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Chapter

Transgenic Technology can Accelerate Cotton Breeding: Transgenic *ScALDH21* Cotton Significantly Improve Drought Tolerance in Southern and Northern Xinjiang

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Abstract

Aldehyde dehydrogenases (ALDHs) contribute to cellular protection against oxidative stress. These enzymes are crucial to organisms' ability to cope with environmental stress. The ALDH21 gene was introduced into upland cotton (*Gossypium hirsutum* L.) from desiccant-tolerant *Syntrichia caninervis* moss, created stable genetic transgenic lines. As a result, drought tolerance is increased and yield penalty is reduced in those transgenic lines. The first study to demonstrate overexpression of *ALDH21* enhances drought tolerance in cotton under multi-location field experiments is presented here. Cotton genotypes containing *ScALDH21* exhibit significant morphological, physiological, and economic benefits. *ScALDH21* functions in the physiology of cotton plants to protect them by scavenging ROS and reducing osmotic stress. The yield of transgenic cotton in northern Xinjiang showed up to 10% improvement under full irrigation and up to 18% improvement in deficit irrigation conditions on fields with purple clay loam soils. Additionally, transgenic cotton can be grown in sandy loam soil in southern Xinjiang with an average yield increase of 40% on different irrigation levels in the desert-oasis ecotone. Using *ScALDH21* as a candidate gene for cotton improvement in arid and semi-arid regions was demonstrated. In addition, we assessed different irrigation protocols and optimized irrigation methods with minimal water requirements for *ScALDH21*-transgenic cotton that could be used in production agriculture.

Keywords: transgenic cotton, molecular breeding, *ScALDH21*, drought tolerance, yield improved

1. Introduction

Plants are restricted in their habitat range and productivity by adverse environmental conditions [1]. Drought is the foremost constraint on agricultural production. Cotton (*Gossypium* spp.) is a major source of textile fibers and oil around the world. More than 32 million ha of cotton are produced in 76 countries [2]. In terms of cotton production, China is ranked among the top two countries in the world [3]. However, cotton production in China, as well as other countries, has recently declined due to increasing drier environments [3, 4]. Chinese agriculture consumes 62% of the country's annual water consumption, and the country is in a moderate water shortage [4]. In agriculture, cotton is the crop with the highest water consumption. In China, cotton is grown mainly in the Xinjiang-Uyghur Autonomous Region, an area characterized by very low air humidity and a severe water shortage.

Cotton is the most important crop in China, accounting for around 25% of global fiber production. There is more than one-third of all agricultural land in the Xinjiang-Uyghur Autonomous Region dedicated to cotton plantations [5]. This region has a warm climate with average temperatures of 11.4°C and 49 mm precipitation annually, low groundwater levels, sandy soils, and severe soil salinization [6–9]. In southern Xinjiang, cotton has low germination rates, low survival rates, and low yields [10].

Plants are able to generate significant amounts of reactive aldehydes when faced with a variety of abiotic stresses (such as salinity, desiccation, and cold) [11], which can impair plant growth and crop productivity. Cotton varieties that survive droughts and other adversities must be developed urgently to combat these conditions. In arid lands where freshwater scarcity is a severe constraint on agricultural production, it is necessary to develop more tolerant varieties of plants. It is often difficult to obtain drought-tolerant crops through traditional breeding programs because of the time and labor involved, in addition to the need for large-scale facilities, such as rainout shelters. Interestingly, biotechnological improvements have been attempted since the 1990s, which is inspiring. However, the majority of transgenic cotton is aimed at controlling insect pest damage by expressing a variety of insecticidal proteins from *Bacillus thuringiensis* (i.e., Bt cotton). In transgenic plants, a number of genes have been discovered and studied that have a high potential for improving drought resistance, and some of these genes have shown promise for crop improvement [12–16]. However, only a few of these genes have been successfully deployed in fields of agriculture [17–20].

Plants that are known as bryophytes (mosses, hornworts, liverworts, etc.) are among the oldest species in the world's flora; they are thought to be small, non-vascular, and green plants. Many bryophytes survive even with a total loss of water in their vegetative tissues [21, 22]. The study of drought-tolerant mosses is of particular interest because their genetic engineering properties can be used to increase drought tolerance in arid-zone crops. The desiccation-tolerant moss *Syntrichia caninervis* is distributed in the Gurbantunggut desert in western China and can survive almost complete water loss and recover within 30 seconds after rehydrating [23]. Thus, *S. caninervis* may be a natural gene base for desiccation tolerance (**Figure 1**).

Aldehyde dehydrogenase (ALDH) genes show promise as candidate genes to increase plant resistance, especially *ALDH21* gene from moss *S. caninervis*, which is not found in seed plants. In a desert-oasis ecotone, non-transgenic cotton has an advantage over plants that overexpress *ALDH21* from desiccant tolerant moss, even under different irrigation practices. In addition, we are seeking the best irrigation scheme to reduce the consumption of irrigating water and increase crop production



Figure 1.
The natural habitat of *S. caninervis* distributed in the Gurbantunggut desert of western China. Different morphology of moss: dried state and recovered state after applied water.

in desert areas. Zhu et al. reported that different cotton planting lands gave different yields for some cotton lines [17]. To evaluate the efficacy of transgenes in cotton, irrigation strategy has a crucial role to play, especially in climate-dependent arid regions.

