Cloning and functional characterization of two abiotic stress tolerance genes, *AtLTP1* and *LeCBF1*, in *Saccharomyces cerevisiae*

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Background
Salinity is one of the most damaging abiotic stress factors in agriculture, it has a negative impact on crop growth, production, and development. It is predicted that salinity will become much more severe due to global climate change. Moreover, soil salinization affects three hectares of agricultural land every minute, increasing the salinity-affected area by 10% annually. The improvement of abiotic stress tolerance in plants was made possible by recent developments involving transgenes and the isolation of some abiotic stress tolerance genes.

Objective
The current study aimed to synthesize, clone and characterize two abiotic stress tolerance genes Lipid transfer protein (*AtLTP1*) of *Arabidopsis thaliana* and Stress-inducible transcription factor C-repeating binding factor (*LeCBF1*) of *Solanum lycopersicum* in *Saccharomyces cerevisiae*.

Materials and methods
The above-mentioned genes were synthesized, cloned into the pYES2 vector then transformed into *Saccharomyces cerevisiae* as a model eukaryotic system. The yeast growth was measured at (OD_{600} nm) in a spectrophotometer, RT-PCR expression analysis and estimation of intracellular proline content after exposure to different salt concentration were performed to characterize and evaluate the physiological roles of the selected genes in the yeast.

Results and conclusion
The *AtLTP1* and *LeCBF1* genes were cloned into the pYES2 vector for *Saccharomyces cerevisiae* expression. After being exposed to increasing concentrations of sodium chloride (0, 1.7, 1.8, 1.9, 2.0, 2., 2.2, and 2.3 M) for 7 days, transgenic yeast cells were tested for their ability to survive under increasing salt-stress conditions and their growth response. A spectrophotometer was used to measure yeast growth at OD_{600}nm. The growth of the control cells was dramatically hindered when the salt content was increased to 1.9 M NaCl. However, two transgenic yeast lines continued to grow well, at a slower rate, up to 2.3 M NaCl. The two genes' expression in transgenic yeast in response to salt stress was verified by RT-PCR. In this transgenic yeast, the precise primers of *LeCBF1* and *AtLTP1* amplified the genes successfully at 633 base pairs and 368 base pairs, respectively. The findings showed that increasing salinization level considerably boosted the transgenic yeast's intracellular proline accumulation. It was suggested that the possibility of utilizing these genes to produce salt tolerant transgenic plants, consequently, increase the amount of land that can be exploited for agriculture.

Keywords:
Arabidopsis thaliana Lipid Transfer Protein and *Solanum lycopersicum* C-repeating Binding Factor genes, expression, Reverse Transcriptase-polymerase chain reaction and proline content, *Saccharomyces cerevisiae* (INVSc1), transgenic

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Statements and Declaration

Abbreviation: AL, Alfin-like gene; *AtLTP1*, *Arabidopsis thaliana* Lipid Transfer Protein; cDNA, Complementary DNA; E. coli, Escherichia coli; INVsc1, Invitrogen *Saccharomyces cerevisiae* yeast strain; *LeCBF1*, *Solanum lycopersicum* C-repeating Binding Factor; NaCl, Sodium Chloride; *nsLTP1s*, nonspecific lipid Transfer Proteins; PCR, Polymerase chain reaction; PEG, Polyethylene glycol; RACE, Rapid amplification of cDNA End; RT-PCR, Reverse Transcriptase polymerase chain reaction; SC, synthetic minimal defined medium; TF, Transcription factors; YPD, yeast extract peptone dextrose.

Introduction

Salinity is one of the main global threats to agricultural growth. Salt stress results in altered physiological processes and several growth-inhibiting factors for plants [1]. About 20% of the world's cultivated land and 33% of its irrigated land is impacted by salt and is deteriorated., while nearly 7% of the land has a high salt content [2,3]. The salinization of soil is typically categorized as primary occurring from natural processes and secondary salinity brought on by human activity, etc. [4]. Increased sodium chloride levels cause hypertonic and hyperosmotic stressors [5,6].

Understanding the mechanisms that contribute to salt tolerance is crucial for reducing the impacts of salinity on plant growth. Ion homeostasis, osmolytes, signalling elements, transcription factors and protective proteins are only a few of the many factors that have been discovered [7].

Genes encoding Tfs (Transcription factors) have frequently been chosen as targets of genetic engineering to successfully develop crops to endure abiotic stresses [8]. The majority of Tfs participate in numerous stresses signaling pathways, making them viable candidates to confer stress tolerance to various stresses. Tfs controls the transcription of stress-responsive genes and restores cellular ion homeostasis as part of its function in abiotic stress tolerance [9,10].

C-repeat binding factors (*CBFs*) encode transcription factors that are crucial for reactions to environmental stimuli, such as salt, drought, fungal infection, freezing and cold stress [11]. The expression of different cold-responsive genes can be regulated by these *CBF* proteins' ability to bind to their promoter regions [12]. Like this, overexpressing *AtCBF13*, *AtCBF12* and *AtCBF11* increased tolerance to salt, freezing and drought by activating a variety of downstream genes, including those that are susceptible to dehydration and cold [13].

Plenty of evidence based on gene expression data showing *LTPs* play a role in adaptation to drought stress, osmotic stress, salt stress, and cold stress. [14]. *LTPs* are encoded by large multigene families, although the lipid transfer activity of just a few of the encoded proteins has been demonstrated. 52 members make up the *LTP* gene family in *Oryza sativa*, 156 in *Triticum aestivum,* and 110 in *Arabidopsis thaliana,* of which 15 are designated *AtLTP1* to *AtLTP15* [15].

The growth, development, and abiotic stress responses of plants are significantly influenced by plant nonspecific lipid transfer proteins (*nsLTP1s/LTP1s*). Numerous *LTP1* homologs have already been found in a wide variety of plants and different plant species have different numbers of *LTP1* genes. Currently, 138 *LTP1* genes in cotton, 77 *LTP1* genes in rice, and 51 *nsLTP1* genes in Arabidopsis have been found [16]. Biotic and abiotic stresses, such as low or high temperature, drought, salinity, exposure to heavy metals, and disease, can cause some *nsLTP1s* to express [17].

Since many of the stress response mechanisms and proteins involved in yeast osmoadaptation are shared by other fungi and plants, yeast serves as an excellent model system for researching osmoadaptation in eukaryotes [18,19]. The expression vector pYES2 is most used for the expression of genes cloned into yeast. [20]. Therefore, this study aimed to clone two genes involved in abiotic stress *LeCBF1* and *AtLTP1* as well as their expression in yeast, as a salt-stressed eukaryotic model organism.

