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Knockdown of 60S ribosomal protein L14-2 reveals their potential regulatory roles to enhance drought and salt tolerance in cotton

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Abstract

Background: Cotton is a valuable economic crop and the main significant source of natural fiber for textile industries globally. The effects of drought and salt stress pose a challenge to strong fiber and large-scale production due to the ever-changing climatic conditions. However, plants have evolved a number of survival strategies, among them is the induction of various stress-responsive genes such as the ribosomal protein large (*RPL*) gene. The *RPL* gene families encode critical proteins, which alleviate the effects of drought and salt stress in plants. In this study, comprehensive and functional analysis of the cotton *RPL* genes was carried out under drought and salt stresses.

Results: Based on the genome-wide evaluation, 26, 8, and 5 proteins containing the RPL14B domain were identified in *Gossypium hirsutum*, *G. raimondii*, and *G. arboreum*, respectively. Furthermore, through bioinformatics analysis, key *cis*-regulatory elements related to *RPL14B* genes were discovered. The Myb binding sites (MBS), abscisic acid-responsive element (ABRE), CAAT-box, TATA box, TGACG-motif, and CGTCA-motif responsive to methyl jasmonate, as well as the TCA-motif responsive to salicylic acid, were identified. Expression analysis revealed a key gene, *Gh_D01G0234* (RPL14B), with significantly higher induction levels was further evaluated through a reverse genetic approach. The knockdown of *Gh_D01G0234* (RPL14B) significantly affected the performance of cotton seedlings under drought/salt stress conditions, as evidenced by a substantial reduction in various morphological and physiological traits. Moreover, the level of the antioxidant enzyme was significantly reduced in VIGS-plants, while oxidant enzyme levels increased significantly, as demonstrated by the higher malondialdehyde concentration level.

Conclusion: The results revealed the potential role of the *RPL14B* gene in promoting the induction of antioxidant enzymes, which are key in oxidizing the various oxidants. The key pathways need to be investigated and even as we exploit these genes in the developing of more stress-resilient cotton germplasms.

Keywords: Abiotic stress, Cotton, Ribosomal protein large, Transcription factor, Virus-induced gene silencing

Highlights

- Cotton is the source of natural fiber. However, drought and salt stress exacerbated by climate change, pose a severe threat to strong fiber and large quantity production.

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- The RPL14B gene was previously identified as a candidate gene for drought stress tolerance in the QTL map.
- Virus-induced gene silencing (VIGS) revealed that the Gh_D01G0234 (RPL14B) knockdown significantly impacted cotton seedling production under drought and salt stress conditions.

Background

Cotton is an essential plant worldwide (Campbell et al. 2010), mainly as a natural source of fiber (Haigler et al. 2012), oil (Singh et al. 2013), and protein for animal feeds (Rogers et al. 2002). However, due to the effects of abiotic stress factors such as cold, drought and salinity, high quantity and quality cotton production is steadily declining (Magwanga et al. 2018a, b). The adverse effect of drought and salinity stress conditions has intensified with ever-changing climate conditions. As a result, improving drought and salinity stress tolerance may mitigate osmotic stress-induced yield loss. Previous studies have demonstrated that drought and salt stresses induce the expression of osmotic stress-associated genes. Ribosomal protein large (*RPL*) is a gene family that was previously thought to be primarily involved in enhancing homeostasis inside the ribosomal complex and protein biosynthesis (Chaillou 2019). However, recent studies have demonstrated that abiotic stress factors regulate the transcription of genes coding for the RPL protein (Horiguchi et al. 2012). For example, *GmRPL37* is highly expressed during cold stress in soybean and positively regulated cold tolerance (Kim et al. 2004). Overexpression of *RPL44* in *Aspergillus glaucus* enhanced drought and salt stress tolerance (Liu et al. 2014). Overexpression of *RPL23A* in transgenic rice increased the water use efficiency and improved its tolerance to drought stress (Moin et al. 2017). In 2008, Rogalski found out that *RPL33* in tobacco plants was not essential when plants are growing in suitable conditions but it was crucial in enhancing acclimation to cold stress (Rogalski et al. 2008). The *RPL* genes are characterized by multiple abiotic stresses and phytohormones *cis*-elements in their transcription regulatory regions, which respond specifically to stress and signal molecules (Moin et al. 2016, 2017; Saha et al. 2017). MBS (Myb binding site) and low-temperature response (LTR), among others, are stress-responsive *cis*-elements widely distributed in the putative promoter regions of *RPL* genes. These responsive elements are associated with genes responsive to drought and cold stress, respectively (Zou et al. 2011). The presence of these *cis*-elements in the *RPL* gene promoter region suggests that they are involved in abiotic stress response and tolerance.

QTL mapping is one of the strategies developed and currently used to identify genes involved in different plant pathways (Kim et al. 2019). A BC_2F_2 population was obtained from *Gossypium tomentosum* as the donor parent, well-known for its drought tolerance, and *G. hirsutum,* as the recurrent parent, widely cultivated due to its high yielding but susceptible to drought and salt stress. A high-density genetic linkage map was developed by adopting genotyping by sequencing (GBS), integrating genotype and phenotype (Magwanga et al. 2018a, b). Several stable quantitative trait loci (QTLs) were identified and grouped into three main clusters focusing on the physiological related QTLs contributed by the donor parent *G. tomentosum* which were cell membrane stability (CMS), chlorophyll content, and saturated leaf weight (SLW). Within the QTL regions, 89 genes were mined, including *Gh_D01G0234 (RPL14B).* Further, they analyzed the 89 genes through RNA sequence data from the public domain database and validation through qRT-PCR under drought and found the genes to be upregulated (Magwanga et al. 2020).

The mined genes were of interest because they were contributed by the donor parent *G. tomentosum*. *G. tomentosum* is a wild cotton species that grows in saline and dry environment, making it resistant to drought and salt stress (Oluoch et al. 2016). Wild plant species are known to have traits that can enhance plant resistance to abiotic stress conditions and increase yield quantity and quality when introgressed into the cultivated cultivars (Des Roches et al. 2018; Wang et al. 2021). Therefore, this study focuses on the characterization and functional validation of the 60S ribosomal protein L14-2 (*RPL14B*) gene in cotton. Moreover, the various bioinformatics analysis about *cis*-regulatory elements, Gene ontology (GO) terms, conserved motif, gene structure, and phylogenetic relationship was also performed. Furthermore, the expression profiles of the *RPL14B* gene family were carried on different tissues under drought and salt stress. Virus-induced gene silencing (VIGS) was used to verify the *Gh_D01G0234* gene, and VIGS-plants were evaluated under drought and salt stress conditions. The results revealed that the *RPL14B* gene could have potential and significant role in stress tolerance. This work provides fundamental steps for future exploration of the *RPL14B* genes in improving cotton germplasm to develop climate-smart cotton varieties resilient to drought and salt stress factors.

Materials and methods

Phylogenetic tree analysis and physio-chemical properties of RPL14B protein

The sequences of the RPL14B were obtained from the three cotton genomes, A, D and AD. The tetraploid (AD)

cotton was *G. hirsutum*, *G. barbadense, G. tomentosum, G. mustelinum,* and *G. darwinii,* while the diploid cotton (A and D) was *G. arboreum* and *G. raimondii*, respectively. The tetraploid cotton protein sequences were obtained from their respective genome databases through the Blastp program, while the diploid protein sequences were downloaded from the cotton functional genomics database (https://cottonfgd.org/CottonFGD). In order to identify the *RLP14B* genes with Pfam domain, all the genes were queried using the Pfam Scan (https://www.ebi.ac.uk/Tools/pfa/pfamscan/) and SMART search (http://smart.emblheidelberg. de/smart/). ClustalX and MEGA 7 programs were used to conduct multiple sequence alignments of the RPL14B protein sequences and construct the phylogenetic tree (Tamura et al. 2013; Thompson et al. 2002). The physical and chemical aspects of the *RPL14B* gene family were determined using the CottonFGD database.