2. Cotton drought tolerance breeding with transgene technology

2.1 The overexpression gene types in current drought-tolerant cotton

It is possible to improve cotton drought tolerance using transgenic technology. The drought tolerance of transgenic cotton has recently been enhanced by using several genes (**Table 1**). As an example, AtLOS5, encoding an aldehyde oxidase cofactor sulfurase; GhAnn1, an annexin gene; isopentenyl transferase (IPT), an enzyme responsible for cytokinin biosynthesis; and 14-3-3 genes involved in plasma membrane H⁺-ATPase activity [17, 24–27]. Increased drought tolerance was also observed in transgenic cotton overexpressing the OsSIZ1 gene from *Oryza sativa*, which encodes a SUMO E3 protein [36]. Researchers have used several transcription factor genes as transgenes in cotton to increase drought tolerance, including AtEDT1/HDG11 (homeodomain-START transcription factor), GhABF2 (bZIP transcription factor), NAC (a transcription factor) in rice (*O. sativa* L.), and AtRAV (for ABA insensitive3/viviparous1) in cotton [28–32]. It has been demonstrated that the expression of the vacuolar proton-pumping pyrophosphatase gene (AVP1) from *Arabidopsis* in cotton results in an increase in fiber yield of 20% compared to non-transgenic cotton [33, 34]. Drought tolerance is further improved by co-overexpression of AVP1 and AtNHX1 in cotton [35]. Nonetheless, only AtEDT1/HDG11, transgenic IPT, and

Gene name	Gene types/function	Gene source	References
<i>AtLOS5</i>	Molybdenum cofactor sulfurase gene/ aldehyde oxidase activity	<i>Arabidopsis</i>	Yue et al. [24]
<i>GhAnn1</i>	Annexin gene	<i>G. hirsutum</i>	Zhang et al. [25]
<i>IPT</i>	Isopentenyl transferase gene/rate-limiting enzyme for cytokinin biosynthesis	<i>Arabidopsis</i>	Kuppu et al. [26]; Zhu et al. [17]
14-3-3	Regulate the activity of plasma membrane H ⁺ -ATPase	<i>Arabidopsis</i>	Yan et al. [27]
<i>GhABF2</i>	bZIP transcription factor family gene	<i>Arabidopsis</i>	Liang et al. [28]
<i>AtEDT1/ HDG11</i>	Homeodomain-START transcription factor gene	<i>Arabidopsis</i>	Yu et al. [29]
<i>AtRAV</i>	Related to ABA insensitive3/viviparous1	<i>Arabidopsis</i>	Mittal et al. [30]
<i>SNAC1</i>	Transcription factor gene	<i>Oryza sativa</i> L.	Liu et al. [31]
<i>AtSAP5</i>	Zinc-finger protein gene	<i>Arabidopsis</i>	Hozain et al. [32]
<i>AVP1</i>	Vacuolar proton-pumping pyrophosphatase (H ⁺ -PPase) gene	<i>Arabidopsis</i>	Pasapula et al. [33]; Zhang et al. [34]
<i>AtNHX1</i>	Vacuolar Na ⁺ /H ⁺ antiporter gene	<i>Arabidopsis</i>	Shen et al. [35]
<i>OsSIZ1</i>	SUMO E3 protein gene/participates in a sumoylation reaction	<i>Oryza sativa</i>	Mishra et al. [36]
<i>ScALDH21</i>	Aldehyde dehydrogenases gene	<i>Syntrichia caninervis</i>	Yang et al. [37, 38]
<i>AtHUB2</i>	Histone H2B monoubiquitination E3 ligase gene	<i>Arabidopsis</i>	[39]
<i>StDREB2</i>	Dehydration-responsive element binding (DREB) transcription factors	<i>Solanum tuberosum</i> L.	[40]
<i>ABF</i>	bZIP AREB/ABF transcription factor orthologs	<i>Arabidopsis</i> ; <i>G. hirsutum</i>	[41]
<i>GHSP26</i>	Heat-shock proteins gene	<i>Gossypium arboreum</i>	[42]

Table 1.
Overexpression of various genes in cotton that reported to enhance drought tolerance.

transgenic *AtAVP1* cotton showed a simultaneous increase in drought tolerance as well as cotton or fiber yield.