Material and methods

Amplification of the *AtLTP1* (NM_001124849) and *LeCBF1* (NM_001247194) genes

The *Escherichia coli* (*E. coli*) strain *DH5α* bacteria were transformed with the recombinant vectors pUC 57-Amp harboring *AtLTP1 and LeCBF1* genes of *Arabidopsis thaliana* and *Solanum lycopersicum*, respectively which synthesized by the Macrogen Company, Germany.

Transformed E. coli were cultured in LB medium supplemented with ampicillin (100 g/ml), IPTG (0.1 M), and X-Gal (50 mg/ml).

PCR colony screening and blue-white selection were used to find the recombinant clones. Recombinant vectors were isolated from white colonies using the QIAprep Spin Miniprep Kit (Cat. nos. 27104, Germany). The gene-specific primers used for the amplification of the *AtLTP1* and *LeCBF1* genes are shown in Table 1.

These primers were designed using sequences from the National Centre for Biotechnology Information's (NCBI) database. To make it easier to clone the amplified genes, *Hind III* restriction enzyme recognition sites were added to the 5' end of the forward primer of *LeCBF1* While the *BamHI* recognition site was added to the 5' end of the

Table 1 The primer used for amplification of the *AtLTP1* and *LeCBF1* genes

Primer's name	Sequence (5'-3')	Expected product length (bp)
AtLTP1	F: GCGCGGATCCATGGCTGGTCTTATGAAGTTAGGA	*368*
	R: GCCGGAATTCTCACCTCACGGTGTTGCACTTG	
LeCBF1	F: GCCCAAGCTTATGAATATCTTTGAAACCTATTATTC	*633*
	R: GCGCGGATCCTTAGATAGAATAATTCCATAAAGTT	

F: forward and R: reverse.

reverse primer of *LeCBF1* and to the 5' end of the forward primer of *AtLTP1*, the *EcoRI* recognition site was added to the 5' end of the reverse primer of *AtLTP1*. These primers were synthesized by the Macrogen Company, Germany.

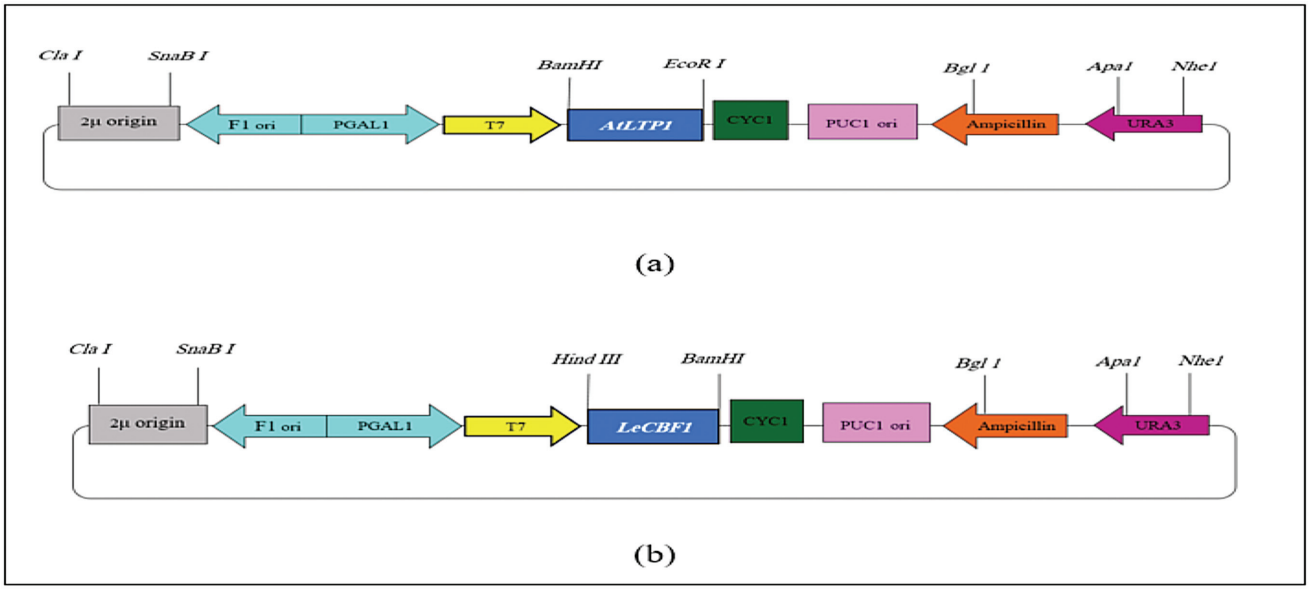
The polymerase chain reaction reactions (PCR) were performed in a total volume of 20 µl containing 1 µl genomic DNA (50 ng/µl), 2 µl each of the forward and reverse primers (10 pmol/1 ml), 1 µl dNTPs mixture solution (10 mM), 2 µl Magnesium chloride (25 mM), 4 µl 5X Green Go Taq Flexi buffer, and 0.5 µl Go Taq DNA polymerase (5 u/µl) Promega. With nuclease free water, the volume was brought up to 20 µl, and conducted with a Biometra DNA thermal cycler.

The PCR temperature profile for the *AtLTP1* and *LeCBF1* genes was as follows: an initial denaturation step at 95°C for 3 min, followed by 30 cycles of 95°C for 30 s, 55°C for 1 min, and 72°C for 1 min, with a final extension of 72°C for 5 min. The PCR products were separated on a 1% (w/v) agarose gel. The PCR products were separated from the gel and purified using the QIA quick gel extraction kit from QIAGEN (Cat. No. 28706, Germany) according to the manufacturer's instructions.

