Agricultural Sciences, 2018, 9, 53-68
http://www.scirp.org/journal/as
ISSN Online: 2156-8561
ISSN Print: 2156-8553

Cloning and Ectopic Expression of *ScYCF*1 Gene from *Saccharomyces cerevisiae* in Cotton

Min Mu*, Na Shu*, Xuke Lu, Xiugui Chen, Shuai Wang, Junjuan Wang, Delong Wang, Weili Fan, Lixue Guo, Chao Chen, Wuwei Ye#

State Key Laboratory of Cotton Biology, Chinese Academy of Agricultural Sciences Cotton Research Institute, Anyang, China
Email: #yew158@163.com

How to cite this paper: Mu, M., Shu, N., Lu, X.K., Chen, X.G., Wang, S., Wang, J.J., Wang, D.L., Fan, W.L., Guo, L.X., Chen, C. and Ye, W.W. (2018) Cloning and Ectopic Expression of *ScYCF*1 Gene from *Saccharomyces cerevisiae* in Cotton. *Agricultural Sciences*, **9**, 53-68.
https://doi.org/10.4236/as.2018.91005

Received: October 12, 2017
Accepted: January 14, 2018
Published: January 17, 2018

Abstract

Yeast cadmium factor 1 (*YCF*1), is a member of the ATP-binding cassette (ABC) transporter family. To explore the functions of *YCF*1 of *Saccharomyces cerevisiae* (*ScYCF*1) in the cotton, *ScYCF*1 was cloned from *Saccharomyces cerevisiae* As2.375, with the full-length of 4548 bp. The bioinformatics analysis revealed that the largest component of *ScYCF*1 protein is leucine (12%). *ScYCF*1 is alkaline and positive charged, stable, and hydrophilic protein. The predictive secondary structure is mainly composed of α-helix areas, random coils and β-sheets. We constructed the pBI121-*ScYCF*1:GFP infusion expression vector and verified it by enzyme ingestion. The transient expression results of cotton pollen showed that the green fluorescence phenomenon of three kinds of upland cotton pollen significantly increased after transforming *ScYCF*1. The salt sensitive material upland cotton CCRI12 was transformed *in vivo* simultaneously, and the germination ability of trans-*ScYCF*1-gene T_0 seeds was much better than the acceptor material CCRI12 under the stress of 100 mM NaCl saline solution. According to the gene nucleotide sequences, four pairs of primers were designed for molecular detection of T_0 generation, and the sequencing results of PCR products of four specific primers evidence that the transgene is successful. Salt tolerance analysis of leaf discs of identified transgenic cotton showed that the chlorophyll content of leaf discs of transgenic cotton was higher than the content of the control cotton under salt stress. *ScYCF*1 gene was cloned and introduced into cotton, showing that *ScYCF*1 plays an important role in improving the salt tolerance of cotton.

Keywords

Saccharomyces cerevisiae, *ScYCF*1, Upland Cotton, Pollen Instantaneous Expression, Molecular Detection

*These authors contributed equally to this work.

1. Introduction

Soil salinization is a phenomenon that the water-soluble salts in soil and groundwater move on with capillary water to surface soil, and the water of the top soil evaporates resulting in accumulation of salts in the soil solum or regolith [1]. Salinization affects about 1/5 of the world's arable land and causes loss of 10 million hectares per year [2] [3] [4].

Numerous studies showed that related salt tolerant genes of halotolerant fungi can increase the salt tolerance of fungal microorganisms and plants [5] [6] [7]. *SOD*2 of yeast was transferred into rice, and the salt tolerance of transgenic plants was improved [8]. *Chaetomium thermophilic SOD* was cloned and transferred into tobacco, and found that it cannot only improve the salt tolerance of transgenic plants but also has certain resistant activity against tobacco brown spot disease and anthracnose [9]. Salt tolerant gene *RPL*44 isolated from *Aspergillus glaucus* and transferred into arabidopsis and tobacco, and showed that tolerance of transgenic plants to salinization was significantly higher than the control wild-types [10].

In 1996, genome sequencing of *Saccharomyces cerevisiae* was completed [11]. Nelson *et al.* [4] showed that there are more than 200 genes in the genome of *Saccharomyces cerevisiae* that are related to salt tolerance. It was found in the genome sequencing results that there are 31 ABC genes in the genome; phylogenetic analysis showed that the yeast ABC proteins can be divided into six different subfamilies, which are named MDR, PDR, MRP/CFTR, ALDp, YEF3 and RLI [12]. *ScYCF*1 is the member of the most studied MRP subfamily. Based on the correlation between *YCF*1 protein and liquid bubble detoxification, the salt tolerance experiment was conducted in arabidopsis under high salt conditions. Three weeks old arabidopsis wild type and transgenic seedlings were treated with 75,100,150 and 200 mM NaCl for 6 h and results showed that after being treated with 200 mM NaCl the accumulation amount of Na^+ in Arabidopsis was 26.5% higher than that in wild type, but under stress of other salt concentrations there was no significant difference between them, indicating that Arabidopsis plants with transformed *ScYCF*1 gene has stronger stress tolerance to NaCl than the wild type, it is because of the transgenic plants can accumulate more Na^+ in vacuoles [13].

Cotton is one of the ideal crops for development and utilization of saline land, and improving salinity tolerance of cotton through genetic engineering and transgenic technology become an urgent demand and important direction for current cotton breeding. This study took *Saccharomyces cerevisiae* As2.375 as the materials, cloned *ScYCF*1 gene and constructed expression vector, used a gene gun *in vivo* conversion technology to transform upland cotton (*Gossypium hirsutum* L.) CCRI12, aiming to explore whether the fungi salt-tolerant gene can successfully play its function in cotton and provide a theoretical basis for obtaining new salt tolerant cotton varieties.

2. Materials and Methods

2.1. Experiment Material

Saccharomyces cerevisiae As2.375 is purchased from Shanghai Industrial Institute of Microbiology. The pMD18-T Vector is purchased from TaKaRa. E. coli strain DH5*α*, expression vector pBI121:GFP are preserved by the research group. CCRI12, GK50, Y2067 and GZ-2 were conserved in the stress-resistance identification research group of Institute of Cotton Research of CAAS.

2.2. Cloning of Targeted Gene and Sequential Analysis

The RNA of *Saccharomyces cerevisiae* AS2.375 was extracted with the Ultra Clean Microbial RNA Isolation Kit (MO BIO) kit, whose concentration and purity was assessed by Nanodrop 2000 ultramicrospectrophotometer. The RNA was reversely transcribed into cDNA with ReverTra Ace qPCR RT Master Mix with gDNA Remover kit. According to the obtained cDNA as the template, the full-length gene primer was designed using the Primer Premier 5 software, 5'-end: *ScYCF*1-F and 3'-end: *ScYCF*1-R (Table 1). The full-length sequence of mRNA was amplified using 2 × TransTaq-T PCR SuperMix. The amplification procedure was 94˚C 5 min, 94˚C 45 s, 55˚C 45 s, 72˚C 1 min, 40 cycles; 72˚C 10 min. The connected pMD-18T vector was transformed into competent Escherichia coli cells DH5*α*, and the positive clone was picked for sequencing verification. The physicochemical property of *ScYCF*1 protein was analyzed using the online-software ProtParam (http://web.expasy.org/protparam/), and the hydrophilic-hydrophobic property of protein was predicted by ProtScale

Table 1. Primers used in the experiments.

Primers name	Sequence (5′- 3′)
*ScYCF*1-F	ATGGCTGGTAATCTTGTTT
*ScYCF*1-R	TTAATTTTCATTGACCAAACCA
In*ScYCF*1-F	CACGGGGGACTCTAGAATGGCTGGTAATCTTGTTT
In*ScYCF*1-R	ACTCATACTAGTCCCGGGATTTTCATTGACCAAAC
*ScYCF*1-F1	ACGGTGTGATCCTAAATCTATCAG
*ScYCF*1-R1	AAGTTCTGCATATAGCCCATGA
*ScYCF*1-F2	GCTATGTTTCTGGTGGGCTT
*ScYCF*1-R2	CTTTTCCCTATAAGGCTTCTCCCA
*ScYCF*1-F3	GGCATAGATACGACGCGGAA
*ScYCF*1-R3	GCAGTTGTTCCAGCTCTCCT
*ScYCF*1-F4	CATCAAGGGAGTTGCGTCGT
*ScYCF*1-R4	GTGGTCTGTGGCCTTCAACT

(http://web.expasy.org/protscale/). The secondary structure of protein was analyzed with SOPMA (http://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/MPSA/npsa_sopma.html).

2.3. Construct Fluorescence Expression Vector pBI121-*ScYCF*1:GFP

The In-Fusion primer was designed on line (http://bioinfo.clontech.com/infusion) 5'-end In*ScYCF*1-F and 3'-end In*ScYCF*1-R (**Table 1**). The target sequence was obtained through amplification of the *ScYCF*1 plasmid as the template. The double cleavage sites Xba I and Sma I were chosen, and double enzyme digestion was performed on the expression vector pBI121:GFP. The fusion expression vector pBI121-*ScYCF*1:GFP was constructed with In-Fusion method, followed by recombinant clone verification and sequencing.

2.4. Transient Expression Analysis of *ScYCF*1 in the Pollen from Upland Cotton

The pollen from upland cotton Y-2067, ZA-23 and GZ-3 were bombarded with portable particle gun GDS-80, and the gene expression was observed under laser scanning confocal microscope FV1000 (Olympus, Japan).

2.5. *In vivo* Transformation of Cotton with Gene Gun

The powders and plasmids were treated as the way of Kong Jingjing [14] and the particle gun transformation *in vivo* was completed.

