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EFFECTS OF ABIOTIC STRESS ON SEED GERMINATION AND ROOT GROWTH OF *ACHYRANTHES BIDENTATA*

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Abstract

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Compared with the control, seed germination and root growth of *Achyranthes bidentata* were obviously inhibited by NaCl, especially seeds stressed with 250mmol/L NaCl colud not germinate. However, germination rate and root length of *Achyranthes bidentata* under drought condition were significantly higher than the controls, germination rate under moderately low temperature condition also enhanced and yet root growth was evidently inhibited. Similarly, seed germination was also promoted by streptomycin which yet restrained root growth of *Achyranthes bidentata*, but germination rate which was higher than the controls reduced along with increase of streptomycin concentration. In addition, under low temperature and streptomycin stress, shape of some cells and the intercellular space in root tip of *Achyranthes bidentata* seedling changed as compared to the controls, and effects of low temperature on root tip were higher than those of streptomycin. On the contrary, cells in meristematic zone of root tip under drought condition arranged orderly and closely and had strong cell division. Therefore, this research results indicated that effects of abiotic stresses on seed germination and root growth of *Achyranthes bidentata* were different, but specific mechanisms of effects are still unclear and need to be further explored.

Key words: abiotic stress, *Achyranthes bidentata*, germination, root

Introduction

Achyranthes bidentata belongs to *Achyranthes L.* of Amaranthaceae, is Perennial herb and mainly originates in qinyang, Wuzhi of Henan province in China. The root tuber of *Achyranthes bidentata* has many functions, such as nourishing liver and kidney, strengthening sinews and bones, falling blood pressure, invigorating blood circulation silt and analgesia, and so is frequently used in cure health (Han et al., 2005; Jin et al., 2007; Chen et al., 2009; Zhu et al., 2012). The main cultivars of *Achyranthes bidentata* are Hetaowen, Fengzhengke and Xiangniuxi in China and suitably grow in fertile and deep sandiness soil, while *Achyranthes bidentata* is not resistant to pathogen, low temperature, drought and salt, which not only influenced quality of *Achyranthes bidentata*, but also brought about serious reduction in output of *Achyranthes bidentata*. At present, the improvement in yield and quality of *Achyranthes bidentata* is only limited to prevention and control of field management, and the effectiveness is not optimistic. Therefore, the important missions in genetic research and breed improvement of *Achyranthes bidentata* are to understand the response and signal transduction mechanism of *Achyranthes bidentata* to adversity stress and enhance stress resistance of *Achyranthes bidentata*.

It is well known, salt, low temperature, drought and other stresses are primary factors influencing growth and development of plant (Boyer, 1982). In this article, Fengzhengke species which is the good variety of *Achyranthes bidentata* was used to study effects of abiotic stress on seed germination and root growth of *Achyranthes bidentata* in order to explore ability of *Achyranthes bidentata* responsing to abiotic stress, meanwhile the experimental results would provide scientific basis and useful information for further understanding effect mechanism of abiotic stress on growth and development of plant.

Materials and Methods

Plant materials: Seeds of *Achyranthes bidentata* (Fengzhengke type) were kindly provided by Jiaozuo Academy of Agricultural Sciences, Henan, P. R. China.

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Experimental design of stress treatments: Seeds of *Achyranthes bidentata* were subjected to various stresses including salt, drought, low temperature and streptomycin after placed in petri dishes to germination. The controls were watered with 2 ml distilled water every day, but seeds were only watered with 0.25 ml, 0.5 ml or 1.0ml distilled water for drought treatment. The low temperature treatment was performed by transferring seeds to incubator for 2h, 4h and 6h every day in which the temperature was set at 4^0C. For other stress conditions, seeds were watered every day with 2 ml NaCl solution (62.5 mmol/L, 125 mmol/L, 250 mmol/L NaCl) or streptomycin solution (25 mg/L, 50 mg/L, 100 mg/L streptomycin), respectively. Furthermore, 300 seeds were treated in each stress experiment, which was repeated three times.