2.2 Transgenic *ScALDH21* cotton significantly improve drought tolerance in southern and northern Xinjiang

A number of fiber quality parameters and yield were improved with cotton *ScALDH21*. Planting transgenic *ScALDH21* cotton lines on purple clay loam soils in northern Xinjiang, field experiments demonstrated an increase in yields of 10.0% under full irrigation and >18.0% under deficit irrigation conditions (**Figure 2**). Compared to the non-transgenic cotton variety “Xin Nong Mian 1,” the transgenic cotton showed an average yield increase of at least 40% grown on sandy loam soil in southern Xinjiang. Compared to the recipient cultivar “Xin Nong Mian 1,”

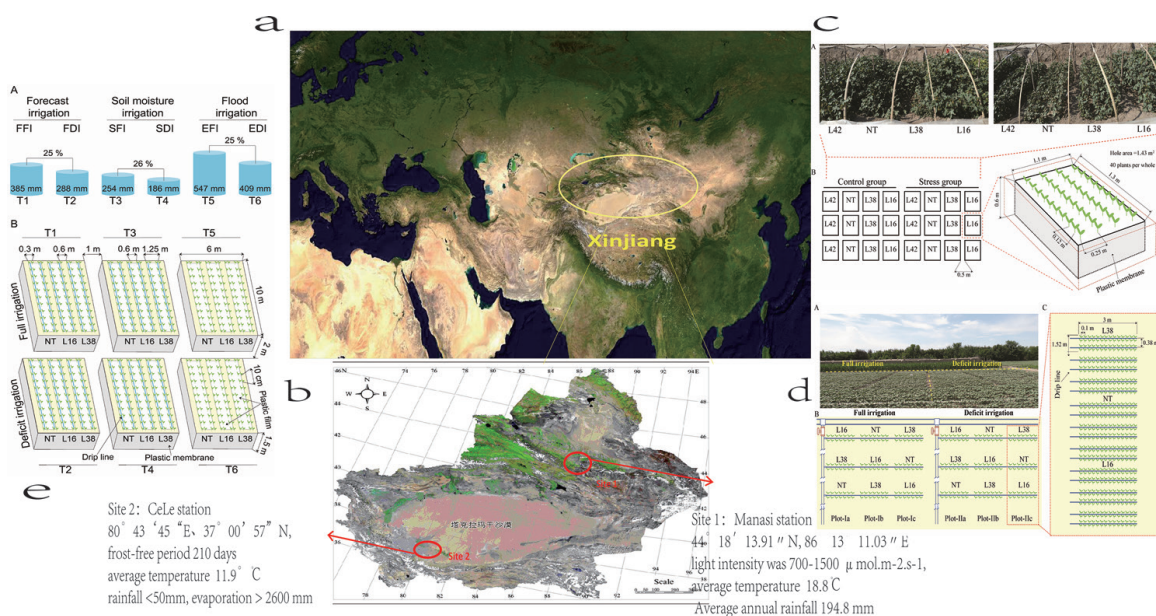


Figure 2. The experiment sites location and experiment design. (a) Site of Xinjiang in the world map; (b) experiment site in Xinjiang map; (c) experiment design in the north of Xinjiang (44° 18'13.91" N, 86° 13'11.03" E, average temperature 18.8°C, average annual rainfall 194.8 mm); (d) experiment design in south of Xinjiang (80° 43'45" E, 37° 00'57" N, frost-free period 210 days, average temperature 11.9°C, rainfall < 50 mm, evaporation > 2600 mm).

ScALDH21-cotton had substantially improved performance under deficit irrigation, ensuring a more sustainable cotton production in the desert-oasis ecotone [37].

A variety of irrigation protocols were evaluated and optimized to use ScALDH21-cotton genotypes in production agriculture with minimum water requirements. The following paragraphs describe the characteristics of transgenic ScALDH21 cotton.

2.2.1 The aldehyde dehydrogenase (ALDH) enzyme superfamily and its functions

As ROS are generated, oxidative stress is induced, lipid membranes are destroyed, and 200 types of aldehydes are accumulated, many of which are highly reactive and toxic. Aldehydes must be effectively removed and detoxified in arid environments to improve plant productivity. Plants have developed many enzymatic and non-enzymatic mechanisms to scavenge these toxic compounds [24]. Aldehyde dehydrogenase (ALDH) superfamily proteins may also play a role in scavenging ROS enzymatically [43]. Aldehyde dehydrogenases (ALDHs) have been found to play a central role in plants exposed to stressful conditions in the detoxification of aldehyde [44]. This superfamily of enzymes metabolizes endogenous and exogenous aldehydes to their carboxylic acids by using the coenzyme NAD(P)⁺, producing NAD(P)H and thereby reducing oxidative/electrophilic stress [45]. ALDHs belong to a group of NAD(P)⁺-dependent enzymes. Based on sequence similarity, the ALDH gene superfamily has been classified into 24 protein families by the ALDH Gene Nomenclature Committee (AGNC) [46]. There are 14 ALDH enzyme families in plants. Two of them (ALDH21 and ALDH23) are unique to bryophytes, and the rest (ALDH10, ALDH11, ALDH12, ALDH19, ALDH21, ALDH22, ALDH23, and ALDH24) are unique to higher plants [11, 47, 48]. ALDH21A1 plays a crucial role in the detoxification of aldehydes generated by desiccation stress, and it is proposed that ALDH21A1 expression is a unique stress resistance mechanism. Two classes of resistance pathways have been linked