2.6. Molecular Detection of Transgenic Cotton

Four pairs of primers were designed for *ScYCF*1 gene sequence, 5'-end primers were *ScYCF*1-F1, *ScYCF*1-F2, *ScYCF*1-F3, *ScYCF*1-F4 and the 3'-end primers were *ScYCF*1-R1, *ScYCF*1-R2, *ScYCF*1-R3, *ScYCF*1-R4 (**Table 1**). DNA from transgenic plants was extracted with CTAB method for PCR amplification, and the program is 94˚C, 5 min; 94˚C 45 s, 58˚C 45 s, 72˚C 30 s, 40 cycle; 72˚C 10 min.

2.7. Analysis of Salt Tolerance of Trans-*ScYCF*1 Cotton

The germination experiment was performed on obtained T_0 seeds with the double walled filter paper method [14], using 100 mM NaCl solution as treatment solution and water as control. Each group repeated for three times. The seeds were sent into the illumination incubator for incubation (condition of culture: 28˚C, illumination for 14 h; 25˚C, darkness for 10 h). The germination results were summarized after 7 d. Measured and compared the content of chlorophyll of the leaf discs, which obtained from the same position of 6 transgenic cotton and acceptor material CCRI12, were treated with 0, 400 and 600 mM

NaCl and cultured at 28˚C under illumination conditions for 72 h to analyze salt tolerance of trans-*ScYCF*1 cotton.

3. Results

3.1. Cloning of *ScYCF*1 Gene and Construction of Expression Vector

The specific primer was designed according to the full-length sequence of *Saccharomyces cerevisiae ScYCF*1 gene retrieved from NCBI database (GenBank: NM_001180442.3). Gene amplification was performed using cDNA as template to obtain a nucleotide sequence with a length of 4584 bp (**Figure 1**), which was connected to vector for sequencing and turned out correct. *ScYCF*1 gene was inserted into a plant expression vector pBI121:GFP to construct green fluorescent fusion expression vector. Digestion by restriction endonuclease Bgl II was verified to be properly inserted (**Figure 2**), indicating that the expression vector was successfully constructed and was named as pBI121-*ScYCF*1:GFP.

3.2. Bioinformatics Analysis

The results of bioinformatics analysis indicated that *ScYCF*1 gene encodes a

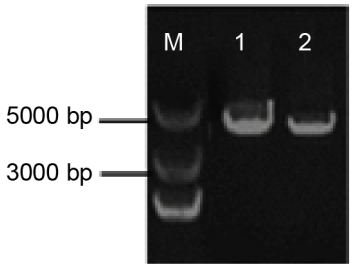


Figure 1. Gene amplification PCR product. M: DNA Marker DL5000; 1- 2: *ScYCF*1 gene.

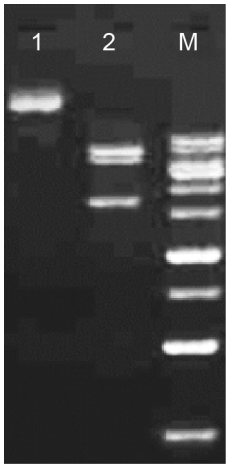


Figure 2. Enzyme digestion of expression vector pBI121-*ScYCF*1:GFP. M: 1 KB ladder; 1: vector plasmid; 2: digested with Bgl II.

protein *ScYCF*1 with 1515 amino acid, the molecular formula of *ScYCF*1 protein was C7777H12253N2031O2199S54, with the molecular weight of 120.34 kDa and the isoelectronic point of 8.64, and located on the cell membrane. Amino acid composition showed that *ScYCF*1 protein leucine (Leu) takes up the largest proportion, 12%; which is followed by valine (Ile) and serine (Ser) taking up 8% each (**Figure 3**). With 156 positively charged alkaline amino acids (Arg + Lys) and 143 negatively charged acidic amino acid (Asp + Glu), the protein is alkaline and positively charged, the instability index is 36.15, with a half-life over 20 h, which belonged stable protein. The hydrophilicity and hydrophobicity analysis of *ScYCF*1 protein revealed that the quantity of hydrophobic amino acids in the protein much larger than that of hydrophilic amino acids (**Figure 4**), of which the GRAVY was 0.064 namely the whole protein was hydrophobic. The secondary structure prediction result (**Figure 5**) showed that in the protein there were 700 amino acids in α helical region, which constituted the main structure of the protein; there were 309 β-pleated sheets, accounting for 20.4%, 375 randomly coiled amino acids, accounting for 24.75%, and β-turn contains 131 amino acids, accounting for 8.65%. Thus, we could speculate that the structural and functional domain might be composed of β-pleated sheets and randomly coils.

3.3. Transient Expression Analysis of Cotton Pollen

Cotton pollen has auto-fluorescence phenomenon. Under excitation light at a wavelength of 488 nm, the auto-fluorescence of cotton pollen is green [14]. According to previous findings, three varieties Y2067, ZA-23 and GZ-2 with weak auto-fluorescence [14] were selected to collect pollen, and gene gun in vivo conversion technology was used to bombard the cotton pollen. The study found that

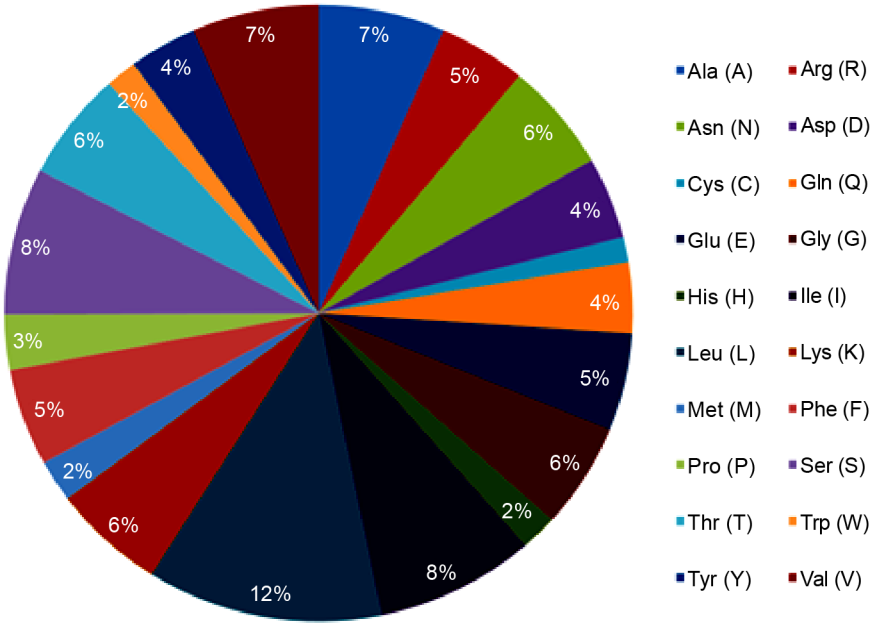


Figure 3. Amino acid composition of *ScYCF*1 protein.

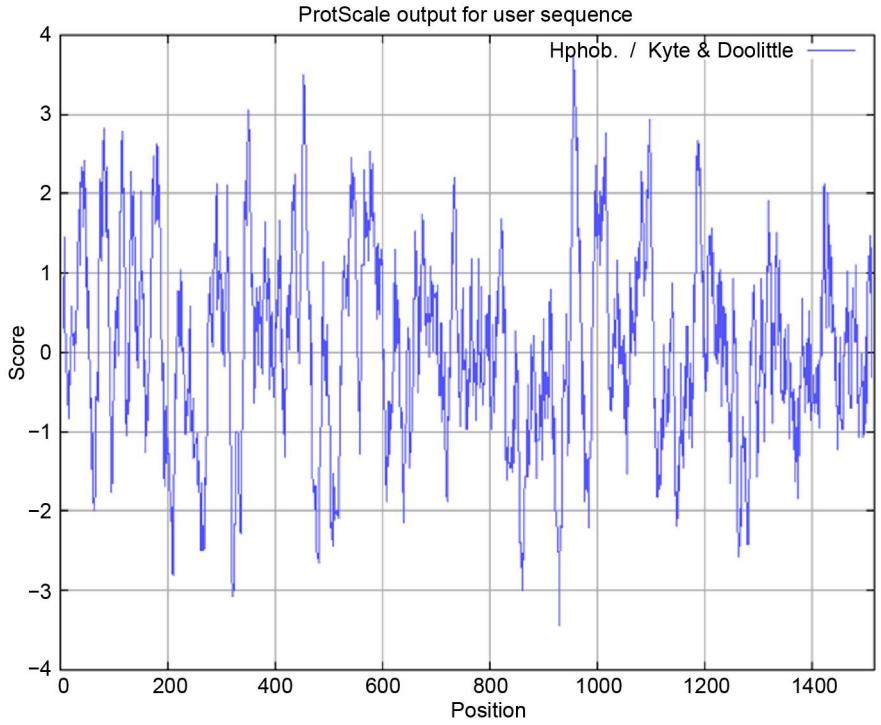


Figure 4. Hydrophilic and hydrophobic characteristics of *ScYCF*1 protein.

after the vector pBI121-*ScYCF*1:GFP was bombarded, the green fluorescence of pollens of three materials was significantly enhanced (**Figure 6**), indicating that *ScYCF*1 can be expressed in cotton pollen, which lay the foundation of using gene gun *in vivo* conversion technology to obtain transgenic salt-tolerant materials in the next step.

3.4. Salt Tolerance Analysis of T_0 Generation of Transgenic Cotton Seeds

Choosing 20 grains of plump and uniform transgenic *ScYCF*1 seeds and CCRI12 inbreds was used for salt tolerance screening experiments. The seeds treated with 100 mM NaCl solution were taken as the experimental group, and those treated with water as the control group. The result (**Figure 7**) shown that under stress from 100 mM NaCl solution, the germination capacity of transgenic seeds was significantly higher than the control seeds, and the growth capacity of the root was significantly stronger than the control group. The root length of CCRI12 inbreds and transgenic seeds in water and under stress from 100 mM NaCl was measured respectively and it was found that under two kinds of treatment, the root length of transgenic seeds was longer than that of CCRI12 inbreds, indicating that the expression of salt tolerant gene *ScYCF*1 can not only enhance salt tolerance of cotton seeds, but also can enhance the growing ability of them.

