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Drought-tolerance Evaluation of Cotton with PEG Water-stress Method

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Abstract: Eight upland cotton cultivars, two sea island cotton ones and three Asian cotton ones with varied levels of drought tolerance were used. Cotton samples at germination, bud-stage, cotyledon-stage and real-leaf stage were exposed to PEG6000 stress for 12 hours. After 12-hour osmotic treatment, the survival rates were calculated, then by statistical method, curves of the mutative level of drought tolerance were obtained, proving that the 3~6-leaf stage is the key period related to cotton drought tolerance. With different PEG6000 concentrations, the rates which 3~6-leaf seedlings revived were calculated to compare with the revival rate in real drought situation. And the results revealed that: With 17% PEG6000 treatment, the rate which seedlings revived corresponded with the results in drought-shed. The results suggested that osmotic adjustment of cotton could be used to evaluate simply the drought tolerance of cotton, though the cotton after PEG-treated differed slightly in physiology with cotton after drought-treated. The method with osmotic adjustment of PEG is simple, fast and easily operated, could be used to evaluate the drought tolerance of cotton in principle, and will establish foundations for study on cotton drought-tolerance molecular biology.

Key words: cotton; PEG water-stress; drought tolerance; revival rate

Drought is one of the main factors which affect plant genetic expression. As most cotton-growing regions in China are water-resource limited, it has great significance to establish an evaluation and identification standard of drought-resistance. Such standard is useful in drought-tolerant cotton culture and breeding.

Since the 1980s, researchers have been working to establish the evaluation and identification system of drought-tolerant plants[1-3], with particular on wheat[4], barley[5], maize[6], sorghum[7] and so on. The main identification methods include field identification method, manual-control drought stress, biochemical identification method and natural desiccation stress method. Field identification method is simple and reliable, but restricted by the season, labor-intensive, slow and poorly repetitive; manual-control drought stress, including drought stress of repeated and continuously drying methods, has the similar shortages to field identification method; biochemical identification method with polyethylene glycol (PEG)[8] to make osmotic stress, can overcome the shortcomings of the two methods above. In 1979, it was the first time to utilize PEG as an inducer and identifier to screen and select drought-resistant tobacco cell lines[9]. Articles about using PEG on study of drought-resistant tomato cells[10], drought-resistant sorghum regeneration and seed[11], anti-PEG stress alfalfa cell lines[12-13] we reported. Chinese researchers used to do cotton drought evaluation and identification by repeated drought method[14]. It is still in the experimental stage to use PEG solution for the identification. PEG6000 was used to establish a rapid and effective cotton-drought toler-

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ance evaluation system for selection and breeding of the drought-tolerant cotton resources.

1 Methods and Materials

1.1 Materials

Eight upland cotton cvs (Jinmian 26, Lumian 6, Jihe 713, Coker 310, Coker 348, CCRI 9, CCRI 12 and CCRI 27), two Sea island cotton cvs (Xinhai 16, Xinhai 17), three Asian cotton cvs (Xinping native cotton, Fengyangzhongmian and Shixiya 1) used in the experiments were provided by national medium-term storage of cotton (Anyang).

Polyethylene glycol (PEG6000) was bought from AMERESCO.

Mature seeds of above cultivars were planted in the nutrial mantle (soil matrix: peat: vermiculite : coarse sand = 4: 3: 2: 1)[15]. Experimental seedlings were washed carefully by distilled water, then were cultivated in MS nutrient solution for two days and blotted up with absorbent paper rapidly.

1.2 Experimental methods

1.2.1 *PEG stress identification at germination.* The shelled seeds were disinfected with 0.1% $HgCl_2$, and then placed on three-tier filter paper in Petri dishes, 150 seeds in each dish. 10 mL of 0, 5%, 10%, 15%, 20%, 25% and 30% PEG6000 solution was added into each dish (distilled water was for comparison treatment). Photos were taken for every 24 hours in order to observe and record the germination when the seeds were cultured at 26 to 28℃ in the dark[16].

1.2.2 *PEG stress identification at bud stage.* The germinated seeds were placed in dishes (with Φ150 mm filter pad at the bottom) soaked with 10 mL PEG6000 solution (0, 5%, 10%, 15%, and 20% gradient treatment), cultured at 26℃ to 28℃ in the dark. Every treatment was repeated six times, then the rates of survival were counted[17].