Gene structure, motif identification and gene ontology enrichment analysis

Online tools, the gene structure displayer server (http://gsds.cbi.pku.edu.cn) and MEME Suite (http://meme-suite.org/) (Bailey et al. 2009; Hu et al. 2015), were used to determine the gene structure and conserved motif of *RPL14B* genes. We employed GO Analysis Toolkit and Database, AgriGO v2.0, to conduct gene ontology annotation of the *RPL14B* genes (www.bioinfo.cau.edu.cn/agriGO) (Tian et al. 2017).

Chromosomal allocation, *cis*-regulatory element prediction, and subcellular localization prediction

The information on *RPL14B* chromosome position was retrieved from the CottonGen website and using the chromosome information, mapping of the genes was done by Tbtools (Chen et al. 2020). The subcellular localization of the RLP14B proteins was determined through Wolfpsort (https://www.wolfpsort.hgc.jp/) (Hortona et al. 2005). *G. hirsutum, G. raimondii,* and *G. arboreum* 2 000 bp (base pairs) nucleotide sequence, retrieved from the cotton FGD database, were submitted to the Plant.

Plant material and treatment

Seeds of *G. hirsutum*, Marie-Galante 85 (MG-85) race were obtained from Institute of Cotton Research, Chinese Academy of Agricultural Sciences (ICR-CAAS). The seeds were first delinted using sulfuric acid then grown on moist absorbent paper for four days. The seedlings were transferred to a hydroponic set up with Hoagland nutrient solution (Vinet and Zhedanov 2011) in the climate-controlled greenhouse with 16 h light/8 h dark and the temperature at 28 °C, day and 25 °C night as previously described (Kirungu et al. 2020). At the three-leaf stage, the cotton seedlings were subjected to osmotic stress by adding to the Hoagland nutrient solution 17% of glycol PEG-6000 and 300 mmol·L^{-1} of sodium chloride for drought and salt treatment, respectively. To ensure the results were reliable, the untreated plants were used as the control. We collected samples in three biological replicates from the leaves, stem, and root tissues for RNA extraction at 0 h, 3 h, 6 h, 9 h, 12 h, 24 h, and 48 h of post-stress exposure.

RNA extraction and RT-qPCR assays

The RNAprep Pure Plant kit (Tiangen, Beijing, China) was used for RNA extraction by following its instructions. Quality and concentration of RNA were determined using agarose gel electrophoresis and spectrophotometric analysis. The RNA with the correct concentration and purity was then converted to cDNA. The cDNA was prepared using EasyScript First-strand cDNA Synthesis SuperMix (TransGene, Beijing, China). The primers were designed using primer 5, list attached (Additional file 1: Table S1), and the cotton *GhActin* gene forward sequence 5'-ATCCTCCGTCTTGACCTTG-3' and reverse sequence 5'-TGTCCGTCAGGCAACTCA T-3' was used as the internal control. The real-time quantitative polymerase chain reaction (RT-qPCR) was performed as previously described (Lu et al. 2019). The fold change was analyzed using the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen 2001).

Validation of *Gh_D01G0234* gene through Virus-induced gene silencing (VIGS), under drought and salt stress conditions.

Fragment of the coding DNA sequence of RPL14B (405 bp) was retrieved from the cotton functional genome database (https://cottonfgd.org/search/) and used to design its specific primer using the primer primer5 tool. The gene-specific primer, forward sequence: CGAGCTCCACGTGTTCCCAAGAAG AAGA, and reverse sequence: CCTCGAG TTGCTT GATGACTCCAGACCT were amplified using *G. hirsutum* cDNA by Bioer LifeECO PCR Thermal Cycler. The products were then cloned into the *Eco*R1 and *Bam*H1 sites of the tobacco rattle virus vector (pTRV) to generate pTRV: RPL14B. The recombinant was then transformed into *Agrobacterium tumefaciens* LBA4404 strain using freeze and thaw method (Dupadahalli 2020). Preparation of the bacteria inoculum and inoculation to the plants' cotyledon was done as described by Corbin et al. (2017). For reliable results, we inoculated some plants with the silenced gene inoculum (pTRV: RPL14B), and phytoene desaturase (pTRV: PDS) to determine the effectiveness of the silencing, while

other plants were inoculated with empty vector (pTRV: 00) and plants without any inoculum were denoted as the wild type and represented the control (Yang et al. 2019). Drought and salt stress simulation was done at the three-leaf stage by adding to the Hoagland solution 300 mmol·L^1 sodium chloride for salt treatment and 17% (W/V) of PEG-6000 for drought treatment (Yang et al. 2019), respectively. Sampling for physiological, morphological, and biochemical analysis was done on the leaf, stem, and root before treatment and 24 h after drought and salt treatments. The samples were then placed in liquid nitrogen. After that, they were kept at -80 °C.

Physiological and morphological analysis under drought and salt stress conditions

Samples in three bio-replicates were collected before treatment and 24 h post-stress treatment. We assessed the susceptibility and tolerance of silenced and non-silenced plants to stress by determining the physiological and morphological parameters. Excised leaf water loss (ELWL), relative leaf water content (RLWC), and cell membrane stability (CMS) were the physiological parameters determined as previously described by Cai et al. (2019). Briefly, to determine ELWL, fresh leaf samples were weighed, put on the bench for 24 h under normal room temperature and then weighed to get the wilted weight (WW). Afterward, the leaves were put inside a 50 °C dry oven for two days then weighed to get the dry weight (DW). ELWL is calculated by (FW − WW)/DW. To determine RLWC, the leaf sample's fresh weight was measured (FW) and then put in distilled water under normal room temperature for 24 h, surface dried, and weighed to get saturated weight (SW). After that, put the sample inside a 50° C dry oven for two days and weighed to get the dry weight. Calculation of RLWC was done using the formula, $RLWC = ((FW - DW)/(SW - DW)) \times 100$. CMS was determined by quantifying plant electrolyte or ion leakage (Cai et al. 2019). Leaves from the silenced plants and the control with uniform diameter and weighing 0.5 g were put in tubes containing 5 mL distilled water and kept in the dark for 24 h. Then we measured the electrical conductivity (L1). The leaves were then autoclaved at 70 °C for 30 min and left to cool, and the electrical conductivity (L2) was measured. The formula used to calculate the cell membrane stability is $(L1 - L0)/(L2 - L0) \times 100$ (L0 is the conductivity of distilled water).

The morphological parameters measured were the plant height (PH), root length (RL), shoot fresh weight (SFW), and root fresh weight (RFW). PH and RL were measured in centimeters, while SFW and RFW were measured in grams.

Evaluation of oxidants and antioxidants enzymes in VIGS plants and the wild types under drought and salt stress conditions

We further evaluated the effect of simulated osmotic stress on the silenced and the control plants by quantifying the oxidant enzyme and antioxidant enzyme activities. We evaluated the antioxidants and oxidants enzyme activities on VIGS plants, plants transfused with empty vector and the wild type under drought and salt stress conditions. According to the manufacturer's protocols, the extraction and spectrometric analysis of the oxidants and antioxidants enzymes activities were carried out using their respective assay kits supplied by Beijing Solarbio Science and Technology, China.

Results

Physio-chemical properties of the RPL14B protein in cotton

In evaluating the physio-chemical properties of the RPL14B proteins encoded by the *RPL14B* genes in G. *hirsutum, G. arboreum* and *G.raimondii* cotton species, the proteins were found to exhibit varied features. However, one common feature to all the proteins obtained from all the species was the grand average of hydropathy (GRAVY) values that ranged between 0.322 and −0.657. The negative GRAVY values denoted that the proteins encoded by the *RPL* genes were hydrophilic. The other properties varied among the cotton species (Additional file 1: Table S1). For instance, molecular weights of *RPL14B* genes in *G. hirsutum* ranged from 6.232 kDa to 15.723 kDa, and isoelectric point (pI) value ranged from 10.86 to 11.55. While in *G. raimondii* and *G. arboreum*, the molecular weight ranged from 10.714 kDa to 18.105 kDa, the pI was between 11.5 and 17. The diploid cotton species had a higher molecular weight and pI compared with the tetraploid cotton species ranging from −0.322 to −0.629 (Additional file 2: Table S2).