Cloning of the *AtLTP1* (NM_001124849) and *LeCBF1* (NM_001247194) genes in the pYES2 expression vector

The PCR products of the *AtLTP1* gene were digested with *EcoRI* and *BamHI* enzymes, while those from the *LeCBF1* gene were digested with *HindIII* and *BamHI* enzymes. To create two recombinant plasmids, the purified PCR products of the two genes (*AtLTP1* and *LeCBF1*) were individually ligated into the (pYES2) DNA vector was performed using Invitrogen (Cat. No. V825-20) after digested with the same restriction enzymes (Fig. 1). The *DH5α E. coli* competent cells were transformed with the recombinant pYES2 plasmids and allowed to multiply. The culture was plated on an LB solid plate containing 50 µg/ml ampicillin and incubated overnight at 37°C. The positive colonies produced recombinant pYES2 vectors, which were extracted

Figure 1



Schematic representation of the transformation vector pYES2 contains the *AtLTP1* (a) or *LeCBF1* (b) genes under the genetic control of T7 promoter.

and cultured overnight at 37°C on LB medium supplemented with 50 µg/ml ampicillin. The recombinant pYES2 expression vector carrying the genes of interest was isolated from the bacterial colonies using the QIAprep Spin Miniprep Kit (Cat. nos. 27104, Germany) and analyzed using PCR, restriction digestion and an automated DNA sequencer (Macrogen Company, Germany). Using the Basic Local Alignment Search Tool, the nucleotide sequence and deduced amino acid sequence of the two cloned genes were checked and validated using databases of DNA and protein sequences (BLAST).

Transformation of competent yeast cells as a model eukaryotic system. Subsequently, the produced constructs that multiplied in *E. coli* have transformed into competent yeast cells using the Li-acetate approach outlined by Gietz and colleagues [21]. Transformed yeast cells were cultured on plates containing the synthetic minimal defined medium (SC) devoid of uracil. The plates were dried and then incubated for 3-5 days at 30°C.

The Colony Pick PCR, a quick and accurate technique for yeast DNA amplification, was used to confirm the transformation of yeast [22]. Fresh single colonies that were less than 2 days old were heated for 15 min in 20 µl of sterile water before being added to the PCR reaction mixture in a volume of 50 µl that contained (0.5 units of Taq polymerase enzyme, 1 µM primer, 200 µM dNTPs, and 1 X Taq polymerase buffer). The PCR temperature profile matched what had been previously mentioned.

Characterization and evaluation of the physiological roles of the selected genes in yeast

Yeast growth measurement

Transgenic yeasts for each of the two genes, negative control (yeast without pYES2) and positive control (yeast with pYES2 only) growing for 2 days in 5 ml of selective synthetic minimal defined medium SC devoid of uracil [23] to select transgenic yeast from nontransgenic as the ura3 gene in pYES2 is used to select transformants in yeast host strains grown in uracil-deficient media. A volume of 300 µl of the cultured samples for each of the two genes and the two controls was inoculated into 25 ml of SC medium supplemented with various sodium chloride concentrations of 0, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, and 2.3 M for 7 days. After that, the yeast growth was measured at OD_{600} using a spectrophotometer (Smartspec 3000).

Reverse transcriptase polymerase chain reaction (RT-PCR) expression analysis

Transgenic yeasts were cultured in YPD (yeast extract peptone dextrose) medium for 2 days YPD is a general nutritious liquid growth medium that is extensively used for the maintenance and propagation of yeasts Saccharomyces cerevisiae. [24], after which 300 µl of the cultivated sample was added to 25 ml of YPD media containing various NaCl concentrations (1.8, 2.0, and 2.3 M) for 7 days. Using the SV total RNA isolation kit (Promega TM, Cat. No. Z3100) total RNA was isolated from yeast that was both transgenic and non-transgenic. Reverse transcription was used to create first-strand cDNA. For the reverse transcription reaction, A 20 µL reaction volume contained 11 µl RNA, 2 µl dNTPs, 1 µl oligo(dT), 1 µl reverse transcriptase, 4 µl 5 x reverse transcription buffer, and 1 µl RNase inhibitor was used for the reverse transcription reaction. The reverse transcriptase was inactivated at 70°C for 5 min after the reverse transcription process had been incubated at 42°C for 60 min Using PCR and *AtLTP1* and *LeCBF1* gene-specific primers, the cDNA template was amplified.

Estimation of intracellular proline content

For two days, transgenic yeasts were grown in YPD medium containing 1% yeast extract, 2% bacto peptone, and 2% glucose. Then, for 7 days, 300 µl of the cultivated sample was inoculated into 25 ml of YPD medium. The proline content was estimated from transgenic and nontransgenic yeast using a spectrophotometer at OD_{520} nm according to Sasano and colleagues [23].

Statistical analysis

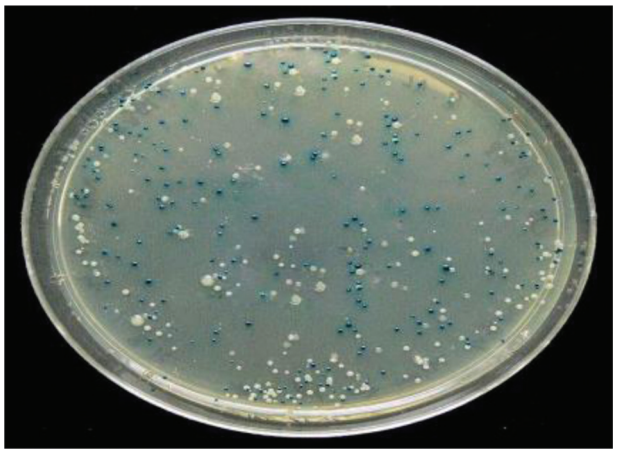
The MSTAT programme was utilized to perform statistical analysis of yeast growth and proline estimates. Factorial experiments with three repetitions were used in the design of the studies. At a significance level of P less than 0.05, the LSD test was performed to analyze differences between treatments.

Results

Amplification of the *AtLTP1* and *LeCBF1* genes in *E. coli*

The blue-white screening and PCR colony techniques were used to choose the positive clones for the *AtLTP1* and *LeCBF1* genes, respectively. White colonies indicate target gene insertion and loss of the cell's capacity to hydrolyze the marker. (Fig. 2).

Figure 2



Transformed *E. coli* DH 5α cells were screened for white-blue colonies using a recombinant pUC 57-Amp vector LB/Amp plate supplemented with IPTG/X-GAL.

Isolated recombinant plasmid DNA was electrophoresed on a 1% agarose gel. after each gene's recombinant colony was selected and cultured in LB broth supplemented with ampicillin. The DNA of the two desired genes represented by the PCR results was purified (Fig. 3a, b) used to transform *E. coli* DH5α cells after being cloned into the pYES vector.

