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SAUSSUREA INVOLUCRATA SIDHN2 GENE CONFERS TOLERANCE TO DROUGHT STRESS IN UPLAND COTTON

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Abstract

Severe water shortage has long been acknowledged as one major limiting factor for global cotton production, and cultivation of cotton varieties with strong drought resistance is of important economic and social significances. In this study, the Xinjiang upland cotton variety Xinluzao 42 was transformed with the *SiDhn2* gene by optimized agrobacterium transformation system. The integration of *SiDhn2* gene into cotton genome was confirmed by PCR and Southern blot hybridization, and the drought resistance of transgenic and corresponding receptor cotton plants and their physiological indexes under drought stress were detailedly analyzed. Multiple physiological and biochemical indexes including soluble sugar content, free proline content, chlorophyll content, relative water content, net photosynthetic rate, transpiration rate, intercellular CO_2 concentration in transgenic cotton expressing *SiDhn2* gene under drought stress were significantly higher than those of receptor cotton. More importantly, the transgenic cotton plants exhibited remarkably decreased boll abscission rate and highly increased seed yield, indicating the significant role of *SiDhn2* gene in cotton drought resistance and its great application potential in agricultural production.

Key words: Drought resistance; *SiDhn2*; Transgenic cotton; Xinluzao 42.

Introduction

Drought and soil salinization are two major factors limiting cotton yield in major cotton-planting regions. Severe drought stress could cause slow growth, square and boll abscission and other abnormal physiological changes that severely affect the production of cotton. Due to the yearly aggravating scarcity of fresh water resources and global warming, it is strategically significant to develop drought-resisting cotton varieties by transgenic technology.

Due to long duration, slow efficiency and limited genetic variation of seed resources, cotton breeding through conventional breeding methods has brought about limited improvement on cotton agronomic characters (Wu *et al*., 2004). It has been gradually recognized that genetic engineering technology might be an effective way to accelerate excellent cotton variety cultivation (Feng *et al*., 2016). So far, the global planting area of transgenic cottons reached 243 thousand hectares, accounting for 81% of the total cotton planting area (James, 2012). The introduction of exogenous genes into cotton by virtue of plant tissue culture technology could greatly improve cotton genetic transformation efficiency, which might result in stable expression of exogenous genes and has been widely used to improve cotton's adaptability to environmental changes and also save money and manpower (Zhao *et al*., 2011). Up until now, plant regeneration by somatic embryogenesis has already been successfully achieved in numerous cotton varieties (Davidonis & Hamilton, 1983; Trolinder & Goodin, 1987; Kumar & Pental, 1998; Zhang *et al*., 2001; Kumria *et al*., 2003). However, the regeneration ability of many other excellent cotton varieties is still very limited (Obembe *et al*., 2011). Therefore, the selection of cotton varieties with high regeneration ability for genetic transformation is one critical step toward efficient transgenic cotton varieties (Furbank *et al*., 2015).

Dehydrin (DHN), one class of hydrophilic proteins widely existing in plants, belongs to the second member of late embryogenesis abundant protein family (Dure *et al*., 1989). During the late stages of plant embryonic development or under stresses such as drought, low temperature and salinity, the expression of *Dehydrin* genes has always been increased in plant cells (Romo *et al*., 2001; Bies-Etheve *et al*., 2008; Chen *et al*., 2011; Fernández *et al*., 2012). Meanwhile, Dehydrin proteins also play important roles in the regulation of cell osmotic adjustment by maintaining cell membrane stability and enhancing antioxidant activity, which finally lead to enhanced plant tolerance to drought stress and low temperature (Hanin *et al*., 2011). Until now, numerous *Dehydrin* genes from multiple plant species have already been cloned and proved to be associated with plant stress resistance. For example, introduction of Barley dehydrin gene *dhn3* and *dhn4* greatly improved the osmotic stress tolerance of *Arabidopsis thaliana* seedlings (Park *et al*., 2006), and expression of wheat *wcor4l0* gene could significantly improve frost resistance of strawberry leaves (Houde *et al*., 2004). Similarly, *Arabidopsis thaliana* Dehydrin gene *dhnx* was revealed to be critical for its drought resistance (Welin *et al*., 1994) and another *Dehydrin* gene in rice, *Rab16A*, remarkably improved the drought and salt resistances of tobacco leaves (Roychoudhury *et al*., 2007). In addition, numerous *Dehydrin* genes from other plant species have also been found to be important for plant response to abiotic stresses. These studies convincingly showed that dehydrin proteins were critical players in plant response to abiotic stresses.

Saussurea involucrata SiDhn2 gene encodes a dehydrin protein that belongs to the KS subtype of dehydrin protein family. Although this protein shares low homology with most previously reported dehydrin proteins, it is still an potential target for improving plant cold tolerance and drought resistance (Qiu *et al*., 2014). In this

study, aiming to cultivate new transgenic cotton varieties with high drought resistance, we introduced *Saussurea involucrata SiDhn2* gene into upland cotton variety Xinluzao 42 cultivated mainly in Xinjiang, and analyzed the drought resistance of these transgenic cotton plants.