3.5. Molecular Detection of Transgenic Seeds

Four pairs of primers were designed based on mRNA of *ScYCF*1 gene for

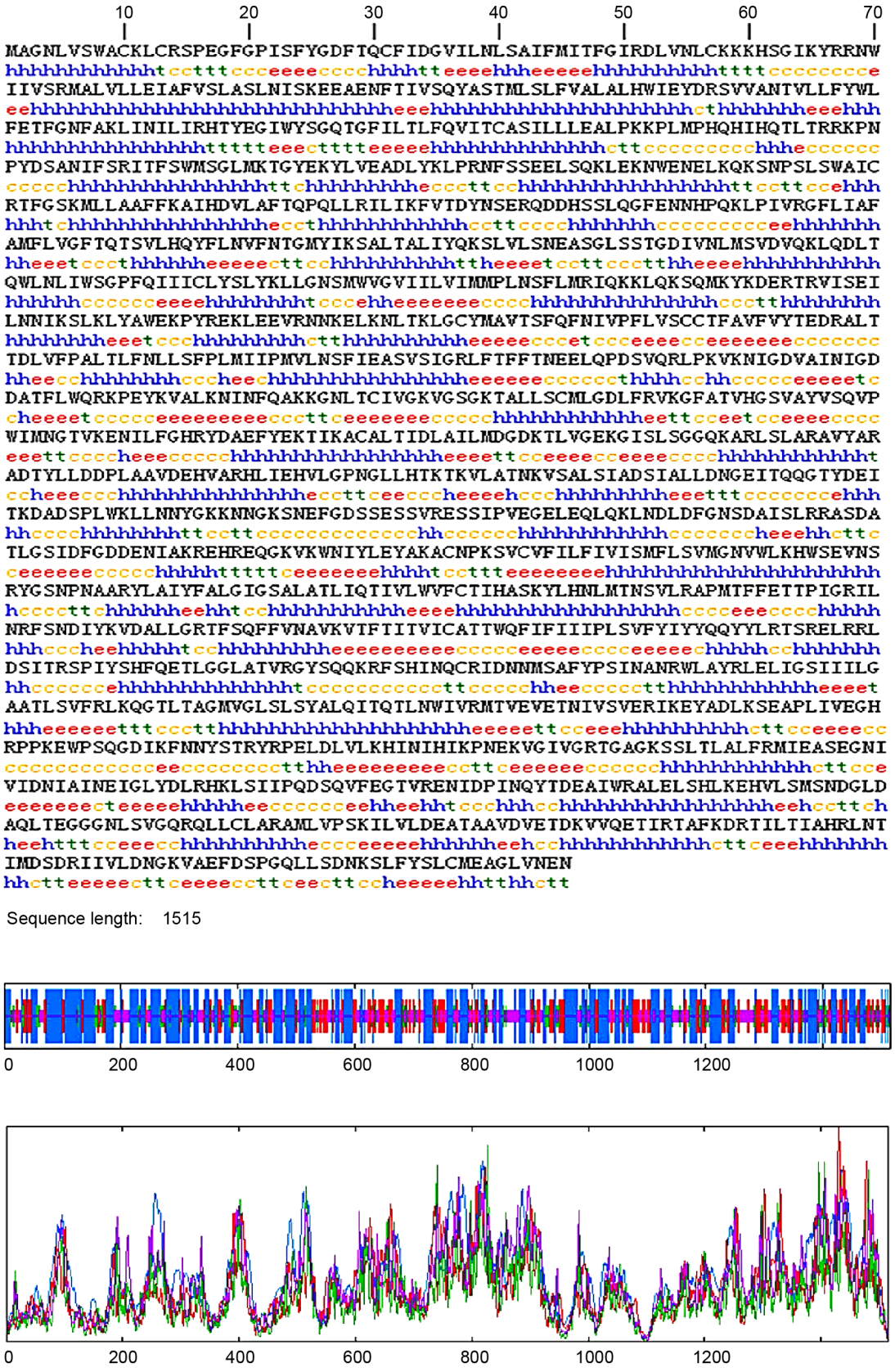


Figure 5. Prediction of secondary structure of *ScYCF*1 protein.

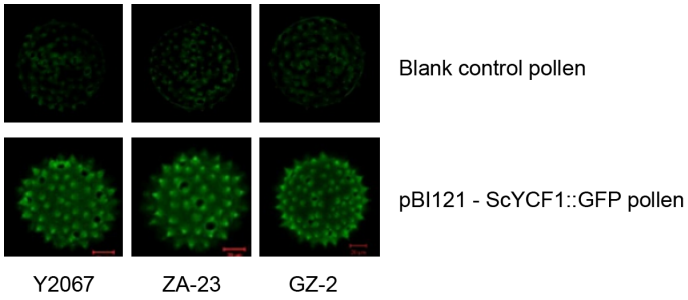


Figure 6. The transient expression analysis of cotton pollen.

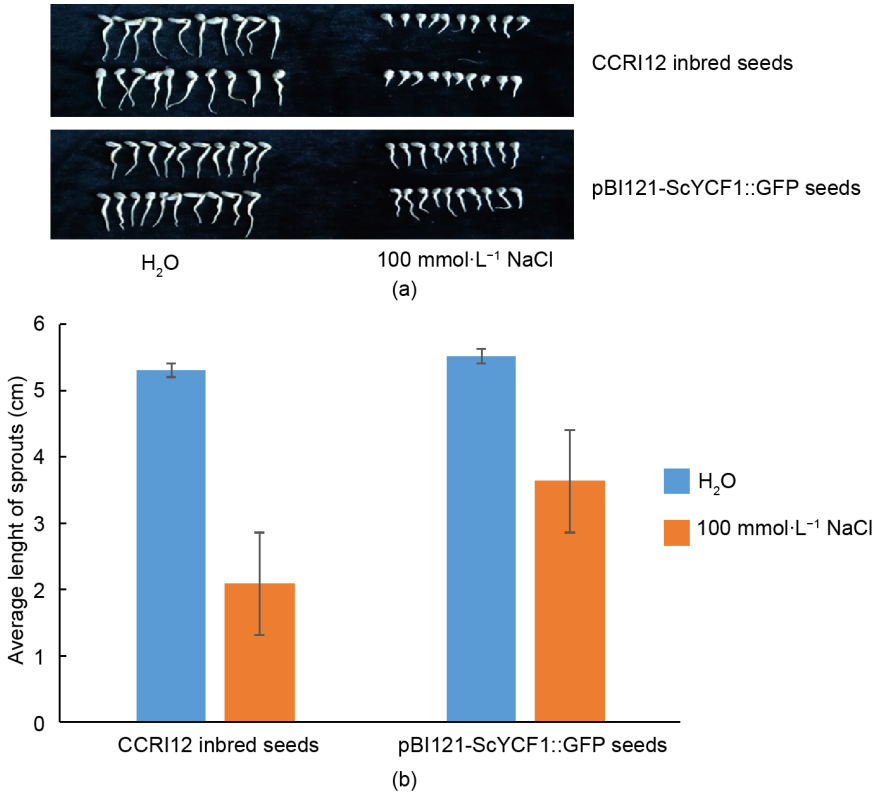


Figure 7. The test of transgenic cotton with (a) seed germination and (b) buds long statistics.

molecular detection of transgenic *ScYCF*1 plants. The length of amplified fragments of primers was 747 bp, 477 bp, 525 bp and 450 bp. The detection results were shown in **Figure 8**, expected band can be amplified from the transgenic plants and plasmid, while in the negative control CCRI12 inbreds there were no band amplified. Combining the sequencing results of PCR products (**Figure 9**), it was considered that the cotton was successfully transformed with *ScYCF*1 gene.

3.6. Salt Tolerance Analysis of Leaf Discs

Salt tolerance experiments of leaf discs were conducted for six transgenic plants that were detected with correct molecular. After being treated with stress from