Phylogenetic analysis and chromosomal distribution of RPL14B protein in cotton

The cotton RPL14B proteins sequences were analyzed to determine its evolutionary pattern. By integrating the use of MEGA7, a phylogenetic tree was constructed after aligning the RPL14B protein sequences using ClustalX. The RPL14B proteins were grouped into three clusters (Fig. 1). Furthermore, the *RPL* genes were mapped into their respective chromosomes by use of Tbtools. The *RPL14B* genes are distributed in thirteen chromosomes in *G. hirsutum.* In the A genome is: A01, A02, A07,

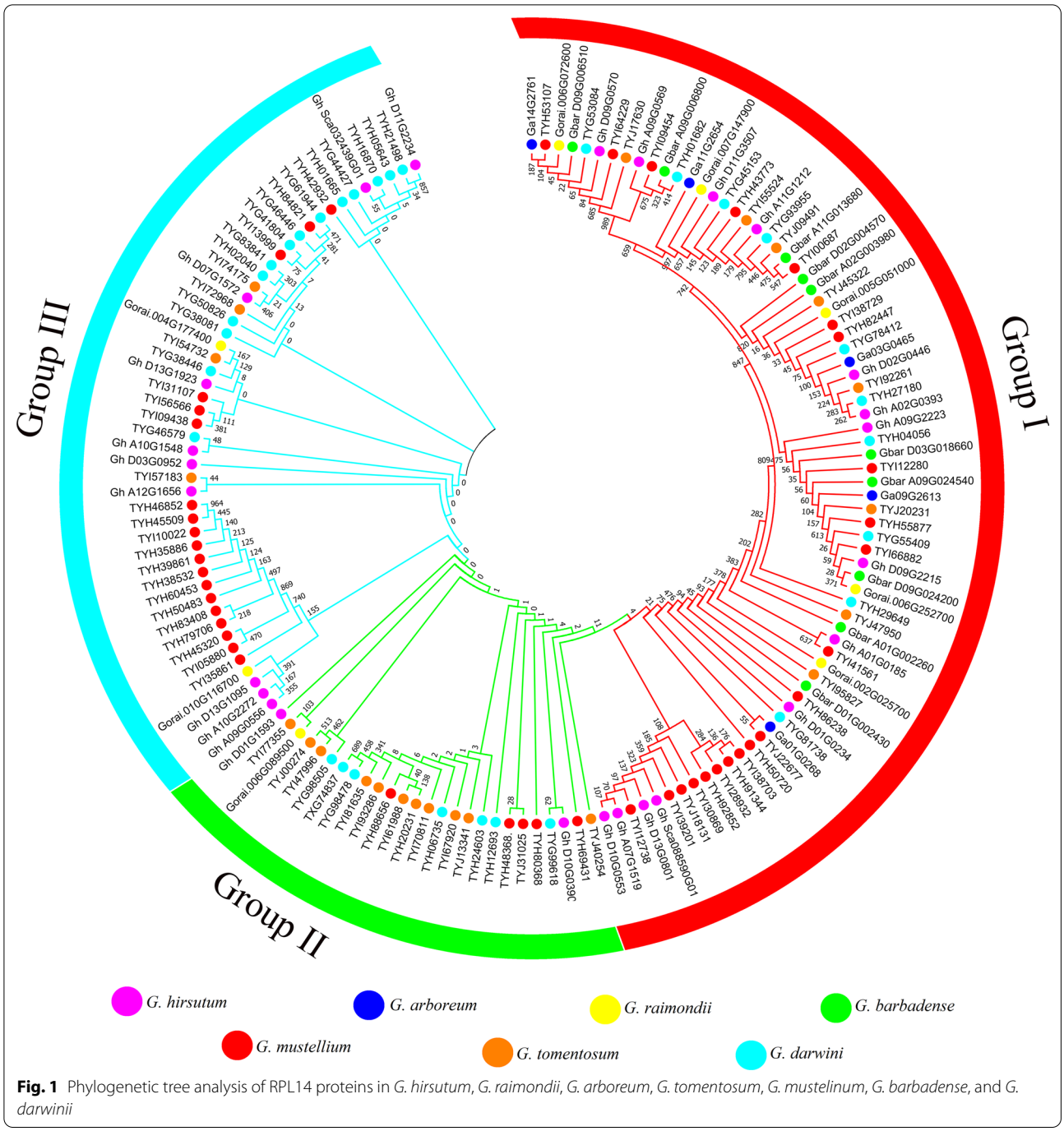

Fig. 1 Phylogenetic tree analysis of RPL14 proteins in *G. hirsutum*, *G. raimondii*, *G. arboreum*, *G. tomentosum*, *G. mustelinum*, *G. barbadense*, and *G. darwinii*

A09, A10, A11, A12, while those in the D genome, D01, D02, D03, D07, D09, D10, D11, and D13 with two genes mapped within the scaffold (Fig. 2).

RPL14B gene structure, domain and conserved motif

The cotton *RPL14B* genes were interrupted by few introns in their gene structure (Fig. 3A–C). Few introns were associated with stress-responsive genes, as seen in previous studies on other stress-responsive genes in cotton, such as dehydrin (Kirungu et al. 2020). Thus, low intron interruption in *RPL14B* genes indicated that they are involved in stress acclimation mechanisms in cotton. Ribosomal domain(s) is an essential component of all ribosomal proteins. Most of the genes had the same type of motif (Fig. 4A). They also have RPL14-KOW conserved domains at their N-terminals that