Cloning into yeast expression vector

To simplify directed cloning, pYES2 yeast expression vector and purified PCR products of *AtLTP1* were restricted with *EcoRI* and *BamHI* enzymes while the yeast expression vector pYES2 and the purified PCR product of *LeCBF1* were restricted with *BamHI* and *HindIII* enzymes (Fig. 4a and b). The two inserts of *AtLTP1* and *LeCBF1* genes were separately ligated into a pYES2 vector, before being transformed into *E. coli* DHα5 cells. Recombinant plasmids that contains either *AtLTP1* or *LeCBF1* genes were further extracted, validated by PCR and subjected to restriction digestion using the *EcoRI, BamHI* for *AtLTP1* gene and *HindIII, BamHI* enzymes for *LeCBF1* gene (Fig. 5a,b).

Sequencing of the cloned genes

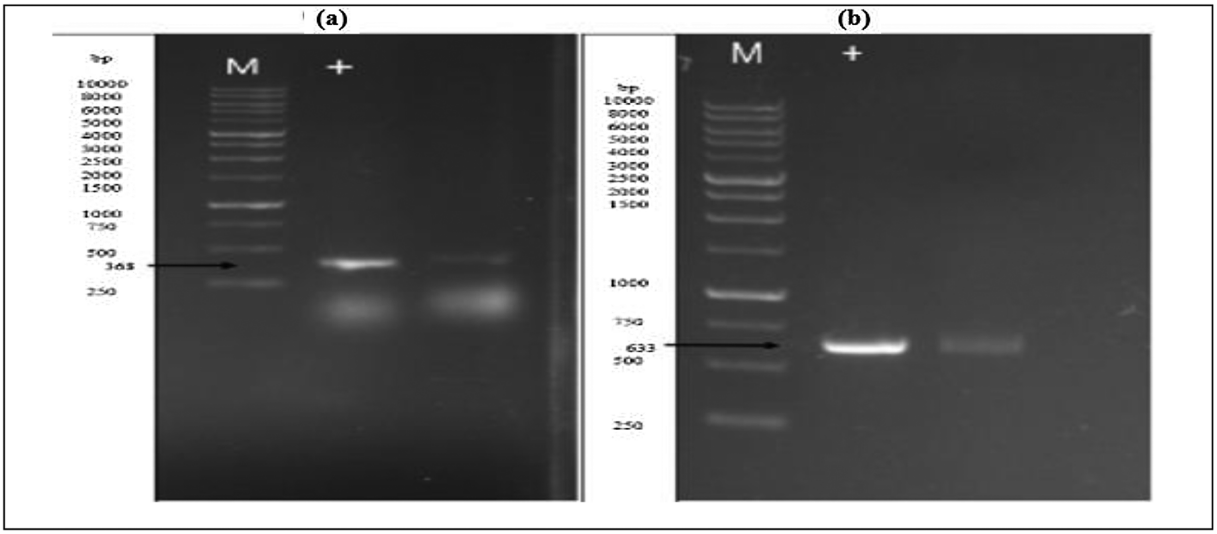
The amplified genes were extracted from the gel and cloned into the pYES2 vector before being sequenced. Blasting was used to analyze the sequences using the sequences that were available at NCBI (National Centre for Biotechnology Information). Figure 6 demonstrate the results, which showed 100% homology between the amplified genes and the database sequences.

The *AtLTP1* and *LeCBF1* genes' nucleotide sequences were converted into protein sequences using the translation tool EMBOSS Transeq. The amino acid alignment was carried out using BLASTP, which showed a homology of 100% for *AtLTP1* and *LeCBF1* homologues. These outcomes demonstrated that it was successful to identify the target genes.

Expression of the cloned genes in yeast

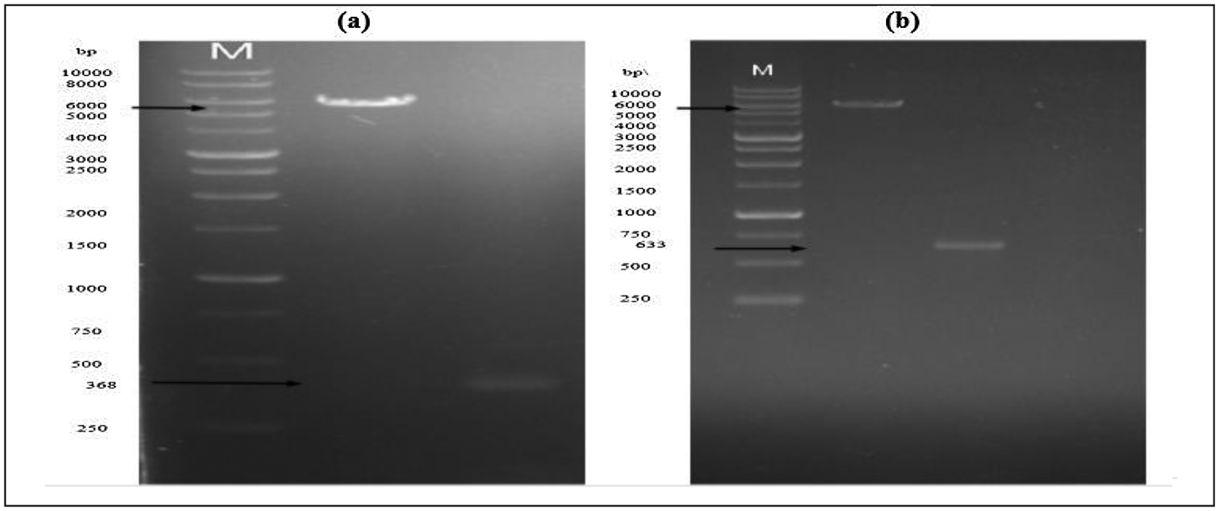
Picked and streaked on SC medium devoid of uracil was transformant yeast. (Fig. 7). Colony Pick PCR was