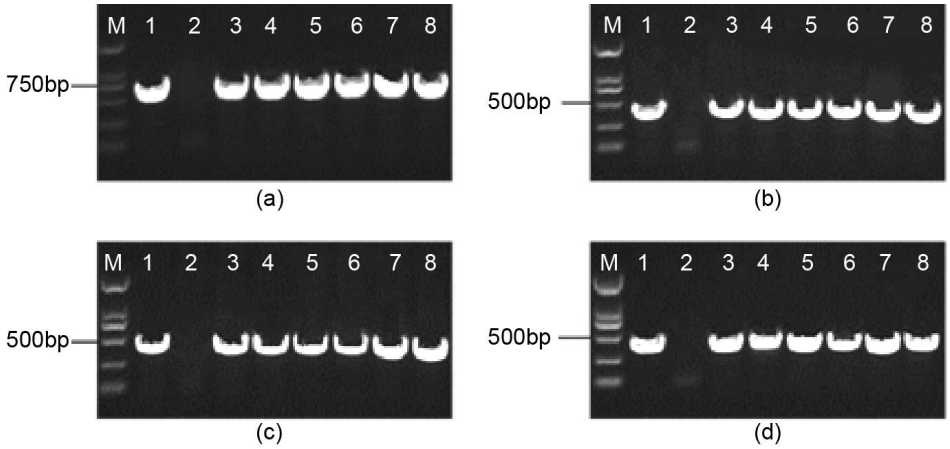
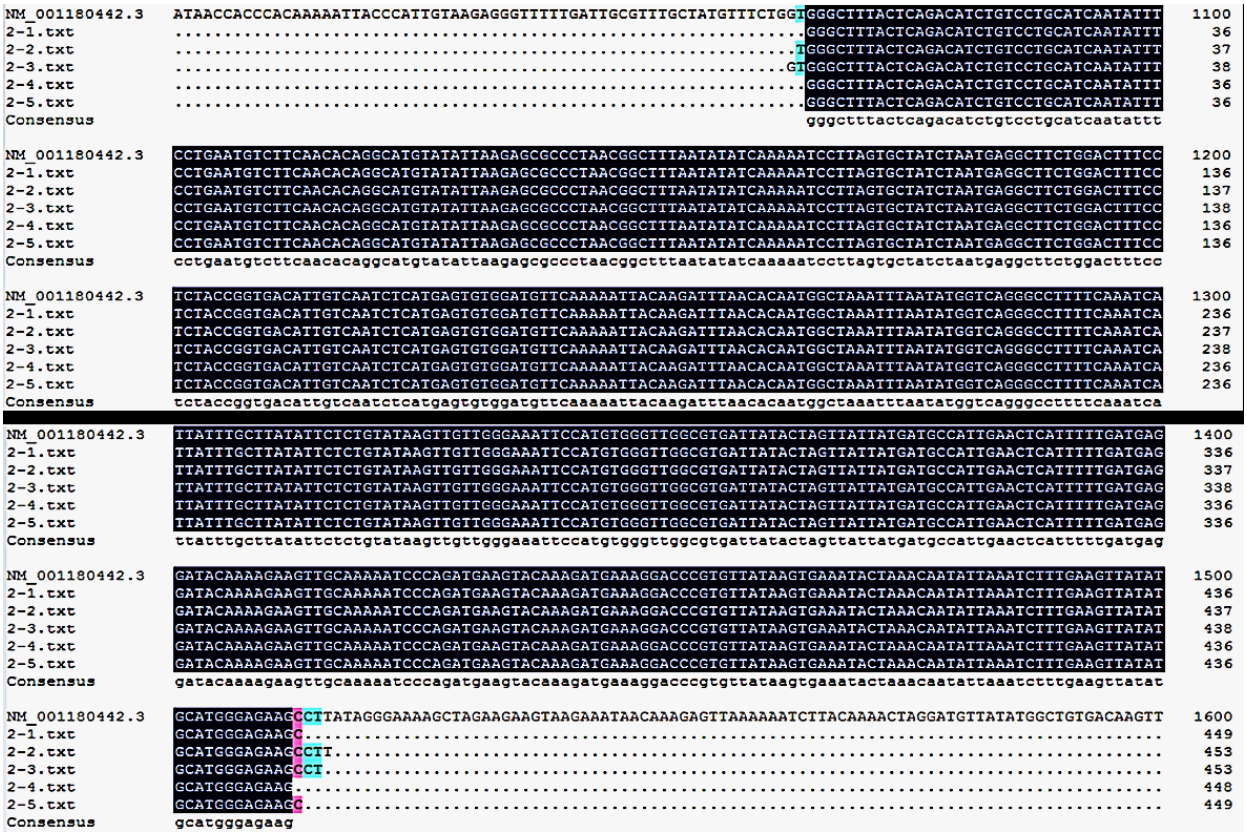


