Transgenic Rice Overexpressing anti-Apoptosis Gene Related with Drought Stress Tolerance and endows to multiple stress tolerance

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Identification of a drought-induced rice gene, that suppresses Bax-induced cell death in yeast

Introduction

- Abiotic stress is the primary cause of crop loss worldwide, reducing average yields for omit major crop plants by more than 50 % (Boyer et al., 1982) .
- This environmental insult leads to a series of morphological, physiological, biochemical and molecular changes that adversely affect plant growth and pro ductivity (Gorantla et al., 2007)
- Abiotic stresses act as messenger can generate reactive oxygen species (ROS) molecules that function at the early stage in signal regulation, stress adaptation and programmed cell death (PCD)(Maki et al., 2005).
- PCD or apoptosis is a cellular suicide process commonly found in organisms that is important for the development and adaptation to environmental stresses (Hulckelhoven, 2004)

Introduction

Cell Death and Differentiation (2011) 18, 1271–1278

www.nature.com/cdd

Review

Bax inhibitor-1: a highly conserved endoplasmic reticulum-resident cell death suppressor

T Ishikawa[1,2,4], N Watanabe[3,4], M Nagano[1], M Kawai-Yamada[*,1,2] and E Lam[*,3]

- Among the important regulators of PCD, BCL2-associated x protein (Bax) has received a great deal of attention as a pro-PCD factor

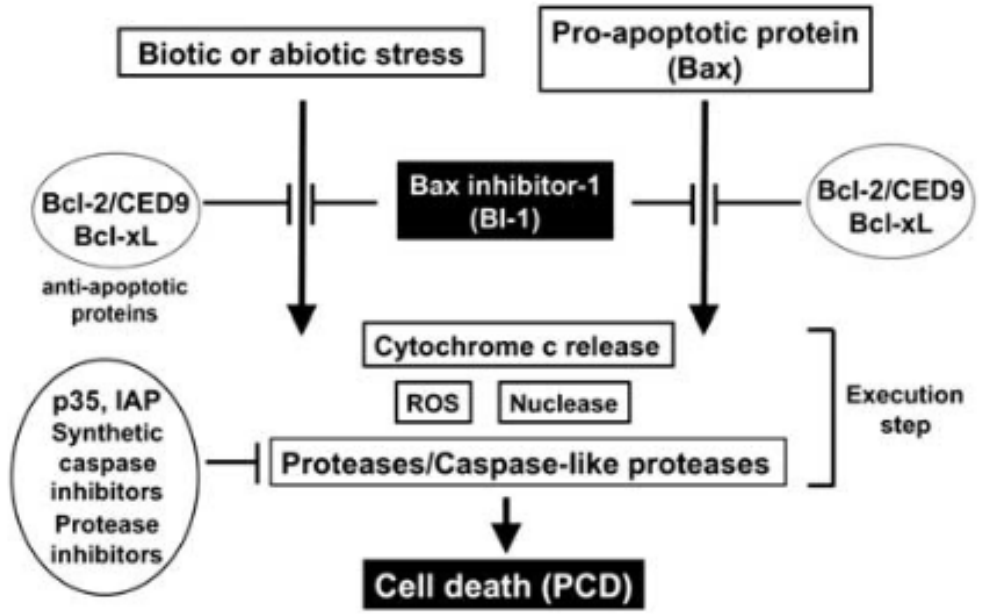


Figure 1 Schematic representation of the effects of heterologously expressed animal and viral PCD regulators and involvement of BI-1 in the control of plant PCD pathway. See text for explanation

Introduction

- Bax inhibitor (BI-1) was found in both animals and plants. Further, over expression of BI-1 can inhibit the activity of Bax protein in plants and yeast, BI-1 has been identified in Arabi dopsis , tobacco and , barley (Eichmann et al., 2004).
- Overexpression of pepper BI-1 in tobacco conveyed multi tolerance to several abiotic stresses (Isbat et al., 2012)
- The gene that acts to prevent apoptosis in plants, might also play role in adaptation mechanisms to various stress tolerance

Introduction

- Most interesting use this strategy to identification gene can direct identify using yeast functional screening. The yeast system has been extensively employed as a model for the genetic analysis of complex pathways and processes

- In this study, we investigate Oryza sativa genes derived from drought stress treatment used yeast functional screening that correlates with anti-apoptosis mechanisms.

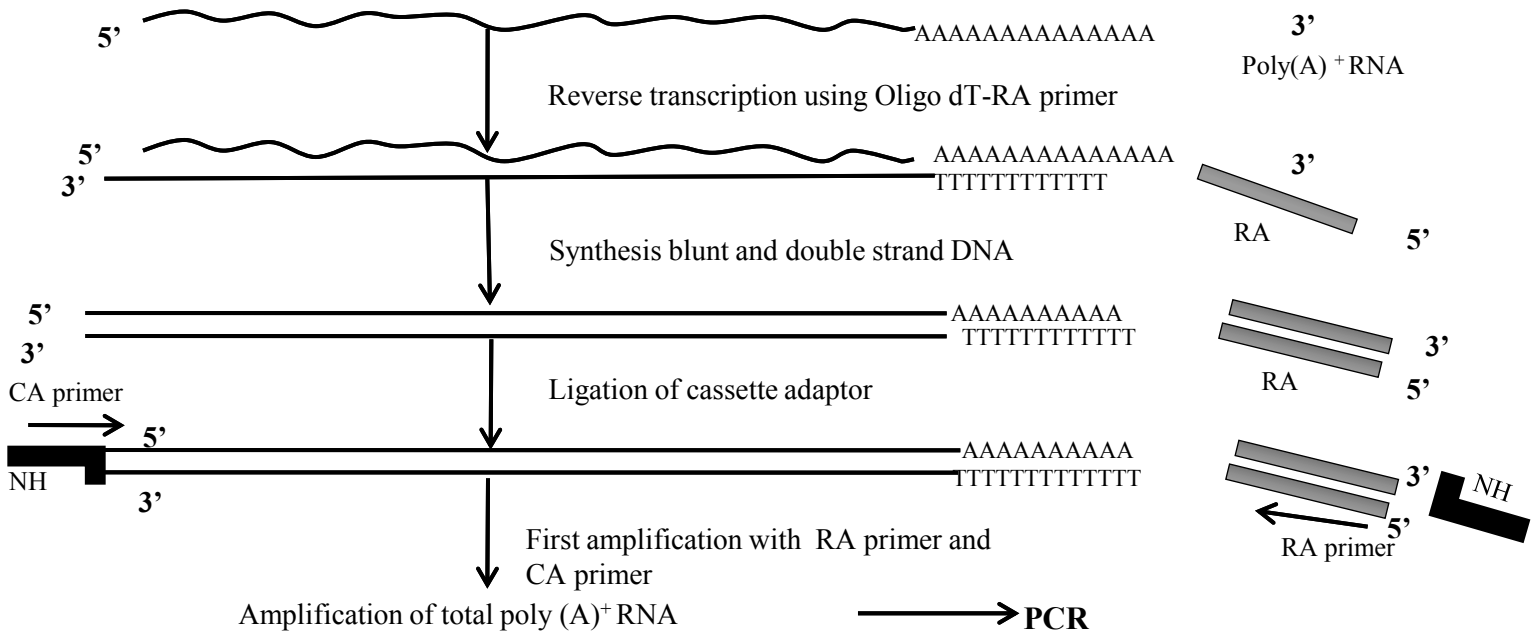
Materials and Method

Design cDNA

cDNA synthesis method using oligo dT-primer

Amplification cDNA by cDNA PCR library method using two primer adaptor

1. oligo dT-RA primer (containing ER site *Xho I*)
2. CA *EcoR I* cassete adaptor (containing ER site *EcoR I*)



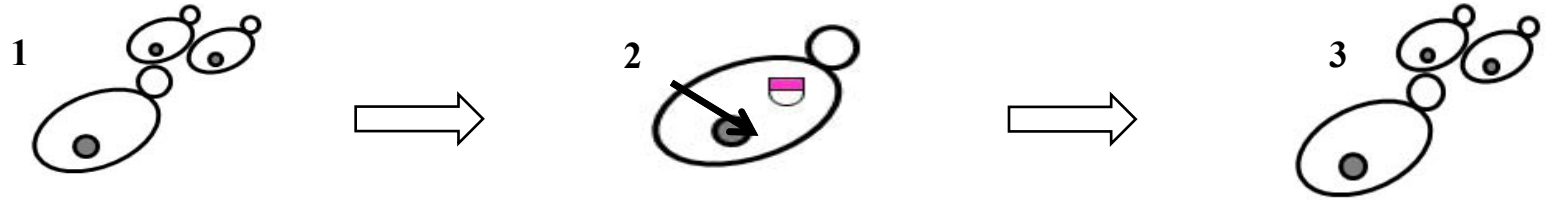
Materials and Method

cDNA construct in the vector pYX112

DNA derived from rice young leaves was inserted into the EcoRI and XhoI sites of vector pYX112 (Ingenius, Wisbaden, Germany), carrying an ARS/CEN Replicon,

Materials and Method

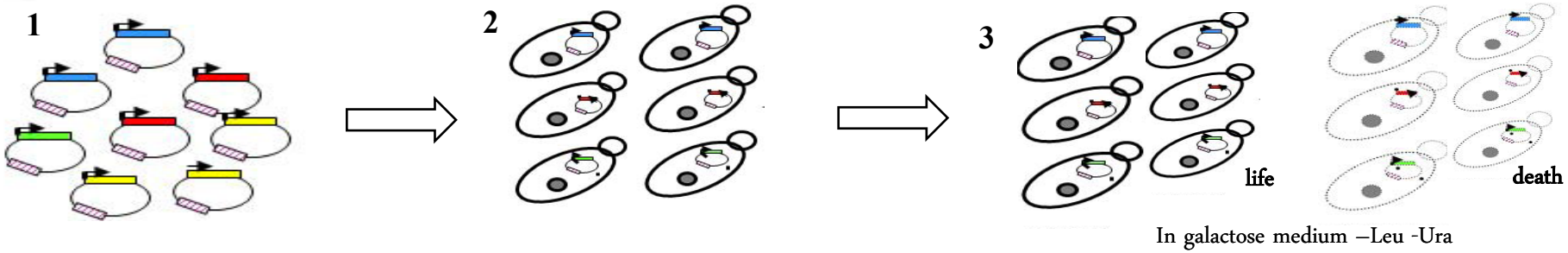
Principle of yeast screening



Yeast BF264-15Dau

Transformed Yeast with YEp 51 (BAX with Gal1 pro Leu marker)

Yeast named QX95001



cDNA libraries in PY112 Ura3 marker

Transformed QX95001

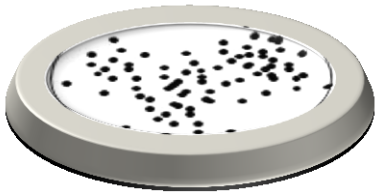
Repressed BAX protein

No repressed BAX protein

Materials and Method

Screening yeast using replica printing method

1



Yeast QX95001 transformed

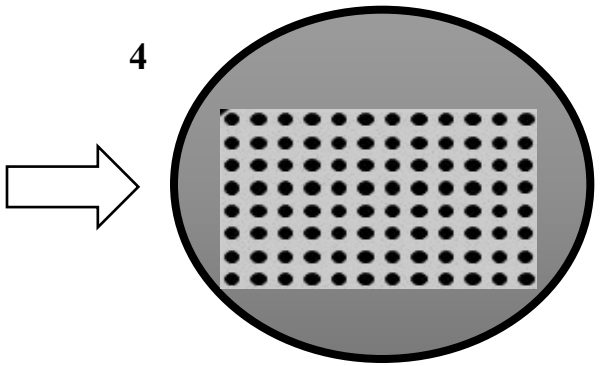
2

96-well microtitre plates

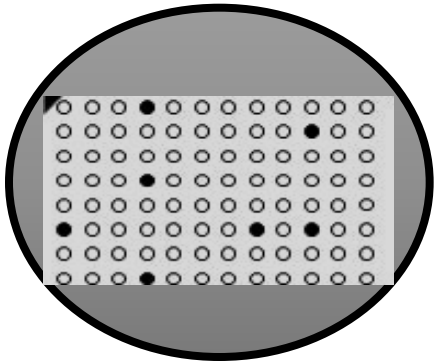
3

96 pin-replicator

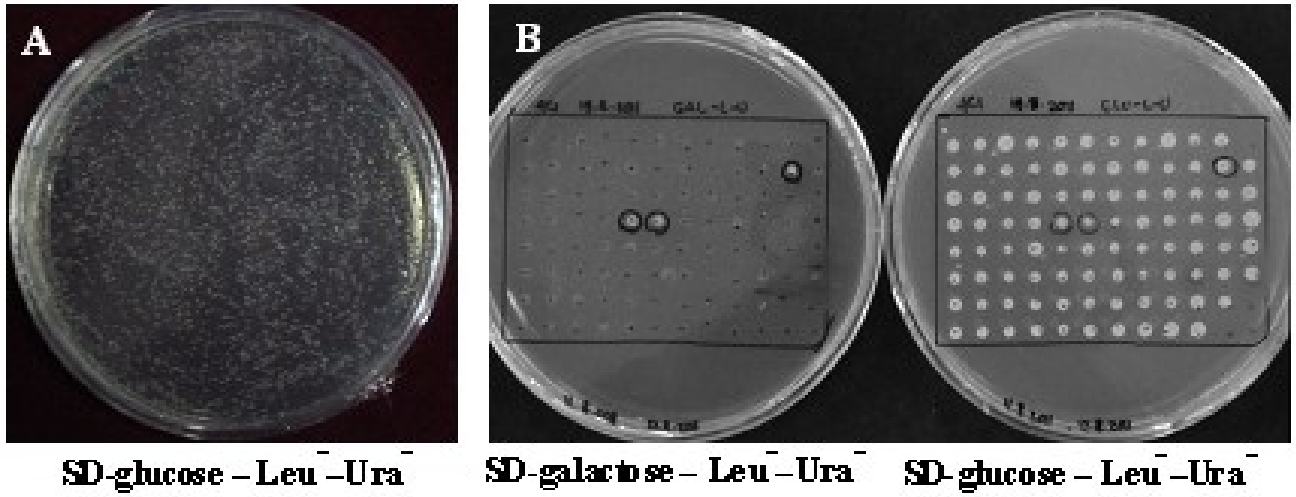
4



SD-glucose – Leu $^-$ Ura $^-$



SD-galactose – Leu $^-$ Ura $^-$



The picture represents a replica plate demonstrating the ability to isolate yeast transformants cultured in SD-galactose–Leu⁻-Ura⁻ and SD-glucose–Leu⁻-Ura⁻ medium. Yeast transformants expressing genes derived from rice tissue libraries demonstrating that yeast survive in SD-galactose–Leu⁻-Ura⁻ medium.