to the ALDHs superfamily as abiotic or biotic resistance genes. An ALDH acts as an ‘aldehyde scavenger’ [49]. The increased activity of the Arabidopsis aldehyde dehydrogenase Ath-ALDH3 and soybean ALDH7 was reported to act as a detoxification mechanism that limits the accumulation of aldehyde and oxidative stress in Arabidopsis [1, 50]. In addition, metabolized ALDH products are directly involved in maintaining cellular osmotic homeostasis by catalyzing the synthesis of osmolytes [51, 52]. POD activity was the primary reason for the reduced peroxide levels in transgenic BADH tomatoes compared with SOD, APX, and CAT [52]. ALDH21 confers tolerance to osmotic and oxidative stress in cotton, according to our data. Under deficit stress, *ScALDH21*-cotton showed lower MDA production, increased POD activity, and higher proline and soluble sugar levels than non-transgenic cotton. This indicates that the *ScALDH21* gene may play a significant role in drought tolerance. Several ALDHs participate in drought-tolerant pathways in plants [53–55]. The transcriptional level is believed to be the mechanism by which ALDHs mediate environmental stress. An et al. [56] reported that the treatment of maize plants with NaCl and mannitol increased levels of *ZmALDH7B6* mRNA transcripts. Results of quantitative real-time PCR revealed that osmotic and H₂O₂ stress increased the expression of the *SiALDH7B1*, *SiALDH12A1*, and *SiALDH18B2* genes of *foxtail millet* (*Setaria italica*) [57]. ALDH overexpression has been shown to positively mitigate environmental stress. In contrast to non-transgenic plants, transgenic Arabidopsis *AtALDH2B8*, *AtALDH3I1*, *AtALDH7B4*, and *SpBADH* were able to survive on media containing high levels of H₂O₂. Moreover, ROS content in detached leaves of ALDH plants was significantly lower than that of WT [58, 59]. Plants overexpressing *ZmALDH22A1* show increased stress tolerance [60]. *SpALDH10* encodes the drought-inducible betaine aldehyde dehydrogenase (BADH) that catalyzes the oxidation of betaine aldehyde to the compatible solute glycine betaine, resulting in enhanced drought and salinity tolerance in potato plants [61]. ALDHs appear to play an important role in cell metabolism and stress physiology, according to these results. Recently, cotton transformed with *ALDH* genes has been reported to be tolerant to drought and salinity [62]. Transgenic cotton harboring the betA gene (part of the ALDH10 family) improved salt tolerance and cotton yield [62]. However, in only a few cases has a member of *ALDH* been reported as performing a specific function. *ALDH21A1* has previously been identified as a novel eukaryotic aldehyde dehydrogenase that is transcriptionally activated by abiotic stress [11]. Recombinant *Escherichia coli* expressing *ScALDH21* showed higher drought tolerance than control *E. coli* [23]. Compared with the control, tobacco overexpressing *ScALDH21* was more drought-tolerant [63]. Abiotic stress tolerance has been demonstrated in transgenic cotton using *ScALDH21* [37, 38, 64].

2.2.2 The genetic background of transgenic *ScALDH21* cotton

2.2.2.1 The plant expression vector and the plant transformation

To make *ScALDH21* expression under CaMV 35S promoter, the open reading frame of *ScALDH21* cDNA (GQ245973) was amplified and cloned into the *Sal* I and *Kpn* I sites of the pCAMBIA2300. A recombinant vector containing selective neomycin phosphotransferase (NPTII) gene was transformed into *Agrobacterium tumefaciens* strain EHA105. Xin Nong Mian 1 (*Gossypium hirsutum*) has been transformed through *Agrobacterium*-mediated transformation. The Economic Crop Research Institute,

Xinjiang Academy of Agricultural Sciences, China, developed this cotton variety for specific arid zones in Xinjian. It displays good agronomic traits and economic characteristics.

2.2.2.2 PCR, RT-PCR detection, and Southern blot analysis

Using the cetyltrimethylammonium bromide method, genomic DNA was isolated from cotton seedlings at the five-leaf stage. PCR was used to detect the *ScALDH21* transgene in cotton plants using gene-specific primers [38]. The PCR amplification conditions were as follows—initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, primer annealing at 60°C for 30 s, and elongation at 72°C for 90 s, with a final elongation at 72°C for 5 min. Electrophoresis of 1% (w/v) agarose gels was used to visualize the PCR products. DNA extracted from cotton seedlings of the T5 generation was digested, run through gel electrophoresis, and transferred to a positively charged nylon membrane (Amersham, USA). Hybridization and chemiluminescence detection were performed according to the manufacturer's protocol (Roche, Germany) using digoxigenin dUTP-labeled probes of the *ScALDH21* gene product. Total RNA was extracted from young cotton leaves to analyze transgene expression. The genomic DNA was removed from the total RNA using DNase I (TaKaRa, Dalian, China). DNA was codified with the cDNA synthesis kit (TaKaRa, Dalian, China) according to the manufacturer's instructions. *ScALDH21* cDNA was used as a positive control for qPCR detection [38]. As an internal control, the *UBQ7* gene (GenBank accession no. DQ116441) was amplified with specific primers [24].