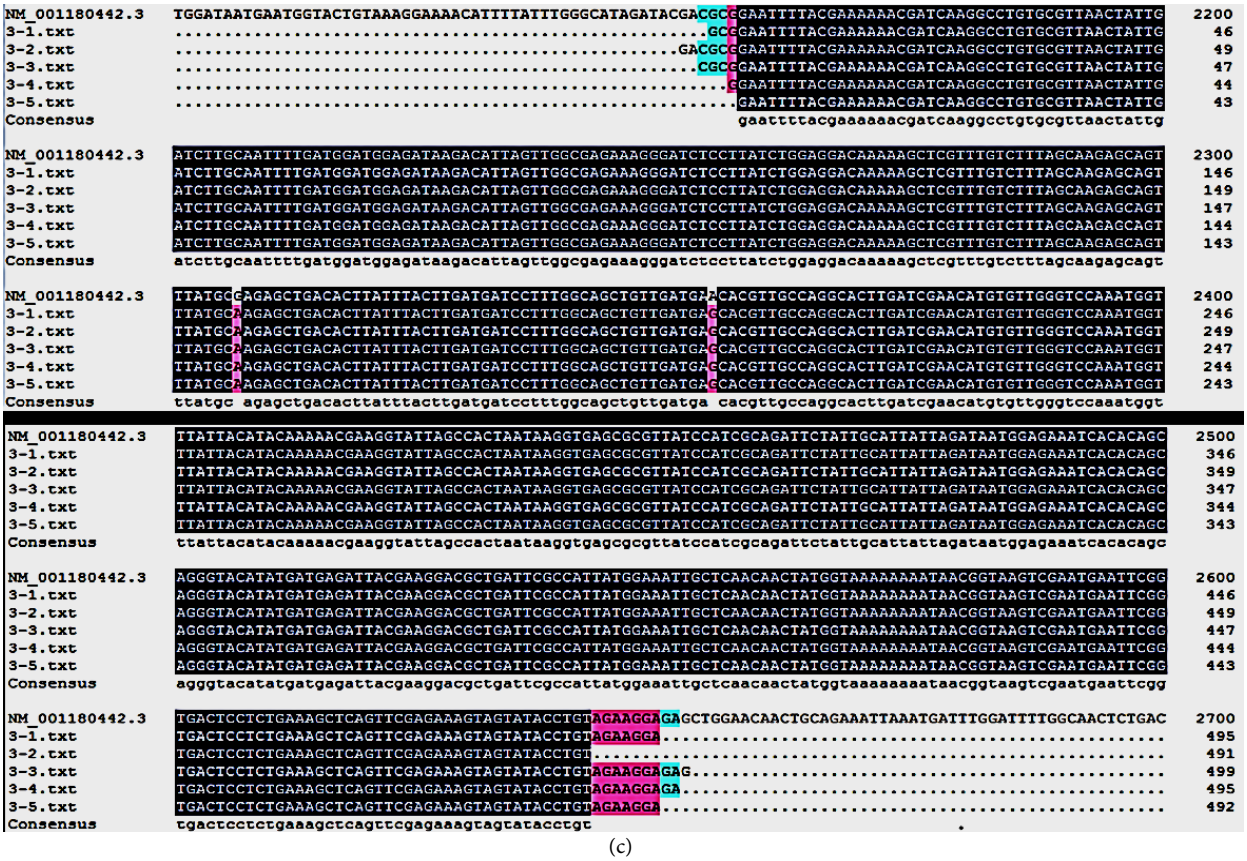
Figure 8. The transgenic plants molecular detection of *ScYCF*1 with (a) *ScYCF*1-F1 and *ScYCF*1-R1; (b) *ScYCF*1-F2 and *ScYCF*1-R2; (c) *ScYCF*1-F3 and *ScYCF*1-R3; (d) *ScYCF*1-F4 and *ScYCF*1-R4. M: DNA Marker DL2000; 1: Vector plasmid; 2: Receptor species of CCRI12; 3 - 8: Transgenic plant.

```
NM_001180442.3  ACGGTGTGATCCTAAATCTATCAGCAATTTTCATGATAACCTTCGGTATCAGAGATTTAGTTAACCTTTGCAAGAAAAAACACTCTGGCATCAAATATAG  200
1-1.txt         ....................................................................TGCAAGAAAAAACACTCTGGCATCAAATATAG   32
1-2.txt         ..................TATCAGCAATTTTCATGATAACCTTCGGTATCAGAGATTTAGTTAACCTTTGCAAGAAAAAACACTCTGGCATCAAATATAG   82
1-3.txt         ................TCTATCAGCAATTTTCATGATAACCTTCGGTATCAGAGATTTAGTTAACCTTTGCAAGAAAAAACACTCTGGCATCAAATATAG   84
1-4.txt         ....................AGCAATTTTCATGATAACCTTCGGTATCAGAGATTTAGTTAACCTTTGCAAGAAAAAACACTCTGGCATCAAATATAG   78
1-5.txt         ................TCTATCAGCAATTTTCATGATAACCTTCGGTATCAGAGATTTAGTTAACCTTTGCAAGAAAAAACACTCTGGCATCAAATATAG   84
Consensus                                                                          tgcaagaaaaaacactctggcatcaaatatag

NM_001180442.3  GCGGAATTGGATTATTGTCTCTAGGATGGCACTAGTTCTGTTGGAGATAGCGTTTGTTTCACTTGCGTCTTTAAATATTTCTAAAGAAGAAGCGGAAAAC  300
1-1.txt         GCGGAATTGGATTATTGTCTCTAGGATGGCACTAGTTCTGTTGGAGATAGCGTTTGTTTCACTTGCGTCTTTAAATATTTCTAAAGAAGAAGCGGAAAAC  132
1-2.txt         GCGGAATTGGATTATTGTCTCTAGGATGGCACTAGTTCTGTTGGAGATAGCGTTTGTTTCACTTGCGTCTTTAAATATTTCTAAAGAAGAAGCGGAAAAC  182
1-3.txt         GCGGAATTGGATTATTGTCTCTAGGATGGCACTAGTTCTGTTGGAGATAGCGTTTGTTTCACTTGCGTCTTTAAATATTTCTAAAGAAGAAGCGGAAAAC  184
1-4.txt         GCGGAATTGGATTATTGTCTCTAGGATGGCACTAGTTCTGTTGGAGATAGCGTTTGTTTCACTTGCGTCTTTAAATATTTCTAAAGAAGAAGCGGAAAAC  178
1-5.txt         GCGGAATTGGATTATTGTCTCTAGGATGGCACTAGTTCTGTTGGAGATAGCGTTTGTTTCACTTGCGTCTTTAAATATTTCTAAAGAAGAAGCGGAAAAC  184
Consensus       gcggaattggattattgtctctaggatggcactagttctgttggagatagcgtttgtttcacttgcgtctttaaatatttctaaagaagaagcggaaaac

NM_001180442.3  TTTACCATTGTAAGTCAATATGCTTCTACAATGTTATCTTTATTTGTTGCTTTAGCCTTACACTGGATAGAATACGATAGATCAGTTGTAGCCAATACGG  400
1-1.txt         TTTACCATTGTAAGTCAATATGCTTCTACAATGTTATCTTTATTTGTTGCTTTAGCCTTACACTGGATAGAATACGATAGATCAGTTGTAGCCAATACGG  232
1-2.txt         TTTACCATTGTAAGTCAATATGCTTCTACAATGTTATCTTTATTTGTTGCTTTAGCCTTACACTGGATAGAATACGATAGATCAGTTGTAGCCAATACGG  282
1-3.txt         TTTACCATTGTAAGTCAATATGCTTCTACAATGTTATCTTTATTTGTTGCTTTAGCCTTACACTGGATAGAATACGATAGATCAGTTGTAGCCAATACGG  284
1-4.txt         TTTACCATTGTAAGTCAATATGCTTCTACAATGTTATCTTTATTTGTTGCTTTAGCCTTACACTGGATAGAATACGATAGATCAGTTGTAGCCAATACGG  278
1-5.txt         TTTACCATTGTAAGTCAATATGCTTCTACAATGTTATCTTTATTTGTTGCTTTAGCCTTACACTGGATAGAATACGATAGATCAGTTGTAGCCAATACGG  284
Consensus       tttaccattgtaagtcaatatgcttctacaatgttatctttatttgttgctttagccttacactggatagaatacgatagatcagttgtagccaatacgg

NM_001180442.3  TACTTTTATTCTATTGGCTTTTTGAAACATTCGGTAATTTTGCTAAACTAATAAATATTCTAATTAGACACACCTACGAAGGCATTTGGTATTCCGGACA  500
1-1.txt         TACTTTTATTCTATTGGCTTTTTGAAACATTCGGTAATTTTGCTAAACTAATAAATATTCTAATTAGACACACCTACGAAGGCATTTGGTATTCCGGACA  332
1-2.txt         TACTTTTATTCTATTGGCTTTTTGAAACATTCGGTAATTTTGCTAAACTAATAAATATTCTAATTAGACACACCTACGAAGGCATTTGGTATTCCGGACA  382
1-3.txt         TACTTTTATTCTATTGGCTTTTTGAAACATTCGGTAATTTTGCTAAACTAATAAATATTCTAATTAGACACACCTACGAAGGCATTTGGTATTCCGGACA  384
1-4.txt         TACTTTTATTCTATTGGCTTTTTGAAACATTCGGTAATTTTGCTAAACTAATAAATATTCTAATTAGACACACCTACGAAGGCATTTGGTATTCCGGACA  378
1-5.txt         TACTTTTATTCTATTGGCTTTTTGAAACATTCGGTAATTTTGCTAAACTAATAAATATTCTAATTAGACACACCTACGAAGGCATTTGGTATTCCGGACA  384
Consensus       tacttttattctattggctttttgaaacattcggtaattttgctaaactaataaatattctaattagacacacctacgaaggcatttggtattccggaca

NM_001180442.3  AACGGGTTTCATACTAACGTTATTCCAAGTAATAACATGTGCCAGTATCCTGTTACTTGAAGCTCTTCCAAAGAAGCCGCTAATGCCACATCAACACATA  600
1-1.txt         AACGGGTTTCATACTAACGTTATTCCAAGTAATAACATGTGCCAGTATCCTGTTACTTGAAGCTCTTCCAAAGAAGCCGCTAATGCCACATCAACACATA  432
1-2.txt         AACGGGTTTCATACTAACGTTATTCCAAGTAATAACATGTGCCAGTATCCTGTTACTTGAAGCTCTTCCAAAGAAGCCGCTAATGCCACATCAACACATA  482
1-3.txt         AACGGGTTTCATACTAACGTTATTCCAAGTAATAACATGTGCCAGTATCCTGTTACTTGAAGCTCTTCCAAAGAAGCCGCTAATGCCACATCAACACATA  484
1-4.txt         AACGGGTTTCATACTAACGTTATTCCAAGTAATAACATGTGCCAGTATCCTGTTACTTGAAGCTCTTCCAAAGAAGCCGCTAATGCCACATCAACACATA  478
1-5.txt         AACGGGTTTCATACTAACGTTATTCCAAGTAATAACATGTGCCAGTATCCTGTTACTTGAAGCTCTTCCAAAGAAGCCGCTAATGCCACATCAACACATA  484
Consensus       aacgggtttcatactaacgttattccaagtaataacatgtgccagtatcctgttacttgaagctcttccaaagaagccgctaatgccacatcaacacata

NM_001180442.3  CATCAAACTTTAACAAGAAGAAAACCAAATCCATACGATAGCGCAAACATATTTTCCAGGATTACCTTCTCTTGGATGTCAGGTTTGATGAAAACTGGCT  700
1-1.txt         CATCAAACTTTAACAAGAAGAAAACCAAATCCATACGATAGCGCAAACATATTTTCCAGGATTACCTTCTCTTGGATGTCAGGTTTGATGAAAACTGGCT  532
1-2.txt         CATCAAACTTTAACAAGAAGAAAACCAAATCCATACGATAGCGCAAACATATTTTCCAGGATTACCTTCTCTTGGATGTCAGGTTTGATGAAAACTGGCT  582
1-3.txt         CATCAAACTTTAACAAGAAGAAAACCAAATCCATACGATAGCGCAAACATATTTTCCAGGATTACCTTCTCTTGGATGTCAGGTTTGATGAAAACTGGCT  584
1-4.txt         CATCAAACTTTAACAAGAAGAAAACCAAATCCATACGATAGCGCAAACATATTTTCCAGGATTACCTTCTCTTGGATGTCAGGTTTGATGAAAACTGGCT  578
1-5.txt         CATCAAACTTTAACAAGAAGAAAACCAAATCCATACGATAGCGCAAACATATTTTCCAGGATTACCTTCTCTTGGATGTCAGGTTTGATGAAAACTGGCT  584
Consensus       catcaaactttaacaagaagaaaaccaaatccatacgatagcgcaaacatatttttccaggattaccttctcttggatgtcaggtttgatgaaaactggct

NM_001180442.3  ATGAAAAATACTTAGTGGAAGCAGATTTATATAAATTACCGAGGAACTTTAGTAGTGAAGAACTCTCTCAAAAATTGGAGAAAAACTGGGAAAATGAGTT  800
1-1.txt         ATGAAAAATACTTAGTGGAAGCAGATTTATATAAATTACCGAGGAACTTTAGTAGTGAAGAACTCTCTCAAA.............................  604
1-2.txt         ATGAAAAATACTTAGTGGAAGCAGATTTATATAAATTACCGAGGAACTTTAGTAGTGAAGAACTCTCTCAAAAATTGGAGAAAAACTGGGAAAATGAGTT  682
1-3.txt         ATGAAAAATACTTAGTGGAAGCAGATTTATATAAATTACCGAGGAACTTTAGTAGTGAAGAACTCTCTCAAAAATTGGAGAAAAACTGGGAAAATGAGTT  684
1-4.txt         ATGAAAAATACTTAGTGGAAGCAGATTTATATAAATTACCGAGGAACTTTAGTAGTGAAGAACTCTCTCAAAAATTGGAGAGAAACTGGGAAAATGAGTT  678
1-5.txt         ATGAAAAATACTTAGTGGAAGCAGATTTATATAAATTACCGAGGAACTTTAGTAGTGAAGAACTCTCTCAAAAATTGGAGAAAAACTGGGAAAATGAGTT  684
Consensus       atgaaaaatacttagtggaagcagatttatataaattaccgaggaactttagtagtgaagaactctctcaaa
```

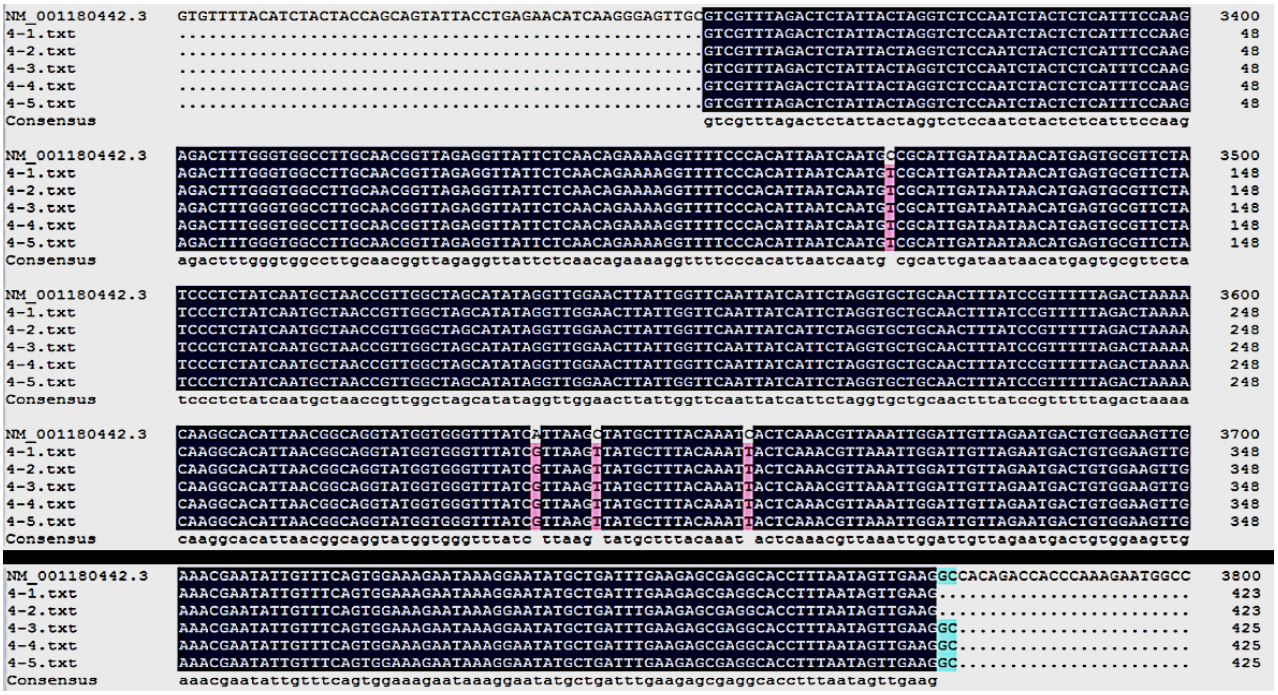
(a)