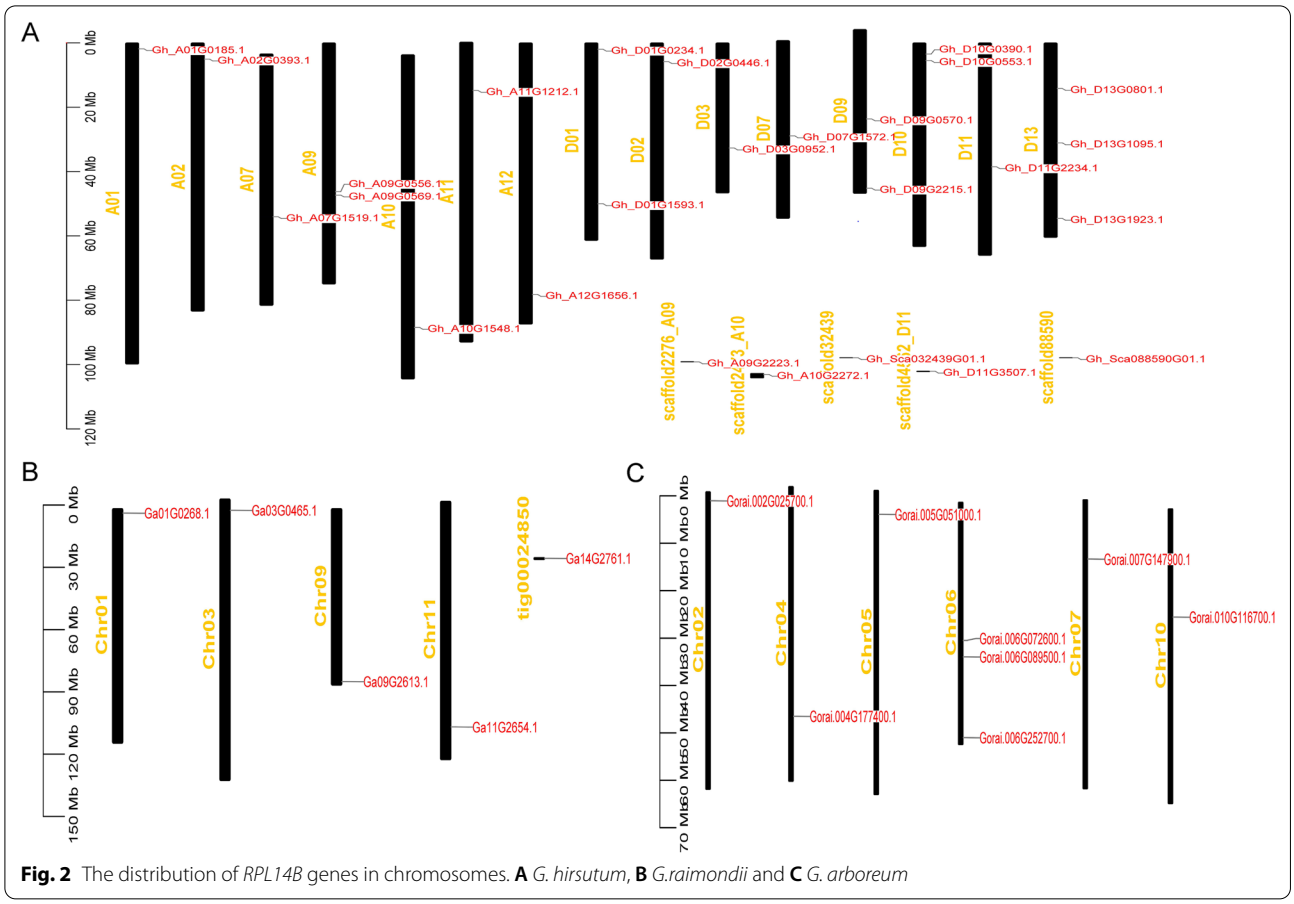

Fig. 2 The distribution of *RPL14B* genes in chromosomes. **A** *G. hirsutum*, **B** *G.raimondii* and **C** *G. arboreum*

enable them to interact with other proteins (Fig. 4B). The motifs contain invariant glycine residues, which are composed of alternating blocks of hydrophilic and hydrophobic residues.

Subcellular localization prediction, gene ontology annotations and *cis*-acting regulatory element

RPL14B genes were predicted to be sub-localized in the mitochondrion, nucleus, and endoplasmic reticulum. However, they are abundant in the nucleus, especially in the *G. hirsutum* (Fig. 5A). GO enrichment analysis showed that these genes have all GO functions: molecular function, biological process, and cellular component. Cellular components associated with this gene are ribosomes (GO:0005840), cell (GO:0005623), cytoplasm (GO:0005737), ribonucleoprotein complex (GO:0030529), and intracellular organelles (GO:0043229). RPL14B is part of metabolic and cellular cell processes. The molecular function is the structural molecule activity of the cell (GO:005198) and structural constituent of ribosome (GO:0003735) (Fig. 5B). The most relevant biological functions are biosynthetic process (GO:0009058), cellular metabolic process (GO:0044237), protein metabolic process (GO:0019538), gene expression (GO:0010467), and translation process (GO:0006412). The *RPL14B* genes in *G. hirsutum*, *G. barbadense*, *G. raimondii,* and *G. arboreum* species have stress-related *cis*-regulatory elements. The *cis*-regulatory elements obtained are shown in Fig. 5C; all the identified *cis*-regulatory elements are involved in phytohormones and abiotic stress response.

Expression of *RPL14B* genes in upland cotton under drought and salt stress

The expression analysis of *GhRPL14B* genes was assayed through RT-qPCR. The expression levels of the genes under drought and salt stress were different (Fig. 6). In the leaf, the expression was higher from 12 to 48 h under both drought and salt stress. While in the roots, the expression level was high from the onset of stress, at 3 h, they were highly expressed, and the expression levels were differential onwards up to 48 h under stress conditions.

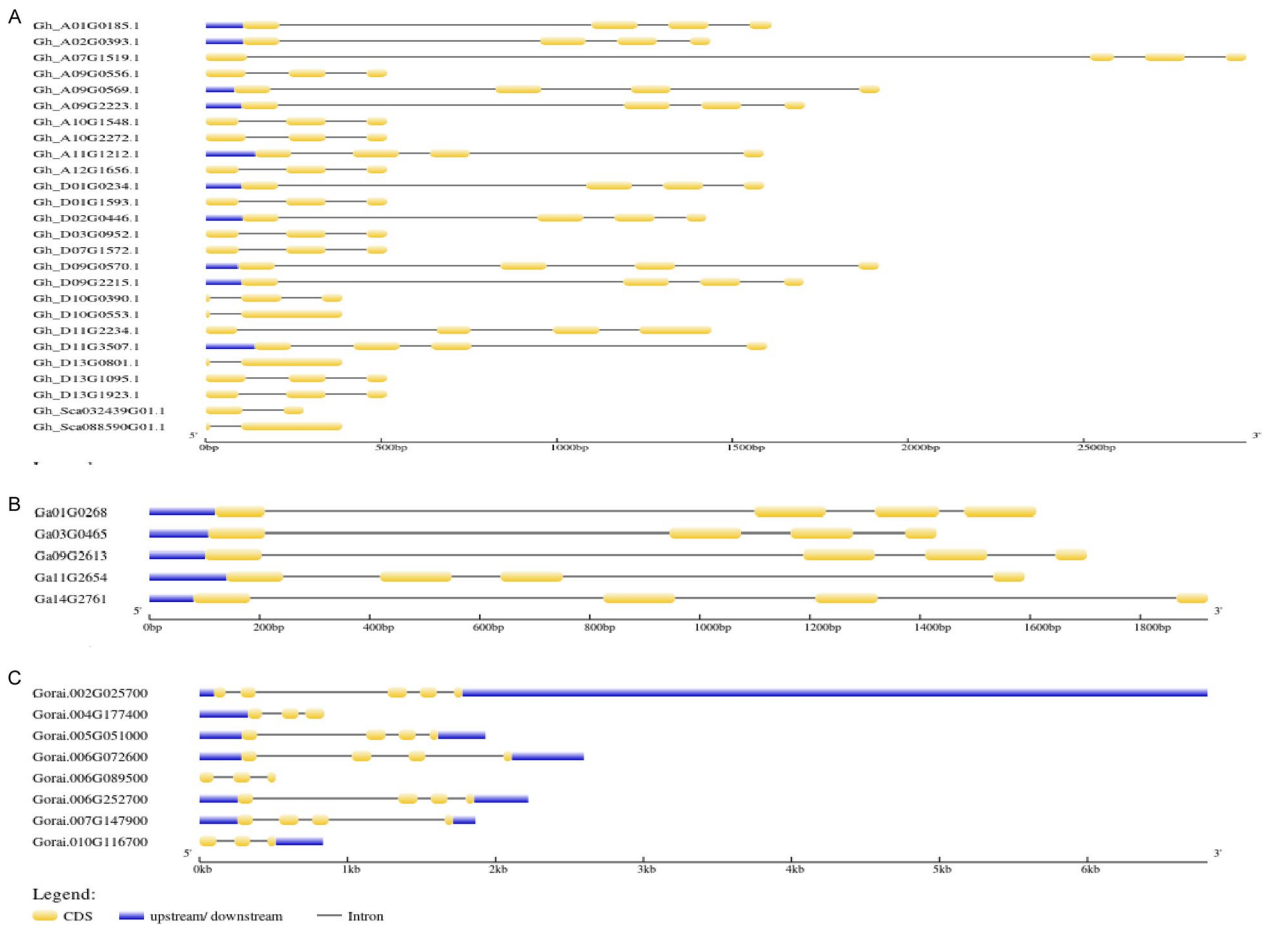

Fig. 3 Gene structure of cotton RPL14B proteins. **A** Gene structure of genes in *G. hirsutum*, **B** gene structure of *G. arboreum* genes, **C** gene structure of *G. raimondii* genes

Evaluation of the efficiency of *RPL14B* gene silencing

The effectiveness of silencing the *RPL14B* gene in cotton was evaluated using the phytoene desaturase (PDS) gene. Previous research has shown that PDS can be used as a positive control to evaluate the effectiveness of silencing a particular gene. Plants infiltrated with PDS tend to exhibit a photobleached leaf phenotype that extends to the stem. In this experiment, the plants infused with pTRV2: PDS showed albino trait after 2 weeks of post-inoculation. The leaves and upper part of the stem exhibited this chlorotic/ bleached phenotype (Fig. 7A). RT-qPCR analysis to determine expression levels of *RPL14B* gene in the silenced plants and the wild type showed lower gene expression levels in the silenced plants relative to the wild type. This demonstrates that this gene's silencing was successful, and the vector used was effective (Fig. 7B).