Material and Method

Cotton seed treatment and culture conditions: Xinjiang upland cotton Xinluzao 42 seeds were provided by Prof. Li Baocheng from the Xinjiang Academy of Agricultural Sciences. Plump seeds with no sign of pest and damage were pre-sterilized with 70% alcohol for 30 s, disinfected with 30% hydrogen peroxide for 2.5-3 h, rinsed with sterile water for 5-6 times and then soaked in sterile water (28ºC) for 24 h. After germination, the seed coats were removed under sterile condition, and the seeds were then inoculated in 1/2MS medium and cultured for 7 d at 28ºC with sunshade. Mediums used in this study were listed as follows: MSB1: mitis-salivarius-bacitracin (MSB) agar medium with 0.05 mg l^{-1}, 2,4-D, 0.05 mg l^{-1} IAA, 0.1 mg l^{-1} Kt and 2.3-2.5 g phytagel; MSB2: MSB agar medium with 0.1 mg l^{-1} 2,4-D, 0.1 mg l^{-1} Kt, 75 mg l^{-1} Kan, 400 mg l^{-1} cb and 2.3-2.5 g phytagel; MSB3: MSB agar medium with 1.9 g l^{-1} KNO3, 75 mg l^{-1} Kan, 400 mg l^{-1} cb and 2.3-2.5 g phytagel; MSB4: MSB agar medium with 0.5 g l^{-1} ammonium aspartate, 0.1 g l^{-1} ammonium nitrate and 2.3-2.5 g phytagel.

Optimization of genetic transformation system: To explore the influence of kanamycin concentration, agrobacterium concentration, infection time, co-cultivation time on the transformation efficiency, different settings in Table 1 were compared to obtain an optimal combination of screening conditions for genetic transformation of cotton variety Xinluzao 42 hypocotyls. Five different kanamycin concentrations ranging from 25 to 125 mg l^{-1} were compared and the optimal concentration was chosen for callus induction. Similarly, cotton hypocotyls were infected for 10 min with the agrobacrerial suspension when OD600 value reaches 0.1, 0.2, 0.3, 0.4 and 0.5 respectively, co-cultivated for 48 h in MSB1 midium, and then cultured in MSB2 medium for 21 days. The callus growth condition and induction rate were evaluated for determination of the optimal agrobacterium suspension concentration. Moreover, infection time and co-cultivation time were separately evaluated as shown in Table 1.

Acquisition of transgenic cotton plants: Agrobacterium containing recombinant plasmid 35S:SiDhn2 was detected by PCR, and positive strains were selected for cotton hypocotyls infection. Segments from the middle of sterile seedling hypocotyls, 5-7 mm long, were infected with agrobacterium suspension (OD600 = 0.2-0.3) for 10 min, co-cultivated for another 48 h, subcultured in MSB2 medium for 3-4 months. Calluses from the ends of hypocotyl segments were subcultured for 1-2 months, and then cultured on MSB3 medium for 2-3 months. Differentiated callus tissues were subcultured in MSB4 medium for selection of well-developed cotton plants, and then cultured in plant culture medium. For successful recovery of transgenic plants, grafting using cotton variety Xinluzao 42 as rootstock were performed.

Cotton DNA extraction and PCR detection: Genomic DNA samples of transgenic cotton leaves were extracted and used as template for PCR detection of the introduction of exogenous gene, with a plasmid containing the target gene as positive control and DNA sample from corresponding non-transgenic receptor cotton variety as negative control. The following primers specific to *SiDHN2* gene were applied in PCR detection: 5-CCATGGATGGCCGGAATCATAAACAAG-3 (forward primer) and the 5-CACGTGCTAATCGCT GTCGCTGCTG-3 (reverse primer). The PCR reaction conditions were set as follows: pre-denaturation at 94ºC for 5 min, followed by 30 cycles of denaturation at 94ºC for 30 s, annealing at 57ºC for 30 s and elongation at 72ºC for 45 s.

Southern blot hybridization: Genomic DNA samples from three PCR-positive strains were extracted, digested with *HindIII* enzymes, separated by electrophoresis on an 8% agarose gel and transferred to Hybond-N+ nylon membranes. The insertion of *SiDhn2* gene into the genome of T0 generation transgenic cotton were analyzed by southern blot hybridization using probes targeting the *SiDhn2* gene sequences and DIG High Prime DNA Labeling and Detection Starter Kit II, as recommended by the manufacturer's instructions (Roche, Switzerland). The sample volume was increased to 80 ug considering the relatively low sensitivity of this kit compared with isotope labeling.

Analysis of physiological and biochemical indexes of transgenic cottons: Wild-type and transgenic cotton plants were respectively subjected to continuous drought stress till seedling stage, bud stage, flowering stage or normal irrigation. The physiological indexes of 10 cotton plants with similar growth tendency including cellular membrane ion leakage, free proline, leaf relative water content, MDA content, chlorophyll content and soluble sugar content were measured as previously described (Lv *et al*., 2007). Agronomic traits of these cotton plants were investigated after boll opening and maturation.

Table 1. Screening conditions for Xinluzao 42 transformation.

Kan concentration (mg l^{-1})	Agrobacterium liquid concentration (OD600)	Infection time (min)	Co-cultivation time (h)
25	0.1	0	12
50	0.2	5	24
75	0.3	10	36
100	0.4	15	48
125	0.5	—	60

Determination of transgenic cotton photosynthetic rate at flowering stage: The photosynthetic rates of cotton plants were measured at full flowering sate (July 15th). Photosynthetic parameters of the top fourth leaf including net photosynthetic rate (PN, μmol·m-2·s-1), transpiration rate (Tr, mmol·m-2·s-1), stomatal conduction (Gs, mmol·m-2·s-1), intercellular CO_2 concentration (Ci, μmol·mol-1) were measured using a Li-6400 portable photosynthesis meter (LI-COR, USA). All measurements were performed at 9:00~11:00 am.