Results and Discussion

Protein prediction from cDNA libraries positive clone at 4h drought stress treatment

No	Clone ID	Homology Protein			Accession number
		Protein prediction	Identity	Match	
1.	R4H25	Putative uncharacterized protein Oryza sativa subsp. indica	75 %	56 AA	EMBLABR26061.1 www.uniprot.org
2.	R4H 36	Retrotransposon proteinOryza sativa subsp. Indica	88.0%	109AA	EMBL ABR26094.1 www.uniprot.org
3	R4H1026	putative uncharacterized protein Oryza sativa subsp. Indica	66.0%	56AA	EMBL ABR25604.1 www.uniprot.org
4	R4H95	unidentified	-	-	-
5	R4H 873	unidentified	-	-	-
6	R4H882	unidentified	-	-	-
7	R4h251	Putative uncharacterized protein Oryza sativa subsp. indica	100.0%	55AA	EMBL ABR25604.1 www.uniprot.org
8	R4H68	Thiol protease aleurain Oryza sativa subsp. Indica	94.0%	35	EMBL ABR25942.1 www.uniprot.org

Results and Discussion

Protein prediction from cDNA libraries positive clone at 8h drought stress treatment

No	Clone ID	Homology Protein			Accession number
		Protein prediction	Identity	Match	
1	R8H90	Cell wall-associated hydrolase, partial(Medicagotruncatula)	44.0%	151AA	EMBL AES84212.1 www.uniprot.org
2	R8H501	Not identified	-	-	-
3	R8H449	50S ribosomal protein L16, chloroplastic(Oryza sativa)	96.0%	27AA	HAMAP MF_01342 www.uniprot.com
4	R8C395	Not identified	-	-	-
5	R8H390	50S ribosomal protein L1(Oryza sativa)	100%	27AA	HAMAP MF_01342 www.uniprot.com

Results and Discussion

Protein prediction from cDNA libraries positive clone at 12h drought stress treatment

No	Clone ID	Homology Protein			Accession number
		Protein prediction	Identity	Match	
1	R12H190	Not identified		-	-
2	R12H389	Not identified	-	-	-
3	R12H900	zinc inducible protein (Oryza sativa japonica Group)	81.0%	58AA),	EMBLADM86848.1 www.uniprot.com)
4	R12H89	Tar1p Medicago truncatula (Barrel medic)	78.0%	100AA	EMBL AES97349.1 www.uniprot.com)
5	R12H2	Serine protease, subtilase family(Providenciarustigianii DSM 4541)	51.0%	31AA	EMBL EFB74095.1 www.uniprot.org
6	R12H941				
7	R12H780	Putative senescence-associated protein Liliumlongiflorum (Trumpet lily)	73.0%	84AA	EMBL ABO20851.1 www.uniprot.org
8	R12H389	Putative uncharacterized protein Sorghum bicolor (Sorghum)	93.0%	108AA	EMBL EES20513.1
9.	R12H145	unidentified	-	-	-

Results and Discussion

Protein prediction from cDNA libraries positive clone at 24h drought stress treatment

No	Clone ID	Homology Protein			Accession number
		Protein prediction	Identity	Match	
1	R24H10	salt stress-induced protein (Oryza sativa Indica Group)	86.0%	33AA	EMBL ADM86855.1 www.uniprot.org
2	R24H84	Apocytochrome f(Oryza sativa Japonica)	98.0%	59AA	HAMAP MF_00610 www.uniprot.org
3	R24H12	Not identified	-	-	-
4	R24H866	Putative uncharacterized protein (Oryza sativa subsp. Indica)	71.0%	49AA	EMBL ABR25314.1 www.uniprot.org
5	R24H891	Not identified	-	-	-
6	R24H120	Cell wall-associated hydrolase, partial (Medicagotruncatula)	95.0%	62AA	EMBL AES84212.1 www.uniprot.org

Results and Discussion

protein prediction

Putative characteristic	Clones
Ribosomal protein	R8H390, R8H449
putative ancharacteristic Rice protein	R4H25, R4H1026, R4h251, R12H780, R24H866
Retrotransposon protein	R4H 36
Thiol protease aleurain	R4H68
zinc inducible protein	R12H900
Putative senescence-associated protein	R12H780
Salt stress-induced protein	R24H10
Tar1p	R12H89
Apocytochrome f	R24H84
Cell wall-associated hydrolase protein	R24H120
Putative uncharacterized protein Sorghum	R12H389
Serine protease, subtilase	R12H2
Unidentified	R4H95, R4H882, R4H882, R8H501, R12H190, R12H389,R12H145 , R24H12, R24H891

Results and Discussion

Sequencing of R12H780 and deduced amino acid

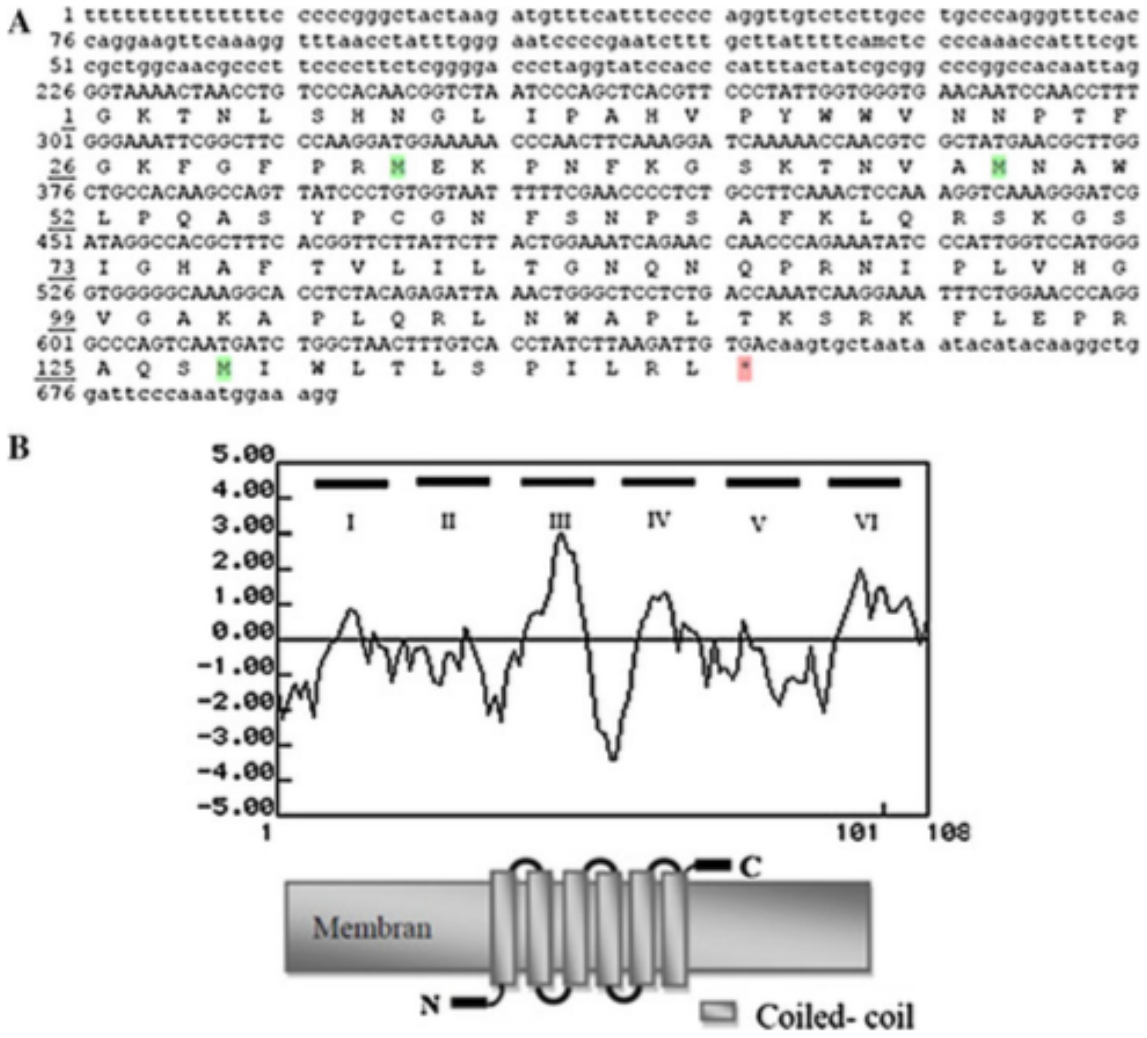


Fig. 1 Sequence analysis of R12H780 (accession no. AB734097). a Nucleotide and deduced amino acid sequences. The protein coding regions are in upper case letters and the 50- and 30-flanking regions are in lower case letters. The putative open reading frame is shown below the nucleotide sequence in one letter symbols of amino acids. Nucleotide numbers are on the left side and amino acid numbers are underlined. The putative termination codon is marked by an asterisk. b Hydrophobicity analysis, coiled coil protein structure. Hydrophobicity was estimated by the method developed by Kyte and Doolottle, Trans- membrane domains were predicted by DNAsis program