Evaluation of morphological and physiological traits of the VIGS-plant and the wild type (WT) under drought and salt conditions

RPL14B silenced plants and the control plants under normal conditions exhibited no physiological or morphological changes. However, when the plants were subjected to drought and/or salt stress conditions, the plants showed some wilting elements and indicated that they were stressed (Fig. 8A, B). The PH, RL, and RFW exhibited significant difference between the VIGS plants and the control (Fig. 8C–E). The silenced Gh_D01G0234 cotton leaves showed a significant reduction in RLWC relative to the wild type and TRV2:00 leaves. Whereas there was a relative increase in ELWL and ion leakage compared with the wild type and TRV2:00 leaves. An increase in ion leakage and ELWL demonstrates that this gene's silencing compromises the plant's effectiveness in tolerating drought stress (Fig. 9A i–iii).

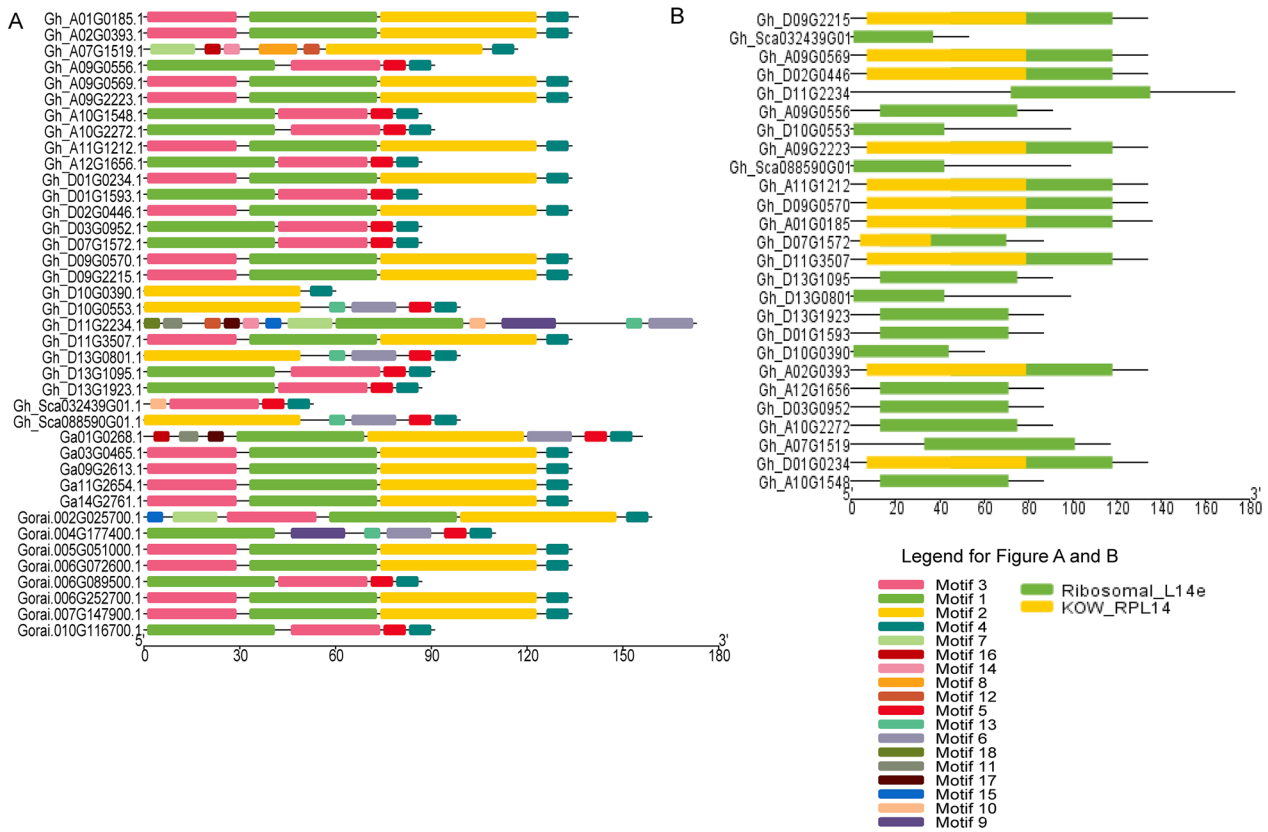

Fig. 4 Motif and conserved domain present in RPL14B proteins. **A** Motif present in *G.hirsurtum, G. arboreum* and *G. raimondii*. **B** Conserved domain of GhRPL14B protein

Oxidant and antioxidant enzymes assay

The plants were further analyzed for the levels of oxidant and antioxidant enzymes. These enzymes were assayed on the VIGS plant's leaf tissue and the wild type under drought and salt stress conditions. The antioxidants peroxidase (POD) and catalase (CAT) level in VIGS plants was significantly reduced compared with the wild type. In contrast, the oxidant MDA level was significantly high in VIGS plants compared with the wild type (Fig. 9B). This result demonstrates that the silencing of RPL14B compromises the plant's ability to tolerate drought and salt stress.

Discussion

Ribosomal protein is involved in abiotic stress tolerance

Abiotic stress factors like drought and salt have exacerbated cotton production with an estimated loss of 70%. Plants being sessile initiates signaling pathways, activation of transcriptional factors and eventually expression of stress-responsive genes, all are to ensure plant survival. It is imperative to identify genes involved in sustaining plant growth and development during abiotic stress to improve their productivity further. The *RPL* gene family has been known to be involved with the housekeeping of the ribosome. However, recent studies have demonstrated that ribosomal protein has evolved (Horiguchi et al. 2012), and they are involved in extra ribosomal activities like biotic (Li 2019) and abiotic stress tolerance (Mukhopadhyay et al. 2011). Under abiotic stress, plants increase protein production as a metabolic response (Song et al. 2014). Under stress conditions, protein in the plant undergoes denaturation, and it is crucial to maintain the homeostasis between protein synthesis and degradation to ensure a normal metabolic process (Byrne 2009). Several studies have been done in several plants, for instance, rice (Moin et al. 2017), tobacco (Liu et al. 2014), and *Arabidopsis* (Sormani et al. 2011) whereby many *RPL* genes are upregulated in response to abiotic stress suggesting that they are involved in maintaining or improving protein biosynthesis enabling plants to acclimatize to stress. All these studies demonstrated that ribosomal protein is involved in abiotic stress tolerance.