Figure 3



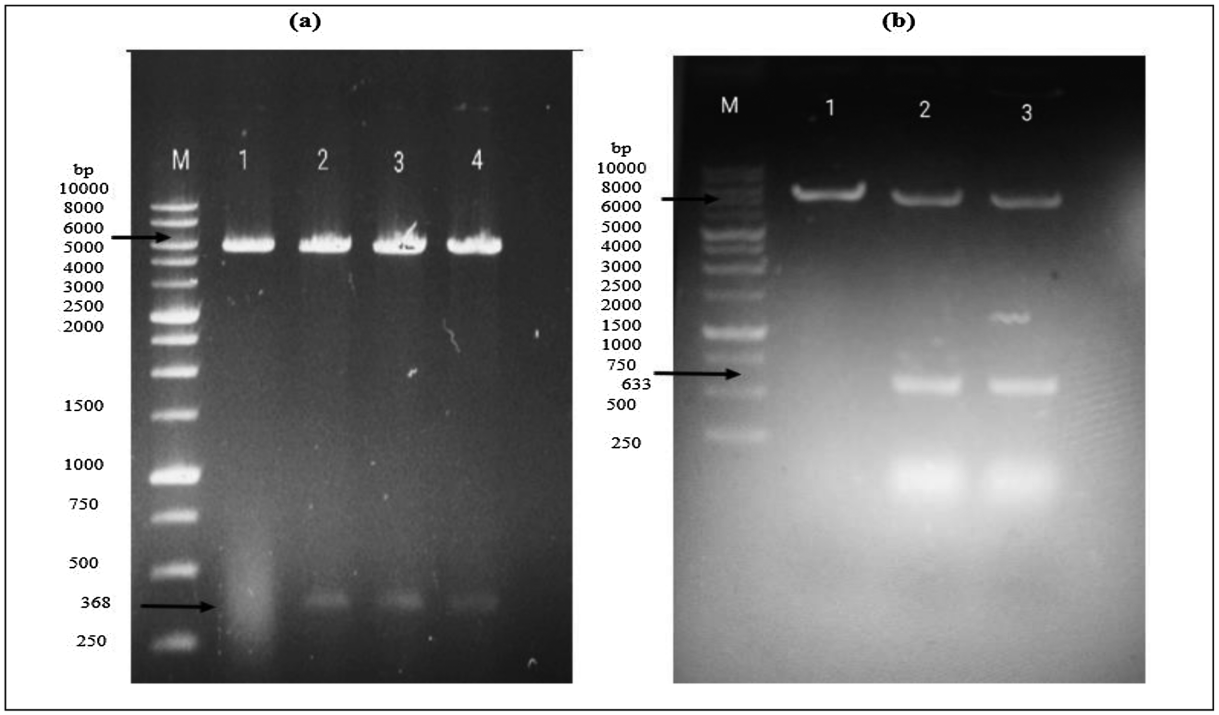
Purified PCR products of the *AtLTP1* (368 bp) (a) and *LeCBF1* (633 bp) (b) genes electrophoresed on an agarose gel. M;1 kb ladder marker.

Figure 4



The directed cloning of *AtLTP1* and *LeCBF1* genes. *AtLTP1* gene (368 bp) and pYES vector (5.9Kbp) after digestion with *BamHI* and *EcoRI* enzymes (a). and *LeCBF1* gene (633 bp) and pYES vector (5.9 Kbp) after digestion with *BamHI* and *HindIII* enzymes (b). M; 1 kb ladder marker.

Figure 5



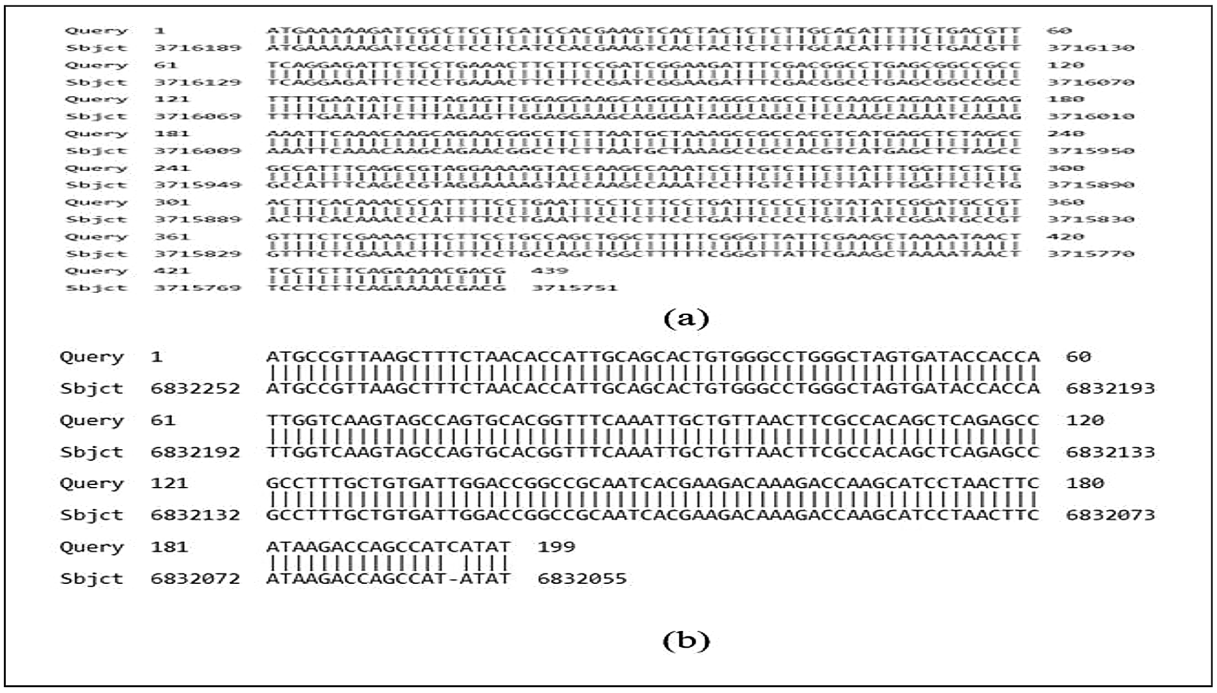
Validation of the recombinant pYES2 plasmids that contain either *AtLTP1* or *LeCBF1* genes. (a): restriction digestion of *AtLTP1* recombinant plasmid with *BamHI* and *EcoRI* restriction enzymes, Lane 1: an empty pYES2 plasmid(5.9Kbp) digested with *BamHI* and *EcoRI* enzymes; lane 2-4: *AtLTP1* recombinant plasmid digested with *BamHI* and *EcoRI* enzymes; M: 1 kb ladder marker. (b): restriction digestion of *LeCBF1* recombinant plasmid with *BamHI* and *HindIII* restriction enzymes; Lane 1: an empty pYES2 plasmid(5.9Kbp) digested with *BamHI* and *HindIII* enzymes; lane 2-3: *LeCBF1* recombinant plasmid digested with *BamHI* and *HindIII* enzymes; M: 1 kb ladder marker.

used to confirm that the insert was present in these clones (Fig. 8 a, b). The amount of intracellular proline accumulated and yeast growth under salt stress were measured to evaluate the expression of the cloned genes in yeast.