Results and Discussion

Comparison deduced amino acid sequence R12H780 with other plant

```
1 Orryza sativa (R12H780)      **************************************************
2 Lilium longiflorum           **************************************************
3 Cupressus sempervirens       **************************************************IKLN 4
4 Zea mays                     **************************************************
5 Arabidopsis thaliana         **************************************************
6 Pisum sativum                TAGARIASSPDSDLEAFSHNPTHGSFAPLAFQPSAMTNCANQRFLSYYVE 50

1 Orryza sativa (R12H780)      *********GKTNLSHNGLIPAHVPYWWVNNPTFGKFGFPRMEKPNFKGS 41
2 Lilium longiflorum           *********GKTNLSHDGLIPAHVPYWWVNNPTLGEFCFTMIGRADIEGS 41
3 Cupressus sempervirens       YYRGADHQQGKTNLSHDGLNPAHVPYWWVNNPTLGEFCFTMIGRADIEGS 54
4 Zea mays                     ****************************************MIGRADIEGS 10
5 Arabidopsis thaliana         ****************************************MIGRADIEGS 10
6 Pisum sativum                LLLRHCHQWGKTNLSHDGLIPAHVPYWWVNNPTLGEFCFTMIGRADIEGS 100
                                                                        : :.:::**

1 Orryza sativa (R12H780)      KTNVAMNAWLPGASYPCGNFSNPSAFKLQRSKGSIGH*FTVLILTGNQNQ 90
2 Lilium longiflorum           KSNVAMNAWLPQASYPCGNFSDTSSFKLRRSKGSIGHAFTVRIR****** 85
3 Cupressus sempervirens       KSNVAM*AWLPQASYPCGNFSDTSSFKFRKSKGSISHAFTVCIR****** 97
4 Zea mays                     KSNVAMNAWLPQASYPCGNFSDTSSFKLRRSKGSIGHAFTVRIRTGNQNQ 60
5 Arabidopsis thaliana         KSNVAMNAWLPQASYPCGNFSDTSSFKFRRSKGSIGHAFTVRIRTENQNQ 60
6 Pisum sativum                KSNVAMNAWLPQASYPCGNFSDTSSFKFRSLKDRLATLSRFVFVLEIRIK 150
                               *:**** **** *********:.*:**::  *. :.    . :

1 Orryza sativa (R12H780)      PRNIPLVH***GVGAKAPLQRLNW*******APLTKSRKFLEP*R*AQSM 128
2 Lilium longiflorum           **************************************************
3 Cupressus sempervirens       **************************************************
4 Zea mays                     TSFYPFVPHEISVLVELILGHLRYLLTDVPPQPNSPPDNVFRPDRPGEAG 110
5 Arabidopsis thaliana         TSFYPFVPHEISVLVELILGHLRYLLTDVPPQPNSPPDNVLRPDRPAEAS 110
6 Pisum sativum                RAFTLLFHTRFLFSLSSSLGHLRYLLTDVPPQPNSPPDNVFRPDRPTKVS 200

1 Orryza sativa (R12H780)      IW**********************************LTLSPILRL 139
2 Lilium longiflorum           **************************************************
3 Cupressus sempervirens       **************************************************
4 Zea mays                     PWSQKEG**RCP*****************************ASDPRNK* 127
5 Arabidopsis thaliana         LGTKNRG**CTPPPIHGVSKITLKVVVFHLRRSSHLSYTSQVISQSRTRV 158
6 Pisum sativum                LWIKKEGQRRPPDSRNKLNNVKSSGISTFAVSSSHLSYTSQVISQSRTRV 250
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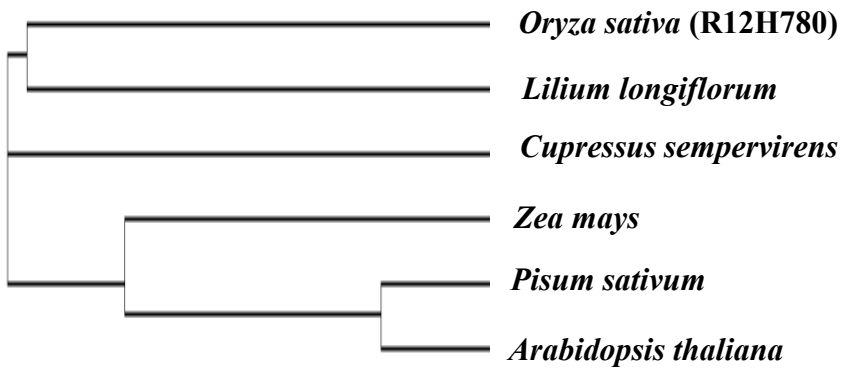


Fig. 2 Comparison of the deduced amino acid sequence of R12H780 with the putative senescence associated protein number of the amino acid residues is shown to the right of each sequence. Bootstrap analysis of the phylogeny using Clustal W. Branch length was drawn to scale and numbers indicate frequencies of the same result. Accession numbers are as follows: L. longiflorum (EMBL ABO20851), C. sempervirens (EMBL ACA30301.1), Z. mays (EMBL ACR36970.1), A. thaliana (EMBL BAF01964), and P. sativum (EMBL BAB33421)

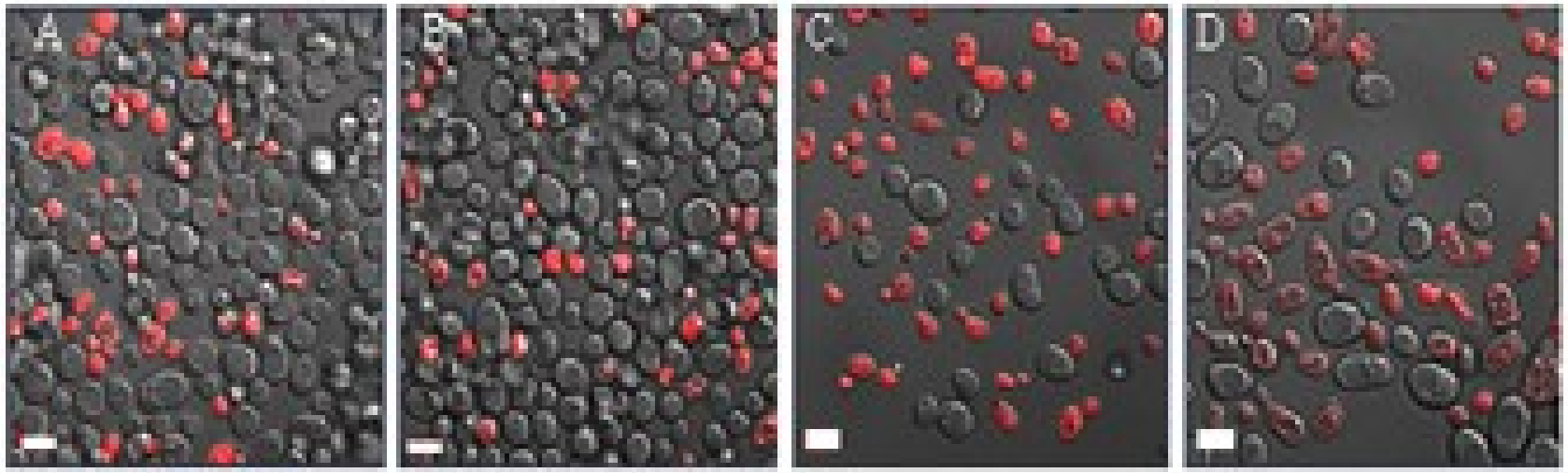


Figure S5. Yeast cell growth defect factor analysis in SD-galactose-Leu$^-$-Ura$^-$ medium determined based on the OD600. Visualization of yeast cell growth in SD-galactose-Leu$^-$-Ura$^-$ medium after 72 h. Red colors show cell death as evaluated by Evan's blue staining (0.05%). Samples were observed by confocal microscopy in conjunction with the RFP program. A: AtBI-1 with a death rate of 11.5%. B: R12H780 with a death rate of 7.8%. C: pYX112-Bax with a death rate of 74%. D: pYX112 vector alone with a death rate of 67%. The white bar is 2 μm.

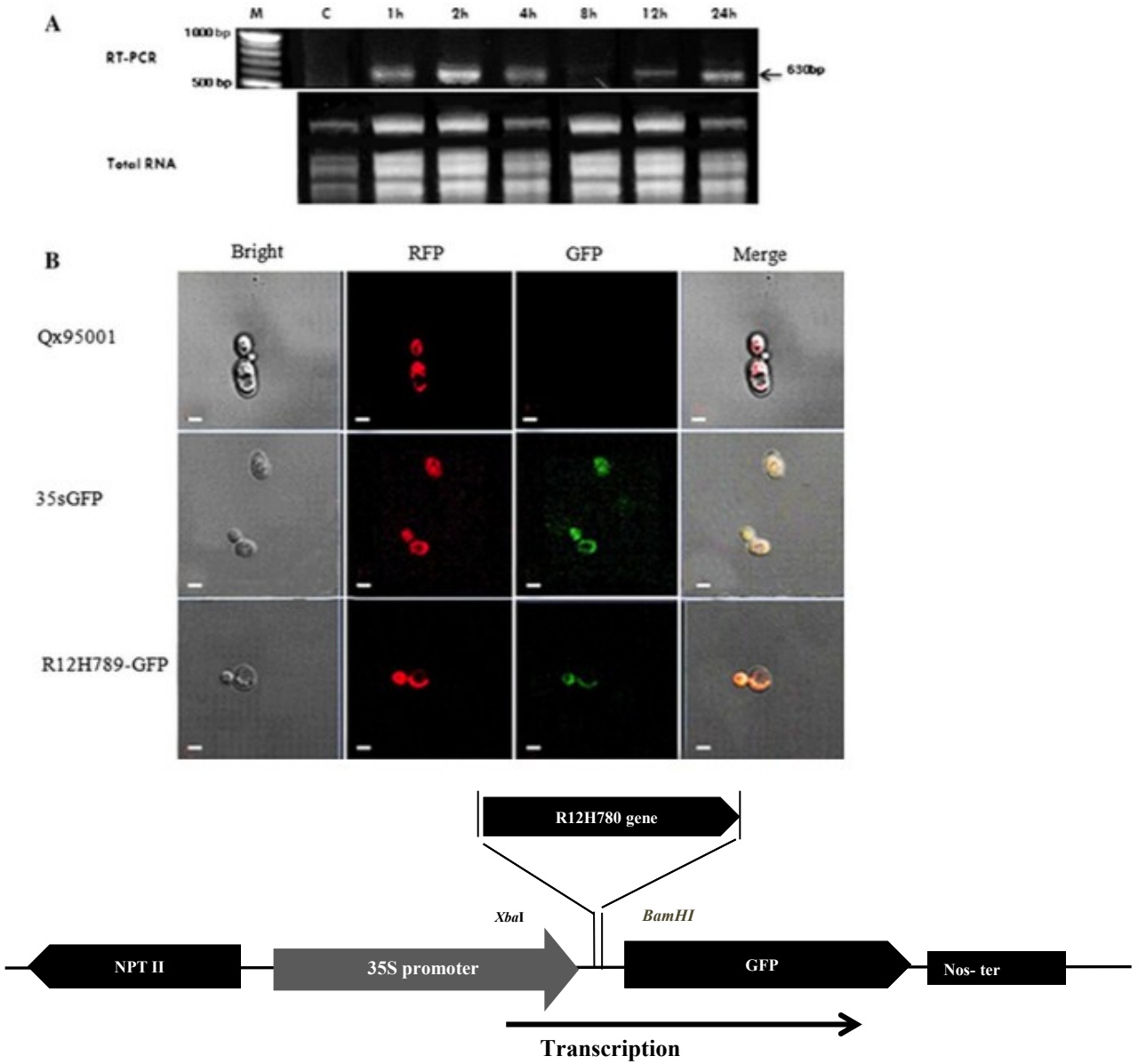
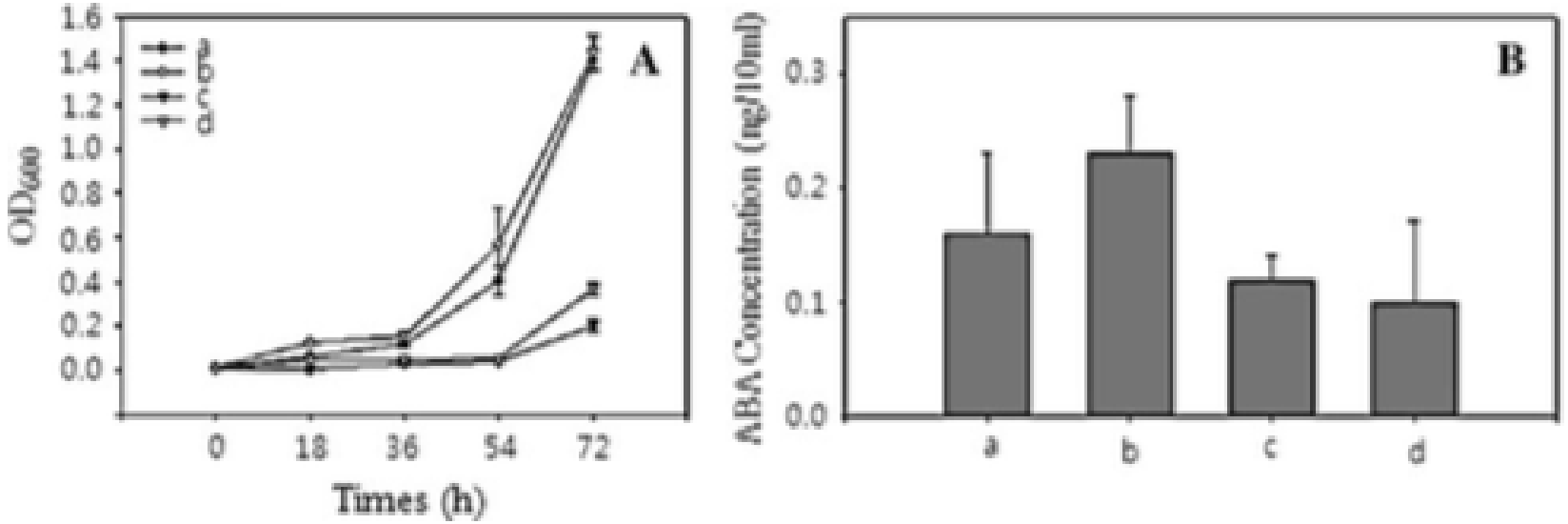


Fig. 3 Analysis of R12H780.
a Effect of drought stress treatment on the level transcript R12H780 on rice total RNA was isolated from the leaf, analysis with RT-PCR. b Distribution of GFP and R12H780-tagged GFP in yeast. Yeast cells cultured for 36 h in SD-glucose-Leu--Ura- medium were imaged at 488-nm to detect GFP. Red represents mitochondrial staining and green is GFP accumulation. The white bar is 2 lm. (Color figure online)