Seed germination of Achyranthes bidentata: Seeds of *Achyranthes bidentata* were surface-sterilized with 0.5% potassium permanganate for 30 seconds and washed several times with sterile water, then soaked for 24h at 24^0C. Subsequently, these seeds were placed in Petri dishes at whose bottom two layers of filter papers were laid out, and there were approximately 100 seeds in per Petri dishes. The seeds in petri dishes were cultured at 26 ± 1^0C with 14h photoperiod of 1000-1200 lux illumination intensity and were watered every day, and then germination rate of seeds and root length of seedlings were countted after seeds of *Achyranthes bidentata* germinated. The computational formula of germination rate is the following, germination rate (%) = (sum of germinated seeds/ sum of experimental seeds) × 100%.

Histological analysis of root tip: After seeds germinated, 0.5-1 cm root tips were cut from root of seedlings under stress conditions in order to explore effects of abiotic stress on root growth of *Achyranthes bidentata*. The root tip had been fixed into 50% FAA solution for 30-50 min, and then was processed according to the following steps: dehydration in a series of ethanol, transparence with xylene, immersion and embedment in paraffin wax. The paraffin-embedded tissue samples were sliced by microtome with 5-10 μm slice and put on the slide, subsequently were dewaxed, and the safranin staining was used to observe histological structure of root tip. Every experimental material was repeated three times and observed with Olympus microscope.

Results

Effects of abiotic stress on seed germination of Achyranthes bidentata

As shown in Table 1, when cultured for 3d, some seeds of *Achyranthes bidentata* in different experimental groups began to bourgeon, compared with 0.29% germination rate of the control group (CK), the germination rate of T10 and T11 groups stressed with streptomycin was higher, yet germination rate of T4 and T5 groups treated by drought and T7 group stressed by 4^0C was also different to the controls. However, as cultured for 5d, germination rate of T4-T6 groups and T10 group was much higher than the controls. At 7d, germination rate of the control group was 10.20%, just 1.42% seeds stressed with 62.5 mmol/L NaCl (T1) began to bourgeon, but seeds stressed with 125 mmol/L (T2) and 250 mmol/L NaCl (T3) still could not germinate. As cultured for 9d, germination rate of seeds stressed with 62.5 mmol/L NaCl or 125 mmol/L NaCl was respectively 2.37% and 0.42%, yet seeds stressd with 250 mmol/L NaCl did not germinate. In addition, germination rate of each group stressed with streptomycin, drought and T7 group treated by low temperature was evidently higher than the control (18.90%), especially the germination rate of T4 and T5 groups can achieve even more than 80%.

Effects of abiotic stress on root growth of Achyranthes bidentata seedling

In this research, it was found that roots of *Achyranthes bidentata* seedling in the control group (CK) were longer and

Table 1
Germination time and germination rate of *Achyranthes bidentata* seed

Experimental group	Germination rate, %			
	3d	5d	7d	9d
CK	0.29%	1.45%	10.20%	18.90%
TI	0.00%	0.00%	1.42%	2.37%
T2	0.00%	0.00%	0.00%	0.42%
T3	0.00%	0.00%	0.00%	0.00%
T4	0.50%	40.00%	75.00%	85.00%
T5	1.00%	35.50%	67.50%	81.00%
T6	0.00%	10.50%	49.00%	70.00%
T7	0.34%	9.16%	40.80%	62.20%
T8	0.00%	2.05%	13.70%	24.70%
T9	0.00%	0.50%	3.50%	9.67%
T10	1.50%	26.00%	51.50%	67.50%
T11	1.02%	5.08%	33.50%	64.00%
T12	0.00%	1.84%	7.37%	26.70%

CK represents the control group; T1-T3 respectively represent seeds stressed with 62.5 mmol/L, 125 mmol/L and 250 mmol/L NaCl; T4-T6 represent seeds treated by drought and watered with 1.0 ml, 0.5 ml and 0.25 ml distilled water per day; T7-T9 represent seeds stressed by low temperature at 4°C for 2h, 4h and 6h per day; T10-T12 represent seeds stressed with 25 mg/L, 50 mg/L or 100 mg/L streptomycin.