Results

Optimization of the agrobacterium-mediated system for cotton hypocotyl transformation: For effective genetic transformation of cotton variety Xinluzao 42, we explored the influence of kanamycin concentration, agrobacterium culture liquid concentration, infection time and co-cultivation time on resistant callus induction rate. To obtain an optimal kanamycin concentration for screening, several kanamycin concentrations were tried in the preliminary experiment, and 75 mg l^{-1} kanamycin were finally chosen as the screening concentration, under which the growth and differentiation of cotton calluses were obviously inhibited. Also, we found that the highest resistant callus induction rate could be achieved when OD600 value of agrobacterium culture liquid was approximately 0.3, the infection time 10 min and the co-cultivation time 48 h. Therefore, 75 mg ml^{-1} kanamycin, agrobacterium culture liquid OD600 0.3, infection time 10 min and co-cultivation time 48 h were combined and used for cotton variety Xinluzao 42 transformation in the following analysis.

Induction of SiDhn2-expressing Cotton Resistant Callus and embryonic callus: Cotton hypocotyls infected with agrobacterium containing recombinant pBI121-SiDhn2 plasmid were inoculated into MSB2 culture medium and subcultured every 21 days. After cultured for 2-3 months, three distinct forms of callus were observed (Fig 1A, 1B and 1C) and only the third form of callus with loose texture and light green color showed strong vitality and was ready to differentiate into embryonic callus in subculture, as shown in Figure 1D. For induction of embryonic callus, the resistant calluses were further cultured in MSB3 culture medium and three types of calluses were formed during subculturing (Fig 1E, 1F and 1G). The first type of callus featured by yellow color and strong differentiation potential, along with the second type of yellow callus with certain differentiation potential surrounded by white hard callus, were subcultured for 3-5 months and finally differentiated into embryogenic callus as shown in Figure 1H.

Somatic embryogenesis and transgenic *SiDn2* cotton regeneration: Those fresh beige or yellow embryonic calluses were further cultured in MSB4 medium and a layer of sterile filter paper was applied to promote the maturity of embryonic calluses. After being selectively subcultured 1-2 times, embryonic calluses gradually developed into different somatic embryoids with various stages (Fig. 2A), such as globular embryo (Fig. 2B), torpedo-shaped embryo (Fig. 2C) and cotyledonary embryo (Fig. 2D). After being further subcultured 1-2 times, cotyledonary embryo developed into seedling accompanying root formation by radicle elongation, which is more conducive to nutrient absorption and seedling regeneration (Fig. 2E). The transgenic cotton seedlings were grown in seedling medium for 15 days and developed into intact plants (Fig. 2F). The grafting technology was applied to improve survival rate of transgenic cotton (Fig. 2G), which reached approximately 95%. Two weeks after grafting, transgenic cotton plants with healed wounds were then cultured in greenhouse for further analysis (Fig. 2H).