Results and Discussion



Cell growth and viability assays. a Yeast cell growth defect factor analysis in SD-galactose-Leu--Ura- medium determined based on the OD600. Visualization of yeast cell growth in SD-galactose- Leu--Ura- medium after 72 h. Endogenous ABA isolated from yeast (OD1.3) transformed with the AtBI-1 gene, R12H780, pYX112-Bax, and vector alone. a pYX112-AtBI-1, b pYX112-R12H780, c pYX112-BAX, d pYX112

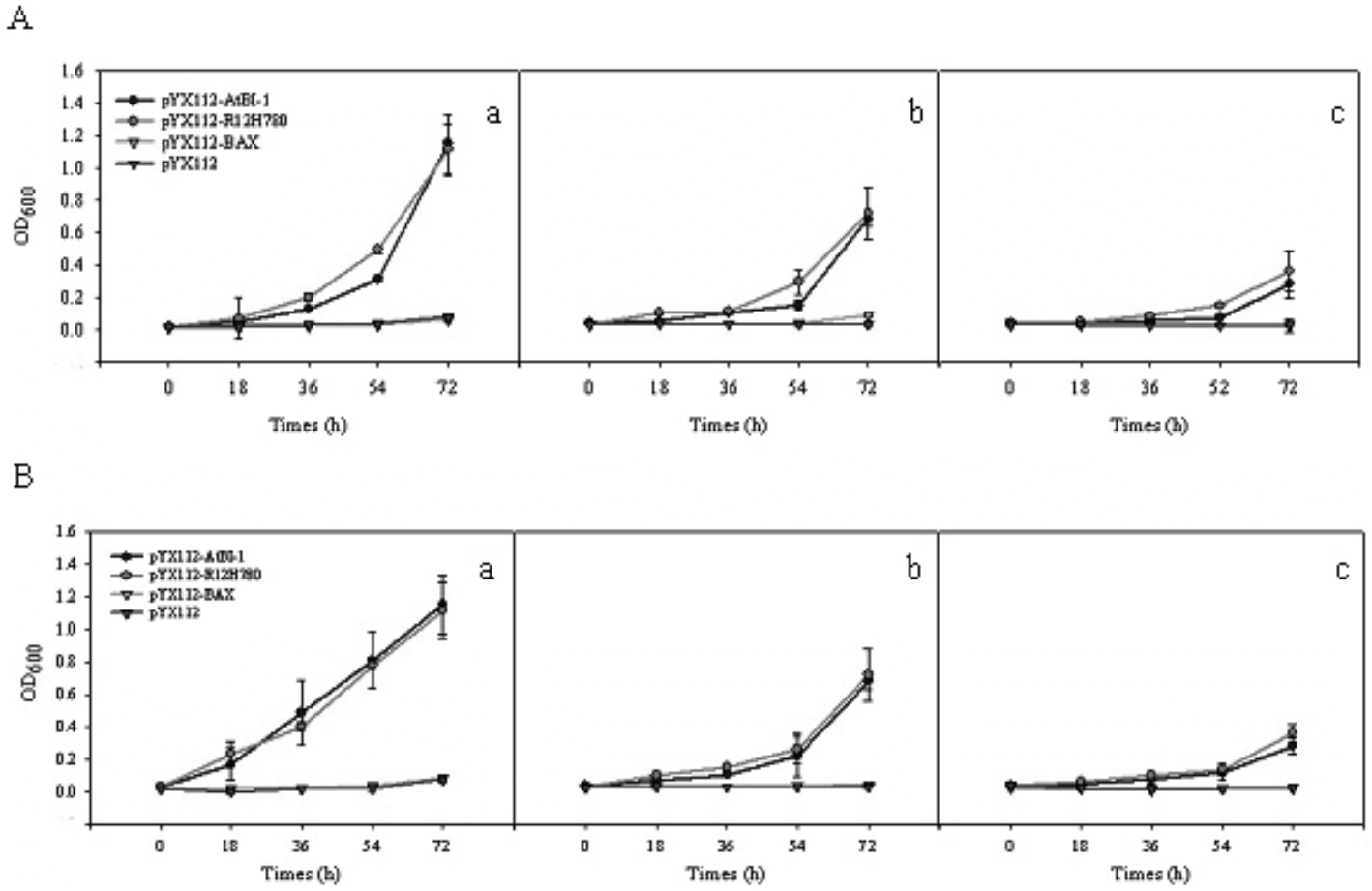


Figure S6. Cell growth and viability assays, PEG and NaCl treatment. A: Effect of PEG on cell division in yeast. OD_{600} of cells incubated on SD-galactose-Leu$^-$-Ura$^-$ during 72 h. a: yeast division on SD-galactose-Leu$^-$-Ura$^-$ medium containing 5% PEG. b:10% PEG. c: 15% PEG. B: Effect of NaCl on cell division in yeast. OD_{600} of cells incubated on SD-galactose -Leu$^-$-Ura$^-$ during 72h. a: yeast division on SD-galactose -Leu$^-$-Ura$^-$ medium containing 50 mM NaCl. b: 100 mM NaCl. c: 200 mM NaCl.

Alteration of Hormone Levels in Transgenic Rice Overexpressing anti-Apoptosis Gene Related with Drought Stress Tolerance

Introduction

- Overexpression of BAX inhibitor-1 BI-1 in conveyed multi tolerance to several abiotic stresses (Isbat *et al*., 2009).
- OsSAP represents a new type of Bax suppressor related gene and endows multiple stress tolerance in yeast (Ubaidillah *et al*., 2013).
- CaMSRB2 confers drought tolerance to rice, as evidenced by less oxidative stress symptoms and a strengthened PSII quantum yield under stress conditions, Methionine oxidation could be a common protein posttranslational modification, MSRB might play a critical role in enzyme recovery by reducing the oxidized proteins (Kim et al 2013)

Introduction

Abiotic stress and phytohormone

- Plant hormones have pivotal roles in plant growth, development and response to biotic and abiotic cue (Pan *et al*., 2010).
- Plant hormones function as central integrators that link and re-program the complex developmental and stress adaptive signaling (Golldack *et al.*, 2014).
- The phytohormone functions as a key regulator in the activation of plant cellular adaptation to drought and salinity and has a pivotal function as a growth inhibitor (Raghavendra *et al*., 2010; Weiner *et al*., 2010).

Introduction

Abiotic stress and phytohormone

- Plant hormones do not act independently, and extensive synergistic or antagonistic interaction between hormonal pathways is observed in development and stress responses after exogenous application, or through mutant analysis (Wolters and Jurgens, 2009).

Plant Cell Rep
DOI 10.1007/s00299-013-1409-2

REVIEW

Gibberellins and abscisic acid signal crosstalk: living and developing under unfavorable conditions

Dortje Golldack · Chao Li · Harikrishnan Mohan ·

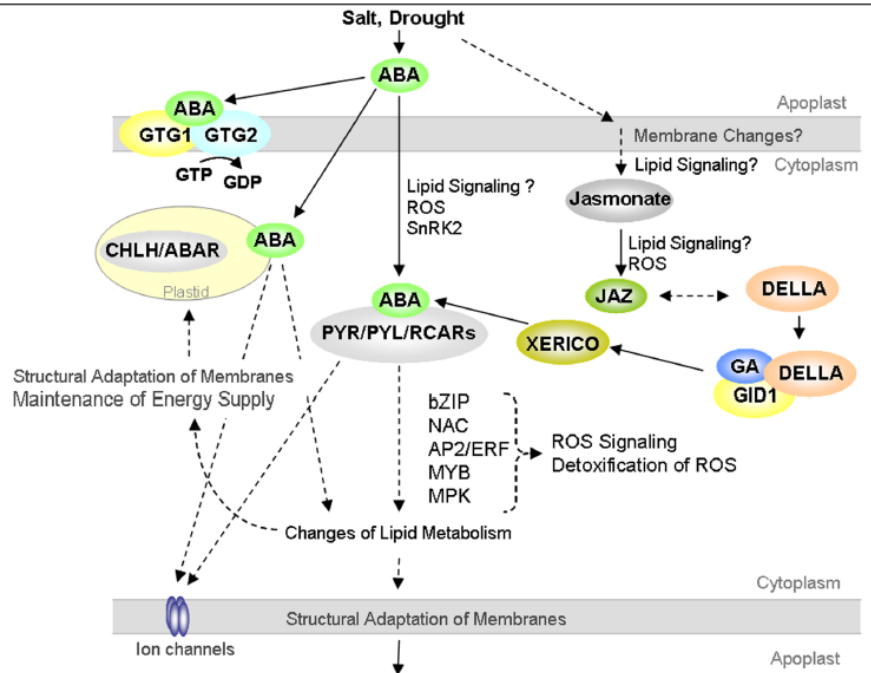


Figure 1. Proposed model on crosstalk of abscisic acid (ABA), gibberellic acid (GA), and jasmonate signalling in plant cellular responses to the abiotic stressors drought and salt.

Golldack. D, *et al*., 2014.

frontiers in
PLANT SCIENCE

REVIEW ARTICLE
published: 19 May 2014
doi: 10.3389/fpls.2014.00207

Enhancing crop resilience to combined abiotic and biotic stress through the dissection of physiological and molecular crosstalk

*Christos Kissoudis, Clemens van de Wiel, Richard G. F. Visser and Gerard van der Linden**

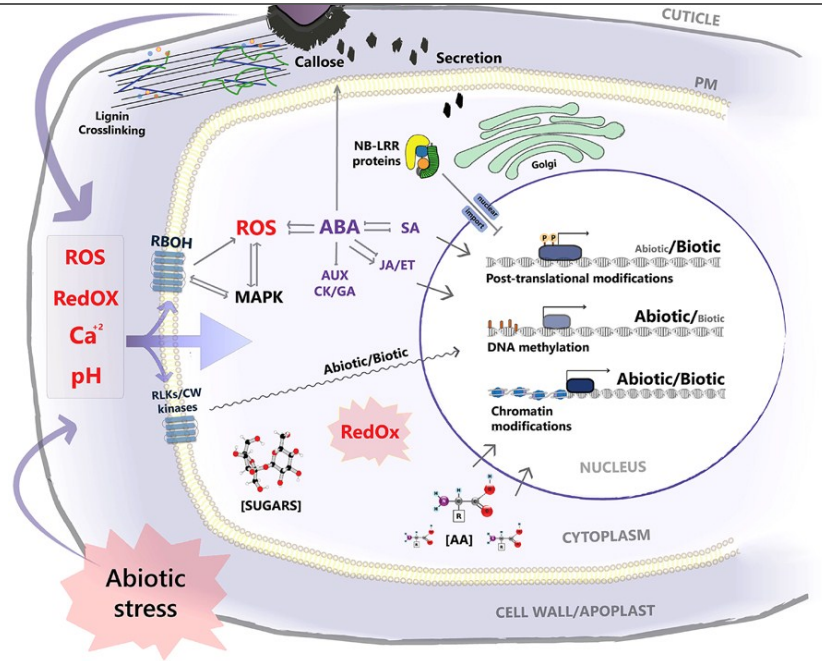


Figure 2. A scheme for the interaction interface and overlapping signaling pathways of abiotic and biotic stress at the cellular level.

Kissoudis. C *et al*., 2014

Introduction

Objectives

- This study was conducted to overexpress OsSAP and AtBI-1 which possesses anti-apoptosis activity, and chachracterization of transformans CaMsBr2 during drought and salinity stress
- Observation of plant hormones level in tranformants plant.

Materials and Method

- Plasmid construction for OsSAP and AtBI-1
- Transformation of OsSAP and AtBI-1 to rice
- Drought and salt tolerance analysis (Transformant rice) OsSAP , AtBI-1 and CaMsBr2
- Treatment of drought and salinity stress
- Polymerase Chain Reaction (PCR)
- Reverse-Transcriptase PCR (RT-PCR) and Quantitative Real Time-PCR (qRT-PCR)