NM_001180442.3 ATAACCACCCACAAAAATTACCCATTGTAAGAGGGTTTTTGATTGCGTTTGCTATGTTTCTGGTGGGCTTTACTCAGACATCTGTCCTGCATCAATATTT 1100
2-1.txt GGGCTTTACTCAGACATCTGTCCTGCATCAATATTT 36
2-2.txt TGGGCTTTACTCAGACATCTGTCCTGCATCAATATTT 37
2-3.txt GTGGGCTTTACTCAGACATCTGTCCTGCATCAATATTT 38
2-4.txt GGGCTTTACTCAGACATCTGTCCTGCATCAATATTT 36
2-5.txt GGGCTTTACTCAGACATCTGTCCTGCATCAATATTT 36
Consensus gggctttactcagacatctgtcctgcatcaatatttt
NM_001180442.3 CCTGAATGTCTTCAACACAGGCATGTATATTAAGAGCGCCCTAACGGCTTTAATATATCAAAAATCCTTAGTGCTATCTAATGAGGCTTCTGGACTTTCC 1200
2-1.txt CCTGAATGTCTTCAACACAGGCATGTATATTAAGAGCGCCCTAACGGCTTTAATATATCAAAAATCCTTAGTGCTATCTAATGAGGCTTCTGGACTTTCC 136
2-2.txt CCTGAATGTCTTCAACACAGGCATGTATATTAAGAGCGCCCTAACGGCTTTAATATATCAAAAATCCTTAGTGCTATCTAATGAGGCTTCTGGACTTTCC 137
2-3.txt CCTGAATGTCTTCAACACAGGCATGTATATTAAGAGCGCCCTAACGGCTTTAATATATCAAAAATCCTTAGTGCTATCTAATGAGGCTTCTGGACTTTCC 138
2-4.txt CCTGAATGTCTTCAACACAGGCATGTATATTAAGAGCGCCCTAACGGCTTTAATATATCAAAAATCCTTAGTGCTATCTAATGAGGCTTCTGGACTTTCC 136
2-5.txt CCTGAATGTCTTCAACACAGGCATGTATATTAAGAGCGCCCTAACGGCTTTAATATATCAAAAATCCTTAGTGCTATCTAATGAGGCTTCTGGACTTTCC 136
Consensus cctgaatgtcttcaacacaggcatgtatattaagagcgccctaacggctttaatatatcaaaaatccttagtgctatctaatgaggcttctggactttcc
NM_001180442.3 TCTACCGGTGACATTGTCAATCTCATGAGTGTGGATGTTCAAAAATTACAAGATTTAACACAATGGCTAAATTTAATATGGTCAGGGCCTTTTCAAATCA 1300
2-1.txt TCTACCGGTGACATTGTCAATCTCATGAGTGTGGATGTTCAAAAATTACAAGATTTAACACAATGGCTAAATTTAATATGGTCAGGGCCTTTTCAAATCA 236
2-2.txt TCTACCGGTGACATTGTCAATCTCATGAGTGTGGATGTTCAAAAATTACAAGATTTAACACAATGGCTAAATTTAATATGGTCAGGGCCTTTTCAAATCA 237
2-3.txt TCTACCGGTGACATTGTCAATCTCATGAGTGTGGATGTTCAAAAATTACAAGATTTAACACAATGGCTAAATTTAATATGGTCAGGGCCTTTTCAAATCA 238
2-4.txt TCTACCGGTGACATTGTCAATCTCATGAGTGTGGATGTTCAAAAATTACAAGATTTAACACAATGGCTAAATTTAATATGGTCAGGGCCTTTTCAAATCA 236
2-5.txt TCTACCGGTGACATTGTCAATCTCATGAGTGTGGATGTTCAAAAATTACAAGATTTAACACAATGGCTAAATTTAATATGGTCAGGGCCTTTTCAAATCA 236
Consensus tctaccggtgacattgtcaatctcatgagtgtggatgttcaaaaattacaagatttaacacaatggctaaatttaatatggtcagggccttttcaaatca
NM_001180442.3 TTATTTGCTTATATTCTCTGTATAAGTTGTTGGGAAATTCCATGTGGGTTGGCGTGATTATACTAGTTATTATGATGCCATTGAACTCATTTTTGATGAG 1400
2-1.txt TTATTTGCTTATATTCTCTGTATAAGTTGTTGGGAAATTCCATGTGGGTTGGCGTGATTATACTAGTTATTATGATGCCATTGAACTCATTTTTGATGAG 336
2-2.txt TTATTTGCTTATATTCTCTGTATAAGTTGTTGGGAAATTCCATGTGGGTTGGCGTGATTATACTAGTTATTATGATGCCATTGAACTCATTTTTGATGAG 337
2-3.txt TTATTTGCTTATATTCTCTGTATAAGTTGTTGGGAAATTCCATGTGGGTTGGCGTGATTATACTAGTTATTATGATGCCATTGAACTCATTTTTGATGAG 338
2-4.txt TTATTTGCTTATATTCTCTGTATAAGTTGTTGGGAAATTCCATGTGGGTTGGCGTGATTATACTAGTTATTATGATGCCATTGAACTCATTTTTGATGAG 336
2-5.txt TTATTTGCTTATATTCTCTGTATAAGTTGTTGGGAAATTCCATGTGGGTTGGCGTGATTATACTAGTTATTATGATGCCATTGAACTCATTTTTGATGAG 336
Consensus ttatttgcttatattctctgtataagttgttgggaaattccatgtgggttggcgtgattatactagttattatgatgccattgaactcatttttgatgag
NM_001180442.3 GATACAAAAGAAGTTGCAAAAATCCCAGATGAAGTACAAAGATGAAAGGACCCGTGTTATAAGTGAAATACTAAACAATATTAAATCTTTGAAGTTATAT 1500
2-1.txt GATACAAAAGAAGTTGCAAAAATCCCAGATGAAGTACAAAGATGAAAGGACCCGTGTTATAAGTGAAATACTAAACAATATTAAATCTTTGAAGTTATAT 436
2-2.txt GATACAAAAGAAGTTGCAAAAATCCCAGATGAAGTACAAAGATGAAAGGACCCGTGTTATAAGTGAAATACTAAACAATATTAAATCTTTGAAGTTATAT 437
2-3.txt GATACAAAAGAAGTTGCAAAAATCCCAGATGAAGTACAAAGATGAAAGGACCCGTGTTATAAGTGAAATACTAAACAATATTAAATCTTTGAAGTTATAT 438
2-4.txt GATACAAAAGAAGTTGCAAAAATCCCAGATGAAGTACAAAGATGAAAGGACCCGTGTTATAAGTGAAATACTAAACAATATTAAATCTTTGAAGTTATAT 436
2-5.txt GATACAAAAGAAGTTGCAAAAATCCCAGATGAAGTACAAAGATGAAAGGACCCGTGTTATAAGTGAAATACTAAACAATATTAAATCTTTGAAGTTATAT 436
Consensus gatacaaaagaagttgcaaaaatcccagatgaagtacaaagatgaaaggacccgtgttataagtgaaatactaaacaatattaaatctttgaagttatat
NM_001180442.3 GCATGGGAGAAGCCTTATAGGGAAAAGCTAGAAGAAGTAAGAAATAACAAAGAGTTAAAAAATCTTACAAAACTAGGATGTTATATGGCTGTGACAAGTT 1600
2-1.txt GCATGGGAGAAGC 449
2-2.txt GCATGGGAGAAGCCTT 453
2-3.txt GCATGGGAGAAGCCT 453
2-4.txt GCATGGGAGAAG 448
2-5.txt GCATGGGAGAAGC 449
Consensus gcatgggagaag

(b)