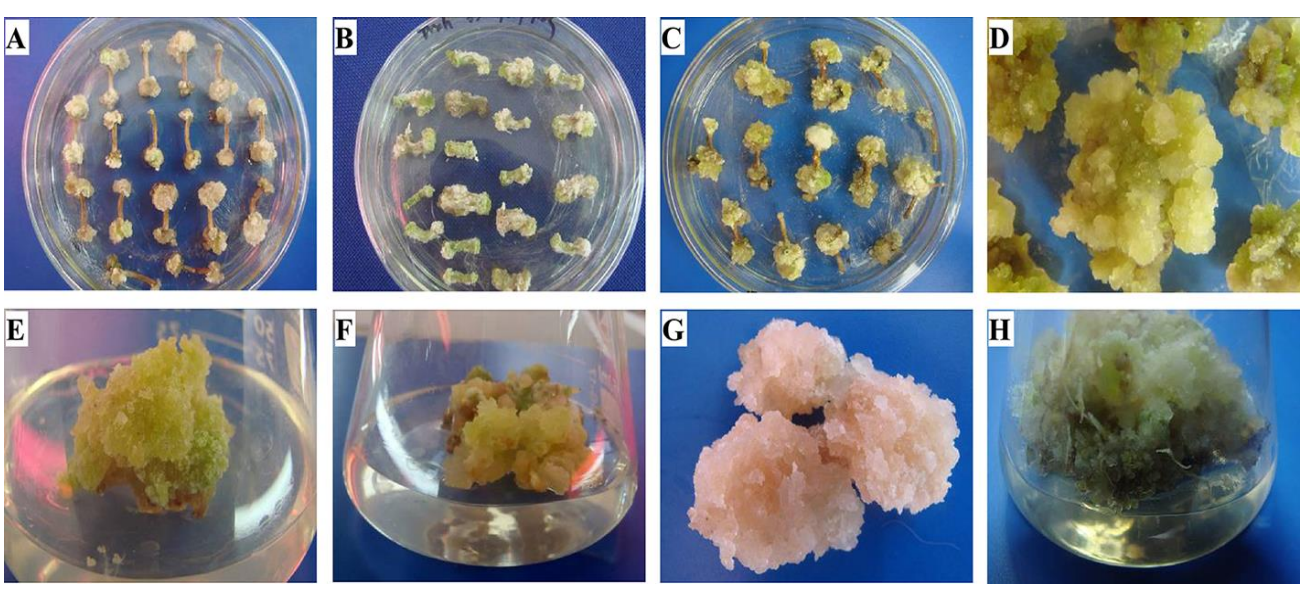

Fig. 1. Induction of SiDhn2-expressing transgenic cotton callus.
A-D: Induction of resistance calluses of SiDhn2-expressing transgenic cotton. (A: hard white callus; B: flocculent callus; C: light green loose callus with light green color; D: generation of beige loose callus). E-H: Induction of embryonic callus of SiDhn2-expressing transgenic cotton. (E: subculture of beige callus; F: beige callus surrounded by hard callus; G: soaring Callus; H: embryonic callus produced form beige callus).

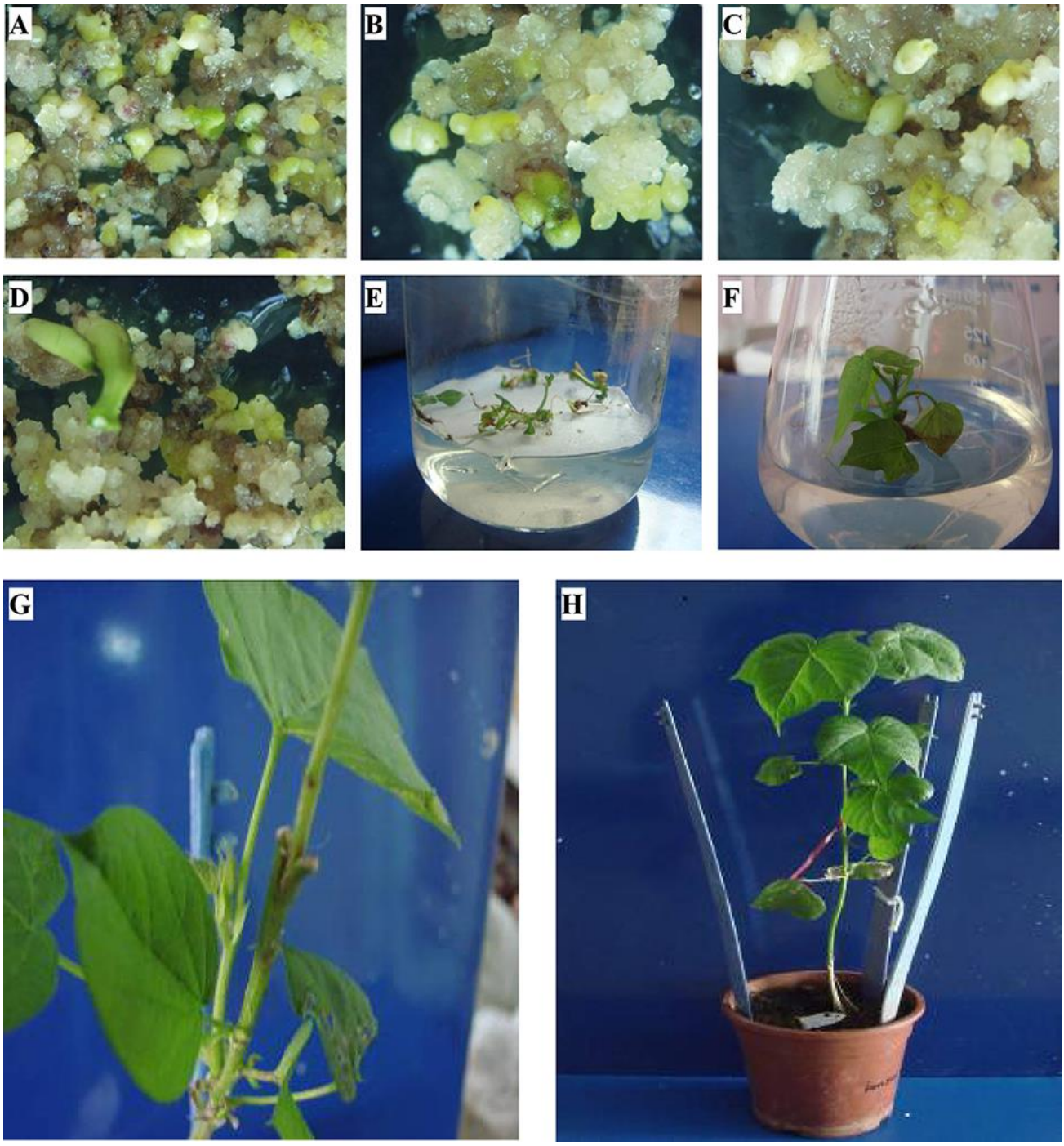

Fig. 2. Somatic embryogenesis and plant regeneration.
A-D: Different embryogenesis stages of SiDhn2-expressing transgenic cotton under microscope. (A: globular stage embryos; B: synthesis of anthocyanin; C: torpedo-shaped embryos; D: cotyledonary embryos). E-H: Plant regeneration of transgenic *SiDhn2* cotton. (E: embryo development of cotton seedlings; F: seedling regeneration; G: grafting for recovery of transgenic plants; H: Intact plant in the greenhouse after grafting).