Transgenic yeast growth was evaluated as an indicator of salt tolerance

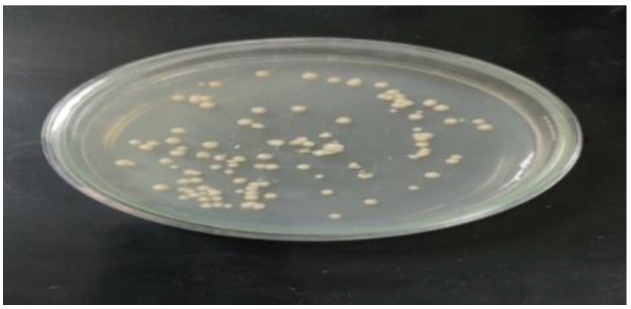
To evaluate whether transgenic yeasts could withstand salt stress, The yeasts were cultivated in SC liquid medium without uracil and supplemented with eight

Figure 6



The alignment of the amplified *AtLTP1* (a) and *LeCBF1* (b) genes with their respective database sequences.

Figure 7



Transformation of yeast using recombinant pYES2.

different NaCl concentrations. (0, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, and 2.3 M) for 7 days. The results revealed that significant variations between the transgenic yeast and control exist in terms of cell growth. The findings in Table 2 show the impact of various salt concentrations on the mean values of growth at OD_{600} in transgenic yeast when compared with the controls (Positive and Negative). The absence of uracil in the negative control prevented the yeast cells under any salt treatments from growing. The mean yeast cell growth values in the second control (positive control) and transgenic yeast at 0 M NaCl were comparable (2.8820 a, 2.9300 a, and 2.9610 a, respectively). The capacity of yeast to withstand salt stress was significantly influenced by the salt concentration of 1.8 M NaCl. At this dose, the mean growth values for the transgenic yeast expressing the *AtLTP1* and *LeCBF1* genes were 1.6386 c and 1.5100 cd, respectively, as opposed to 0.8553 fg for the control. The growth of the control cells was severely hindered when the salt content was increased to 1.9 M NaCl. While maintaining decent growth, the transgenic yeast cells for the two genes continued to develop, albeit at a slower rate, up to a concentration of 2.3 M NaCl.

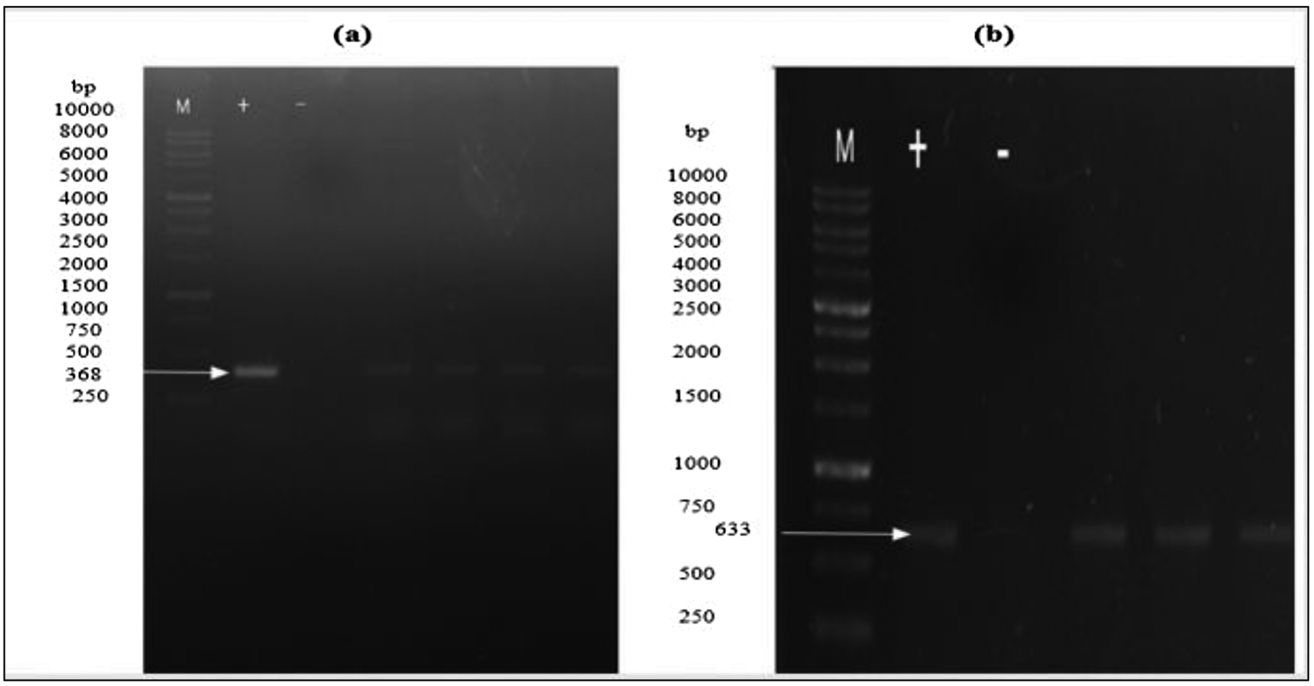
Reverse transcriptase PCR expression analysis

RT-PCR was used to confirm the transcriptional expression of both genes *LeCBF1* and *AtLTP1* in transgenic yeast. As shown in (Fig. 9a, b) the expression of both genes was detected in transgenic yeast at the expected size for *LeCBF1* gene (633 bp) and *AtLTP1* gene (368 bp). While these bands were absent from the control (transgenic yeast with the empty pYES vector).

Measurement of intracellular proline contents

An essential role is played by the amino acid proline in plants. It also aids in protecting the plants from various stresses, increases speed of stress recovery in plants. Intracellular proline buildup has been found in the current work to evaluate the transgenic yeast's reaction to salt stress. The results shown in Table 3 demonstrated that increasing salinization level greatly decreased the mean values of the controls'

Figure 8



Amplification of *AtLTP1* and *LeCBF1* gene using colony pick PCR. (a) The amplification of *AtLTP1* gene (368 bp) and (b) *LeCBF1*gene (633 bp). M: 1 kb ladder Marker and (–) negative control transformed yeast cells for empty pYES2 vector.