NM_001180442.3 TGGATAATGAATGGTACTGTAAAGGAAAACATTTTATTTGGGCATAGATACGACGCGGAATTTTACGAAAAAACGATCAAGGCCTGTGCGTTAACTATTG 2200
3-1.txt GCGGAATTTTACGAAAAAACGATCAAGGCCTGTGCGTTAACTATTG 46
3-2.txt GACGCGGAATTTTACGAAAAAACGATCAAGGCCTGTGCGTTAACTATTG 49
3-3.txt CGCGGAATTTTACGAAAAAACGATCAAGGCCTGTGCGTTAACTATTG 47
3-4.txt GGAATTTTACGAAAAAACGATCAAGGCCTGTGCGTTAACTATTG 44
3-5.txt GAATTTTACGAAAAAACGATCAAGGCCTGTGCGTTAACTATTG 43
Consensus gaattttacgaaaaaacgatcaaggcctgtgcgttaactattg
NM_001180442.3 ATCTTGCAATTTTGATGGATGGAGATAAGACATTAGTTGGCGAGAAAGGGATCTCCTTATCTGGAGGACAAAAAGCTCGTTTGTCTTTAGCAAGAGCAGT 2300
3-1.txt ATCTTGCAATTTTGATGGATGGAGATAAGACATTAGTTGGCGAGAAAGGGATCTCCTTATCTGGAGGACAAAAAGCTCGTTTGTCTTTAGCAAGAGCAGT 146
3-2.txt ATCTTGCAATTTTGATGGATGGAGATAAGACATTAGTTGGCGAGAAAGGGATCTCCTTATCTGGAGGACAAAAAGCTCGTTTGTCTTTAGCAAGAGCAGT 149
3-3.txt ATCTTGCAATTTTGATGGATGGAGATAAGACATTAGTTGGCGAGAAAGGGATCTCCTTATCTGGAGGACAAAAAGCTCGTTTGTCTTTAGCAAGAGCAGT 147
3-4.txt ATCTTGCAATTTTGATGGATGGAGATAAGACATTAGTTGGCGAGAAAGGGATCTCCTTATCTGGAGGACAAAAAGCTCGTTTGTCTTTAGCAAGAGCAGT 144
3-5.txt ATCTTGCAATTTTGATGGATGGAGATAAGACATTAGTTGGCGAGAAAGGGATCTCCTTATCTGGAGGACAAAAAGCTCGTTTGTCTTTAGCAAGAGCAGT 143
Consensus atcttgcaattttgatggatggagataagacattagttggcgagaaagggatctccttatctggaggacaaaaagctcgtttgtctttagcaagagcagt
NM_001180442.3 TTATGCAAGAGCTGACACTTATTTACTTGATGATCCTTTGGCAGCTGTTGATGAACACGTTGCCAGGCACTTGATCGAACATGTGTTGGGTCCAAATGGT 2400
3-1.txt TTATGCAAGAGCTGACACTTATTTACTTGATGATCCTTTGGCAGCTGTTGATGAGCACGTTGCCAGGCACTTGATCGAACATGTGTTGGGTCCAAATGGT 246
3-2.txt TTATGCAAGAGCTGACACTTATTTACTTGATGATCCTTTGGCAGCTGTTGATGAGCACGTTGCCAGGCACTTGATCGAACATGTGTTGGGTCCAAATGGT 249
3-3.txt TTATGCAAGAGCTGACACTTATTTACTTGATGATCCTTTGGCAGCTGTTGATGAGCACGTTGCCAGGCACTTGATCGAACATGTGTTGGGTCCAAATGGT 247
3-4.txt TTATGCAAGAGCTGACACTTATTTACTTGATGATCCTTTGGCAGCTGTTGATGAGCACGTTGCCAGGCACTTGATCGAACATGTGTTGGGTCCAAATGGT 244
3-5.txt TTATGCAAGAGCTGACACTTATTTACTTGATGATCCTTTGGCAGCTGTTGATGAGCACGTTGCCAGGCACTTGATCGAACATGTGTTGGGTCCAAATGGT 243
Consensus ttatgc agagctgacacttatttacttgatgatcctttggcagctgttgatga cacgttgccaggcacttgatcgaacatgtgttgggtccaaatggt
NM_001180442.3 TTATTACATACAAAAACGAAGGTATTAGCCACTAATAAGGTGAGCGCGTTATCCATCGCAGATTCTATTGCATTATTAGATAATGGAGAAATCACACAGC 2500
3-1.txt TTATTACATACAAAAACGAAGGTATTAGCCACTAATAAGGTGAGCGCGTTATCCATCGCAGATTCTATTGCATTATTAGATAATGGAGAAATCACACAGC 346
3-2.txt TTATTACATACAAAAACGAAGGTATTAGCCACTAATAAGGTGAGCGCGTTATCCATCGCAGATTCTATTGCATTATTAGATAATGGAGAAATCACACAGC 349
3-3.txt TTATTACATACAAAAACGAAGGTATTAGCCACTAATAAGGTGAGCGCGTTATCCATCGCAGATTCTATTGCATTATTAGATAATGGAGAAATCACACAGC 347
3-4.txt TTATTACATACAAAAACGAAGGTATTAGCCACTAATAAGGTGAGCGCGTTATCCATCGCAGATTCTATTGCATTATTAGATAATGGAGAAATCACACAGC 344
3-5.txt TTATTACATACAAAAACGAAGGTATTAGCCACTAATAAGGTGAGCGCGTTATCCATCGCAGATTCTATTGCATTATTAGATAATGGAGAAATCACACAGC 343
Consensus ttattacatacaaaaacgaaggtattagccactaataaggtgagcgcgttatccatcgcagattctattgcattattagataatggagaaatcacacagc
NM_001180442.3 AGGGTACATATGATGAGATTACGAAGGACGCTGATTCGCCATTATGGAAATTGCTCAACAACTATGGTAAAAAAAATAACGGTAAGTCGAATGAATTCGG 2600
3-1.txt AGGGTACATATGATGAGATTACGAAGGACGCTGATTCGCCATTATGGAAATTGCTCAACAACTATGGTAAAAAAAATAACGGTAAGTCGAATGAATTCGG 446
3-2.txt AGGGTACATATGATGAGATTACGAAGGACGCTGATTCGCCATTATGGAAATTGCTCAACAACTATGGTAAAAAAAATAACGGTAAGTCGAATGAATTCGG 449
3-3.txt AGGGTACATATGATGAGATTACGAAGGACGCTGATTCGCCATTATGGAAATTGCTCAACAACTATGGTAAAAAAAATAACGGTAAGTCGAATGAATTCGG 447
3-4.txt AGGGTACATATGATGAGATTACGAAGGACGCTGATTCGCCATTATGGAAATTGCTCAACAACTATGGTAAAAAAAATAACGGTAAGTCGAATGAATTCGG 444
3-5.txt AGGGTACATATGATGAGATTACGAAGGACGCTGATTCGCCATTATGGAAATTGCTCAACAACTATGGTAAAAAAAATAACGGTAAGTCGAATGAATTCGG 443
Consensus agggtacatatgatgagattacgaaggacgctgattcgccattatggaaattgctcaacaactatggtaaaaaaaataacggtaagtcgaatgaattcgg
NM_001180442.3 TGACTCCTCTGAAAGCTCAGTTCGAGAAAGTAGTATACCTGTAGAAGGAGAGCTGGAACAACTGCAGAAATTAAATGATTTGGATTTTGGCAACTCTGAC 2700
3-1.txt TGACTCCTCTGAAAGCTCAGTTCGAGAAAGTAGTATACCTGTAGAAGGA 495
3-2.txt TGACTCCTCTGAAAGCTCAGTTCGAGAAAGTAGTATACCTGT 491
3-3.txt TGACTCCTCTGAAAGCTCAGTTCGAGAAAGTAGTATACCTGTAGAAGGAGAG 499
3-4.txt TGACTCCTCTGAAAGCTCAGTTCGAGAAAGTAGTATACCTGTAGAAGGAGA 495
3-5.txt TGACTCCTCTGAAAGCTCAGTTCGAGAAAGTAGTATACCTGTAGAAGGA 492
Consensus tgactcctctgaaagctcagttcgagaaagtagtatacctgt

(c)



(d)

Figure 9. The gene sequencing result of trans-*ScYCF*1: (a) The sequencing results of *ScYCF*1-F1 and *ScYCF*1-R1; (b) The sequencing results of *ScYCF*1-F2 and *ScYCF*1-R2; (c) The sequencing results of *ScYCF*1-F3 and *ScYCF*1-R3; (d) The sequencing results of *ScYCF*1-F4 and *ScYCF*1-R4.

400 and 600 mM NaCl saline for 72 h, (Figure 10) the color of the cotton leaf discs gradually turned yellow, and the leaf margin turned white, while the leaf discs under 0 mM NaCl solution remained green; Under the same concentration of saline, the color and shape of leaf discs in *ScYCF*1 transgenic plants were more obvious than the control leaf discs. The chlorophyll content of the leaf discs processed was measured and results showed that under three kinds of treatment, the chlorophyll content of leaf discs in transgenic plants was significantly higher than that in CCRI12; but after treated with 400 mM NaCl, there wasn't much difference in chlorophyll content between the two groups, so it is speculated that *ScYCF*1 gene of *Saccharomyces cerevisiae* can increase the chlorophyll content of cotton and plays a significant role in the tolerance of the cotton to salt stress.

4. Discussion

In recent years, domestic and foreign experts have used transient expression technology to establish corresponding expression systems in wheat (*Triticum monococcum*), corn (*Zea mays*), rice (*Oryza sativa*), petunia (*Petunia hybrid*), periwinkle (*Catharanthus roseus*) and green algae (*Chlorophyta*) and other plants [15] [16] [17] [18] [19]. Kong Jingjing selected 26 cotton varieties to study the auto-fluorescence phenomenon of cotton pollen and results showed that the auto-fluorescence of cotton pollen is green under 288 nm excitation light, and

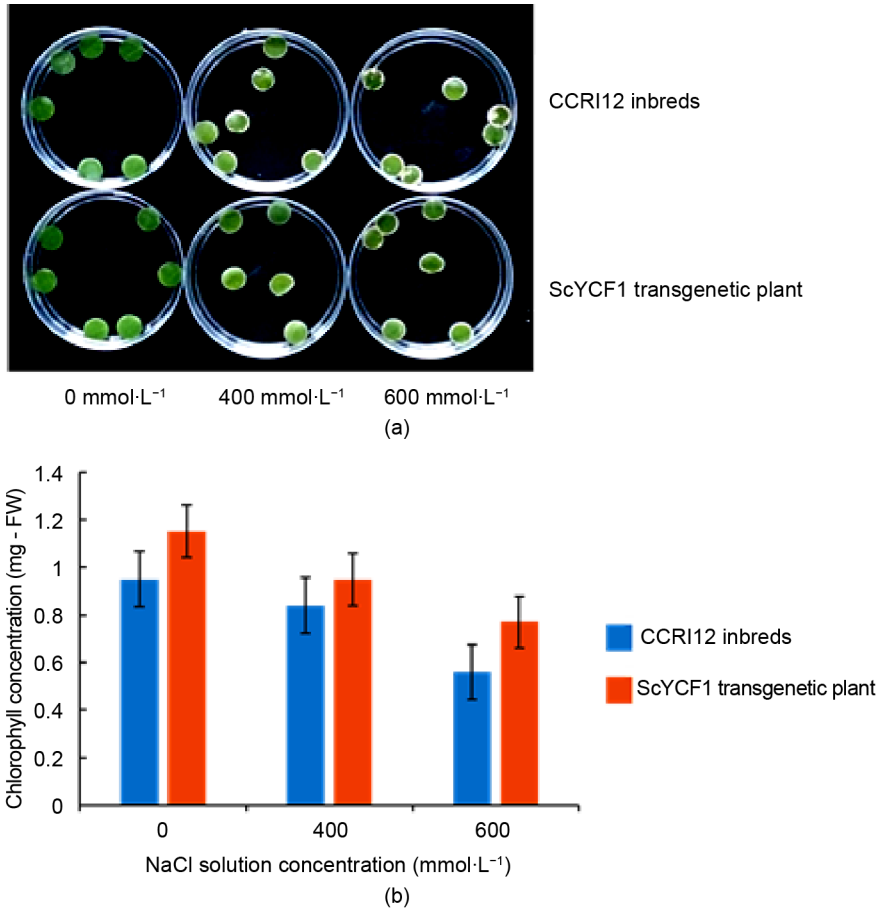


Figure 10. Transgenic cotton semal salt resistance analysis: (a) The color and form of the *ScYCF*1 transgenic plant and CCRI12 in different concentrations of salt-stress in solution after 96 hours; (b) The chlorophyll of semal (six slices).