Molecular detection of SiDhn2-expressing transgenic cotton: Genomic DNA from T0 generation SiDhn2-expressing transgenic cotton plants were extracted using CTAB method and analyzed by PCR, and genomic DNA from corresponding receptor variety and pGM-SiDhn2 plasmid were used as negative and positive controls respectively. PCR results showed that *SiDhn2* gene has been successfully inserted into the cotton genome (Fig. 3A). For further confirmation, RNA samples were extracted and used for cDNA synthesis, and the integration of target gene was verified by the detection of the target bands during RT-PCR analysis (Fig. 3B). Furthermore, Southern blot hybridization showed positive signals in 2 transgenic strains that were tested positive by PCR (Fig. 3C). More importantly, two positive transgenic plants exhibited bands with different sizes, indicating that these strains were produced by independent transformations with distinct insertion sites, which further proved that the *SiDhn2* gene has been integrated into the cotton genome.

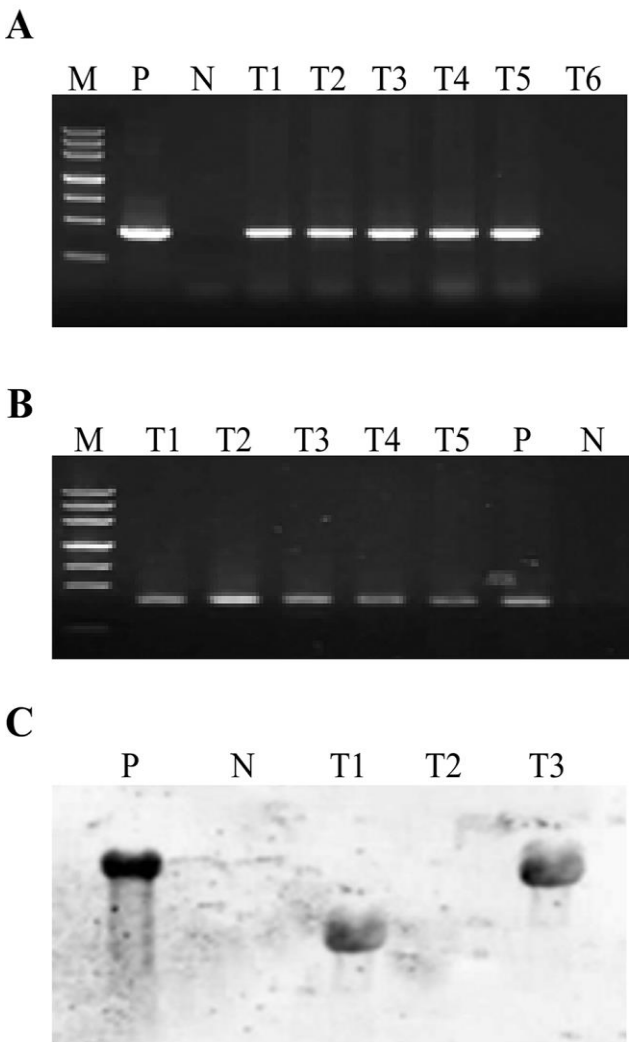

Fig. 3. Molecular identification of SiDhn2-expressing transgenic cotton plants.
A: PCR analysis of transgenic plants (M: DNA marker; P: positive plasmid; N: negative control; T1-6: transgenic cotton plants); B: RT-PCR analysis of the transgenic cotton plants (M: DNA marker; T1-5: transgenic cotton plants; P: positive plasmid; N: negative control); C: Southern blot analysis of cotton transgenic plant (P: positive control; N: Non-transgenic negative control; T1-5: transgenic cotton plants).

Physiological and biochemical indexes of SiDhn2-expressing transgenic cotton under drought stress: As shown in Fig. 4, the cell membrane ion leakage rates of SiDhn2-expressing transgenic cotton plants were significantly lower than the wild-type plants under continuous drought stress at budding and full-bloom stages (Fig. 4A), the relative water content of transgenic plants was markedly higher than wild-type plants under continuous drought stress (Fig. 4B), the MDA content in transgenic cotton plants was much lower than the wild-type plants (Fig. 4C), the chlorophyll content in transgenic cotton plants was also greatly higher than the wild-type plants (Fig. 4D). Furthermore, we found that soluble sugar content was significantly increased by integration of the *SiDhn2* gene under both drought stress and normal irrigation condition (Fig. 4E), and the proline content in SiDhn2-expressing transgenic cotton plants was also markedly increased in transgenic cotton plants under drought stress compared with wild-type cotton plants (Fig. 4F). It is noteworthy that most physiological indexes except chlorophyll and soluble sugar content were not influenced by the genetic transformation under normal irrigation condition, suggesting the specific role of *SiDhn2* gene in plant drought stress response. In summary, changes of these physiological indexes under drought stress indicate that SiDhn2-expressing transgenic cotton plants have significantly enhanced resistance to the drought stress compared with the wild-type plants.