- Quantitative analysis of major plant hormones Analysis of rice hormones using HPLC–electrospray ionization–tandem mass spectrometry (HPLC– ESI–MS/MS)

Materials and Method

Plasmid Construction

- The OsSAP encoding senescence associated protein and AtBI-1 were cloned into binary vector pBIN19 under the control of CaMV 35s (p35s) promoter in-frame using *Sal*I and *Nco*I.
- The *Agrobacterium* strain LBA4404 was used for transformation.

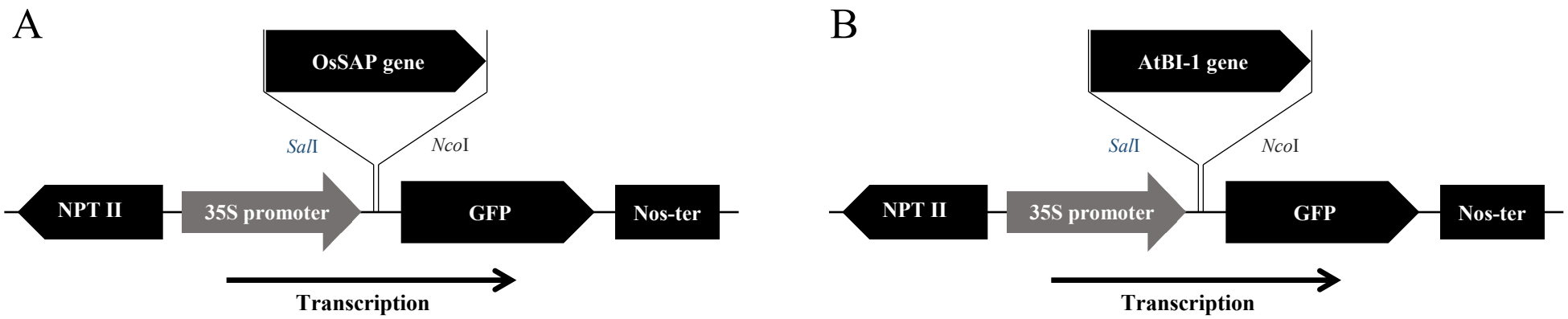


Figure 3. Plasmid construction for transformation to rice A: Construction of OsSAP, B: Construction of AtBI-1.

Materials and Method

- A pepper (Capsicum annuum) methionine sulfoxide reductase B2 gene (CaMSRB2) the CaMSRB2 gene was cloned into the pSB- Rab21

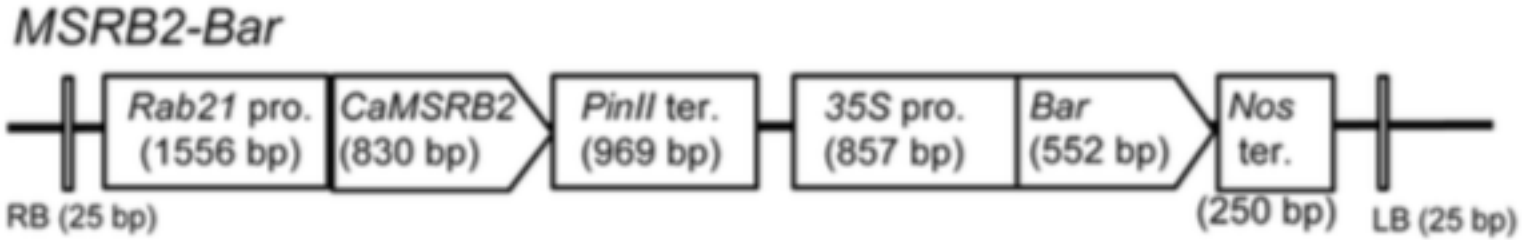
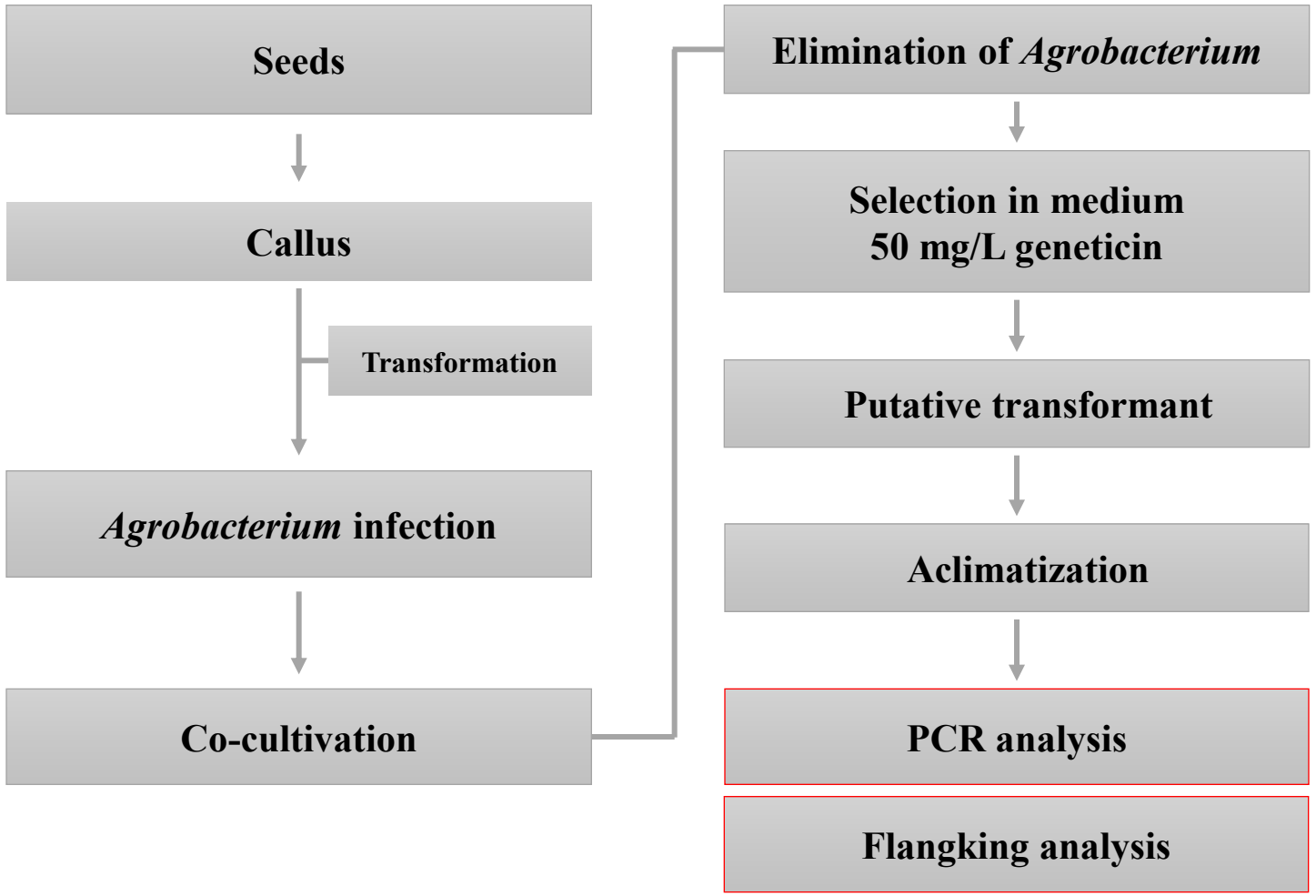


Figure 3. Plasmid construction for transformation to rice, Construction of CaMSRB2

Materials and Method

Rice transformation



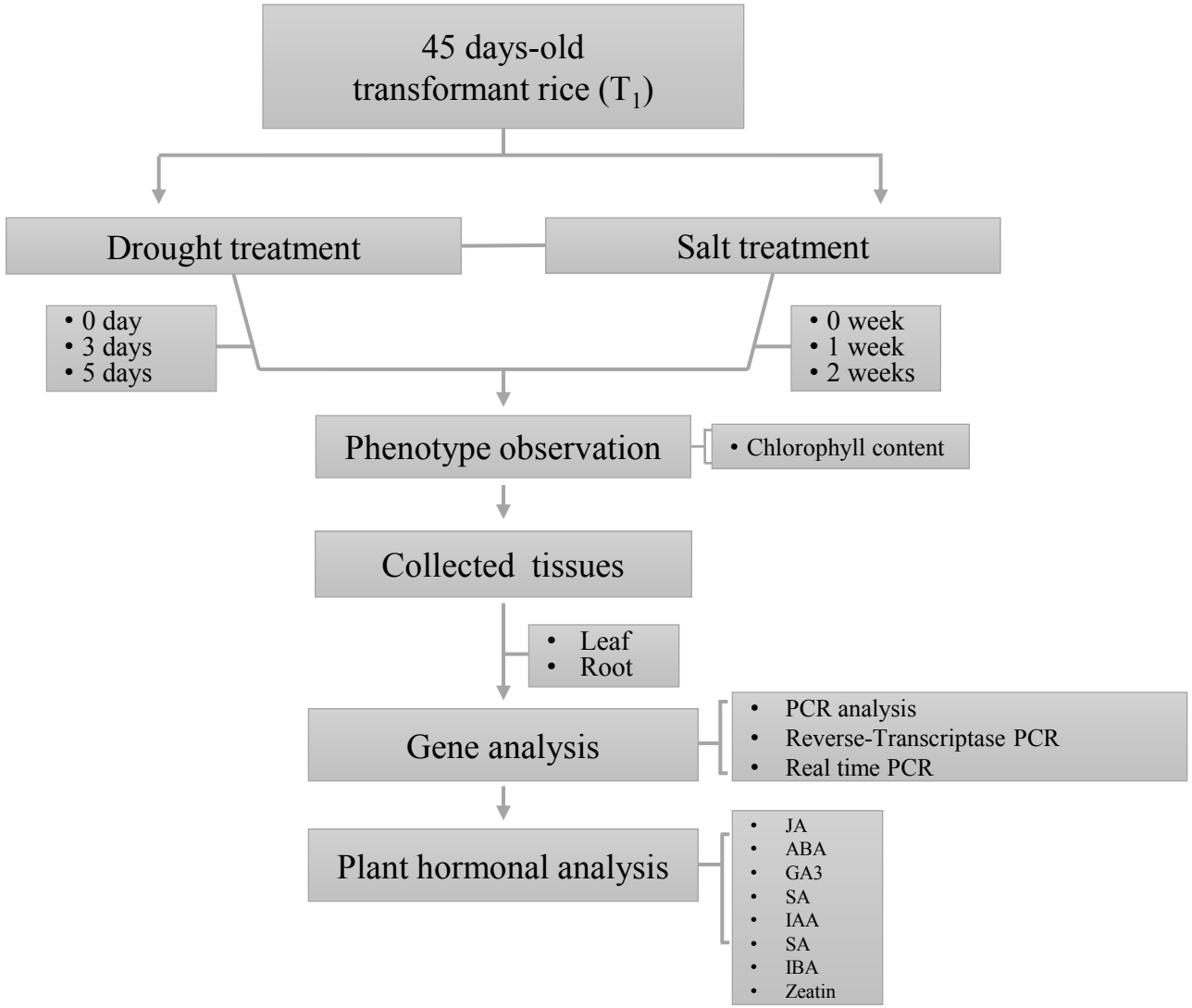
Materials and Method

Drought and salinity tolerance analysis with transformants

- Transformants (T_2) were planted in pot on nursery bed.
- Drought stress was conducted for 4 days on 45 days old transformants and non transformants by decanting all of remained water in the pot. The survival rate was measured 5 days after treatment.
- Salinity stress was conducted for 14 days on 45 days old transformants and non transfromants by NaCl addition to water until it reached the final concentration of 200mM.
- The unit of chlorophyll content was measured by chlorophyll meter SPAD502 (Japan).

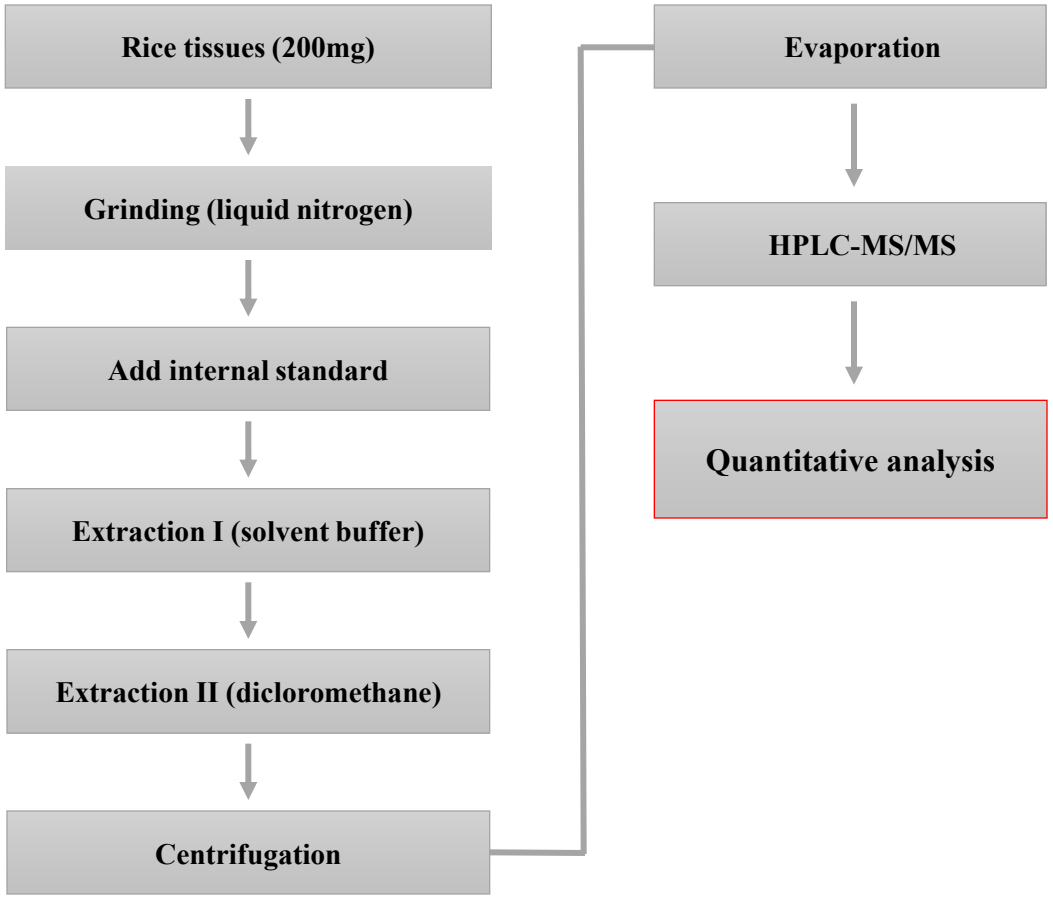
Materials and Method

Treatment of drought and salt stress



Materials and Method

Analysis of rice hormones using HPLC–electrospray ionization–tandem mass spectrometry (HPLC– ESI–MS/MS)



PROTOCOL

Quantitative analysis of major plant hormones in crude plant extracts by high-performance liquid chromatography–mass spectrometry

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Table 1. Selected reaction monitoring condition for protonated or deprotonated rice hormones (Pan *et al*. 2010)

PH	SM	Q1	Q3	Q2 (V)	RT[a]	IS	SM	Q1	Q3	Q2 (V)	RT[a]
GA3	-	345.1	142.7	-40	4.29	d_2-GA3	-	347.1	142.7	-40	4.31
ICA	-	160.0	115.8	-22	4.47	d_5-IAA	-	179.0	134.8	-14	53.22
IAA	-	174.0	129.6	-14	5.22	d_5-IAA	-	179.0	134.8	-14	5.22
ABA	-	262.8	152.6	-16	7.96	d_6-ABA	-	269.1	158.8	-16	7.98
SA	-	136.6	92.8	-24	9.45	d_6-SA	-	141.9	97.8	-22	9.48
IBA	-	202.0	157.5	-16	10.17	d_5-IAA	-	179.0	134.8	-14	5.22
JA	-	209.0	59.0	-24	10.44	H_2-JA	-	211.0	58.8	-24	11.94
Zeatin	-	220.0	136,2	29	2.91	d_5-Zeatin	-	225.2	136.2	29	2.92

ABA: 2-*cis*,4-*trans*-abscisic acid, d6-ABA: 2-*cis*,4-*trans*-abscisic acid-[2H6]ABA, d5-BA: benzoic acid-[2H5], acidαd2-GA3: [2H2]gibberellic acid, d2-GA4: [2H2] gibberellin A4, d5-IAA: indol-3-acetic-2,2, IAA: indole-3-acetic acid, IBA: indole-3-butanoic acid, ICA: indole-3-carboxylic acid, IS: internal standards, JA:jasmonic acid.

Results and Discussion

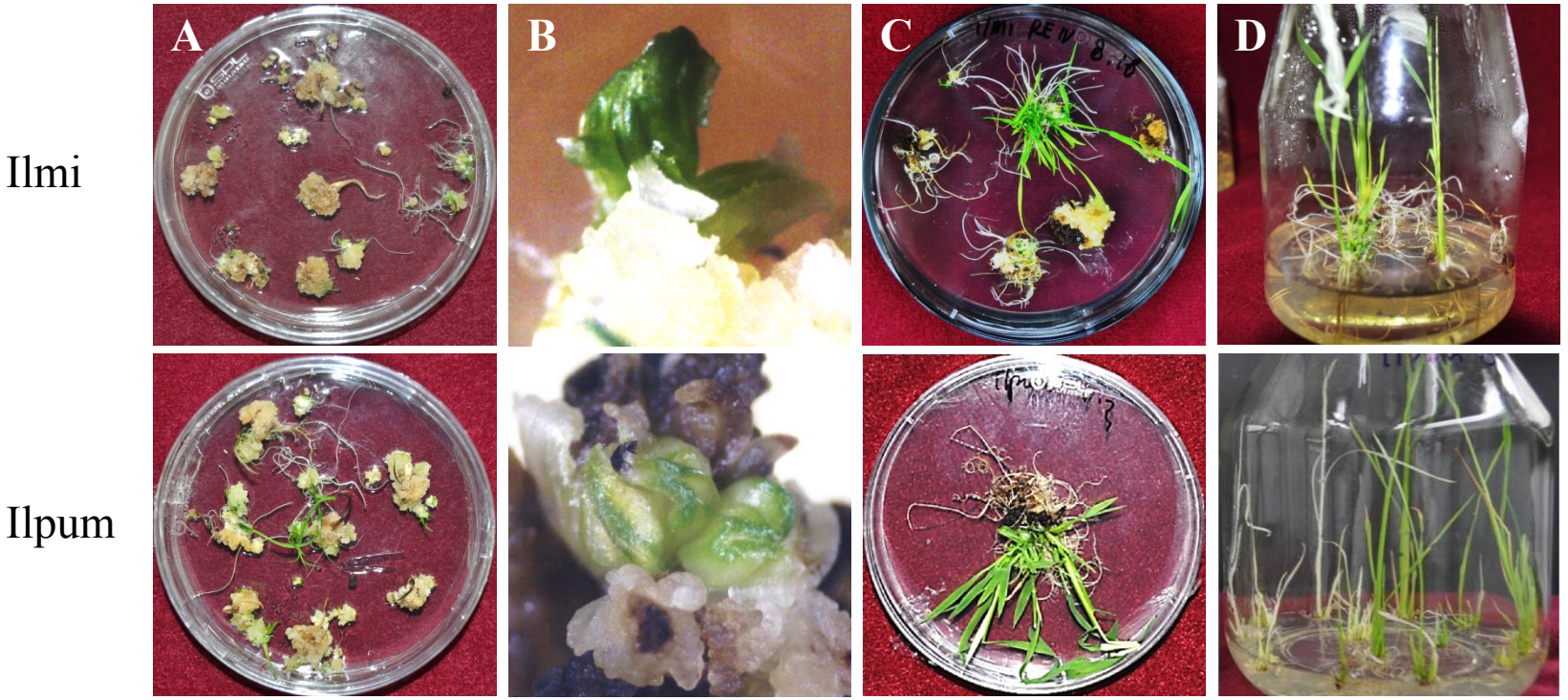
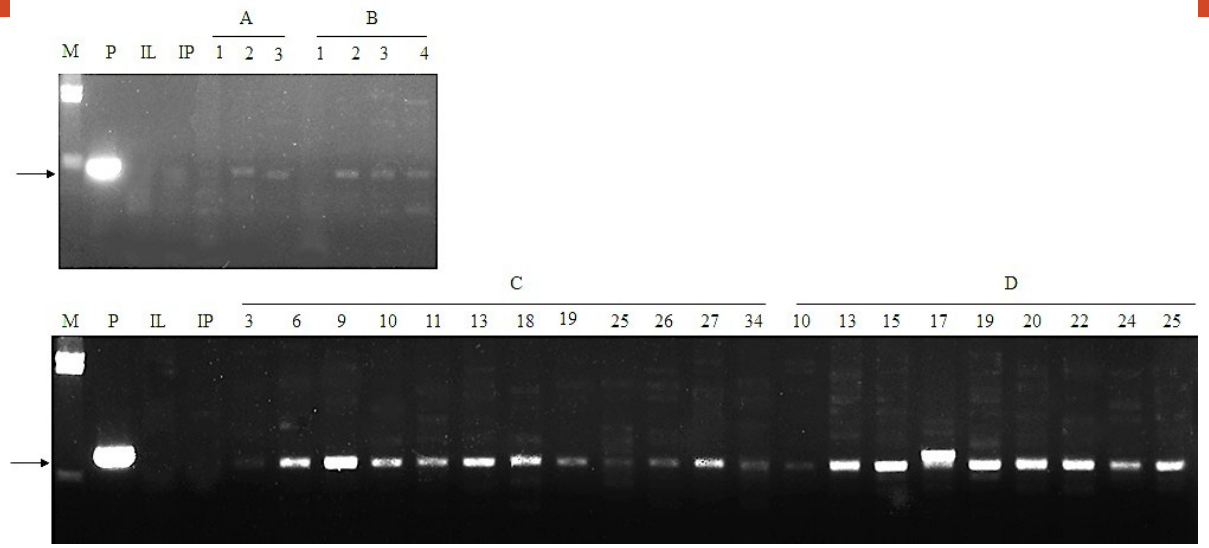
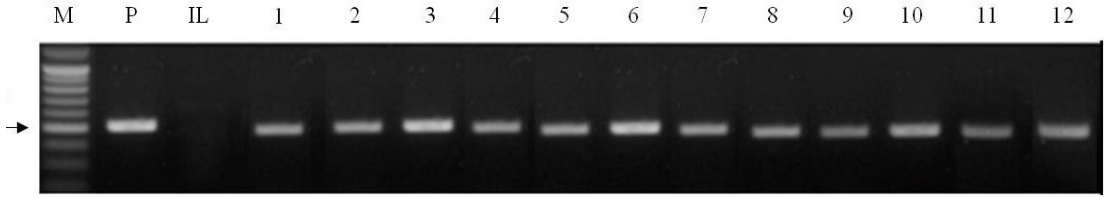


Figure 4. Regeneration of transgenic rice from mature seeds derived embryogenic calli in Ilmi and Ilpum. A: Callus formation on the selection medium, B: Shoot regeneration from transgenic calli, C: Shoot elongation on MS selection containing carbenecillin and geneticin with plant hormone, D: Rooting of putative transgenic plants in half-strength MS medium without plant hormone.

Results and discussion



Supplementary Fig. 1 The validation of T_0 transformants through genomic PCR analysis. Amplicons were separated on 1% agarose gel. M: Lambda *Hind* III marker (Fermentas, USA), P: plasmid, IL: Ilmi, IP: Ilpum, Numeric number of A: Ilmi transformed with *OsSAP*, Numeric number of B: Ilpum transformed with *OsSAP*. Numeric number of C: Ilmi transformed with *AtBI*-1, Numeric number of D: Ilpum transformed with *AtBI*-1



Supplementary Fig. 2. Amplification of PCR result to confirm insertion in 12 transgenic plants, *CaMsrB2-8* and *CaMsrB2-23*. DNA was isolated and PCR reactions were performed using *CaMsrB2* primers. *Lane M* Lambda DNA/*Hind* III marker, *Lane P* Plasmid DNA, *Lane IL* Ilmi, *Lane 1-6 CaMsrB2-8* lines, *Lane 7-12 CaMsrB2-23* lines

Results and discussion

Table 2. Transformation efficiency of OsSAP and AtBI-1 on rice cv. Ilmi and Ilpum