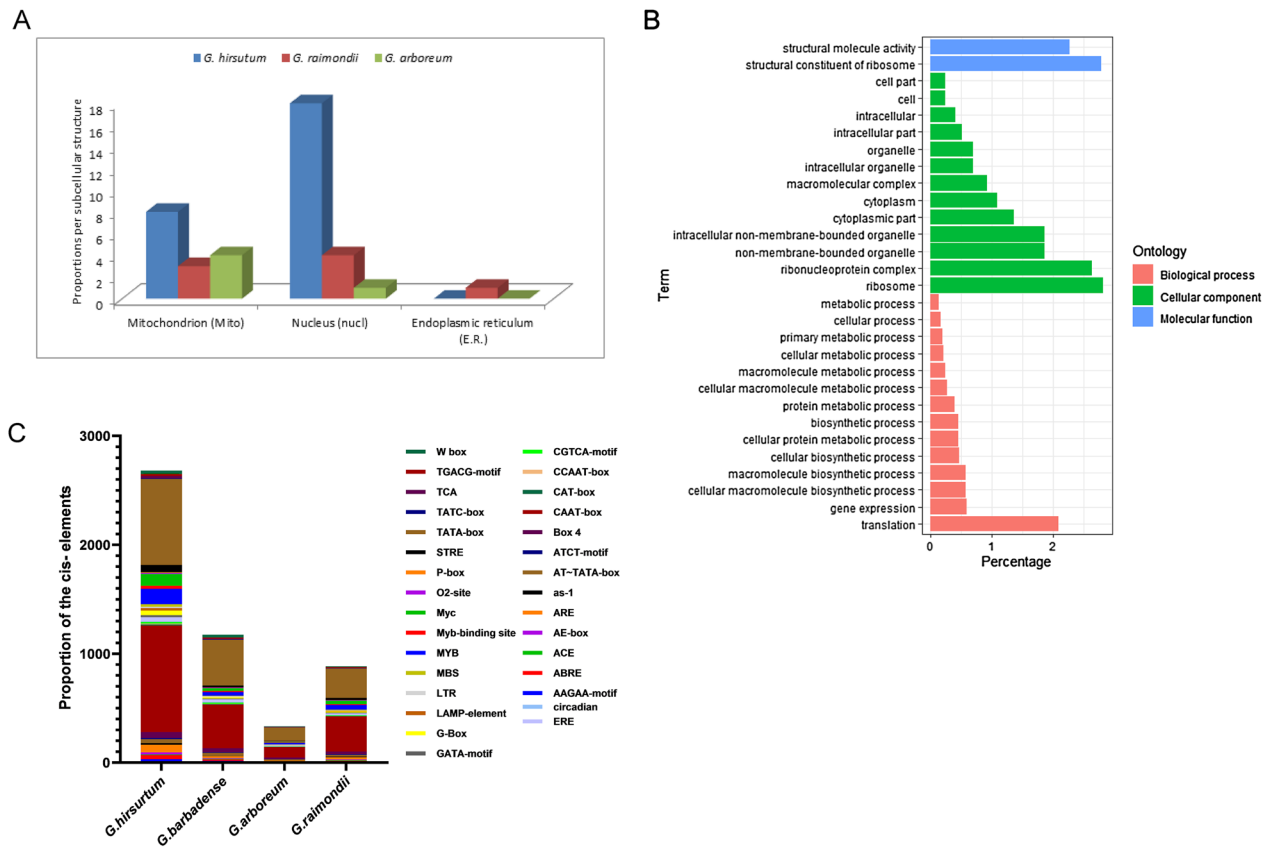

Fig. 5 Subcellular localization, GO analysis and *cis*-regulatory element. **A** Subcellular localization of *RPL14B* genes in *G. hirsutum*, *G. raimondii*, and *G. arboreum*. B Go annotation of biological process, cellular component, and molecular functions identified in *GhRPL14B* genes. C *Cis*-regulatory elements obtained from *G. hirsutum*, *G. raimondii*, *G. arboreum*, and *G. barbadense*

Evolution analysis and physicochemical properties of the proteins encoded by the *RPL14B* genes in cotton

In this study, the phylogenetic tree results showed that the RPL proteins have diverse distribution and could perhaps have a common origin. Similar results have been shown in various subtypes of the Late embryogenesis abundant (LEA) proteins, in which the various classes showed wider distribution across the three cotton genomes, A, D, and AD (Magwanga et al. 2018a, b). Three cotton species had different numbers of genes, which had the RPL14B functional domain. Twenty-six, 5, and 8 genes were found in *G. hirsutum* (AD), *G. raimondii* (D), *and G. arboreum* (A), respectively. Moreover, evaluating the proteins encoded by the *RPL14B* genes, all were found to have negative GRAVY values, which implied that the proteins encoded by the *RPL14B* genes were hydrophilic. The GRAVY values are important protein property because it indicates the protein's behavior in relation to water. Hydrophilic genes have been correlated with the role of enhancing the survival of plants and animals in periods of stress, putatively through safeguarding enzymatic function and prevention of aggregation in times of dehydration and or heat stress. For instance, researches on the *LEA2* gene in cotton found that they were hydrophilic and conferred to enhance drought stress acclimation (Magwanga et al. 2018a, b).

Subcellular localization and motif identification of RPL14B protein

The nucleus has an integral role in cell functioning. This involves regulating of gene expression under different internal and external conditions and regulates the synthesis of proteins. The majority of *RPL14B* were located in the nucleus. RPL14B has the RPL14-KOW motif at its N-terminal. KOW motif has been identified in some large ribosomal proteins (Kyrpides et al. 1996). This motif contains invariant glycine residue, which is composed of alternating blocks of hydrophilic and hydrophobic residue. The KOW motif is common among other ribosomal protein families like RPL 19, 21.2, 24b, and 26; this shows the evolutionary relationship between this gene and the KOW motif family. KOW motif is involved in

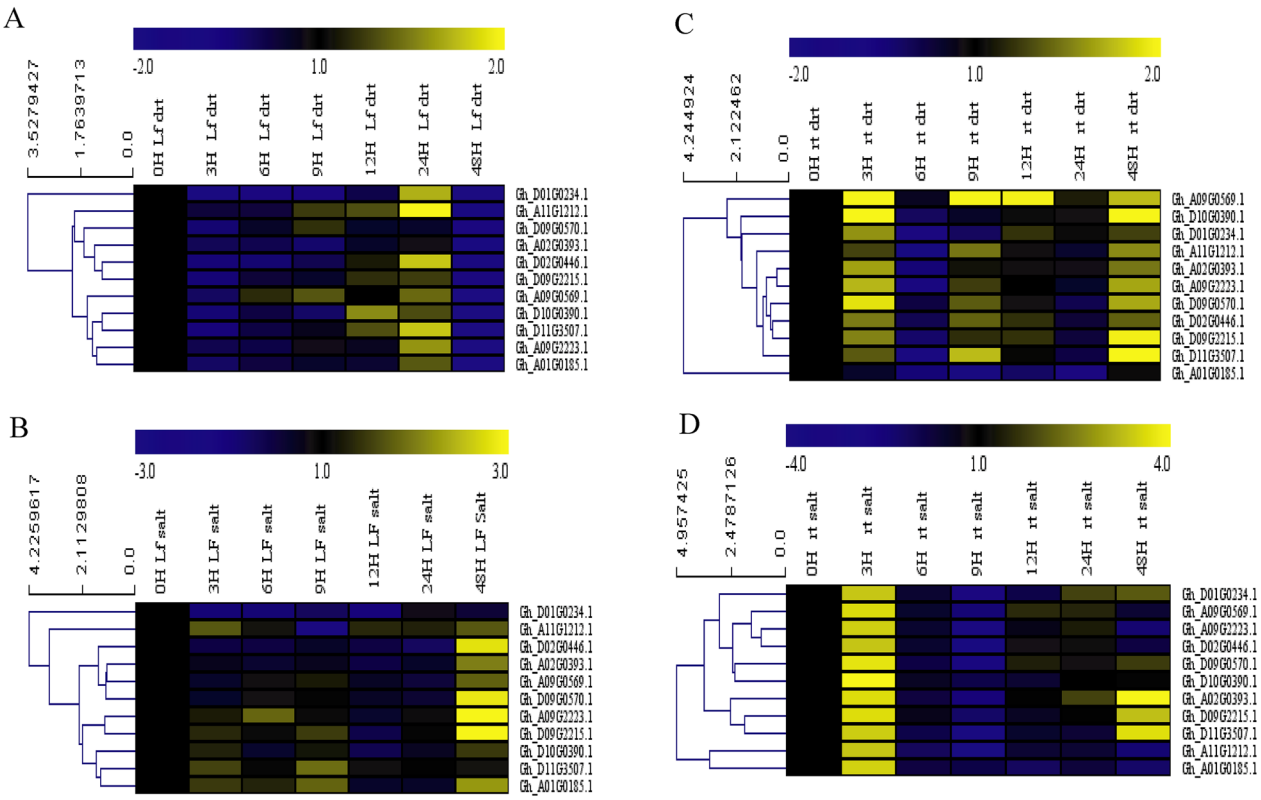

Fig. 6 Differential expression of *GhRPL14B* genes under drought and salt stress. **A** Heat map of the *RPL14B* gene expression in the leaf under drought stress conditions. **B** Heat map of the *RPL14B* gene expression in the leaf under salt stress condition. **C** Heat map of the *RPL14B* gene expression in the root under drought stress conditions. **D** Heat map of the *RPL14B* gene expression in the root under salt stress condition. Yellow mark depicts a high expression level of the genes, and blue mark depicts a low expression level of genes. Black mark depicts no expression of the genes at a particular time