Photosynthetic rate of SiDhn2-expressing transgenic cotton plants under drought stress: To address the effect of drought stress on photosynthesis in cotton plants, the photosynthetic parameters of transgenic and wild-type cotton plants were measured at the full flowering stage, as shown in Table 2. Under normal irrigation condition, the Pn, Ci and Tr value of SiDhn2-expressing transgenic cotton plants were not significantly different from the wild-type cotton plants. Under drought stress lasting until different growth stages, photosynthetic parameters of both the transgenic and wild-type cotton plants were decreased compared with plants under normal irrigation conditions. Also from this assay, we observed that the longer the drought stress lasted, the greater the decrease of these photosynthetic parameters in both the transgenic and wild-type cotton plants. However, the net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration and transpiration rate of the transgenic cotton plants subjected to drought stress lasting through various growth stages were still remarkable higher than those of wild-type plants under the same drought or irrigation treatments, indicating that integration of the *SiDhn2* gene significantly enhances the photosynthetic potential of cotton plants especially under drought stress.

The morphology and yield of SiDhn2-expressing transgenic cotton under drought stress: Water availability is one of the key limiting factors affecting cotton yield by physiological metabolism and other mechanisms. The agronomic traits of SiDhn2-expressing transgenic cotton plants were investigated shortly before the beginning of boll opening stage, including the average height, branch number, boll number, stem diameter, boll abscission rate and seed cotton yield, etc. As shown in Table 3, although plant heights of both the transgenic and wild-type cotton plants were obviously decreased under drought stress compared with those under normal irrigation conditions, the transgenic cotton plant height remains remarkably higher than the wild-type plants under the same drought treatment. Similarly, the branch number, stem diameter and seed cotton yield of transgenic cotton plants were remarkably higher than the wild-type plants under the same drought treatment, while the boll shedding rate of transgenic plants were lower than the wild-type, indicating that SiDhn2 protein could enhance cotton drought resistance by promoting plant growth and reducing abscission rate.

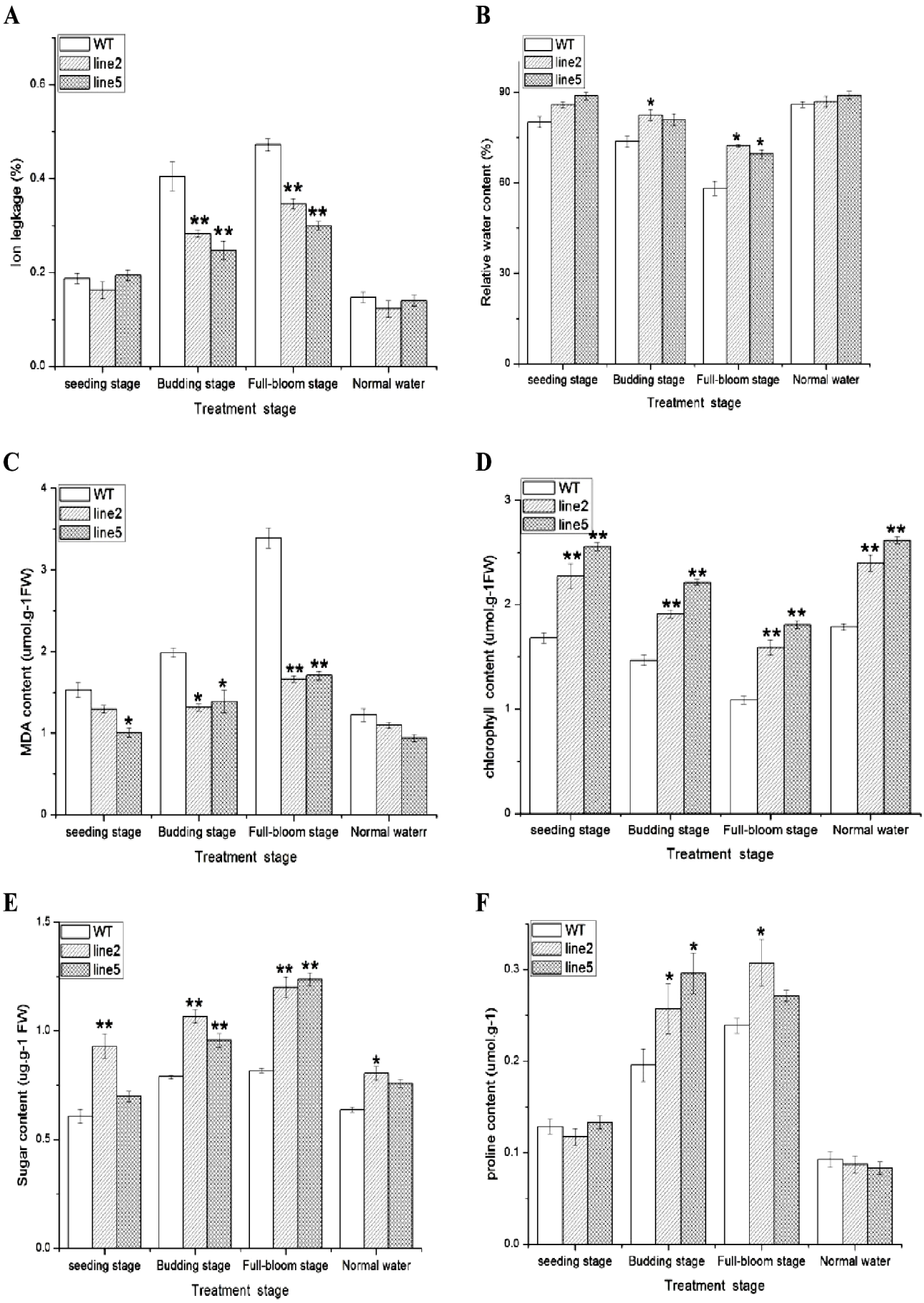

Fig. 4. Physiological and biochemical indexes of transgenic cotton under drought stress.
Note: ** and * mean the significance of differences at 1% ($P < 0.01$) or 5% ($p<0.05$), respectively.