Cultivars	Vector	Callus infection	Putative transformants (T_0)	Positive transformants (T_0)
Ilmi	pBIN-OsSAP	500	11	3
	pBIN-AtBI-1	2,000	53	12
Ilpum	pBIN-OsSAP	500	8	2
	pBIN-AtBI-1	1,000	32	9

Mapping results of T-DNA flanking sequences

Query Name	Read length (bp)	T-DNA end	Insert from	Insert to	Adaptor start	Insert length (bp)	Chromosome	matching (%)	matching length	query start	query end	chr start	chr end	type	copies
OsSAP_H2_	885	-1	1	885	-1	885									
OsSAP_R_R	603	-1	1	591	592	591									
OsSAP_L	436	-1	1	424	425	424	chr04	98.58	424	1	424	24411733	24412156	5'Upstream-1000	1
OsSAP_R	507	-1	1	495	496	495	chr04	99.19	495	1	495	24411663	24412256	5'Upstream-1000	
Atbi-1 L_L	865	-1	1	865	-1	865									
AtBi-1R-L	603	-1	1	591	592	591									
Atbi-1I_L	766	-1	1	766	-1	766	chr10	99.6	759	1	759	5777560	5776802	Intergenic	1
AtBI-1I_R	778	-1	1	778	-1	778	chr10	99.74	774	1	774	5777561	5776789	Intergenic	
5_43_H2_A	298	-1	1	298	-1	298									
5_43_H2_B	152	-1	1	152	-1	152									
5_43_R	307	-1	1	307	-1	307									
5_43_H2_A	354	47	48	342	343	295									

Mapping results of T-DNA flanking sequences

Query Name	Read length	A/NA	T-DNA end	Adaptor start	matching length	query start	query end	Chr.	chr start	chr end	type	copies
MSRB2-Bar-1-R1	623	A	72	600	299	301	599	10	15728411	15728709	650bp at 5nd of Os10g0429500	1
MSRB2-Bar-1-L1	620	Vector	18	597								
MSRB2-Bar-3-R1	188	Vector	71	155								2
MSRB2-Bar-3-R2	126	NA	72	-1								
MSRB2-Bar-3-L1	502	A	-1	469	299	170	468	11	17171622	17171324		
MSRB2-Bar-3-L2	278	A	-1	249	248	1	248	4	21629644	21629891		
MSRB2-Bar-4-R1	256	A	40	223	161	62	222	2	33365046	33365206	intergenic region	1
MSRB2-Bar-4-L1	586	A	28	564	538	26	563	2	33365042	33364505		
MSRB2-Bar-8-R1	836	A	29	-1	601	29	629	1	41711122	41711722		1
MSRB2-Bar-8-L1	472	A	-1	-1	333	140	472	1	41713700	41713368	intergenic region	

Role of plant hormone in transformant rice (*Oryza sativa* L.) overexpression anti-apoptosis gene during drought stress

Results and discussion

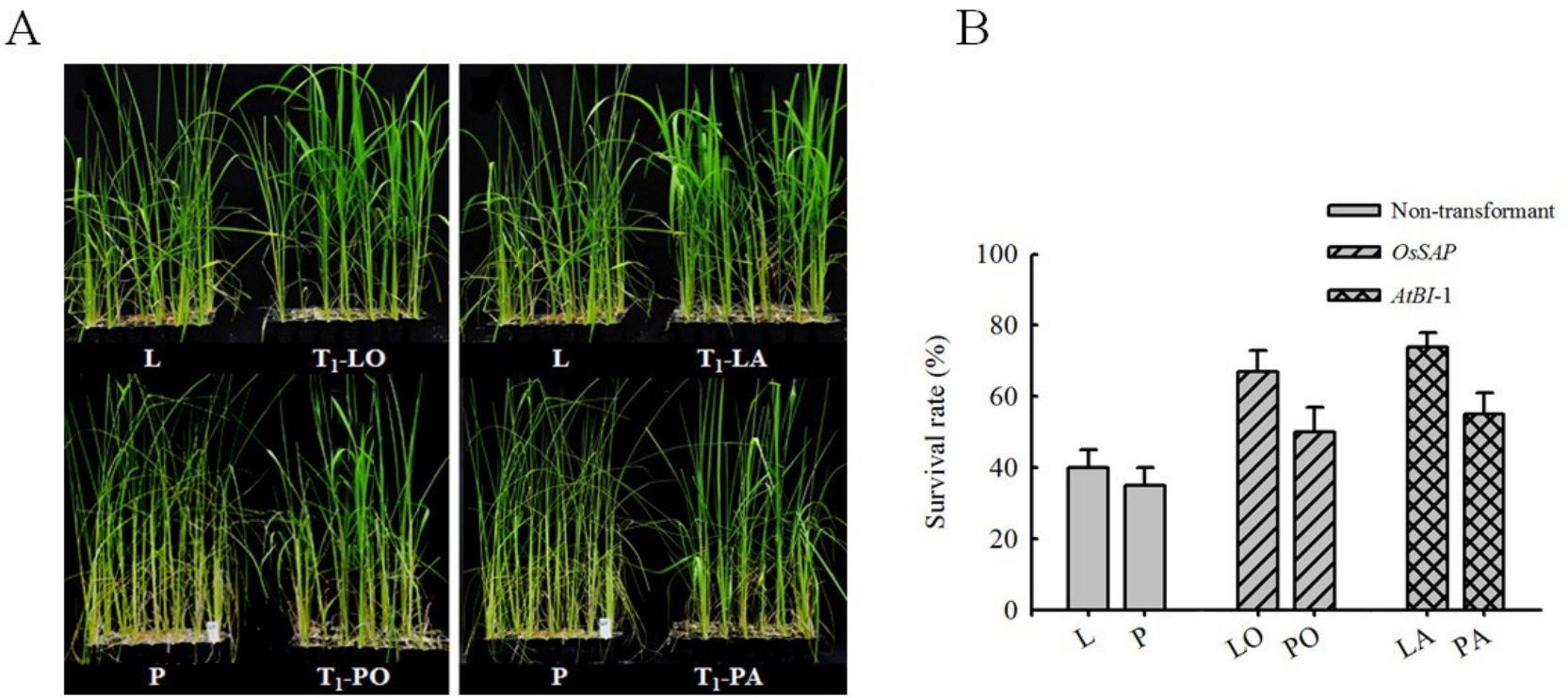


Fig. 1 The *OsSAP* and *AtBI-1* tranformants tolerance to drought stress compared with control. A:Phenotype after drought stress in transgenic rice overexpressing *OsSAP* and *AtBI-1*. L:Ilmi, P:Ilpum, T_1-LO:*OsSAP* T_1 transformants in Ilmi, T_1-PO:*OsSAP* T_1 transformants in Ilpum, T_1-LA:*AtBI-1* T_1 transformants in Ilmi, T_1-PA:*AtBI-1* T_1 transformants in Ilpum

Results and discussion

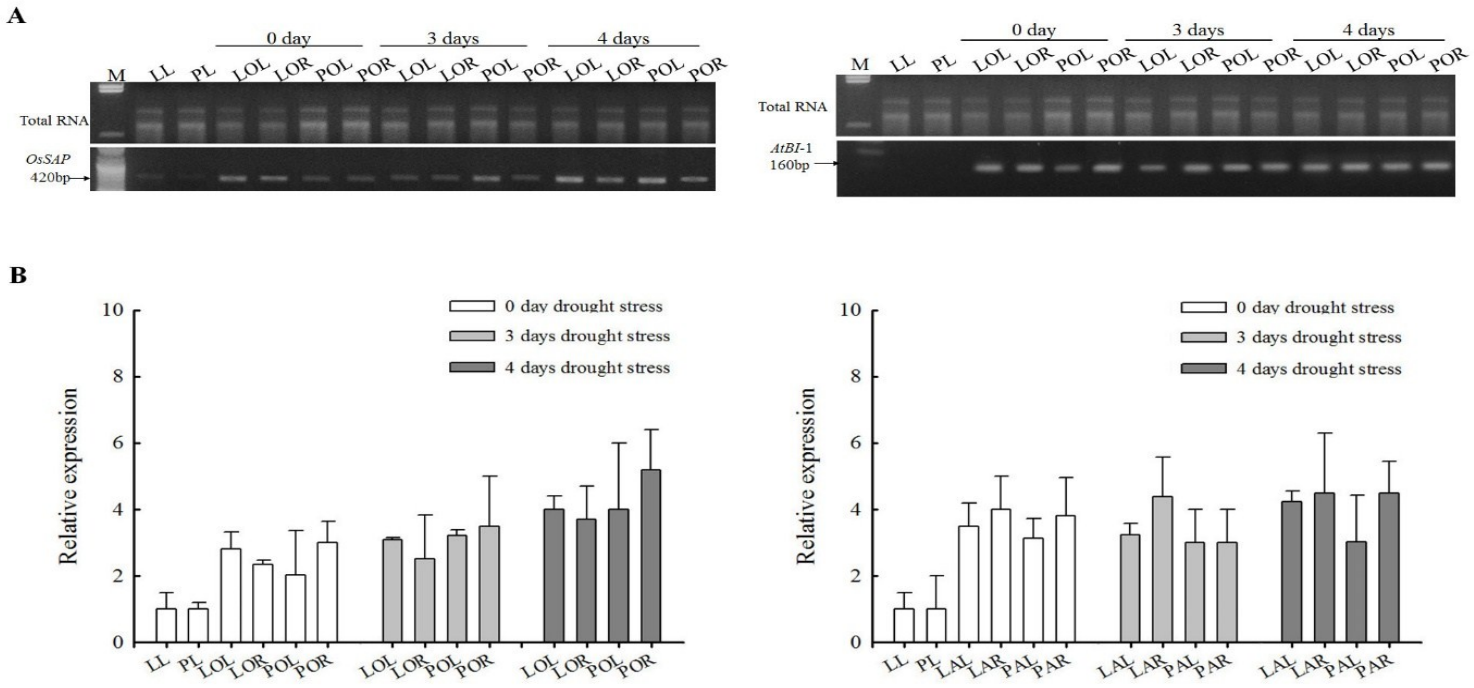


Fig. 2 Gene expression analysis under drought stress condition. A : RT-PCR analysis of *OsSAP* (leaft) and *AtBI-1* (right) transformants, B : Relative expression of *OsSAP* (left) and *AtBI*-1 (right) transformants under drought stress for 3 and 4 days. Mean values ± Standard Deviation are given. LL : control Ilmi-leaf, LL : control Ilpum-leaf, LOL : *OsSAP* Ilmi transformants-leaf, LOR : *OsSAP* Ilmi transformants-root, POL : *OsSAP* Ilpum transformants-leaf, POR : *OsSAP* Ilpum transformants-root, LAL : *AtBI-1* Ilmi transformants-leaf, LAR : *AtBI-1* Ilmi transformants-root, PAL : *AtBI-1* Ilpum transformants-leaf, PAR : *AtBI-1* Ilpum transformants-root

Results and discussion

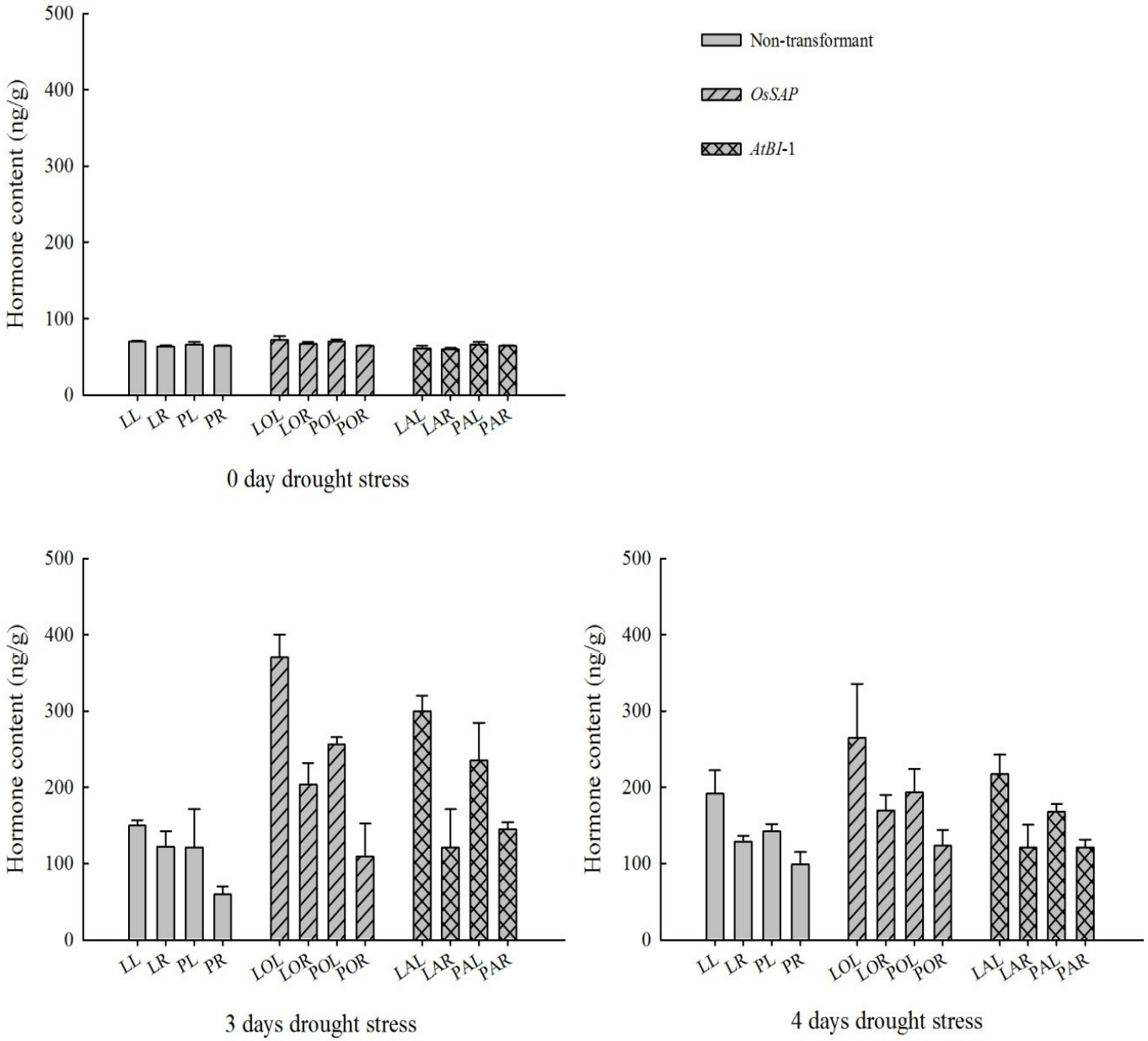


Fig. 3 Abscisic acid (ABA) level of transformants compared with control under different drought stress conditions. Mean values ± Standard Deviation are given

- These hormones generate a signal transduction network that leads to a cascade of events responsible for the physiological adaptation of plants to stress. It was evident that ABA level under drought stress increases in all leaf and root tissues in both *OsSAP* and *AtBI*-1 transformants.
- It have been known ABA positively contributes toward adaptation to osmotic stress, a major component of several abiotic stresses (Kissoudis et al., 2014).
- On the other hand, it has been reported that ABA can stimulate production of anti-apoptotic protein and reduces the expression of number of pro-apoptotic proteins (Scarfì et al., 2009).
- This implies that the relation of ABA and *OsSAP* and *AtBI*-1 overexpression to anti apoptosis could have connected pathways.

Results and discussion

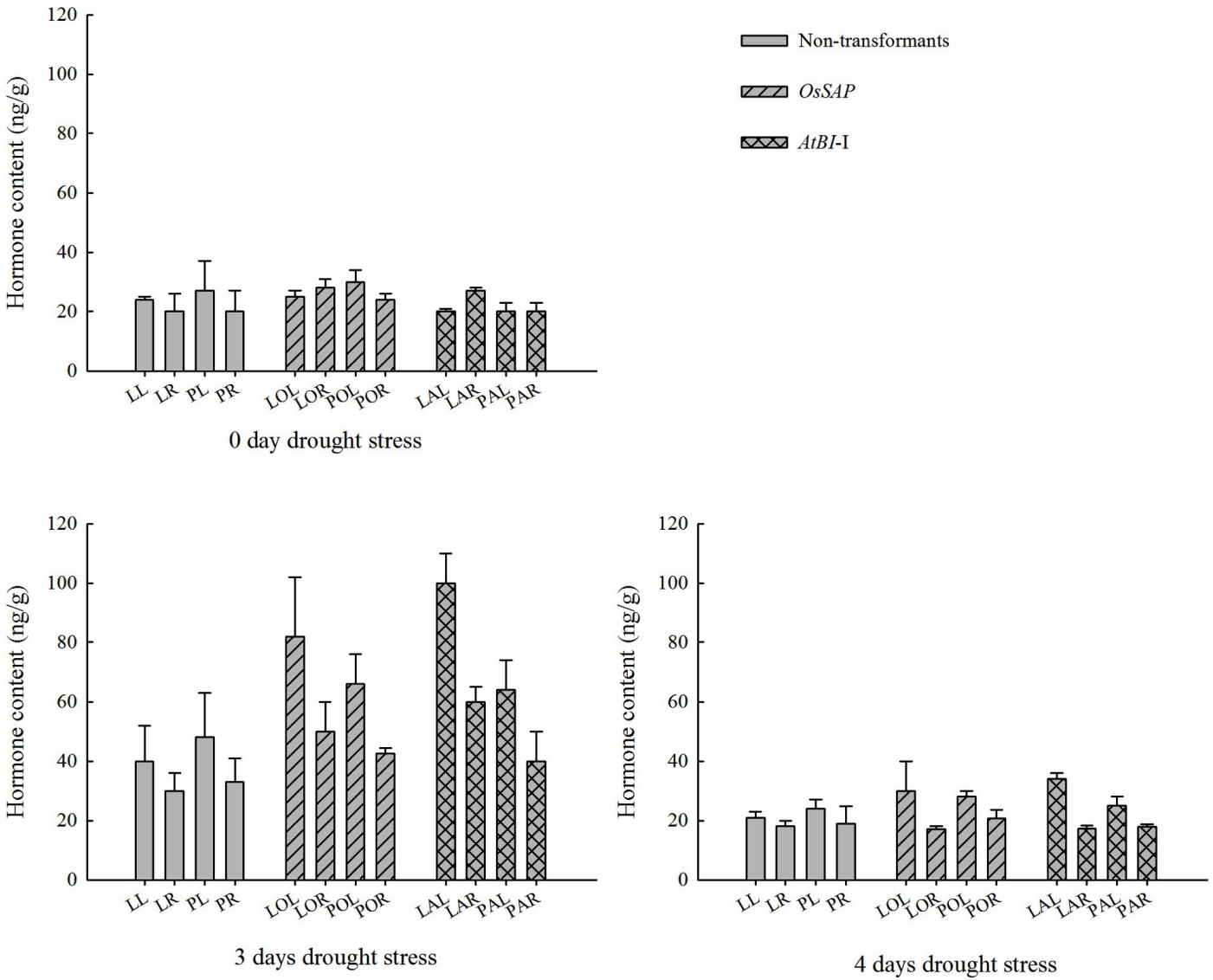


Fig. 4 Jasmonic acid (JA) level of transformants compared with control under different drought stress conditions. Mean values ± SE are given