the auto-fluorescence of pollens of three cotton varieties (Y2067, GZ-2 and ZA-23) is weaker than it of other cotton varieties [14]. This study chose the three cotton varieties with weak pollen auto-fluorescence, bombarded the target gene to the pollen by a gene gun and results showed that the fluorescence of three kinds of cotton pollens was strengthened. If to transform upland cotton with genes of *Saccharomyces cerevisiae*, the distant genetic relationship between the two may lead to that the genes of *Saccharomyces cerevisiae* cannot be expressed in the upland cotton, while in the transient expression assay of cotton pollen, the expression of yeast genes was observed in a short time, which laid a foundation for successful transformation of cotton with yeast genes.

T_0 cotton seeds were harvested after transforming with a gene gun, but whether the yeast salt-tolerant gene can enter and be integrated into the genome of upland cotton, the size of integrated fragments, and functional performance of the target gene in the transgenic plants all needs to be further explored, therefore, detection of transgenic cotton materials is a critical step. Given that more T_0 generation of seeds ask for arduous testing work and the growing cycle is

quite long, this study started from verification of the gene function, coerced the transgenic seeds with 100 mM NaCl solution, used double-filter paper method to filter its salt tolerance, and combined with PCR method for amplification of target gene sequences, and directly sequenced the purified fragments after amplification, which greatly reduced the time of a series of experiments including molecular detection and function verification of target genes and sped up the progress of the experiment.

Given that *ScYCF*1 protein is related to vacuole detoxification, salt tolerance experiments were conducted for *ScYCF*1 transformed arabidopsis plants under high salt conditions. Three weeks old arabidopsis wild type and transgenic seedlings were treated with 75, 100, 150 and 200 mM NaCl for 6 h and results showed that after being treated with 200 mM NaCl the accumulation amount of Na+ in Arabidopsis was 26.5% higher than that in wild type, but under stress of other salt concentrations there was no significant difference between them, indicating that Arabidopsis with transformed *ScYCF*1 gene has stronger stress tolerance to NaCl than the wild type, which is because that the transgenic plants can accumulate more Na+ in vacuoles [13]. This study conducted salt tolerance germination experiment for transgenic cotton plants with 100 mM NaCl solution and results showed that the bud length of transgenic seeds under salt stress was significantly shorter than that in water of the control group, but was longer than that of non-transgenic seeds under salt stress. Salt tolerance analysis of leaf discs in transgenic plants showed that transforming the cotton with yeast *ScYCF*1 improves the salt tolerance of leaf discs and plays an important role in improving the salt tolerance under high salt concentrations. The transgenic plants will be further explored in subsequent trials.

5. Conclusion

The *ScYCF*1 gene of *Saccharomyces cerevisiae* were transformed and expressed in cotton and its pollen, which not only enhanced salt tolerance of cotton seeds and the growing ability of them, but also increased the chlorophyll content of cotton and played a significant role in the tolerance of the cotton to salt stress.

Acknowledgements

This work was supported by National High-technology Research and Development Program (863 Program) (2013AA102601). Min Mu, Na Shu and Wuwei Ye participated in the design of the study, and performed major experiments, the data interpretation and drafted the manuscript. Xuke Lu, Xiugui Chen and Shuai Wang participated in part of the experiments. Junjuan Wang, Delong Wang, Weili Fan, Lixue Guo and Chao Chen performed part of proteomics analysis.

References

[1] Rengasamy, P. (2006) World Salinization with Emphasis on Australia. *Journal of Experimental Botany*, **57**, 1017-1023. https://doi.org/10.1093/jxb/erj108

[2] Boyer, J.S. (1982) Plant Productivity and Environment. *Science*, **218**, 443-448. https://doi.org/10.1126/science.218.4571.443

[3] Flowers, T.J., and Yeo, A.R. (1995) Breeding for Salinity Resistance in Crop Plants: Where Next? *Australian Journal Plant Physiology*, **22**, 875-884. https://doi.org/10.1071/PP9950875

[4] Nelson, D.E., Shen, B. and Bohnert, H.J. (1998) Salinity Tolerance—Mechanisms, Models and the Metabolic Engineering of Complex Traits. *Genetic Engineering*, **20**, 153-176. https://doi.org/10.1007/978-1-4899-1739-3_9

[5] Mai, Z., Yang, J., Tian, X., Li, J. and Zhang, S. (2013) Gene Cloning and Characterization of a Novel Salt-Tolerant and Glucose-Enhanced *β*-Glucosidase from a Marine *Streptomycete*. *Applied Biochemistry and Biotechnology*, **169**, 1512-1522. https://doi.org/10.1007/s12010-012-0080-3

[6] Jin, Y., Weining, S. and Nevo, E. (2005) A MAPK Gene from Dead Sea Fungus Confers Stress Tolerance to Lithium Salt and Freezing-Thawing: Prospects for Saline Agriculture. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 18992-18997. https://doi.org/10.1073/pnas.0509653102

[7] Kultz, D. (2003) Evolution of the Cellular Stress Proteome: From Monophyletic Origin to Ubiquitous Function. *The Journal of Experimental Biology*, **206**, 3119-3124. https://doi.org/10.1242/jeb.00549

[8] Zhao, F.Y., Guo, S.L., Zhang, H. and Zhao, Y.X. (2006) Expression of Yeast *SOD2* in Transgenic Rice Results in Increased Salt Tolerance. *Plant Science*, **170**, 216-224. https://doi.org/10.1016/j.plantsci.2005.08.017

[9] Zhang, L.Q., Guo, F.X., Xian, H.Q., Wang, X.J., Li, A.N., *et al.* (2011) Expression of a Novel Thermostable Cu, Zn-Superoxide Dismutase from Chaetomium Thermophilum in *Pichia pastoris* and Its Antioxidant Properties. *Biotechnology Letters*, **33**, 1127-1132. https://doi.org/10.1007/s10529-011-0543-6

[10] Liu, X.D., Xie, L., Wei, Y., Zhou, X., Jia, B., *et al.* (2014) Abiotic Stress Resistance, a Novel Moonlighting Function of Ribosomal Protein RPL44 in the Halophilic Fungus *Aspergillus glaucus*. *Applied and Environmental Microbiology*, **80**, 4294-4300. https://doi.org/10.1128/AEM.00292-14

[11] Mewes, H.W., Albermann, K., Bahr, M., Frishman, D., Gleissner, A., *et al.* (1997) Overview of the Yeast Genome. *Natur*e, **387**, 7-65. https://doi.org/10.1038/42755

[12] Bauer, B.E., Wolfger, H. and Kuchler, K. (1999) Inventory and Function of Yeast ABC Proteins: About Sex, Stress, Pleiotropic Drug and Heavy Metal Resistance. *Bba-Biomembranes*, **1461**, 217-236. https://doi.org/10.1016/S0005-2736(99)00160-1

[13] Koh, E.J., Song, W.Y., Lee, Y., Kim, K.H., Kim, K., *et al.* (2006) Expression of Yeast Cadmium Factor 1 (YCF1) Confers Salt Tolerance to *Arabidopsis thaliana*. *Plant Science*, **170**, 534-541. https://doi.org/10.1016/j.plantsci.2005.10.007

[14] Kong, J., Lu, X., Zhao, X., Yin, Z., Wang, S., *et al.* (2016) Cloning of *SjCA* Gene and Its Expression Analysis on Upland Cottons. *Journal of Biomedical Engineering and Informatics*, **2**, 150. https://doi.org/10.5430/jbei.v2n2p150

[15] Vickers, C.E., Xue, G.P. and Gresshoff, P.M. (2006) A Novel Cis-Acting Element, ESP, Contributes to High-Level Endosperm-Specific Expression in an Oat Globulin Promoter. *Plant Molecular Biology*, **62**, 195-214. https://doi.org/10.1007/s11103-006-9014-1

[16] Scheffler, B., Franken, P., Schutt, E., Schrell, A., Saedler, H., *et al.* (1994) Molecular Analysis of C1 Alleles in *Zea mays* Defines Regions Involved in the Expression of This Regulatory Gene. *Molecular Genetics and Genomics*, **242**, 40-48.

[17] Lange, P.D., Boer, G.J.D., Mol, J.N.M. and Kooter, J.M. (1993) Conditional Inhibition of β-Glucuronidase Expression by Antisense Gene Fragments in Petunia Protoplasts. *Plant Molecular Biology*, **23**, 45-55. https://doi.org/10.1007/BF00021418

[18] Di Fiore, S., Hoppmann, V., Fischer, R. and Schillberg, S. (2004) Transient Gene Expression of Recombinant Terpenoid Indole Alkaloid Enzymes in Catharanthus roseus Leaves. *Plant Molecular Biology Reporter*, **22**, 15-22. https://doi.org/10.1007/BF02773344

[19] Teng, C.Y., Qin, S., Liu, J.G., Yu, D.Z., Liang, C.W. and Tseng, C.K. (2002) Transient Expression of lacZ in Bombarded Unicellular Green Alga *Haematococcus pluvialis*. *Journal of Applied Phycology*, **14**, 497-500. https://doi.org/10.1023/A:1022314815045