2.2.2.3 The transcriptome background of transgenic *ScALDH21* cotton

We collected root samples from cotton seedlings after 1 month of growth. Three biological replicates of each treatment were carried out. The total RNA was extracted using the RNAPrep Pure Plant Kit (Tiangen, Beijing, China) following the manufacturer's instructions. In accordance with the manufacturer's instructions, sequencing libraries were prepared using the NEB Next Ultra RNA Library Prep Kit for Illumina (NEB, Beverly, CA, USA). Illumina HiSeq 4000 platform was used for sequencing with 150 bp paired-end reads. Based on the length of the gene and the number of reads mapped to the gene (Novogene company, China), the expected fragments per kilobase of genes per million mapped reads (FPKM) of each gene were calculated. Differentially expressed genes were defined using the DESeq R package with an adjusted P-value (q-value) of 0.05. We used the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (<http://www.genome.jp/kegg/>) to test the statistical enrichment of DEGs in KEGG pathways. Five hundred and seventy-eight co-expressed genes were detected in the two *ScALDH21* transgenic lines, which were differentially expressed from NT and indicate that the target gene *ScALDH21* affected gene expression (**Figure 3a**). In **Figure 3b**, transcription expression patterns for those genes are shown. On the basis of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG), 578 genes were identified as overlapping across two transgenic lines compared to NT (**Figure 3c and d**). GO shows that ADP binding, O-methyltransferase activity, sulfotransferase activity, and transferase activity are significantly different from those of NT (**Figure 3c**). KEGG annotation shows no significant differences compared with that of NT (**Figure 3d**). Photosynthesis-antenna proteins, phenylpropanoid biosynthesis, and plant-pathogen interactions are the top

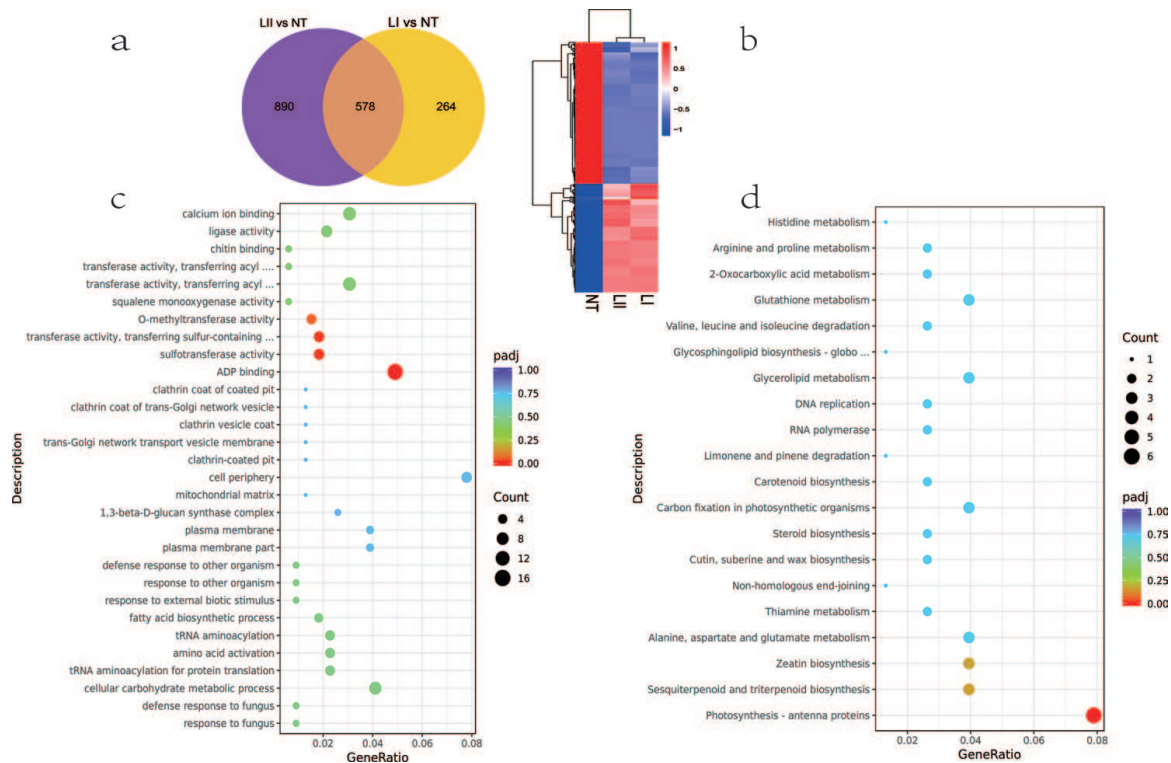


Figure 3.

The analysis of the difference between two *ScALDH21* transgenic lines and non-transgenic cotton based on transcriptomic data. (a) Show the different expression gene numbers in two transgenic lines compared with that in NT in Venn graphs (\log_2 foldchange > 2, $p_{adj} < 0.05$). (b) Gene Ontology (GO) annotation of the 578 overlap genes in two transgenic lines after compared with NT separately. (c) Kyoto Encyclopedia of Genes and Genomes (KEGG) of annotation the 578 overlap genes in two transgenic lines after being compared with NT separately. NT, non-transgenic cotton; LI and LII are the two *ScALDH21* transgenic lines.

three different pathways. These differences may have contributed to the different biofunctions and phenotypes.