longer along with increase of culture time, but root length was influenced by various abiotic stresses (Table 2), in which effects of NaCl on root growth were the largest and root length was shorter with increase of NaCl concentration, especially root could hardly grow under 125 mmol/L NaCl condition (results were not shown). Root growth was also restrained by 4^0C, the speed of root growth was slower and roots were shorter as the treatment time at 4^0C was longer, however root length exhibited growth trend along with the extension of culture time (Figure 1 d-f). As well as, streptomycin inhibited root growth and the inhibition degree was more obvious along with increase of streptomycin concentration, but root growth was on the increasing trend with the elongation of culture time (Figure 1 g-i), moreover, the inhibition action of streptomycin to root growth was lighter compared to low temperature. On the contrary, the speed of root growth under drought condition was relatively faster than the control, especially length of root in T4 and T5 groups was much longer than the control, and roots in T6 group were longer (Figure 1 a-c).

Effects of abiotic stress on ultra structure of root tip

Root elongation is accomplished by the primary growth of meristematic zone in root tip which was studied in order to understand effects of stress on root elongation of *Achyranthes bidentata* seedling in this article. As shown in Figure 2 (a), in the root tip of *Achyranthes bidentata* seedling under non-stress condition, the root cap which covers and protects the meristematic tissue was intact and consisted of more layers of parenchyma cells which were irregularly arranged, the meristematic zone looked like taper and was composed of apical meristem which consists of promeristem and primary meristem, and the stratification characteristics were found in the array and exhibited division activity of promeristem. Under various stress conditions, composition of cell structure in the root tip of *Achyranthes bidentata* seedling did not alter, but shape of some cells and the intercellular space changed (Figure 2 e-j). In the meristematic zone of root tip exposed to low temperature, the cell wall of some cells invaginated and the intercellular space enlarged which was obvious along with increase of stress time at 4^0C (Figure 2 e-g), besides effects of streptomycin on structure of root tip were similar to low temperature, but just were lower (Figure 2 h-j). Other than effects of low temperature and streptomycin on root tip of *Achyranthes bidentata*, cells in meristematic zone of root tip under drought condition arranged orderly and closely, contained a large nucleus and dense cytoplasm, and had strong cell division (Figure 2 b-d).

Table 2
Effects of stress on root length of *Achyranthes bidentata*

Experimental group	Root length, cm		
	5d	7d	9d
CK	0.80±0.05	1.31±0.04	2.32±0.03
T4	1.12±0.06	2.15±0.03	3.51±0.04
T5	0.93±0.04	2.06±0.04	3.48±0.04
T6	0.81±0.03	1.85±0.05	2.65±0.06
T7	0.75±0.03	1.25±0.06	2.15±0.05
T8	0.62±0.06	0.83±0.07	1.34±0.03
T9	0.50±0.07	0.65±0.05	1.15±0.04
T10	0.75±0.02	1.25±0.03	2.08±0.06
T11	0.63±0.06	1.12±0.05	1.90±0.03
T12	0.51±0.04	0.95±0.06	1.81±0.04

CK represents root length of the control; T4-T6 respectively represent root length of seedlings watered with 1.0 ml, 0.5 ml and 0.25 ml distilled water per day; T7-T9 respectively represent root length of seedlings at 4°C for 2h, 4h and 6h per day; T10-T12 respectively represent root length of seedlings stressed with 25 mg/L, 50 mg/L or 100 mg/L streptomycin.