1.2.3 *PEG stress identification at seedling stage.* The roots of prepared cotton seedlings were cleaned carefully, put into PEG6000 solution at 26℃ to 28℃ for 12 hours under light. Then roots were cleaned with distilled water, and immerged into MS nutrient solution again. The state of the seedling growing in MS nutrient solution and the survived numbers were record[15] (keep water fresh). Every treatment was repeated six times (under five treatments).

1.2.4 *Measurement of leaf water content.* Seedlings at cotyledon and real-leaf stage were separately dealt with six gradient treatments (0, 0.5%, 1%, 1.5%, 2% and 2.5%) and three gradient treatments (5%, 7.5%, 10%) of PEG6000 solution for 12 hours. The leaves were washed several times with water, three times with distilled water, and blotted up. After the fresh weight (W_f)[18] were recorded, leaves were immerged into distilled water immediately, and weighed once every 2 hours till the weight didn't increase as it is saturated (W_t), then fixed 15 min at 100～102℃, dried about 8 hours at 80 ℃ to dry weight (W_d). According to the formula, the leaves water contents (RWC) were calculated, each treatment was repeated four times.

$$RWC = (W_f - W_d) / (W_t - W_d) \times 100\%.$$

1.2.5 *Measurement of leaf cell membrane permeability.* cotyledon or real-leaves were deal with as same as **1.2.4**, cut into pieces after airing, weighed 0.5 g and put into 100 mL distilled water. Refering to the "Handbook of Plant Physiology"[19]: the resistivity R_1 was measured after infiltration for 24 hours at 25℃; boiled in boiling water for 15 min, the resistivity R_2 was measured after natural cooling to room temperature (repeat three times). According to the formula, the electrolyte relative permeability was calculated.

$$\text{Electrolyte relative permeability} = (R_1 / R_2) \times 100\%.$$

2 Results

2.1 Results of PEG stress on cotton during the different initial growing stages

The survival rates of a drought-resistant cotton variety (Shixiya 1) and a drought-sensitive variety (Fengyangzhongmian) at germination, bud, cotyledon, and leaf stage under the stress of PEG were measured. The results showed that the semi-lethal concentration(LC_{50}) varied with varieties and growth stages under PEG stress. Semi-lethal concentration of Shixiya1 was higher than that of Fengyangzhongmian, especially at the 1st leaf stage (24% and 17%)(Figure 1). Similar results were obtained in repeated drying identification experiment: when the soil moisture reached 3%, the cotton seedlings were re-watered, then, were seared until the soil moisture reach 3% again. The survival rate of Shixiya1 was more than 70 % (drought-resistant variety level[19]), while the survival rate of Fengyangzhongmian was less than 50%(nondrought-resistant variety level) in repeated drying identification experiment.

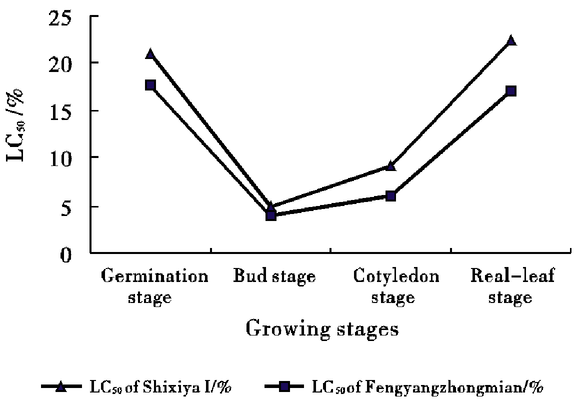

Fig. 1 Variation of median lethal concentration (PEG6000) on cotton during different stages

The drought-tolerant capacity of cotton started to decline from the early germination stage, the lowest was at bud stage, then gradually increase till the real-leaf stage tending to be stable. The repeated drying identification method mainly target on the leaves stage seedlings in coincidence. Furthermore, it is relatively easy to operate at the real-leaf stage. We predict that the real-leaf stage is a key stage of cotton drought tolerance identification experiment by PEG stress. The previous assessment that the botanic growing stage for drought-tolerant experiment should be real-leaf stage seedlings also applies to cotton.

