datura seeds was 1.47 and decreased gradually with the increase of drought stress intensity and reached the minimum at 25%, 77% of the control, indicating that drought stress seriously affected the Datura seed germination.

3.3 Effects of Exogenous Calcium on Datura Seed Germination

The temperature of the seed germination period of the limestone mountain was simulated, i.e. the temperature of 15/25°C, under which condition the Datura seed germination test was conducted with different $CaCl_2$ concentrations. The results analysis of the germination rate of the Datura seeds are shown in Table 3. With the changing of $CaCl_2$ concentration, the germination rate, germination potential and germination index of the seeds vary sharply, that is, first increase and then decrease. The germination rate varies little among the treatment groups, and the other two indexes reduced to the lowest at 30 mmol/L of $CaCl_2$, which was significantly different from the other control groups. The results showed that exogenous calcium had little effect on the Datura seed germination, but inhibited it when the concentration reached to be 30%.

3.4 Effects of Interaction between Exogenous Calcium and Drought on the Germination Rate of Datura Seed

The two factors analysis of variance on the three indicators of Datura showed that the interaction between exogenous calcium and drought had an effect on the germination rate, germination potential and germination index of Datura seeds, and all the three indicators reached a significant level (P<0.05).

According to Table 2, under the same drought stress gradient, the germination rate, germination potential, germination index and vigor index of Datura all rised first and then reduced with the increase of exogenous calcium concentration. When the drought stress was 5%, all the four indexes significantly decreased when the exogenous calcium concentration was 30mmol/L (P<0.05). When the drought stress was 10%, the germination rate and germination potential index reached the maximum when the exogenous calcium concentration was 20mmol/L, which were 0.64 and 0.34, respectively, reaching a significant difference (P<0.05). With a further

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increase in the exogenous calcium concentration, the three indicators began to decline, and the four indicators were significantly lower than the value at 0% when the exogenous calcium concentration was 30 mmol/L. When the drought stress was 15%, the three indexes reached the maximum when the exogenous calcium concentration was 20mmol/L, which was 1.23, 1.29, and 1.14 times those at 0%, respectively. When the exogenous calcium concentration was 30mmol/L, the three indexes decreased sharply, 0.56, 0.76, and 0.59 times those at 0%, respectively.When the drought stress was 25%, the three indicators reached the maximum when the exogenous calcium concentration was 20 mmol/L, with no significant difference from the control group under their own drought gradient, and then reduced with the increase of exogenous calcium concentration, the three indexes were significantly lower than those at 0% of the drought gradient ($P<0.05$).It can be seen that the exogenous calcium of appropriate concentration (20mmol/L) can promote the Datura seed germination under drought stress, especially the moderate drought level (10%, 15%), while that of high concentration (25~20mmol/L) would further inhibit the Datura seed germination under drought stress.

4. Results

Datura has a high medical, agroforestrial and ornamental value. The first important problem that must be faced in the cultivation and application of Datura is that the germination rate of Datura seeds is relatively low. Datura seeds are naturally in dormant state, with a dormancy period of about 6-8 months. The Datura seeds selected for this experiment were collected in early October 2017 and the experiment started in mid-March 2018, so the seeds had been stored for nearly 6 months, and the substances inhibiting germination in the seeds had almost been decomposed. The dormant state had been broken, so no mechanical friction or other processing of the seed was needed during the test.

Calcium is one of the essential elements for the growth of advanced plants. As a signal substance, Ca^{2+} involves in the physiological processes such as plant growth and development, seed dormancy and germination, etc. Also, Calcium is an activator for some enzymes like ATP, hydrolysis, dehydrogenation of succinic acid, etc. The exogenous Ca^{2+} of an appropriate concentration can promote the increase of endogenous free Ca^{2+} content in seeds, while the intracellular free Ca^{2+} and calmodulin are combined to directly or indirectly regulate the activity of relevant enzymes and cell function within the cell, thus to a certain extent reduce the damage of the adversity to the seed and improve the seed germination and vitality. In this study, under normal moisture conditions, 15%-20% of exogenous calcium showed no significant effect on the germination rate, germination potential and germination index of Datura, with differences not reaching a significant level compared with the control group, indicating that exogenous calcium had no positive effect on the Datura seed germination, which varies from the results of previous studies. In the experiment, when the concentration of exogenous calcium ion was 30%, the difference between the treatments reached a significant level, implying that exogenous calcium of high concentration inhibited the Datura seed germination. Exogenous calcium and drought have a significant interactive impact on the Datura seed germination.Under drought stress, exogenous calcium of an appropriate concentration (20%) could promote the Datura seed germination. Under moderate drought conditions (10%, 20%), the promotion was noticeable; severe drought ruined the physiological mechanism of the Datura seed, so exogenous calcium showed no significant effect. Therefore, for the production and utilization of Datura, 20% of exogenous calcium can be applied to soak the seeds before sowing to enhance the germination rate in the moderate arid areas.