Table 2 The average values of transgenic yeast growth under salt stress compared with controls (both positive and negative), evaluated at OD_{600}

Salt concentration (M NaCl)	negative control (–)	positive control pYES2 (+)	*AtLTP1*	*LeCBF1*
0 M	0.0926 l	2.8820 a	2.9610 a	2.9300 a
1.7 M	0.0776 l	1.1733 e	1.7813 b	1.5430 cd
1.8 M	0.0733 l	0.8553 fg	1.6386 c	1.5100 cd
1.9 M	0.0656 l	0.7036 h	1.5286 cd	1.4166 d
2.0 M	0.0563 l	0.6516 hi	1.1436 e	0.9836 f
2.1 M	0.0476 l	0.5003 j	0.9133 f	0.8936 f
2.2 M	0.0426 l	0.3286 k	0.7553 gh	0.6770 hi
2.3 M	0.0370 l	0.2863 k	0.7086 h	0.5540 ij

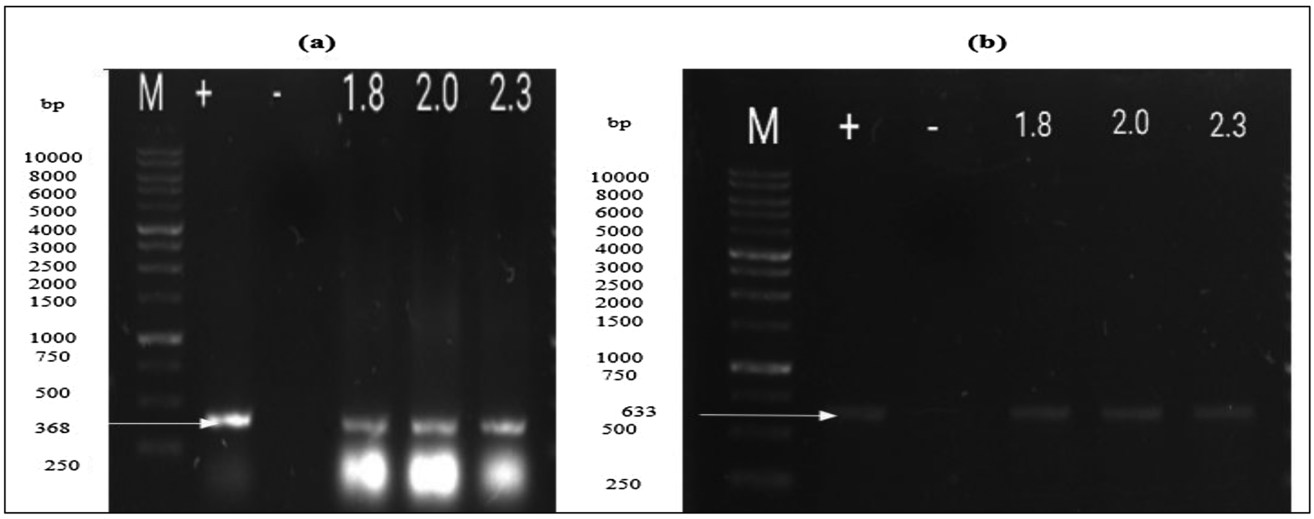
Numbers with the same letters do not differ appreciably at 1%.

intracellular proline concentration. While in the transgenic yeast, the salinization level was raised while the proline concentration increased considerably. The general mean values (OD_{520}) of proline measured from several salt treatments in transgenic and control yeast demonstrate that the proline content of transgenic cells was greater than the controls (1.8177 for *LeCBF1* and 1.9687 for *AtLTP1* verses 0.9276 for the positive control and 0.7917 for the negative control). the transgenic cells' proline content was significantly greater than that of the controls (1.831 for *LeCBF1* and 1.977 for *AtLTP1* verses 0.9476 for the positive control and 0.8160 for the negative control).

Discussion

Sequence analysis of *AtLTP1* and *LeCBF1* demonstrated complete similarity between the amplified genes and the database sequences and showed that *AtLTP1* contained A 122-amino-acid protein while *LeCBF1* contained A 211-amino-acid protein with sequence similarity (100%) for both genes. In this respect, Zhu and colleagues [25] identified the Alfin-like (AL) gene from *Conringia planisiliqua*, a plant tolerant for salt and drought stress. Using the RACE technique (Rapid amplification of cDNA end) and the roughly 187 bp long core fragments, a full-length cDNA (complementary DNA) with an open

Figure 9



RT-PCR analyses showed the amplification of *AtLTP1* and *LeCBF1* genes. (a): The amplification of *AtLTP1* bands at their expected sizes 368 bp;(b): The amplification of *LeCBF1* bands at their expected sizes 633 bp. (+): gene. (–): transgenic yeast cells for an empty pYES vector. M; ladder marker with 1 kb marker.

Table 3 Shows the mean proline values (OD_{520}) in transgenic and control yeasts at varioussalt concentrations (NaCl)

Salt concentration (M NaCl)	Negative control (–)	Positive control pYES2 (+)	*AtLTP1*	*LeCBF1*
0 M	1.5016 l	1.6153 j	1.647 ij	1.626 j
1.7 M	0.9733 n	1.2433 m	1.740 h	1.685 i
1.8 M	0.8266 o	0.9773 n	1.921 f	1.771 h
1.9 M	0.8160 o	0.9476 n	1.977 de	1.831 g
2.0 M	0.7306 p	0.8206 o	2.018 cd	1.968 e
2.1 M	0.5980 r	0.6583 q	2.079 b	2.033 c
2.2 M	0.5016 s	0.6280 qr	2.122 b	2.081 b
2.3 M	0.3866 t	0.5310 s	2.245 a	1.546 k

Numbers with the same letters do not differ appreciably at 1%.

reading frame of 735 bp was produced. The protein that this cDNA encodes is made up of 245 amino acids. And Yang and colleagues [26] discovered and cloned (TtLEA2-1) a specific *LEA* gene that was expressed during salt stress. From 'Y1805' Sequence analysis showed that TtLEA2-1 contained a 151-amino-acid protein with the highest level of sequence similarity (77.00%) to *Thinopyrum elongatum* was encoded by the 453-bp open reading frame of TtLEA2-1. Compared with wild type seedlings, wheat '1718' plants with overexpression of TaLEA2-1 grew taller, had stronger roots, and had increased catalase activity. The authors suggested that the *TaLEA2-1* gene may be useful for agricultural genetic engineering because it improves transgenic wheat's salt tolerance.