2.2.3 The phenotype of *ScALDH21*-transgenic cotton

Following the identification of *ScALDH21* transgenic cotton, the growth performance of non-transgenic cotton (NT) and transgenic cotton (TC) under drought stress was examined and compared in northern Xinjiang from 2011 to 2014 and in southern Xinjiang from 2016 to 2018. Plants of all three independently transformed *ScALDH21* transgenic lines grew significantly taller than NT recipient plants in both full irrigation (26% higher) and deficit irrigation (23% higher). Transgenic and non-transgenic lines did not differ in leaf shape (length/width ratio) in either condition. However, the *ScALDH21* cotton had a greater leaf area compared to the NT plants in both full irrigation (79% increased) and deficit irrigation (51% increased). A significant difference was not observed between transgenic and NT plants in the full irrigated group. However, 24% more branches and 32% more bolls were observed in the deficit stress group. In general, the results showed that transgenic plants outperformed NT in morphological features like plant height, leaf area, leaf number, stem diameter, and root length. Similarly, morpho-physiological traits like fresh weight and dry weight of transgenic plants were greater than those of recipient plants. In transgenic plants, drought stress triggered lateral roots and increased leaf area significantly [38, 64]. Under drought stress conditions, *ScALDH21* overexpression appeared to

enhance plant growth in TC. Thus, overexpression of *ScALDH21* in cotton significantly increased the number of lateral roots, which consequently accelerated leaf growth following drought stress compared to NT. The height, leaf area, and leaf color of the transgenic *ScALDH21* cotton were all enhanced under normal and stress conditions in addition to the root system. The phenotypic results were consistent with the performance of other transgenic cotton [25, 31]. Under normal and drought conditions, overexpression of the rice NAC gene improves the root system [31]. In cotton, overexpressing a vacuolar pyrophosphatase gene increased root length and lateral root number, which improved the plant's water-absorbing abilities [33, 65]. After a 90-day water deficit, expression of the isopentenyl transferase gene IPT in cotton led to increased cotton height and roots. Overexpression of the Arabidopsis 14-3-3 protein gene GF14 in cotton results in a "stay-green" phenotype [27]. A potato sucrose synthase gene ectopically expressed in cotton accelerates leaf expansion and vegetative growth [66]. Additionally to demonstrating drought-tolerant phenotypic characteristics in *ScALDH21* transgenic cotton, our data also explain cotton's stress memory in terms of phenotype and physiology with two continuous water retention experiments that showed double water deficiency was worse than single water deficiency [38].

2.2.4 The physiological character of ScALDH21-transgenic cotton

Various biotic and abiotic stresses trigger ROS accumulation in plant cells, which leads to oxidative stress with lipid peroxidation, which also causes free radical reactions involving membrane polyunsaturated fatty acids [67]. ROS are produced when toxic aldehydes accumulate from lipid peroxidation [68]. Aldehyde-detoxifying enzymes ALDH3I1 and ALDH7B4 are both significant ROS scavengers and proteins that inhibit lipid peroxidation in Arabidopsis. In Arabidopsis, overexpression of these genes reduced lipid peroxidation under drought and salt stress [60].

It remains to be seen whether overexpression of *ScALDH21* improves overall plant health and performance under deficit irrigation. As a result of the toxicity of ROS produced by environmental stress, plants experience reduced growth, delayed development, and decreased yield. The *ScALDH21* cotton lines in this study responded to deficit irrigation with an increase in POD activity. This is consistent with previous reports [69], and therefore, it is reasonable to assume that they are better equipped to negate drought-induced ROS production. The transgenic cotton plants also showed decreased levels of MDA, an indicator of peroxidation [70] that indicates an improved ability to combat oxidative stress. Additionally, the *ScALDH21* cotton lines exhibit a more pronounced proline accumulation response to deficit irrigation, which is a well-distributed, multifunctional osmolyte that aids osmotic stress tolerance. *ScALDH21* overexpression reduced ROS-induced membrane peroxidation (lower MDA), increased ROS protection (elevated POD activity), and increased proline levels. It is likely that *ScALDH21* maintains a more intact cell system to counteract the negative effects of water deficiency. The level of proline and soluble sugars in transgenic cotton was increased by overexpression of *ScALDH21*. After one or two continuous water-withholding treatments, we measured the levels of free proline and soluble sugar in the leaves of seedlings and flowers [38]. It was found that proline accumulation increased after two continuous water withholdings, compared with other treatments. As a result of all drought treatments, NT and TC accumulate more soluble sugar, but TC accumulates more.

ScALDH21 cotton lines are able to maintain photosynthetic homeostasis and chlorophyll levels despite drought-induced oxidative stress. Southern Xinjiang

experienced an increase in SPAD values in either 2017 or 2018 [37]. Furthermore, the photosynthetic characteristics of plants under drought stress conditions were investigated at different stages of development (germination and flowering). As a result of full irrigation, the TC plants had a greater photosynthetic rate than the NT plants. The net photosynthetic rate of both TC and NT cotton was significantly reduced under drought stress, however, the TC cotton still maintained a significantly higher rate than NT cotton. The TC showed higher stomatal conductance and transpiration rates than the NT cotton [38]. Those can provide insight into the possible reason for the increase in biomass in *ScALDH21*-cotton plants.