Discussions

Germination rate could reflect the field emergence rate of seed to some extent and is connected with seed vitality, but the suitable environments are needed for seeds to sprout, such as temperature, light, moisture and so on. In this research, seed germination of *Achyranthes bidentata* was influenced by NaCl, drought and low temperature, especially was obviously restrained under NaCl stress condition, the germination rate of seeds stressed with 62.5 mmol/L and 125 mmol/L NaCl was far less than the control, and seeds stressed with 250 mmol/L NaCl could not germinate. The primary reason of salt stress restraining germination is inferred that salt stress might reduce the osmotic pressure difference between seed and external environment which would bring about reduction of seed water absorption (Werner and Finkelstein, 1995), thus seed germination was restrained . Furthermore, germination time was also delayed when seeds of *Achyranthes bidentata* were put at 4^0C for 4-6h, inferring longer time of low temperature might influence enzyme activity which makes seed germination delay, however germination rate evidently increased when seeds were treated for 2-4h at 4^0C, similarity, germination rate of seed treated with streptomycin was higher than the control and yet reduced along with increase of streptomycin concentration. In addition, it is most remarkable that germination rate of seeds under drought condition increased and was much more than the control, perhaps the requirement for water is few. Accordingly, the above research results demonstrate that *Achyran-*

thes bidentata is suitably grown in sandy soil under sunny and dry environment, but inappositely sowed in saline-alkali soil (Yang et al., 2002).

It is well known, roots of plant have many functions, such as support and fixation, uptake, transmitting and storage, synthesis, secretion, and so on (Nasholm and Persson, 2001; Karthikeyan and Kulakow, 2003; Kirk and Kronzucker, 2005), therefore, root development directly affects growth of plant and is closely relevant with agricultural production. The root tuber of *Achyranthes bidentata* is famous herbal medicine and could promote protein synthesis, remove cholesterol, reduce blood-fat and control increase of blood sugar. In this research, growth speed of *Achyranthes bidentata* root was faster, root was longer and longer along with increase of culture time, but root growth was influenced by abiotic stress, especially effects of NaCl were the most obvious, speed of root growth was slower and even hardly grew when NaCl concentration increased. Moreover, root growth was obviously inhibited when *Achyranthes bidentata* seedlings were put at 4^0C for 2-6h every day; however, drought condition promoted root growth, declaring drought condition is beneficial to growth of *Achyranthes bidentata*, or the volume of water and other solution used in this experiment maybe a litter more than the needed sum of *Achyranthes bidentata*. In addition, root growth was also inhibited by streptomycin and the inhibition action increased along with increase of strepto-