2.2 Determination of the concentration of PEG6000 in identification experiments

Researches showed that botanic drought-resistance varid with its growth stage[4, 6]. According to the results from repeated drying identification experiments in many years, the cotton variety Jinmian 26 (with 50% survival rate in average) was used in the experiment to confirm the concentration of PEG6000 in identification experiments. The survival rate of Jinmian 26 was obtained under 0, 5%, 10%, 15%, and 20% PEG6000 solution treatment (Figure 2). The LC_{50} was between 16% and 17%. Further data showed that, the survival rate of Jinmian 26 seedling (3 to 6 leaf stage) under 17% concentration of PEG6000 was 50%. So the concentration of PEG6000 in identification experiments is exactly 17%.

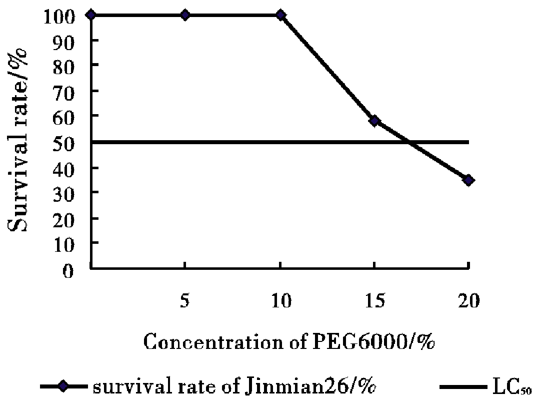

Fig. 2 The effect of different PEG6000 concentration on the survival rate of Jinmian 26 during the real-leaf stage

2.3 Study on drought-tolerance evaluation of cotton with PEG water-stress method

The evaluation results of 13 cottons with 17% PEG6000 solution (Table 1) were compared with the results by repeated drying method. According to the standards in "Germplasms and Agronomic Traits of Chinese Cotton "[20]: the survival rates at 0～49%, 50%～69%, 70%～89%, and above 90% were respectively named as NT (non-drought-tolerant), below 10% is S (drought-sensitive), T(drought-tolerant), R (drought-resistant), and HR (high resistant) varieties. The evaluation results by

PEG water-stress method and repeated drying method were statistically analyzed by univariate fitting: r = 0.9626; a = 1.1653; b = -7.8966 (Table 2).

Table 1 The comparison on the identification results by two identification methods

Cotton variety	Survive/ individual	Individual number	Survival rate (PEG) /%	Level(PEG)	Survival rate (RD)* /%	Level(RD)
Jinmian 26	50	100	50	T	50	T
Lumian 6	12	36	33.3	NT	11.5	NT
Jihe 713	57	90	63.3	T	67.9	T
Coker 310	71	96	74.0	R	85	R
Coker 348	60	160	37.5	NT	39.4	NT
CCRI 9	10	13	76.9	R	92.9	HR
CCRI 12	23	30	76.7	R	76.6	R
CCRI 27	2	57	3.5	NT	0	NT
Xinhai 16	38	66	57.6	T	68.9	T
Xinhai 17	3	40	7.5	NT	5.8	NT
Xinping native cotton	16	51	31.4	NT	23.2	NT
Fengyangzhongmian	46	100	46	NT	48	NT
Shixiya 1**	86	100	86	R	80.5	R

Note: * the data of repeated drought are the mean of many years' experiment by the Chinese Academy of Agricultural Sciences cotton varieties of cotton resource room Resistance Group; ** : the results of identifications of same Shixiya 1 by the two methods in the same year are consistent, verifying that identification the drought resistance of cotton by 17% PEG6000 is feasible.

Table 2 The statistical analysis on the identification results in two identification methods

Variable	SS	DF	MS	F	$F_{0.05}$	$F_{0.01}$
Regression	11256.0792	1	11256.0792	126.3293	4.9646	10.0443
Partial regression	891.0106	10	89.1011			
Total variable	12147.0898	11				

There was a positive relationship between identification results by the two methods, with high coefficient (r) and significant variance analysis by F-test. So, the drought-tolerance evaluation of cotton with PEG water-stress method is simple, rapid, and reliable.