The yeast *Saccharomyces cerevisia*e has been employed to study the expression and function of *AtLTP1* and *LeCBF1* genes for salt stress tolerance, because it possesses a number of well conserved mechanisms that mediate the salt stress response. The data obtained in this study agree with Li and colleagues [27] cloned the transcription factor *SlDREB* from the cultivated tomato M82 and discovered that it affects tomato plant architecture negatively and increases drought and salinity tolerance. Gangadhar and colleagues [28] investigated that expression analysis of *StnsLTP1* gene showed differential expression under heat, water deficit and salt stresses. In this regard, Awaly and colleagues [29] tested the transgenic yeast carrying *nhaS2* and *nhaS4* for their capacity to endure salt stress situations as well as their development response to rising sodium chloride levels (0, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, and 2.3 M) over the course of seven days. The yeast growth was evaluated at OD600 nm the transgenic yeast and the control

showed clear disparities in the results. Additionally, Sun and colleagues [30] confirmed the effectiveness of *PePIP2;7* in preserving the equilibrium of hydraulic conductivity and water using a yeast expression system. After a 10^{-7} dilution, the original INVSc1 cells and transgenic yeast cells carrying the pYES2-PePIP2;7 or pYES2 vector could both grow well on the YPD medium. On the YPD medium supplemented with 4% PEG6000 (Polyethylene glycol), the cells harbouring the pYES2 vector only grew until 10^{-5} dilution, but those harbouring pYES2-PePIP2;7 grew well until 10^{-7} dilution. A similar outcome was obtained using the YPD medium in addition to 200 mM NaCl. However, in the YPD medium with 4% polyethylene glycol and 200 mM sodium chloride, none of the INVSc1 yeast cells could grow. According to these findings, they concluded that, *PePIP2;7* can promote drought and salt tolerance by preserving the equilibrium between water and hydraulic conductivity of yeast cells.

To detect the transgene transcript in the transcriptome of transgenic yeast, reverse transcriptase-PCR (RT-PCR) is commonly used. the expression of both genes was detected in transgenic yeast at the expected size for *LeCBF1* gene (633 bp) and *AtLTP1* gene (368 bp). The results agree with those of Awaly and colleagues [29] who evaluated the expression of the *nhaS2* and *nhaS4* alleles in transgenic yeast, the results showed that, the genes successfully amplified at 1623 bp and 1233 bp in the transgenic yeast using the specific *nhaS2* and *nhaS4* primers, these results indicate that the *nhaS2* and *nhaS4* genes are expressed in the transgenic yeast after exposure to salinity tolerance. In addition, Cai and colleagues [31] used 2-week-old CS wheat seedlings to study the expression of *TabZIP6* transcripts under various stressors. These seedlings were exposed to 200 mM NaCl, 16.7% PEG6000, or 4°C for 0, 15 min, 2, 6, 12, and 24 h. The results indicated that the RT-PCR of the *TabZIP6* transcripts were substantially activated at 4°C. While there were no evident alterations following salt or PEG stress. Also, Karthik and colleagues [32] modified soybean cv. *PUSA 9712* by direct organogenesis with marker-free construct of *p68* gene *via Agrobacterium*-mediated genetic transformation to address the salt stress faced by commercially significant soybean crop. Reverse transcriptase-PCR (RT-PCR) was used to test the potential transgenic plants, revealed that three of the five southern positive (T1) plants expressed the p68 gene. While nontransformed (NT) plants could not withstand irrigation with more than 150 mM of NaCl, the transformed (T1) soybean plants were able to withstand irrigation with up to 200 mM. In contrast Wang and colleagues [33] indicate that the expression of *Th-LTP1*, *Th-LTP2*, and *Th-LTP3* genes of *Tamarix hispida* was repressed by NaCl but induced by NaHCO3. Moreover Moraes and colleagues [34] found that *LTP18, LTP23*, and *LTP26* genes were negatively correlated with the response to salinity. Zhang and colleagues [35] discovered that the tomato *LeCBF1* gene could be induced by cold. *LeCBF1-3* transcripts did accumulate in response to mechanical agitation, similar to *Arabidopsis CBF1-3*, but not in response to drought, ABA, or high salinity.

An essential role is played by the amino acid proline in plants. It shields plants from various challenges and aids in their quicker recovery after experiencing stress. Our results revealed that the proline concentration increased considerably when the salinization level was raised. In this respect, the rice variety (*dhan54)* displayed greater salt tolerance with a higher level of proline accumulation Hasanuzzaman and colleagues [36]. Our results agree with those of Awaly and colleagues [29] regarding the proline. They stated that the proline accumulated in transgenic yeast expressing the salt-tolerant genes (*nhaS2* and *nhaS4*) under various NaCl concentrations. The findings showed that increasing the salinization level considerably boosted the intracellular proline accumulation in the transgenic yeast. Moreover, In *S. cerevisiae* strains, Takagi and colleagues [37] studied the function of intracellular proline during ethanol treatment stress. They found that, the (put1) mutant strain, which lacks proline oxidase, accumulated proline significantly in the stationary phase when grown in YPD media. Also, Skrzeszewska and Rokicka [38] analyzed the growth and symbiotic relationships of 1-year-old *Populus nigra 'Italica'*, under conditions of water shortage and soil salinity (50 mM, 150 mM, 250 mM) of NaCl. As a result of increasing soil salinity, leaves contained more sodium and chlorine but less potassium, magnesium, calcium, phosphorus, and nitrogen. Significant proline accumulated in the leaves suggests that *P. nigra 'Italica'* was subjected to salt stress after being treated with 250 mM sodium chloride, and that proline contributed to the plant's defense responses. In another study, Nguyen *et al.* [39] investigated at 150 mM NaCl for 14 days, the relationship between proline accumulation and salt tolerance in *O. australiensis* seedlings as well as those of the salt-sensitive and salt-tolerant rice cultivars *Nipponbare* and *Pokkali*. At a relatively early stage of salt stress, *O. australiensis* was able to rapidly accumulate free proline and had a lower osmotic potential. The qRT-PCR results also revealed that

O. australiensis wild rice increased the proline synthesis genes *OsP5CS1*, *OsP5CS2,* and *OsP5CR* while suppressing the expression of the proline degradation gene *OsProDH*. These findings imply that *O. australiensis* wild rice can use a rapid accumulation of free proline as a coping mechanism for salinity stress.

Conclusion

In conclusion, in the current experiment the two genes, *AtLTP1* and *LeCBF1* were successfully cloned and expressed in *S. cerevisiae,* as a model eukaryotic organism, which has been used to demonstrate the effectiveness of these genes in granting salt tolerance to eukaryotic species. Because of this, the current findings point to the potential for using these genes to create transgenic salt-tolerant plants, which would increase the amount of land that can be used for farming and provide food for the world's expanding population.

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Conflicts of interest

The authors state they have no competing interests.

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