Table 2. Photosynthesis of SiDhn2-expressing transgenic cotton under drought stress.

Drought stage	Plant lines	Net photosynthesis ($\mu molCO_2\ m^{-2}\ s^{-1}$)	conductance stomatal ($mmolH_2Om^{-2}\ s^{-1}$)	Intercellulat CO_2 concentration ($\mu molCO_2\ mol^{-1}$)	Transpiration rate ($mmol\ H_2Om^{-2}\ s^{-1}$)
Seeding stage	Ck	18.71 ± 4.55	0.35 ± 0.11	205.06 ± 7.97	6.24 ± 1.05
	Lin2	25.05 ± 2.92**	0.45 ± 0.09	219.53 ± 15.12**	6.92 ± 0.93*
	Lin5	23.79 ± 3.40*	0.36 ± 0.10	212.47 ± 18.76*	6.75 ± 1.17
Budding stage	Ck	18.66 ± 2.36	0.27 ± 0.11	202.1 ± 28.28	4.64 ± 1.17
	Lin2	22.36 ± 1.67*	0.37 ± 0.07	229.13 ± 10.39*	5.71 ± 0.43*
	Lin5	21.98 ± 2.44*	0.25 ± 0.06	227.33 ± 13.36*	5.51 ± 0.56*
Ful-bloom stage	Ck	20.3 ± 2.58	0.25 ± 0.09	172.37 ± 31.08	4.35 ± 1.19
	Lin2	25.07 ± 0.95**	0.46 ± 0.07**	216.89 ± 8.39**	6.49 ± 0.53**
	Lin5	21.79 ± 1.48	0.39 ± 0.02*	199.8 ± 6.39**	6.97 ± 0.15**
Normal watering	Ck	29.09 ± 2.1	0.91 ± 0.15	254.76 ± 10.57	8.76 ± 0.34
	Lin2	31.81 ± 1.66	1.02 ± 0.15	253.27 ± 10.56	9.02 ± 0.49
	Lin5	31.08 ± 1.90	1.05 ± 0.14	254.83 ± 5.84	8.82 ± 0.54

Note: ** and * mean significant differences with p<0.01 or 0.05 respectively

Table 3. Agronomic characters of transgenic cotton under drought stress.

Drought stage	Plant lines	Height	Branch number	The first branch height (cm)	Boll number	Boll shedding rate (%)	Stem diameter (mm)	Cotton yield (g) (10 plants)
Seeding stage	Ck	51.84±1.26	4.67±0.29	23.67±6.51	2.83±0.29	68.15±6.63	9.58±1.18	121.24±0.97
	Lin2	60±0.87**	6±1*	19±1.5	2.83±0.58	67.29±5.76	10±1.32*	135.76±1.31*
	Lin5	62.5±0.5**	7.16±1.15 *	16.5±1.32*	3.33±0.41	63.85±6.74	9.08±0.76	130.89±1.13*
Budding stage	Ck	40.88±4.65	2.33±0.58	26.67±3.33	1.50±0.50	80.58±2.69	6.92±1.26b	79.89±1.67
	Lin2	53.5±1.82 **	5±0.87 **	21.67±6.17	2.17±0.29	79.78±1.05	8.67±0.76*	94.71±1.02**
	Lin5	52.67±0.76**	4.83±0.58**	19.67±2.75*	2.0±0.5	76.83±3.79*	8.42±0.38	90.07±1.75*
Ful-bloom stage	Ck	20.67±3.33	1.33±0.29	15.75±1.52	1.33±0.58	81.42±3.95	5.67±0.76	47.99±1.89
	Lin2	*27.5±1***	2.09±0.5*	21.67±2.47	1.33±0.29	76.61±3.04	6.07±0.32	59.16±0.73*
	Lin5	31.50±1.5**	2.67±0.76**	22.5±3.12*	1.50±0.55	78.63±4.96	7.75±1.39*	59.67±1.68*
Normal watering	Ck	61.33±0.29	6.75±0.43b	18.67±2.25	6.33±0.28	54.20±0.76	9.08±0.95	257.51±1.47
	Lin2	59±5.20	6.17±0.58	16.17±1.04	4.17±0.58**	53.99±2.19	8.17±0.14	258.32±1.27
	Lin5	67.13±5.79	8.38±1.6*	18.62±2.62	6.88±1.75	59.09±4.83	9.68±1.25*	257.19±1.41