2.2.5 The yield and fiber performance of *ScALDH21*-transgenic cotton

From 2013 to 2018, transgenic lines of cotton were grown in northern and southern Xinjiang to determine whether the *ScALDH21*-cotton was effective in the field.

TC lines in northern Xinjiang have been found to be better in growth and development than NT lines to some extent, after applying six different water retention treatments at different growth stages. Water deficit stress during the bud stage will cause the plant stalk length and boll number to decrease. Cotton yields were significantly decreased if twice deficit stresses were met during the bud or flower stage. Cotton growth and yield are critically dependent on water availability during the bud stage. Fiber parameters such as fiber strength, ginning out-turn of the fibers, fiber length, and length uniformity of the *ScALDH21*-cotton lines were not significantly different from NT plants. Comparatively to NT plants, TC lines produced stronger, more uniform, and longer fibers. TC had a micronaire value similar to or slightly lower than NT [38].

During harvesting season, boll weight, seed index, cotton yield, and fiber yield were measured in managed treatment plots under full and deficit irrigation conditions in northern Xinjiang in 2014 and 2016. Under drought stress, both *ScALDH21* transgenic cotton lines and NT lines had greatly reduced boll weight. Compared with NT plants, the seed index was higher in transgenic lines under both full and deficit irrigation conditions, and it reached up to 22% under stress. In both conditions, cotton yield per hectare and fiber yield did not differ significantly [64]. Under both full and deficit irrigation conditions in both managed treatment plots and production fields, the fiber length, uniformity, strength, elongation, and micronaire value of *ScALDH21* transgenic lines were improved or significantly improved compared with NT.

To determine the performance of the *ScALDH21* transgenic cotton under oasis field conditions in southern Xinjiang, three kinds of irrigation schedules were used (root zone model-simulated forecast irrigation (F), soil moisture sensor-based irrigation (S), and flood irrigation based on experience estimates (E) and two full (FI) and deficit (DI) irrigation conditions were employed [37]. Under all deficit irrigation conditions, the number of bolls and cotton yield of TC plants decreased compared to full irrigation, however, they were higher than those of NT plants. Over 3 years of experiments, TC plants showed a significant increase in cotton yields of up to 58.7% compared to NT plants [37]. Furthermore, in soil moisture sensor-based deficit irrigation (SDI) treatment, cotton yields are the lowest. In years, the cotton yield of *ScALDH21*-cotton lines grown under forecasted full irrigation (FFI) increased from 37–73% compared to NT plants. NT and Forecasted deficit irrigation (FDI), Soil moisture sensor-based full irrigation (SFI), SDI, Experience-based full irrigation (EFI), and Experience-based deficit irrigation (EDI) differed for all 3 years.

Furthermore, yield increases of transgenic lines were highest in SDI (from 67.5% to 92.3% in 3 years), compared to NT plants. Average data for 3 years in the SDI showed a large increase in cotton yield with a smaller deviation (83.5%).

In addition to treatments, cotton yields vary by year. As with the boll number per plant, the cotton yield per hectare, fiber yield per hectare, and cotton yield per plant were significantly higher in the transgenic lines than in the NT lines. The average seed yield for all treatments was 68% (variable from 14–128%) and 41% (variable from 10–102%) in 2017 and 2018, respectively [37]. Fiber elongation was increased in transgenic lines. Fiber strength also increased in transgenics after irrigation. There were no significant differences in fiber uniformity and micronaires between genotypes.

2.2.6 The irrigation strategy of ScALDH21-transgenic cotton

Cotton productivity and yield are largely influenced by a variety of factors, including genetics and irrigation methods. In this study, the TC lines performed better than the NT lines, despite soil, air humidity, and temperature affecting plant yield [71–73]. This study evaluated the drought tolerance ability of *ScALDH21*-cotton lines at field stations located in southern Xinjiang, China, a region classified as a desert-oasis ecotone with sandy loam soils, as well as at Manas Experimental Station, northern Xinjiang (**Figure 2**). The results indicated that our transgenic cotton had improved performance and could adapt to a wide range of cotton culture environments.

The lack of rainfall makes irrigation vital for agricultural production in arid and semi-arid lands. In arid zones, for example, normal irrigation above 600 mm during the vegetation period is sufficient for stable cotton harvesting [5]. Our desert oasis drought experiments in southern Xinjiang with sandy loam soils designed the 75% deficit irrigation and less than 600 mm different irrigation strategies to conserve more irrigation water and keep cotton yield constant. We used three irrigation schedules: DSSIS (Decision Support System for Irrigation Scheduling) forecasts (F), soil moisture sensors (S), and experience irrigation (E). Full irrigation (FI) and deficit irrigation (DI, 75% of full) were applied from 2016 to 2018 (**Figure 2**). Different irrigation protocols and water consumption affected the growth and yield of cotton, and the “Smart Irrigation” irrigation scheme based on the Root zone water quality model (RZWQM2) was found to be the best irrigation scheme for sustainable cotton production in an arid land. The results indicated that deficit irrigation schemes can be utilized in the desert-oasis ecotone, and in conjunction with the use of *ScALDH21*-cotton lines, the yields are sufficient for viable and sustainable agriculture.