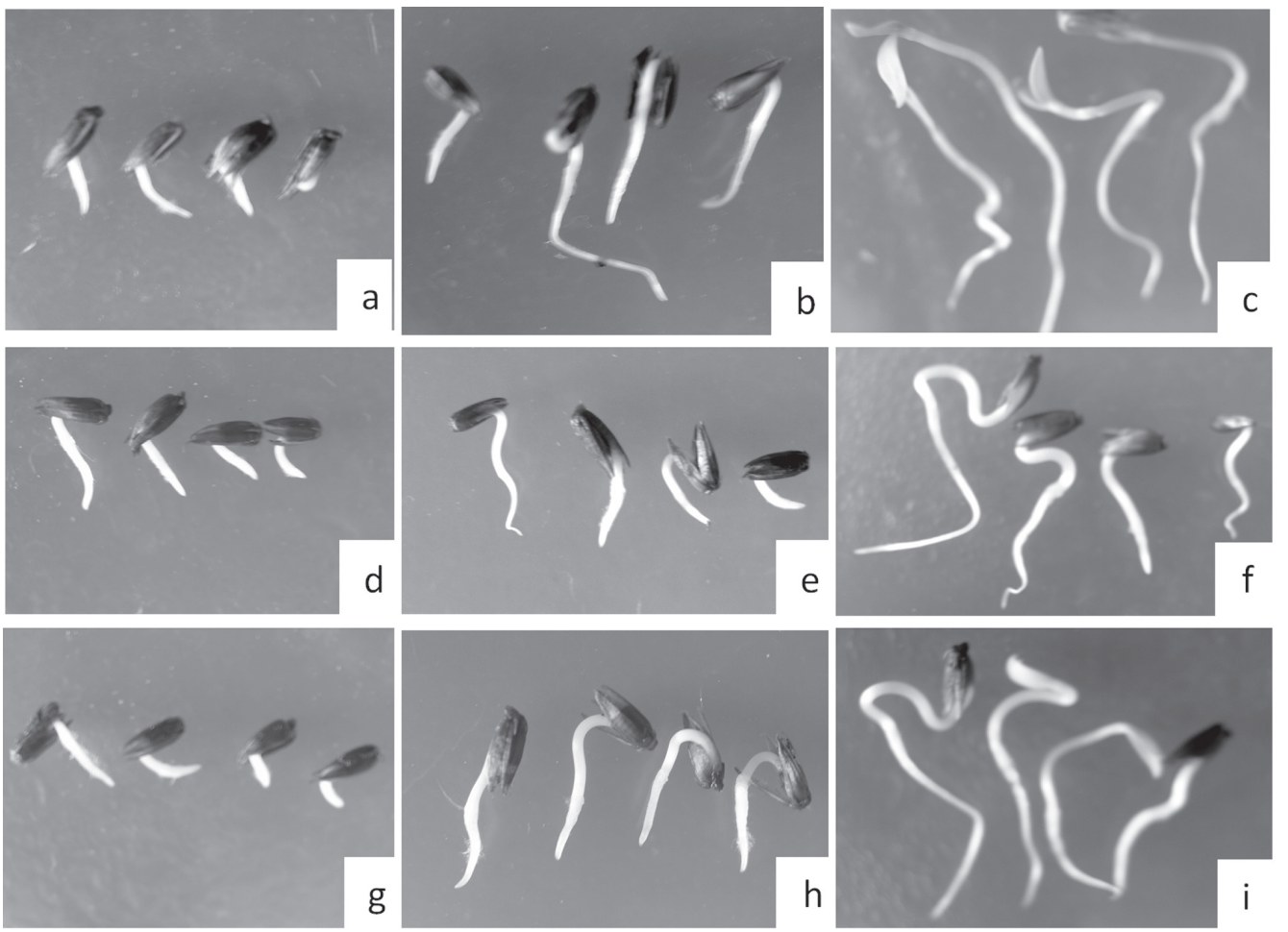

Fig. 1. Effects of stress on root growth of *Achyranthes bidentata*

(a), (b) and (c) represent roots cultured for 4d, 6d, 10d under drought stress, in which roots were respectively watered with 2.0 ml, 1.0 ml, 0.5 ml and 0.25 ml distilled water per day; (d), (e) and (f) represent roots cultured for 6d, 7d, 10d under low temperature stress, in which roots were respectively put at 4°C for 0h, 2h, 4h and 6h per day; (g), (h) and (i) represent roots cultured for 5d, 7d, 10d under streptomycin stress, in which roots were respecitvely watered with 25 mg/L, 50 mg/L or 100 mg/L streptomycin.