Note: ** and * mean significant differences with p<0.01 or 0.05 respectively

Discussion

Agrobacterium-mediated genetic transformation system, featured by good repeatability, high gene copy number, low possibility of gene silencing and high stability, has been widely applied in the plant genetics research and molecular breeding. Cotton genetic transformation is highly dependent on the receptor cotton genotype, which is still one major limiting factors affecting somatic embryogenesis and plant regeneration. Fortunately, cotton transformation efficiency could be greatly improved by optimizing culture condition and hormone ratio (Firoozabady & Deboer, 1993). In this study, cotton variety Xinluzao 42 was used as the genetic transformation receptor, and the somatic embryogenesis and plant regeneration system for this cotton variety has already been established. Agrobacterium strain GV3101 has been widely used in cotton genetic transformation due to its strong infection ability. For example, expression of *Arabidopsis* vacuolar H^+-pyrophosphatase gene (AVP1) in cotton plants by agrobacterium-mediated transformation significantly improved cotton tolerance to drought stress in the field conditions (Pasapula *et al*., 2011). It has been well acknowledged that bacteria concentration, infection time and co-cultivation time great influence the transformation efficiency. During the process of cotton genetic transformation, inappropriately high concentration of agrobacterium will produce toxic substances which might lead to cotton cell death (Sunilkumar *et al*., 2002). In this study, we found that the transformation system with an agrobacteria culture of OD600 = 0.2-0.3, combined with a kanamycin concentration of 75 mg/L, an infection time of 10 min and a co-cultivation time of 48 h, could produce the ideal transformation efficiency, which is consistent with previous research (Ikram-ul-Haq, 2004). This optimization of cotton transformation system provided useful basis for improving cotton agronomic traits by genetic transformation.

Different cotton explants have distinct potentials of somatic embryogenesis and young explants tend to show strong embryonic differentiation and plant regeneration ability during genetic transformation (Sun *et al*., 2006). Previous studies have revealed that cotton hypocotyls have strong callus differentiation and embryogenesis potentials, which could greatly shorten the embryogenesis time (Trolinder & Goodin, 1988). Therefore, in the present study, cotton hypocotyls were applied for induction of embryogenic callus, and the callus induction rate reached 84.5%. In addition, sterile filter papers were applied in this study to mimic drought stress, which effectively promoted embryonic callus differentiation, as well as formation of globular embryos and somatic embryos. Also in this study, we used MSB4 culture medium to promote globular embryo formation and

obtained ideal gene transformation efficiency. These technical explorations provided useful information for improvement of cotton agronomic traits and stress resistance by genetic methods.

SiDhn2, one dehydrin gene from *Saussurea involucrate* involved in the regulation of plant responses to freezing and drought stresses, was used for cotton genetic transformation in this study. In order to enhance the drought resistance of cotton cultivars and also to investigate the application value of this gene in agricultural production, *SiDhn2* gene was transformed into cotton variety Xinluzao 42 and the SiDhn2-expressing transgenic cotton plants exhibited markedly higher drought resistance compared with corresponding non-transgenic receptor cotton. Drought stress always imposes harmful effects on plant growth development and photosynthesis, causing severe leaf wilting, slow growth and other adverse alteration in plant growth and development. The adverse effects of drought stress were mainly mediated by cell membrane structure destruction induced by water deficiency, which could cause the increase of cell ionic leakage rate and promote membrane lipid peroxidation (Meloni *et al*., 2003). Dehydrin proteins are rich in glycine and lysine residues and lack of cysteine and tryptophan, which confers these proteins high hydration ability and strong thermal stability that makes them stable even in boiling water (Allagulova Ch *et al*., 2003). Under drought stress, Dehydrin proteins can bind to the surface of the biological membranes, thus maintaining structure and stability of intracellular membranes, which play an important protective role for normal metabolism in plants under stress (Hanin *et al*., 2011). In this study, we found that SiDhn2-expressing transgenic cotton plants showed significantly lower ion leakage rate under continuous drought stress in contrast to the corresponding receptor cotton variety, further illustrating the roles of dehydrin proteins in preventing membrane system destruction caused by water deficiency and maintaining normal membrane function. Previous studies have showed that dehydrin proteins can scavenge harmful free radicals such as hydroxyl radical and oxygen free radical, thus preventing cell membrane structure destruction induced by reactive oxygen species. For instance, transgenic tobacco plants expressing *CuCOR19* gene exhibited lower malondialdehyde (MDA) content than the wild-type tobacco plants at low temperatures (Hara *et al*., 2003). Consistently, we found the MDA content in SiDhn2-expressing transgenic cotton was significantly lower than corresponding receptor cotton plants in drought stress conditions, also showing that SiDHN2 protein can effectively scavenge free radicals and maintain the stability of the membrane system in cotton.

In addition, we observed that the proline content, soluble sugar content, relative water content and chlorophyll content in SiDhn2-expressing transgenic cotton plants were higher than corresponding receptor cotton under drought stress, which could further promote active accumulation of nutrients and improve water retention ability of plant cells. It has been well established that drought stress could cause significant decrease of plant photosynthetic rate and stomatal conductance, meanwhile increasing respiration rate and active oxygen species accumulation. In this study, the decrease extent of net photosynthetic rate in transgenic cotton plants was significantly lower than receptor cotton, which was beneficial to accumulation of photosynthetic products under stress. Plant height, branch number, boll number and boll abscission rate are very important for cotton production. In this study we found that fruit branch number and boll number of transgenic cotton expressing *SiDhn2* gene were higher than the receptor cotton, while abscission rate of transgenic cotton was significantly lower than receptor cotton, thus leading to significantly increased seed yield in transgenic cotton.

In summary, *Saussurea involucrata SiDhn2* gene was successfully introduced into Xinjiang cotton cultivar Xinluzao 42 through agrobacteria-mediate genetic transformation system to produce transgenic cotton plants. The transgenic cotton plants expressing *SiDhn2* gene exhibited markedly improved water use efficiency and enhanced drought resistance, which finally contributed to the remarkably elevated seed yield in transgenic cotton plants, showing the great application potential of transgenic cotton plants in agricultural production.

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