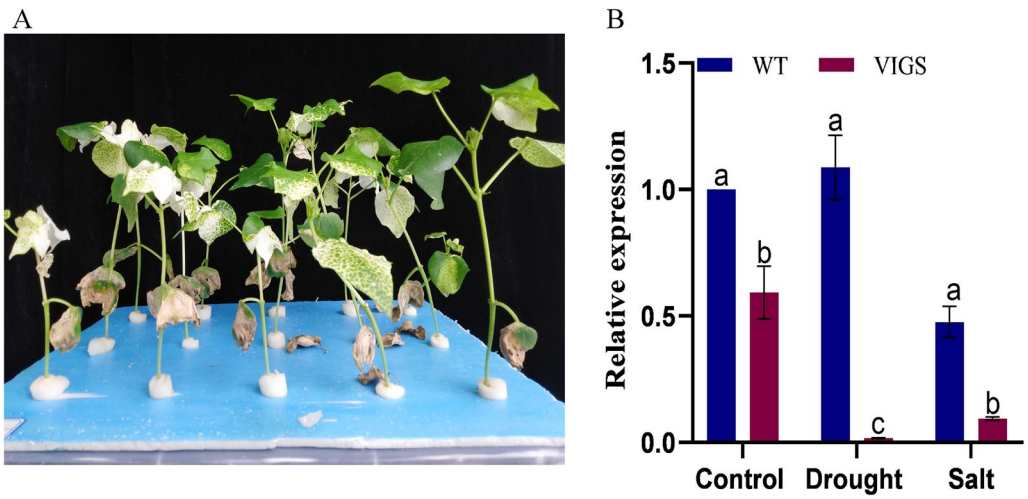

Fig. 7 The efficiency of virus-induced gene silencing in the cotton seedlings. **A** The albino change on the plants under being inoculated with TRV: PDS after 14 days. **B** Expression levels of the knocked gene in WT and VIGS-plants under normal conditions (control), drought and salt stress. Bar indicates the standard error (SE). Different letters indicate the significant differences between the wild type (WT) and the VIGS-plants (ANOVA; $P < 0.05$)

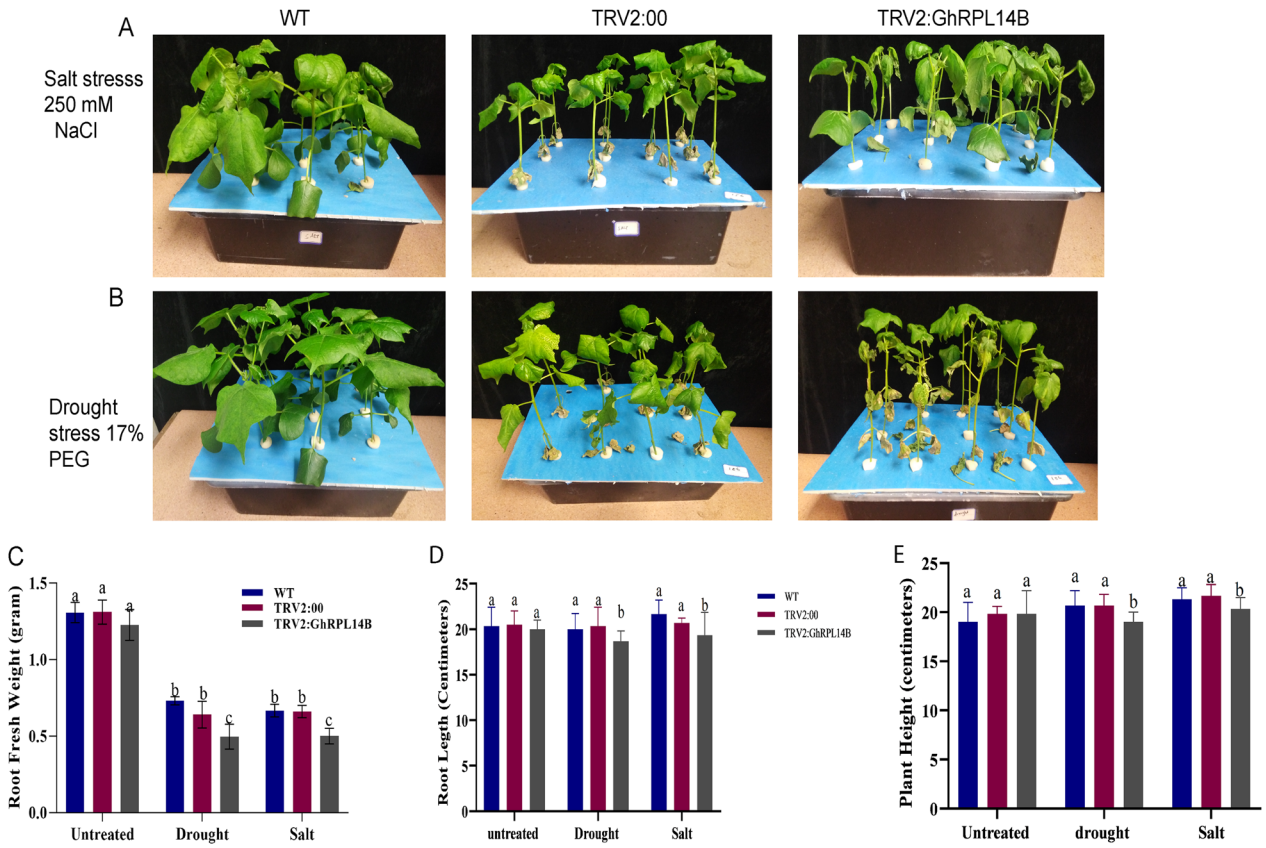

Fig. 8 Morphological evaluation of the VIGS-plants and the wild types (WT) under drought and salt stress conditions. **A** Phenotype observation of TRV2: GhRPL14B, TRV2:00 and WT 24 h after (250 mmol·L^{-1} NaCl) salt treatment. **B** Phenotype observation of TRV2: GhRPL14B, TRV2:00 24 h after 17% PEG treatment. Morphological parameters, **C** root fresh weight (RFW), **D** root length (RL), and **E** plant height (PH). Bar indicates the standard error (SE). Different letters indicate the significant differences between the wild type (WT) and VIGS-plants (ANOVA; $P < 0.05$)

protein–protein interaction and links ribosomal protein with transcription factors that respond to abiotic stress (Moin et al. 2016).

RPL14B protein interacts with other RPLs in response to abiotic stress

This protein interacts with others like RPL 3, 4, 18, 19, 23, and ubiquitin. Some of these genes are involved in stress responses. For instance, overexpression of RPL23A in rice enhanced the water use efficiency of the plants under abiotic stress (Moin et al. 2017). Several studies have demonstrated that ubiquitin is an abiotic stress signaling molecule in plants, and they promote protein interaction in response to abiotic stress (Stone 2014). RPL14 interacts with RPL3 and RPL19 together with the rRNA of the RPL and upholds the ribosome's stability (Tiller et al. 2012). Previous studies have shown that RPL19 was upregulated and enhanced tolerance to drought stress. It is also involved in the thymidylate synthase gene splicing and the regulation of protein synthesis during photosynthesis (Semrad and Schroeder 1998). For plant adaptive responses to drought and salinity, transcription of stress-related genes associated with tolerance mechanisms and pathways is essential. Under drought and salt stresses, stress-related proteins interact with others and induce their transcription to initiate appropriate responses. Therefore the interaction of these genes enhances stress response and tolerance.