- The participation of JA in response to abiotic stress, such as drought has been reported in several species. Stimulated water stress increased endogenous content of JA, followed by synthesis of jasmonate-induced proteins (Lehmann et al., 1995).
- These studies indicate that JA is an important component of a pathway that negatively regulates cell death and lesion formation. JA is believed to cause this effect by attenuating the O3-induced ROS production, as wounding or treatment of plants with JA has been shownto reduce O3-induced cell death and O3-induced ROS levels (Overmyer et al., 2000).
- The response of altered JA level in the *OsSAP* transformants during stress suggests the involvement of *OsSAP* and AtBI-1 in JA physiological pathway. Under drought, plants have developed complex mechanisms

- Overexpression of *OsSAP* and *AtBI*-1, can improve the endogenous level of ABA and JA during early stage of moderate drought. We suggest that these anti-apoptosis genes involved in increasing the endogenous ABA and JA level can sufficiently stimulate the preparatory response needed for drought acclimation.

Results and discussion

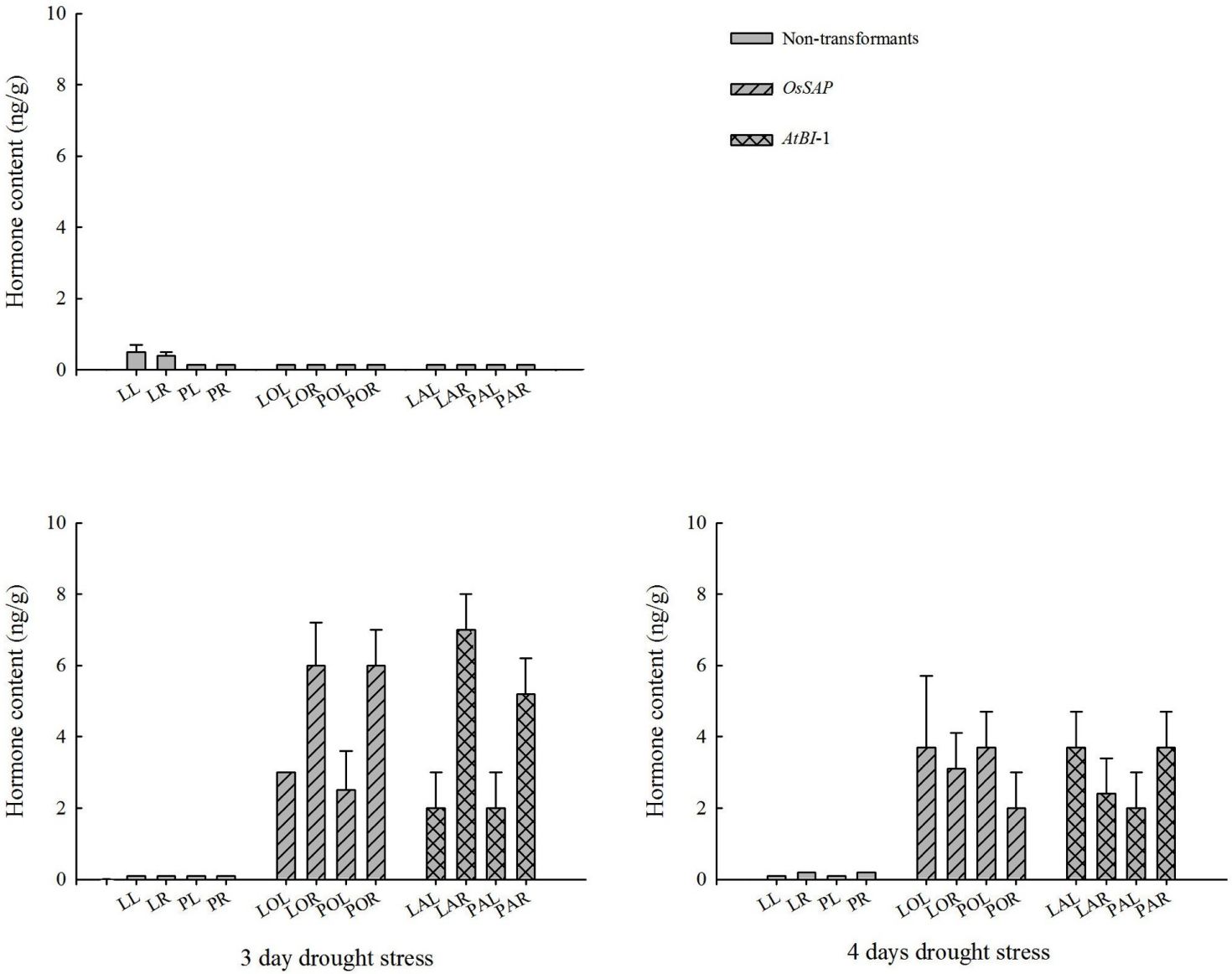


Fig. 5 Giberallic acid (GA_3) level of transformants compared with control under different drought stress conditions. Mean values ± SE are given

- Investigation of GA_3 level in transformed rice overexpressing *OsSAP* and *AtBI*-1, under drought stress, showed high accumulation level of this hormone, while in contrast GA_3 level was not detected or was at very minute level in control. However, these results suggested that GA_3 is involved in the response to drought stress condition only
- Moreover, altered levels of GA_3 due to the overexpression of anti-apoptosis genes *OsSAP* and *AtBI*-1 confirmed their roles in drought stress tolerance mechanism. Particularly, convergence and functional modulation of ABA signalling by GA_3) has a key regulatory function in the plant cellular network of stress and developmental signalling (Golldack et al., 2013).
- It was also reported that exogenous application of GA_3 can reduce cell damage and improve growth of maize seedlings subjected to water stress (Wang et al., 2008).
- Therefore, we suggest that over expression of anti-apoptosis gene *OsSAP* and *AtBI*-1 increase the levels of endogenous GA_3. GA_3 was demonstrated to be effective in alleviating cell damage of rice subjected to drought stress condition. Thus, the results imply that *OsSAP* and *AtBI*-1 can increase GA_3 level and might help to maintain cell membrane stability and increase the tolerance to drought stress.

Results and discussion

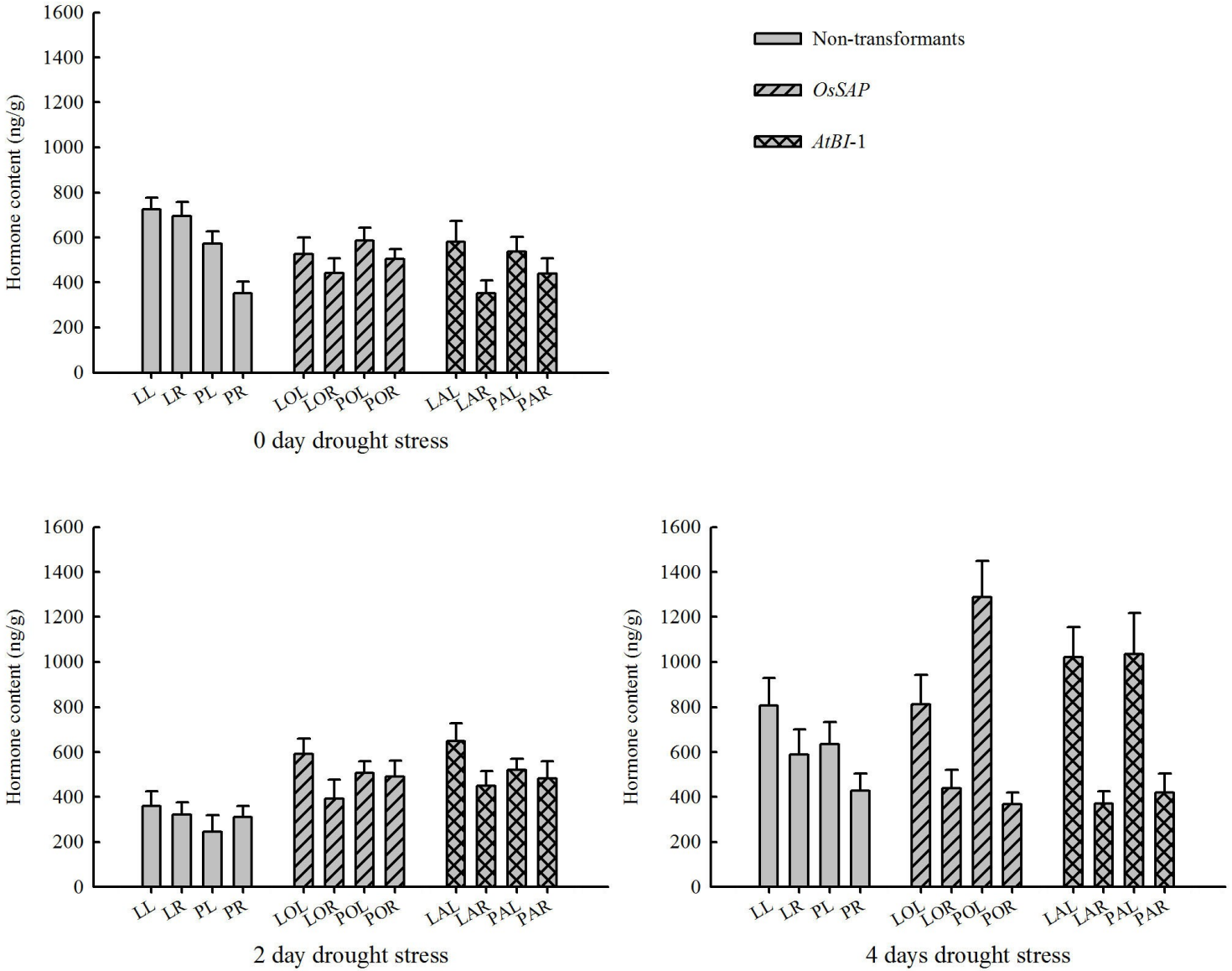


Fig. 6 Zeatin level of transformants compared with control under different drought stress conditions. Mean values ± SE are given

- Observation of cytokinin, zeatin level in transformed rice overexpressing *OsSAP* and *AtBI*-1, under moderate drought stress, decreased accumulation level of this hormone in all genotype and again slight increased after prolonged of stress.
- We found zeatin level was higher in transformant plant compared with control plant under drought stress condition. Decreased of cytokinin content in response to drought stress has been observed, while leaf zeatin concentration declined under osmotic stress in tomato (Walker and Dumbroff, 1981). This also indicating that the relation of zeatin and *OsSAP* and *AtBI*-1 overexpression to anti-apoptosis could have connected pathways.

Results and discussion

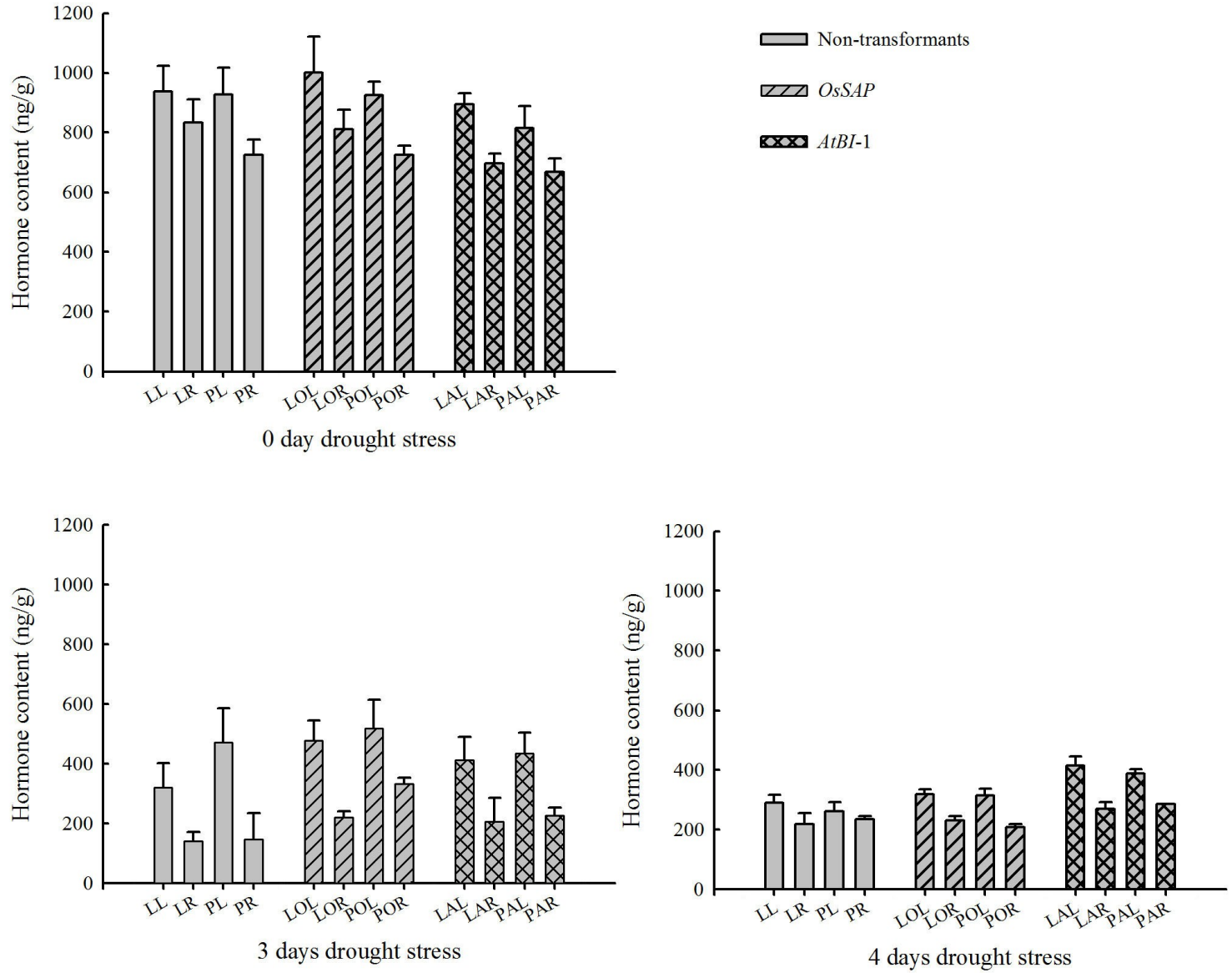


Fig. 7 Indole-3-carboxylic acid (ICA) level of transformants compared with control under different drought stress conditions. Mean values ± SE are given

- Investigation of and auxin ICA were differently affected in leaf and root. Drought stress condition dramatically decreased of ICA level but no evidence different between control and transformants plant. Other hand have been reported that auxin is involved in the attenuation of defence responses in plants.
- In contrast, blocking auxin responses has been shown to increase resistance in plants (Bari and Jones, 2009). In addition, this finding suggests these hormones are not related to abiotic stress response in rice.

Role of plant hormone in transformant rice (*Oryza sativa* L.) overexpression anti-apoptosis gene under salinity stress

Results and discussion

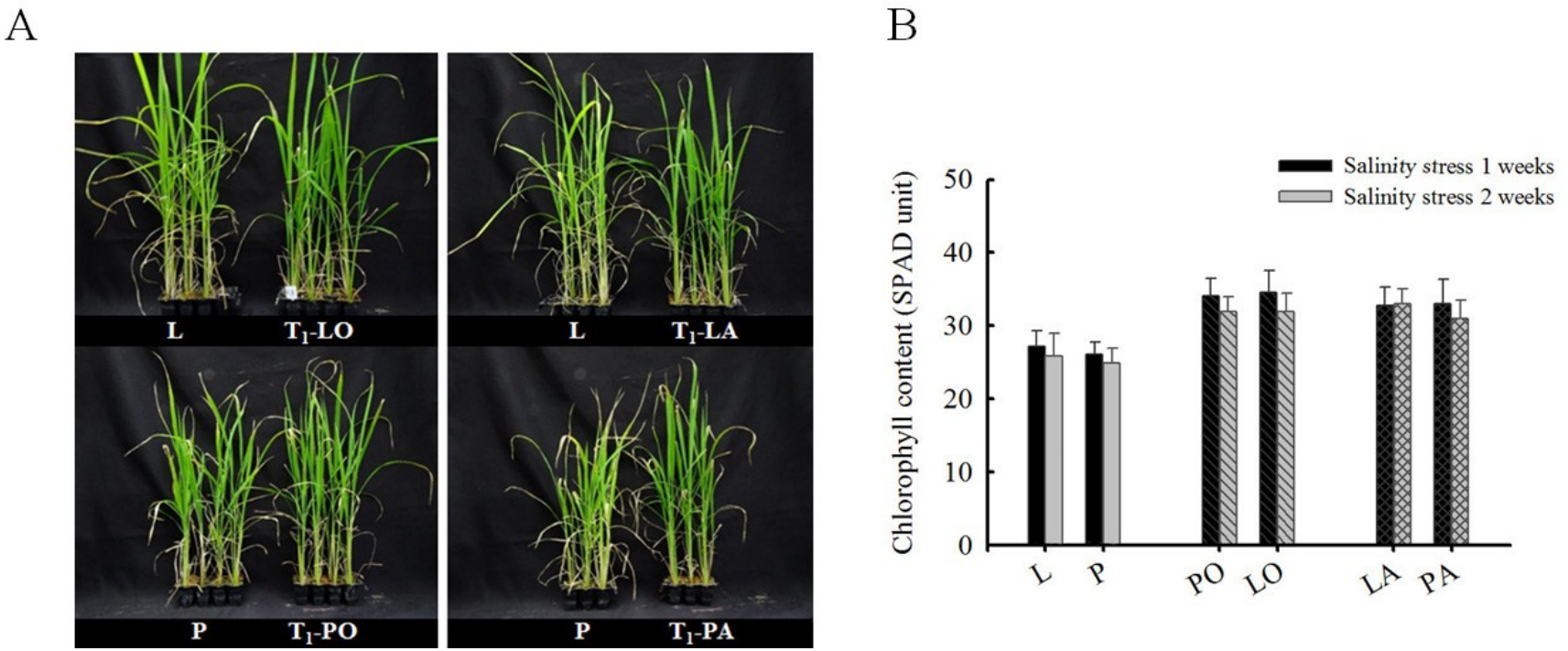


