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DREB类转录因子介导的烟草抗非生物胁迫特性研究

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摘 要：检测并分析了转*BnDREB1-5*基因烟草叶片的持水性能，上、下表皮气孔大小和密度及叶绿素含量，并在自然失水7 h后检测了细胞的离子泄漏状况。结果表明：转基因烟草叶片单位时间内每平方厘米的失水量是野生型烟草叶片的62%；上表皮的气孔大于野生型，而气孔开度小于野生型；野生型烟草叶片上的气孔密度接近转基因烟草的1.5倍；野生型烟草叶片的叶绿素含量比转基因烟草叶片高29%；野生型烟草叶片的质膜相对透性是转基因烟草叶片的1.4倍。

关键词：烟草；DREB转录因子；抗非生物胁迫；生物学性状
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The Tolerance to Abiotic Stresses Mediated by DREB-like Transcription Factors in *Nicotiana tabacum*

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Abstract: The biological properties on tobacco with 35S:*BnDREB1-5* transformation were analyzed which included the holding water capacity, the size and density of upper and lower epidermis stomata, the content of chlorophyll, and plasma membranes permeability after physical drought stressed 7 h. The results indicated that water-holding capacity of 35S:*BnDREB1-5* transgenic tobacco leaves was 62% of that of wild-types. The upper epidermis stomata apparatus was bigger in transgenic tobacco than wild-type, but the opening of stoma was smaller in upper epidermis than wild-types. The stomata density was 1.5 times in wild-type leaves as large as in transgenic leaves. The content of chlorophyll was 29% higher in leaves of wild-type than in transgenic leaves, and the plasma membranes permeability were 1.4 times as much as in transgenic tobacco.

Key words: Tobacco (*Nicotiana tabacum*); DREB transcription factor; Abiotic stresses; Biological properties

Drought, high-salt and low-temperature are stresses that cause adverse effects on growth and development of tobacco resettling stage and result in abloom stage ahead, which bring tobacco farmers about serious loss of economy. To deal with this problem, tobacco scientists have taken many measures in planting and fertilization technique for a long time, but that is ideal. That is because the characters of plant stress-resistance are far more complicated compared with the correlated characters of plant insect-resistance and disease-resistance. The capability of plant to tolerance of drought, high-salt and cold-stress is independent of a single functional gene. The character of plant stress-resistance is influenced by many functional genes[1]. Thus, it is important to enhance the control capability of one key regulatory factor to make the plant achieve ideal, multiple and functional improvement for stress-resistance[2].

DREB (dehydration responsive element-binding protein) transcription factor plays an important role in regulating gene expression in that response to drought, high-salt and cold-stress[3,4]. It may be a more effective strategy to improve or enhance the control capability of the key transcription factor for the ideal and multiple effects compared with the conventional methods which

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a single functional gene is transferred for improving a single trait. We transferred the *BnDREB1-5* from *Brassica napus* to tobacco plant and investigated the abiotic stress tolerance and some biological properties of transgenic tobacco leaves.

1 Materials and methods

1.1 Materials

Transgenic tobacco with 35S: *BnDREB1-5* was used in this experiment.

1.2 Methods

1.2.1 Leaf water loss (LWL)

The areas of leaves of transgenic and wild type tobacco (WT, hereinafter the same) were measured by PLANT LEAF C1-202 (made in U. S. A.). Then leaf water loss of *in vitro* transgenic and wild type tobacco was determined at room temperature and weighed every 30 min for 7 h. Leaf water loss rate was calculated.

1.2.2 The upper and lower epidermis stomata of transgenic tobacco leaves

After being incubated on humid filter paper for one hour, the upper and lower epidermis were isolated from the leaves and then the stomata parameters (including density, length, width, etc.) in that were examined with a Olympus microscope.

1.2.3 The contents of chlorophyll

The method of it accord to reference [5].

1.2.4 The plasma membrane permeability

The method of it accord to reference [5].

2 Results

2.1 The analysis of leaf water loss

The rate of LWL was obviously slower in transgenic tobacco than wild type for 7 h (Fig. 1). In the first hour, the rate of wild type is 1.62 times as much as it

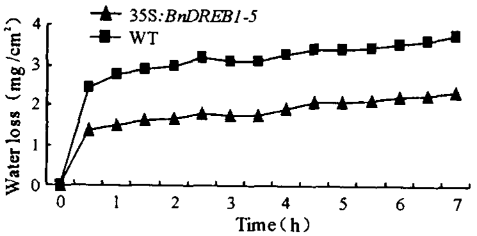

Fig. 1 Analysis of LWL of transgenic tobacco and wild type

of transgenic tobacco. And then the rate of transgenic tobacco was lower than wild-types during all the treated time. 7 hours later, the rate of transgenic tobacco was 0.33 mg H_2O/cm^2 per hour, and the rate of wild-types was 0.54 mg H_2O/cm^2 per hour. The amount of lost water from transgenic tobacco leaves was 62% as much as it of wild-types.