Abiotic stress *cis*-regulatory elements identified in the promoter region of *RPL14B* genes

The *cis*-regulatory elements upstream of the transcription factor region play an active role in activating and suppressing genes in response to stress conditions (Zhao et al. 2014). The presence of several stress-responsive *cis*-regulatory elements in the putative promoter regions of the *RPL14B* gene reveals that this gene activity alleviates the plant's stress effects. In addition to abiotic stresses, elements that respond to phytohormones were identified. ABRE (Abscisic acid-responsive element), TGACG-motif and CGTCA-motif responsive to MeJa, TCA-motif responsive to salicylic acid, and TGA-motif responsive to

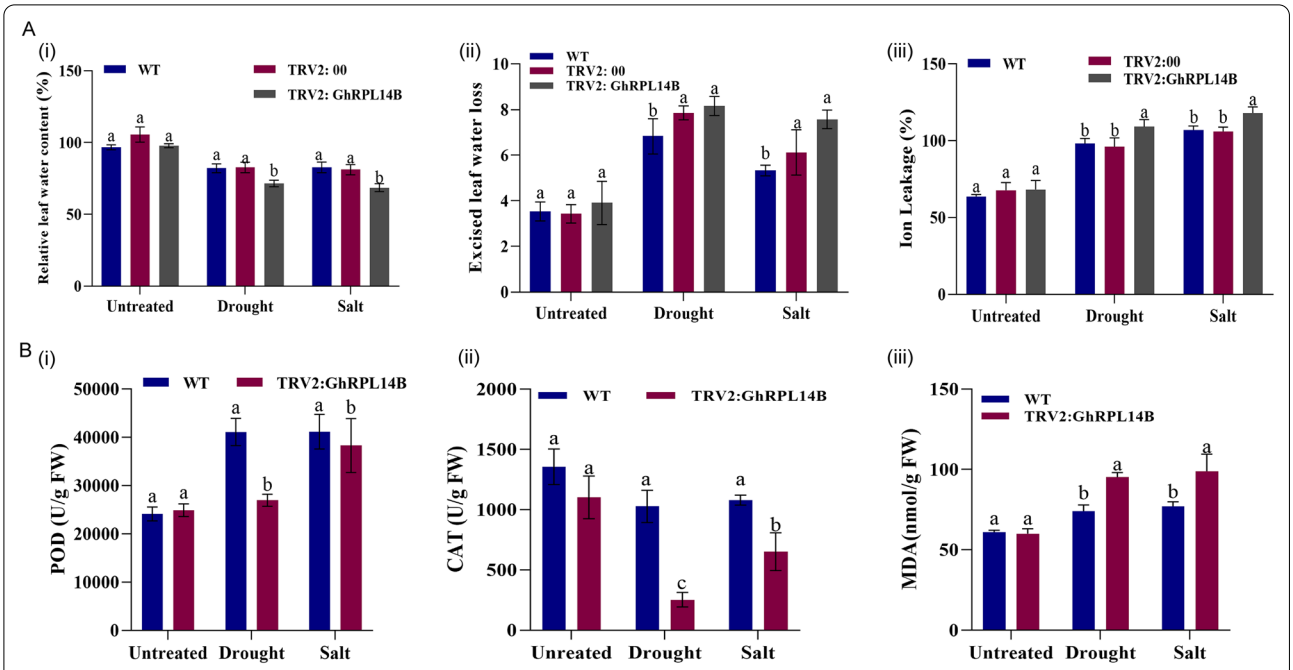

Fig. 9 Physiological analysis and Biochemical assays of the oxidant and antioxidant in VIGS plants under drought and salt stress conditions. **A** (i) Quantitative determination of relative leaf water content (RLWC), (ii) excised leaf water loss (ELWL), and (iii) Quantitative determination of ion leakage as a measure of cell membrane stability (CMS). **B** Biochemical analysis (i) Quantitative determination of peroxidase (POD), (ii) Quantitative determination of catalase (CAT), and (iii) quantitative determination of Malondialdehyde (MDA). The bar indicates a standard error (SE). Different letters indicate the significant differences between the wild type and the VIGS-plants (ANOVA; $P < 0.05$)

Auxin were identified. Previous researches on *RPL* stress-responsive gene families in rice identified similar *cis*-regulatory (Moin et al. 2017; Saha et al. 2017). This suggests *RPL14B* gene enhances plant's adaptation and tolerance to abiotic stress and participates in signal pathways during abiotic stress conditions.

Knockdown of *GhRPL14B* gene increases the sensitivity of upland cotton to drought and salt stresses

VIGS is a versatile tool for functional characterization and has been extensively utilized to study gene function in different plants (Corbin et al. 2017). Moreover, the *RPL14B* (*Gh_D01G0234*) gene was knocked down in upland cotton through VIGS for further evaluation. The silenced *Gh_D01G0234* plants exhibited a susceptibility phenotype compared with the control. The FLW, SFW, FRW, RLWC, and chlorophyll content of the VIGS plants were lower than that of the control (empty vector and wild plants), while ELWL and ion leakage were higher in VIGS plants compared with the control plants. Similar observations were observed in which plants exhibited wilting behaviors when exposed to either osmotic or salinity stress conditions (Fathi and Tari 2016). This results indicated that the VIGS plants experienced reduced water retention and photosynthetic activities. Thus, they were more susceptible to drought and salt stress compared with the control plants. The transpiration rate in plants under stress increase when its stress tolerance mechanisms are compromised (Suzuki et al. 2014). Biochemical analysis showed a higher concentration level of MDA and a lower level of POD, and CAT, in VIGS plants relative to the control plants. A higher amount of oxidant means VIGS plants were experiencing oxidative stress under drought and salt stress conditions. Drought stress results in the upregulation of oxidants due to the lack of homeostasis between oxidants and antioxidants. Oxidative stress results in the production of reactive oxygen species (ROS). The ROS are incredibly toxic and can cause damage to the plant tissues and eventually cell death. Plants use ROS to aid in the signal transduction process in response to various stimuli and offer the plant defense to abiotic stress (Mehla et al. 2017). Oxidants and antioxidants have been used as biochemical markers for drought stress in various studies; upregulation of oxidants and downregulation of antioxidants indicate the plant is under stress.

Conclusions

This study provides an insight into the role of the *RPL14B* gene during drought and salt stresses conditions. The *RP* genes are involved in stabilizing the

ribosomes and interacting with other genes, enhancing plant acclimation to unfavorable conditions. The presence of *cis*-regulatory elements and increased expression of the *RPL14B* gene during drought and salt stress proves that *RPL* genes have evolved and are involved in extra ribosomal activities. The ability to tolerate the effects of osmotic and salt stress of VIGS-plants was significantly compromised. The VIGS plants recorded significantly higher concentrations of oxidant enzymes and a reduction in the concentration levels of the antioxidant enzymes, which revealed that the VIGS plants suffered more severe oxidative stresses than the wild types under osmotic and salt stress conditions. This work lays the very first foundation for further investigations of the specific functions of these RPL14B proteins in cotton about drought stress and other abiotic stress factors.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42397-021-00102-7.

Additional file 1: Table S1. Physiochemical properties of the proteins encoded by the *RPL14B* genes.

Additional file 2: Table S2. List of primers for the RT-qPCR profiling of the cotton *RPL14B* gene under drought and salt stress conditions.

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Not applicable.

Authors' contributions
Shiraku ML, Magwanga RO and Liu F designed the study. Shiraku ML performed the experiment and collected data. Shiraku ML, Magwanga RO, Cai XY, Xu YC, and Mehari TG analyzed the data. Shiraku ML wrote the manuscript. Kirungu JN, Hou YQ, Wang YH, Peng RH, Wang KB review the manuscript. Supervision: Liu F and Zhou ZL. All authors read and approved the final manuscript.

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Declarations

Ethics approval and consent to participate
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Consent for publication
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Competing interests
The authors declare that they have no competing interests.

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