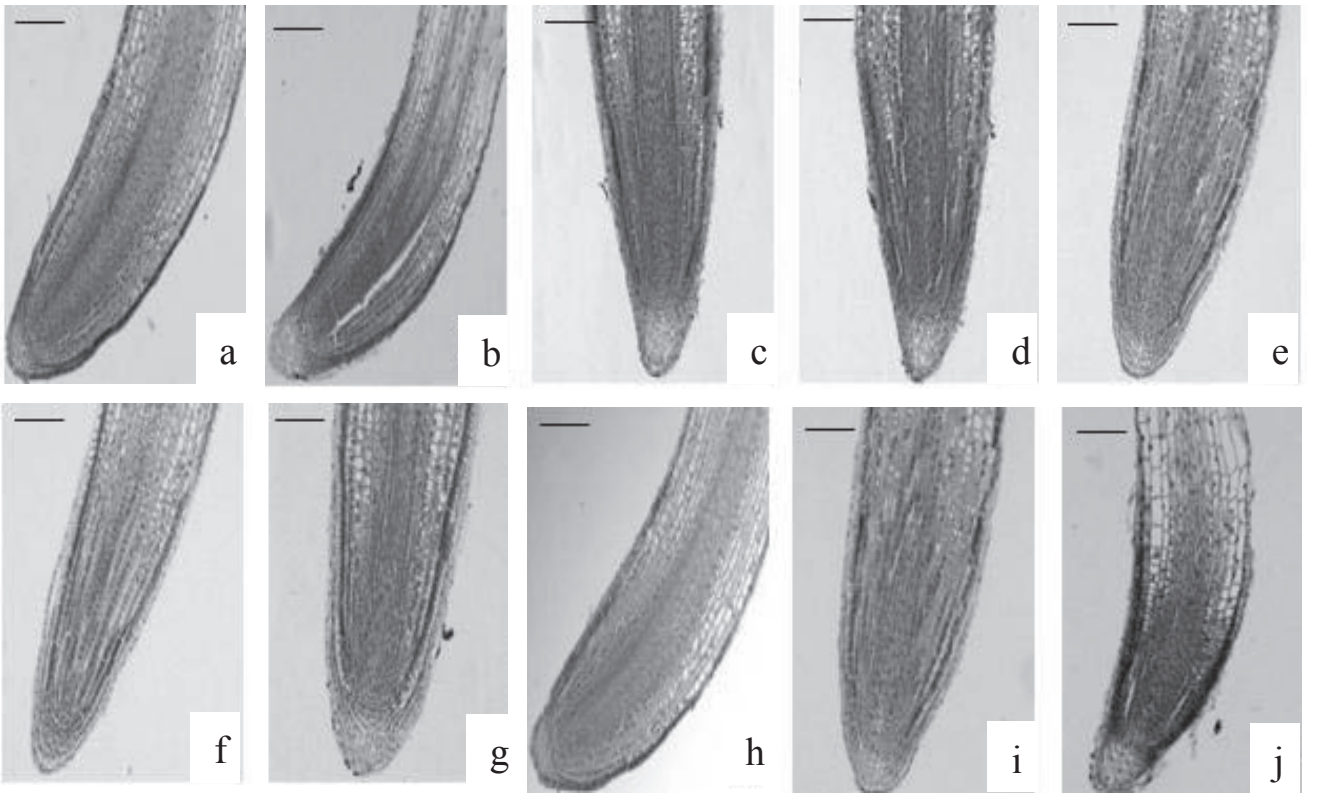

Fig. 2. The ultrastructure of *Achyranthes bidentata* seedling root tip

(a) The part vertical section of root tip from the control; (b), (c) and (d) represent the part vertical section of root tip from seedlings respectively watered with 1.0 ml, 0.5 ml and 0.25 ml distilled water per day; (e), (f) and (g) respectively represent the part vertical section of root tip from seedlings put at 4°C for 2h, 4h and 6h per day; (h), (i) and (j) represent the part vertical section of root tip from seedlings stressed with 25 mg/L, 50 mg/L or 100 mg/L streptomycin. The scales represent 50 μm.

mycin concentration, while inhibition degree of streptomycin was lighter than that of low temperatur, streptomycin could also evidently inhibit root growth of rape seedling (Luo et al., 2005). Furthermore, growth and development of *Arabidopsis* seedling root were distinctly inhibited by Hygromycin and kanamycin (Duan and Ding, 2007; Duan et al., 2009), and cells in the meristematic zone of *Arabidopsis* root tip exhibited abnormal array, weak division ability, evident differentiation and large intercellular space (Duan and Ding, 2007). In order to further explore effects of abiotic stress on root of *Achyranthes bidentata* seedling, structure of root tip was studied by paraffin method. Compared with the control, the cell wall of some cells invaginated and the intercellular space enlarged in the meristematic zone of root tip exposed to low temperature, which was obvious along with increase of stress time at low temperature, besides effects of streptomycin were similar to low temperature. However, cells in meristematic zone of root tip under drought condition arranged orderly and closely and had strong cell division.

Conclusion

In brief, under drought, low temperature or streptomycin conditions, germination rate of *Achyranthes bidentata* seeds all increased, but root growth were obviously inhibited by NaCl, low temperature and streptomycin, only drought condition could promote root growth of *Achyranthes bidentata*. Therefore, this research results indicated that effects of abiotic stresses on seed germination and root growth of *Achyranthes bidentata* were different, which might provide theory basis for field planting, variety selection, drug property and pharmacology and other studies of *Achyranthes bidentata*.

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