2.2 The analysis of stomata parameters

Compared with wild types, the stomatal apparatus of upper epidermis in transgenic tobacco were bigger, and the width of the pore was smaller (Fig. 2: A). But in the lower epidermis, they are both bigger than wild type (Fig. 2: B).

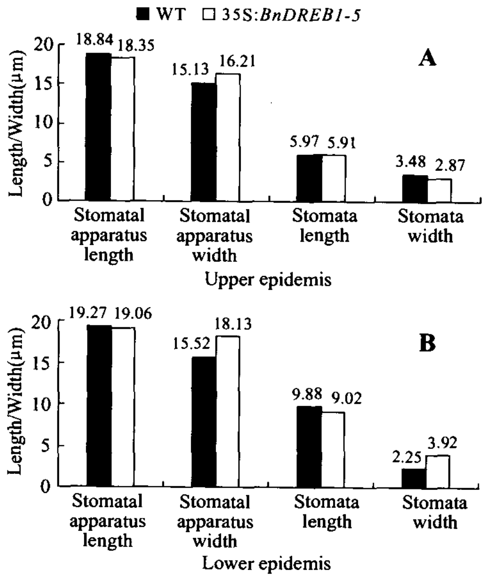

Fig. 2 The stomata size and degree of opening of upper and lower epidermis

Compared with wild types, the stoma densities in upper and lower epidermis of transgenic tobacco were lower. But the stomata density in upper epidermis was different obviously, that of wild-type leaves was 1.5 times as much as transgenic plants (Table 1).

Table 1 Stoma density of leaves between transgenic and WT

Basma	Upper epidermis	Lower epidermis
35S: *BnDREB1-5*	8.1	11.2
WT	12.5	12.4

2.3 The contents of chlorophyll

The contents of chlorophyll in wild-type leaves were 29% more than that of transgenic tobacco (Table.2).

Table 2 The contents of chlorophyll of leaves of transgenic tobacco and WT

Basma	Chlorophyll content(mg/g)
35S:*BnDREB1-5*	2.09
WT	2.70

2.4 The analysis of plasma membrane permeability

The relative plasma membrane permeability of wild-type leaves was 1.4 times as much as that of transgenic leaves after drought stress for 7 h (Fig. 3). The result showed that the ability of transgenic tobacco drought tolerance was obviously strengthened.

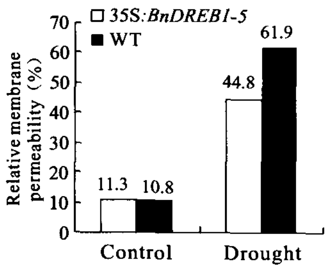

Fig. 3 Relative plasma membrane permeability of leaves of transgenic tobacco and wild-type

2.5 Growth and development of transgenic tobacco

Table 3 showed the growth and development of transgenic tobacco and wild-type after being transplanted for 22 days. The leaf number of transgenic tobacco was more than that of wild-type, the stalk length and the height was shorter than that of wild-type. These data showed that the growth of transgenic tobacco plants had been limited.

Table 3 Growth and development of transgenic and WT

Basma	Number of leaves(piece)	Plant height (cm)	Average stalk distance(cm)
35S:*BnDREB1-5*	17	15.3	0.9
WT	14	16.8	1.2

3 Discussion

Many studies of molecular biology have shown that abiotic stress-tolerance mediated by DREB-like transcription factors mainly was some substances which can improve the osmotic regulation in cells[6,7]. The studies on the biological properties and physiological process in transgenic plant are not revealed. The reports of biological properties and physiological process in transgenic plant are seldom. Comparing with the wild type tobacco, we found that the holding water capacity of leaf was more powerful in transgenic tobacco. Naturally, the size and opening of stomata in upper and lower epidermis were observed because water in leaves was transpired relating to the opening of stomata. The results showed that the stomata density in transgenic tobacco was less than that in wild-type tobacco.

Stoma in leaf is an important channel which water and air pass in and out. The opening and closure of stomata has a significant effect on photosynthesis and transpiration. The number, size and opening degree of stomata have directly relation to stoma conductivity. The stoma conductivity influences the amount of CO_2 from environment to photosynthetic tissues so that it positively relates to photosynthetic rate[8].

The absorption and accumulation of potassium is the crucial regulation factor in guard cell for stomata opening. Sucrose maintains the osmotic potential and regulate stomata closure in guard cell[9]. Because the leaf water loss *in vitro* was less in transgenic tobacco than in wild type, the stomata parameter and the chlorophyll contents were compared after 7 h of leaf water loss. In transgenic tobacco the stomata density was less, the stomatal apparatus was larger and the pore was smaller but in wild-type it was contrary. The results revealed that the development of stomatal apparatus in transgenic tobacco was better than in wild-type. The damage degree in wild-type was more serious than in transgenic tobacco after 7 h of leaf water loss, which brought out decrease of sensitivity to regulation of stomata opening and closure in wild-type. Some researches indicate that the stomata density has relationship with photosynthesis pathway and with different life-type of plant[8]. Whether the metabolism process and the structure of tissue have changed in transgenic tobacco will be studied further.

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