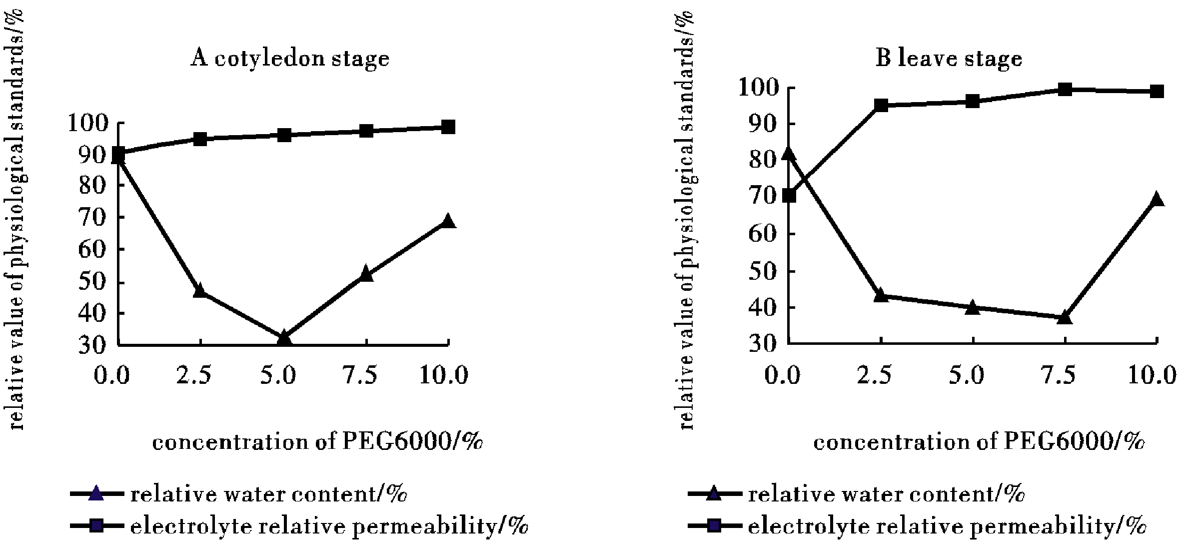

Fig. 3 variation of relatively moisture and electrolyte relative permeability in leaves of seedling under PEG water-stress

2.4 Physiological changes under PEG water-stress

Seedlings of Shixiya 1 were dealt as described in 1.2.4 and 1.2.5. The relative water content

(RWC) and the plasma membrane permeability rate of leaf were measured. According to Hsiao standards, the stresses with RWC decreased 8% ~10%, 10% ~ 20% and 20% were assessed as mild, moderate and severe stress[18].

The changes of RWC and electrolyte relative permeability in cotyledon stage and real-leaf stage seedlings under different concentrations of PEG water-stress were respectively shown in A, B of Figure 3. RWC of real-leaf decreased gradually, while electrolyte relative permeability is slightly rose with the increasing of concentration of PEG6000. At otyledon stage, the RWC dropped to the lowest level when PEG concentration is 5%, while at real-leaf stage, the RWC reached the minimal value when PEG concentration is 7.5%. The results that RWC of cotyledon stage leaves decreased 23.8% (serious stress), while RWC of real-leaf stage leaves decreased 17% (moderate stress) equally under 5% PEG6000 water-stress, is consistent with the result that cotton drought-tolerance at real-leaf stage is higher than cotyledon stage in 2.1. It is appropriate to operate drought-tolerance evaluation of cotton with PEG water-stress at real-leaf stage.

3 Discussion

Cotton drought-tolerance at real-leaf stage is higher than that at cotyledon stage when we operated the drought-tolerance evaluation of cotton with PEG water-stress at different initial growing stages. Maybe the root at real-leaf stage developed more completely leading to the stronger capacity of water-absorbing, so that relative water loss is less than cotyledon seedlings. On the other hand, drought-tolerance improvement may because, cells at cotyledon stage has greater water loss rate than at real-leaf stage under PEG solution stress due to infantility, while structure of stomata at real-leaf stage has become fairly developed. Whereas the true reason needs further researches.

Varied with the increase of PEG concentration, there was a rising of RWC both in cotyledon and real-leaf after reached the lowest. The damage to cell membrane structure caused by prolonged stress might have brought refluence of water.

This evaluation method is well repetitive, labor-spare, and fit for initial identification and evaluation on drought-tolerance of cotton. However, when the method was utilized in production, in order to ensure results are accurate and reliable, it is necessary to be integrated with traditional methods.

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