Moreover, through mixed model analysis, we found that the cotton line always has a significant effect on plant phenotype, physiology, and yield components in southern Xinjiang, and cotton line and irrigation scheduling both have significant effects on cotton growth and development separately. In addition, irrigation scheduling and irrigation levels have a significant interaction effect. The relationship between yield and crop water use was calculated as overall water use efficiency (WUE). The EI schedule consumed more water (EFI, 547 mm, and EDI, 409 mm) than either the FI (FFI, 385 mm, and FDI, 288 mm) or SI (SFI, 254 mm, and SDI, 186 mm) schedules. Compared to NT plants, WUE was higher in *ScALDH21*-cotton lines each year. A high WUE was observed in the forecast, with drip irrigation leading to the highest WUE, and flood irrigation leading to the lowest WUE. In all treatments, the WUE value of *ScALDH21*-cotton lines increased by 59.6% compared to NT.

The individual irrigation level and timing significantly affected vegetative growth parameters, plant height, and leaf area, but the differences did not differ substantially between years despite differing precipitation levels. In each irrigation treatment, the TC, and especially the L16, grew significantly higher than the NT controls from 2016 to 2018. There was a dramatic reduction in the leaf area of NT under SDI in all years, but there was no difference in the leaf area of TC [37]. Therefore, the irrigation treatments can be ranked as forecast irrigation > flood irrigation > soil moisture irrigation based on their ability to maintain high instantaneous water use efficiency (IWUE).

We also used managed treatment plot experiments and field-scale in purple clay loam soil sites at Manas Experimental Station, northern Xinjiang. The two experiments differed in terms of growth space and water consumption. In the managed plot experiment, 50% less of full irrigation significantly reduced cotton vegetative growth and cotton yield (*50% loss of cottonseed and lint yield compared with full irrigation), whereas, in the field, 30% less of full irrigation did not affect cotton vegetative growth or yield. The reason for this can be explained by the amount of water used, which was 675 L of water m⁻² with full irrigation and 472 L m⁻² with deficient irrigation in the field, 566 L m⁻² (control), and 283 L m⁻² (stress) in the managed treatment areas.

The study also provides guidelines for optimal irrigation protocol and minimum water requirements for the use of *ScALDH21*-transgenic cotton lines in arid regions.

3. Conclusions and perspectives

ScALDH21-transgenic cotton exhibits improved plant growth and developmental phenotype through sustained net photosynthetic rate, greater tolerance to osmotic and oxidative stress, and improved cotton yield and fiber quality. Transgenic cotton can also be grown in sandy loam soils in southern Xinjiang and purple clay loam soils in northern Xinjiang that are more productive than non-transgenic recipients. This transgenic variation of *ScALDH21* is significantly better than recipient cultivars that can be commercially exploited when irrigation is scarce, enabling a more sustainable cotton production in the desert oasis ecotone. For *ScALDH21* transgenic cotton that can be used in agricultural production, we evaluated various irrigation protocols and optimized irrigation regimes with minimal water requirements. Using multiple growing seasons and multiple studies, ectopic expression of the moss *ScALDH21* gene in cotton improves drought tolerance and reduces yield penalties. *ScALDH21* overexpression enhances drought tolerance in cotton, suggesting that *ScALDH21* could be a candidate gene for improving cotton in arid-arid regions. To ensure the safety of transgenic lines, they must be tested with a GMO undergone a biosafety protocol. Currently, biosafety and risk assessment based on GMO requirements are being tested. To breed the next generation of crop varieties, updated germplasm, knowledge, and breeding techniques will be needed [74]. There are numerous reports of genes that confer stress tolerance, most of which are from Arabidopsis, very few of which have been successfully tested in crop plants due to potential side-effects of candidate genes on growth and morphology. It is therefore urgent to develop many drought-tolerant gene resources from drought-resistant plants. Reports indicate that introducing foreign genes from xerophytic plants or overexpressing certain cotton genes improve its performance under drought conditions [35, 36, 38]. Therefore, it is reasonable to assume that an in-depth comparative study of the expression and function of members of the same gene families in extreme xerophytes will eventually aid in breeding drought-tolerant crop plants.

It has been widely used in plant biotechnology to improve crop traits with CRISPR/Cas9 technology [75, 76] and it will be applied to crop breeding in the near future [77], especially for gene knock out in crops. To overcome complex and changing adversity, crop breeding must be multi-resistant because climate change leads to an increase in both biotic and abiotic stresses. The pursuit of homologous genes from extreme xerophytes plants, but with a low degree of identity to crop, will significantly increase drought tolerance. *ScALDH21* orthologs were not found in cotton genomes, but transgenic lines exhibited better growth and development, as well as greater photosynthesis ability in a water-scarce environment. Furthermore, this gene was also discovered to be salt-tolerant as well as resistant to *Verticillium*, which indicates that moss as the first landing plant may be an excellent resistant gene resource library, especially the extreme drought-tolerant moss.

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