Thanks to the weathering effect and some of human activities in the Huoshan Mountain area, a special geological condition---saline-alkali land---formed. The saline-alkali land features calcium ions of a high concentration and drought. Datura seeds have certain tolerance to calcium ions and can germinate normally under the soil conditions in the Huoshan Mountain area. This has a positive effect on improving soil quality of the Huoshan Mountain area.

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Supplements

Table 1. The length diameter, short diameter, thickness and TKW of Datura seed

	length diameter (mm)	short diameter (mm)	thickness (mm)	mass of 1000 seeds/g (g)
max	3.49	2.77	1.40	6.472
min	2.77	2.46	1.20	6.151
ave	3.12	2.64	1.31	6.227

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Table 2. Effects of drought on seed germination rate, germination potential and germination index of Datura seed

PEG-6000 %	Germination rate	Germination potential	Vigor index
CK	0.64 ± 0.07^{a}	0.37 ± 0.04^{a}	1.47 ± 0.36^{a}
5	0.58 ± 0.09^{ab}	0.31 ± 0.05^{b}	1.36 ± 0.23^{ab}
10	0.53 ± 0.04^{b}	0.29 ± 0.03^{b}	1.33 ± 0.13^{ab}
15	0.30 ± 0.08^{e}	0.17 ± 0.05^{c}	1.29 ± 0.09^{ab}
25	0.14 ± 0.06^{d}	0.08 ± 0.06^{d}	1.14 ± 0.09^{b}

Note: different letters in the table indicate significant differences in data (P<0.05).

Table 3. Effects of Calcium Chloride on Germination Rate, Germination Potential and Germination Index of Datura Seed

$CaCl_2$mmol/L	Germination percentage	**Germination energy**	**Germination index**
CK	0.64 ± 0.07^{a}	0.37 ± 0.04^{ab}	1.47 ± 0.36^{bc}
15	0.63 ± 0.07^{a}	0.40 ± 0.05^{ab}	1.78 ± 0.42^{ab}
20	0.68 ± 0.03^{a}	0.42 ± 0.03^{ab}	1.88 ± 0.27^{a}
25	0.61 ± 0.11^{a}	0.49 ± 0.11^{a}	1.46 ± 0.20^{bc}
30	0.57 ± 0.05^{a}	0.34 ± 0.10^{b}	1.33 ± 0.14^{c}

Note: different letters in the table indicate significant differences in data (P<0.05).

Table 4. Effects of interaction of drought and calcium chloride on seed germination rate, germination potential and germination index of Datura seed

PEG-6000 %	$CaCl_2$mmol/L	Germination rate	Germination potential	Vigor index
0	CK	0.64 ± 0.07^{a}	0.37 ± 0.04^{a}	1.47 ± 0.36^{a}
5	0	0.58 ± 0.09^{ab}	0.31 ± 0.05^{ab}	1.36 ± 0.23^{a}
	15	0.62 ± 0.08^{a}	0.36 ± 0.05^{a}	1.47 ± 0.22^{a}
	20	0.64 ± 0.07^{a}	0.37 ± 0.04^{a}	1.47 ± 0.36^{a}
	25	0.57 ± 0.07^{ab}	0.33 ± 0.06^{ab}	1.35 ± 0.32^{a}
	30	0.53 ± 0.06^{b}	0.30 ± 0.04^{ab}	1.31 ± 0.33^{a}
10	0	0.53 ± 0.04^{b}	0.29 ± 0.03^{ab}	1.61 ± 0.19^{a}
	15	0.56 ± 0.04^{ab}	0.30 ± 0.03^{ab}	1.45 ± 0.22^{a}
	20	0.61 ± 0.05^{a}	0.34 ± 0.05^{a}	1.38 ± 0.08^{ab}
	25	0.52 ± 0.07^{b}	0.31 ± 0.03^{ab}	1.42 ± 0.40^{b}
	30	0.44 ± 0.06^{bc}	0.26 ± 0.02^{b}	1.08 ± 0.32^{b}
15	0	0.30 ± 0.08^{c}	0.17 ± 0.05^{c}	0.92 ± 0.29^{b}
	15	0.33 ± 0.05^{c}	0.18 ± 0.03^{c}	0.68 ± 0.14^{bc}
	20	0.37 ± 0.03^{bc}	0.22 ± 0.02^{bc}	1.05 ± 0.22^{b}
	25	0.28 ± 0.03^{cd}	0.17 ± 0.02^{c}	0.59 ± 0.09^{c}
	30	0.17 ± 0.06^{d}	0.13 ± 0.03^{cd}	0.55 ± 0.17^{c}
25	0	0.14 ± 0.06^{d}	0.08 ± 0.06^{de}	0.40 ± 0.19^{c}
	15	0.17 ± 0.05^{d}	0.11 ± 0.05^{d}	0.47 ± 0.16^{c}
	20	0.19 ± 0.07^{d}	0.12 ± 0.04^{d}	0.46 ± 0.17^{c}
	25	0.10 ± 0.03^{e}	0.08 ± 0.03^{de}	0.36 ± 0.13^{c}
	30	0.04 ± 0.03^{e}	0.04 ± 0.03^{f}	0.15 ± 0.10^{e}

Note: different letters in the table indicate significant differences in data (P<0.05).

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