Fig. 3 The *OsSAP* and *AtBI-1* tranformants tolerance to salinity stress compared with control. A; Phenotype after salinity stress in transformant rice overexpressing compared with control, B; cholorphyll content after salinity stress in transformantrice compared with control. Mean values ± SE are given

Results and discussion

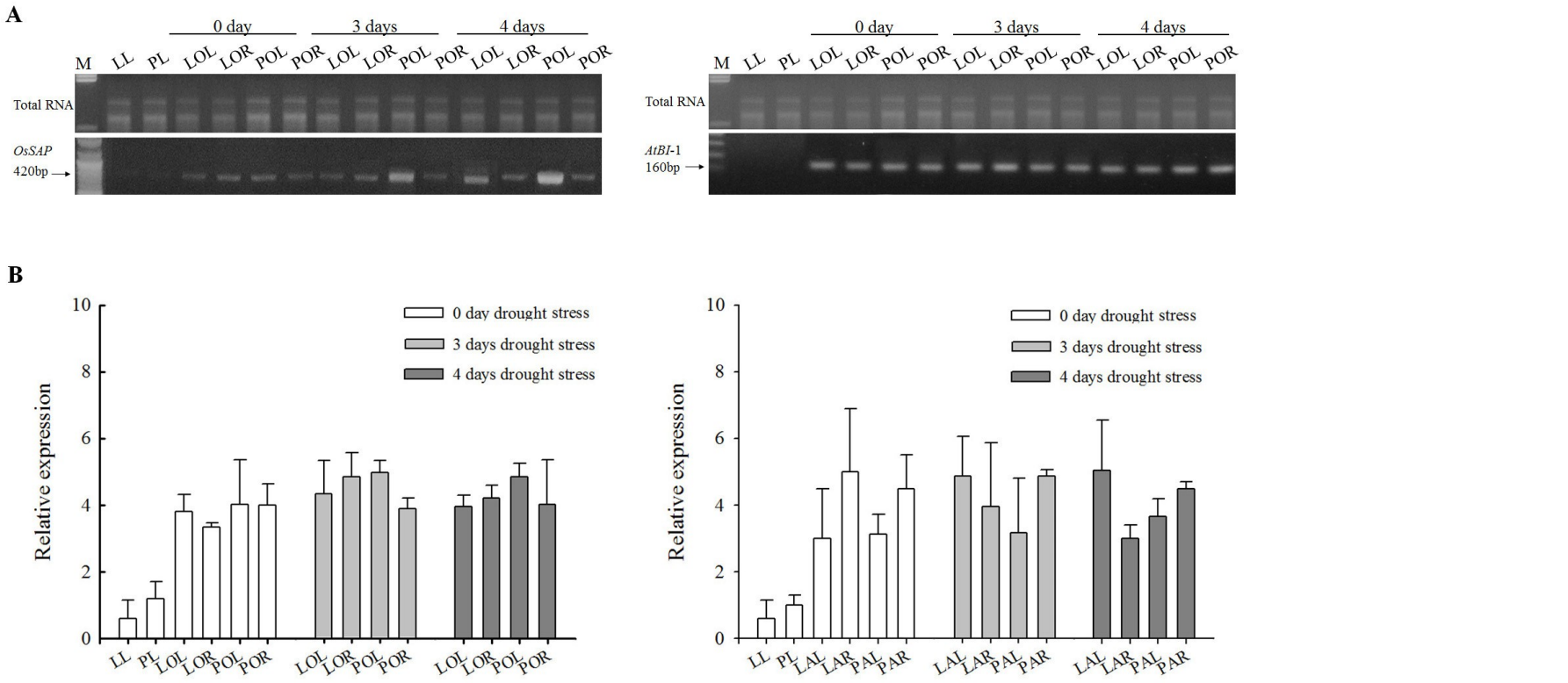


Fig. 4 Gene expression analysis under salinity stress condition. A: RT-PCR analysis of *OsSAP* (leaft) and *AtBI-1* (right) transformants, B: Relative expression of *OsSAP* (leaft) and *AtBI*-1 (right) transformants under salinity stress 1 weeks and 2 weeks. Mean values ± SD are given

Results and discussion

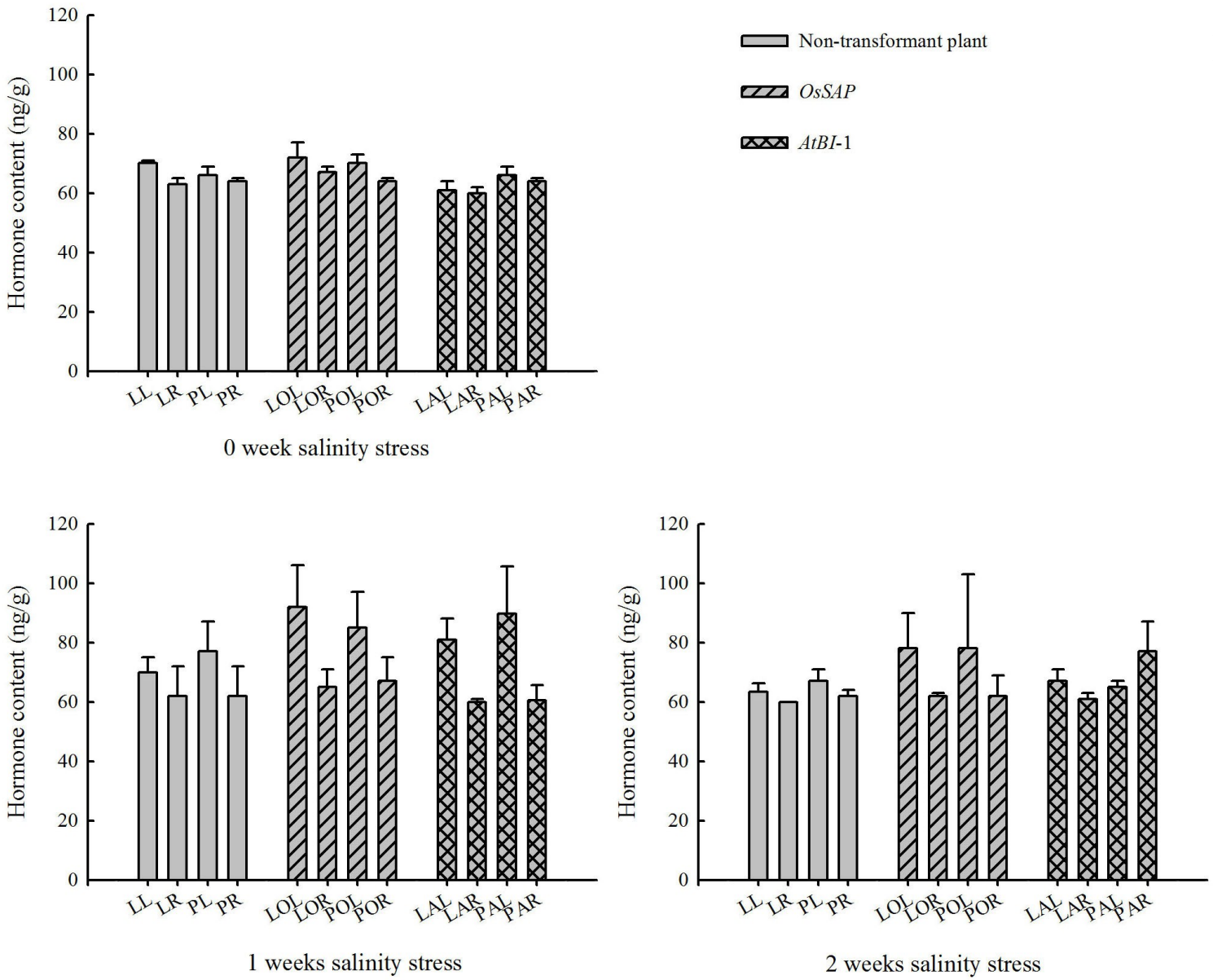


Fig. 5 Abscisic acid (ABA) level of transformants compared with control under different salinity stress conditions. Mean values ± SE are given

- Under salinity stress, ABA level was not affected at the beginning but it slightly increased during prolonged stress of 1 weeks, in leaf tissue of *OsSAP* and *AtBI*-1 transformant. ABA hormone is known for its regulatory role of integrating environmental adversity with developmental program of plant (Christmann et al. 2005).
- ABA is a vital cellular signal that modulates the expression of a number of salt and water deficit-responsive genes (Fukuda and Tanaka, 2006). Synthesis, degradation, and transport processes dynamically maintain ABA levels in plant cells. Therefore, plants maintain their developmental programs and stress responses by modulating endogenous ABA levels (Schwartz et al. 2003).
- On the other hand, it has been reported that ABA can stimulate production of anti-apoptotic protein and reduces the expression of number of pro-apoptotic proteins (Scarfì et al. 2009). This implies that the relation of ABA and *OsSAP* and *AtBI*-1 overexpression to anti-apoptosis could have connected pathways.

Results and discussion

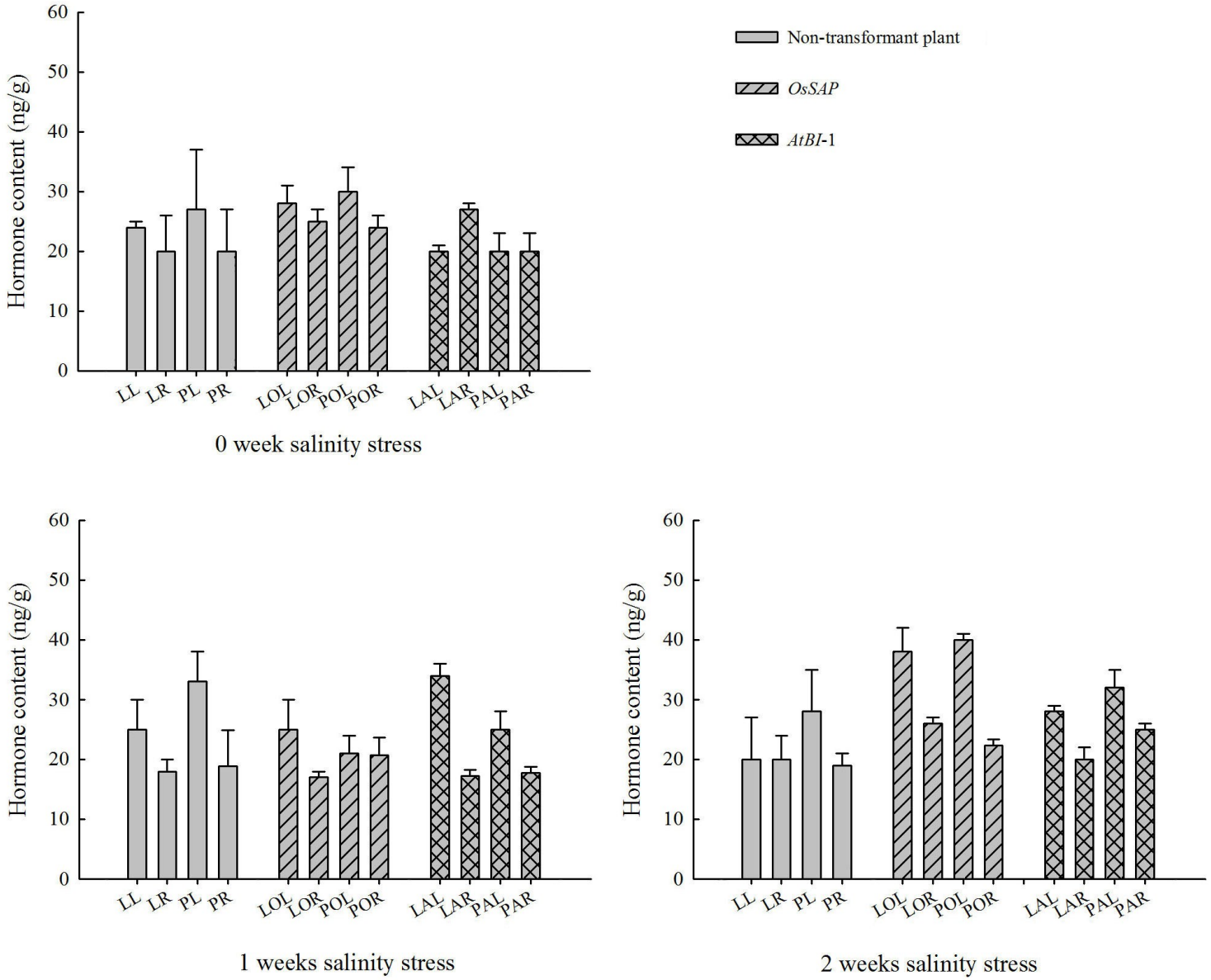


Fig. 6 Jasmonic acid (JA) level of transformants compared with control under different salinity stress conditions. Mean values ± SE are given

- In the investigation of JA levels in the transformants overexpressing *OsSAP* and *AtBI*-1, and control, under salinity stress, there was no difference in JA levels between the transformants and the control, even after prolonged treatment.
- Jasmonic acid (JA) is a well-known signalling molecule in plant defence and stress responses (Hoeberrichts and Woltering, 2003). The participation of JA in response to abiotic stress, such as drought and salinity, has been reported in several species. Stimulated water stress increased endogenous content of JA, followed by synthesis of jasmonate-induced proteins (Lehmann et al. 1995).
- Together, these studies indicate that JA is an important component of a pathway that negatively regulates cell death and lesion formation. However, the precise mechanisms by which JA signalling regulates anti-apoptosis remain to be elucidated. The response of altered JA level in the *OsSAP* and *AtBI-1* transformants during critical condition of salinity stress initially hypnotized the involvement of *OsSAP* in JA physiological pathway.

Results and discussion

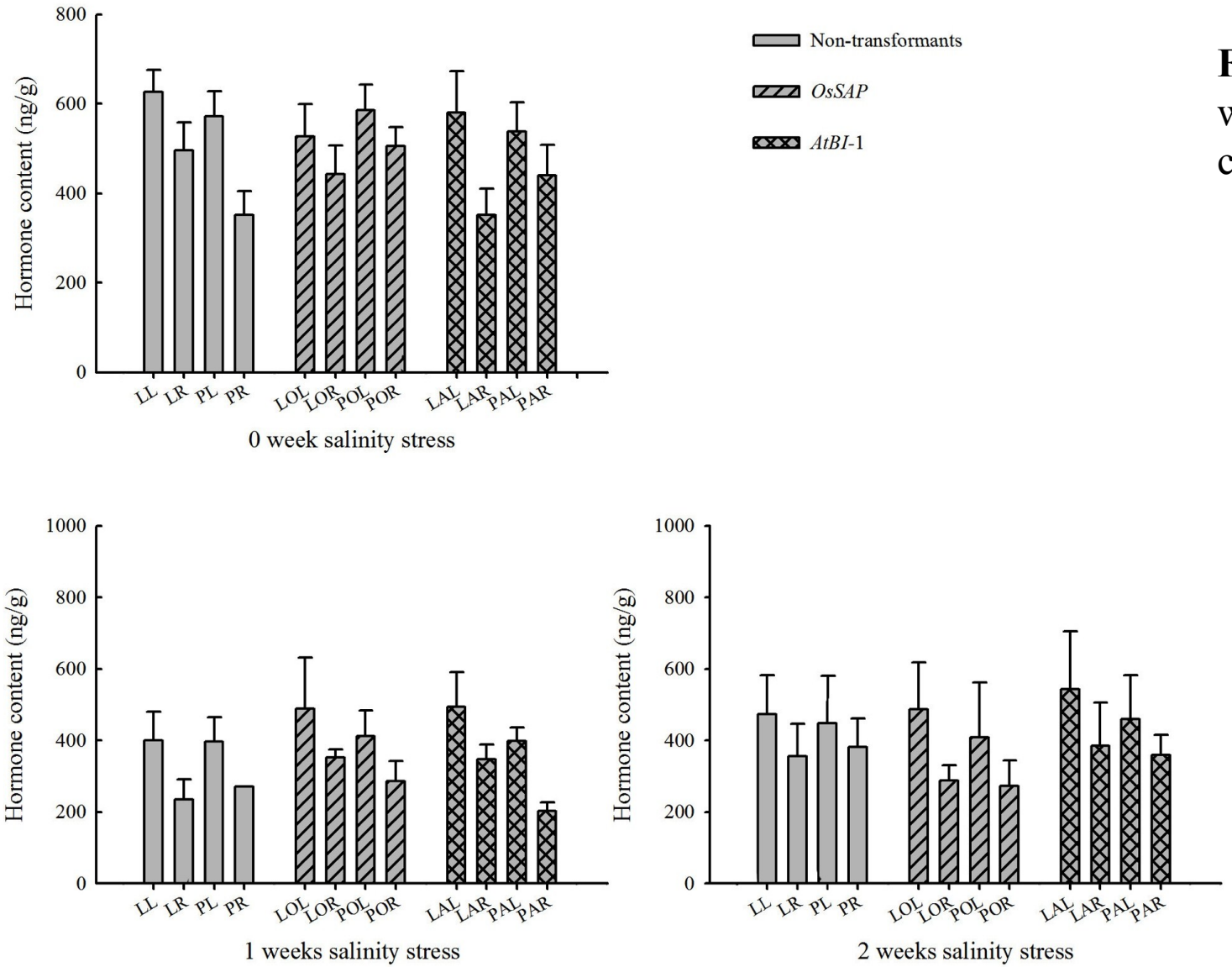


Fig. 7 Zeatin level of transformants compared with control under different salinity stress conditions. Mean values ± SE are given

- Investigation of cytokinin, zeatin were differently affected in leaf and root. Drought stress condition dramatically decreased of ICA level but no evidence different between control and transformants plant.
- Other hand have been reported that auxin is involved in the attenuation of defence responses in plants. In addition, this finding suggests these hormones are not related to abiotic stress response in rice.

- Investigation of GA_3 level in transformed rice overexpressing *OsSAP* and *AtBI*-1, under drought stress, showed high accumulation level of this hormone, while in contrast GA_3 level was not detected or was at very minute level in and control
- under salinity stress, GA_3 was not detected in any of the transformants and control. This might have been due to the level of the hormones being too low to be extracted from rice plant tissue. In addition, this finding suggests that all these hormones are not related to abiotic stress response in rice.

Results and discussion

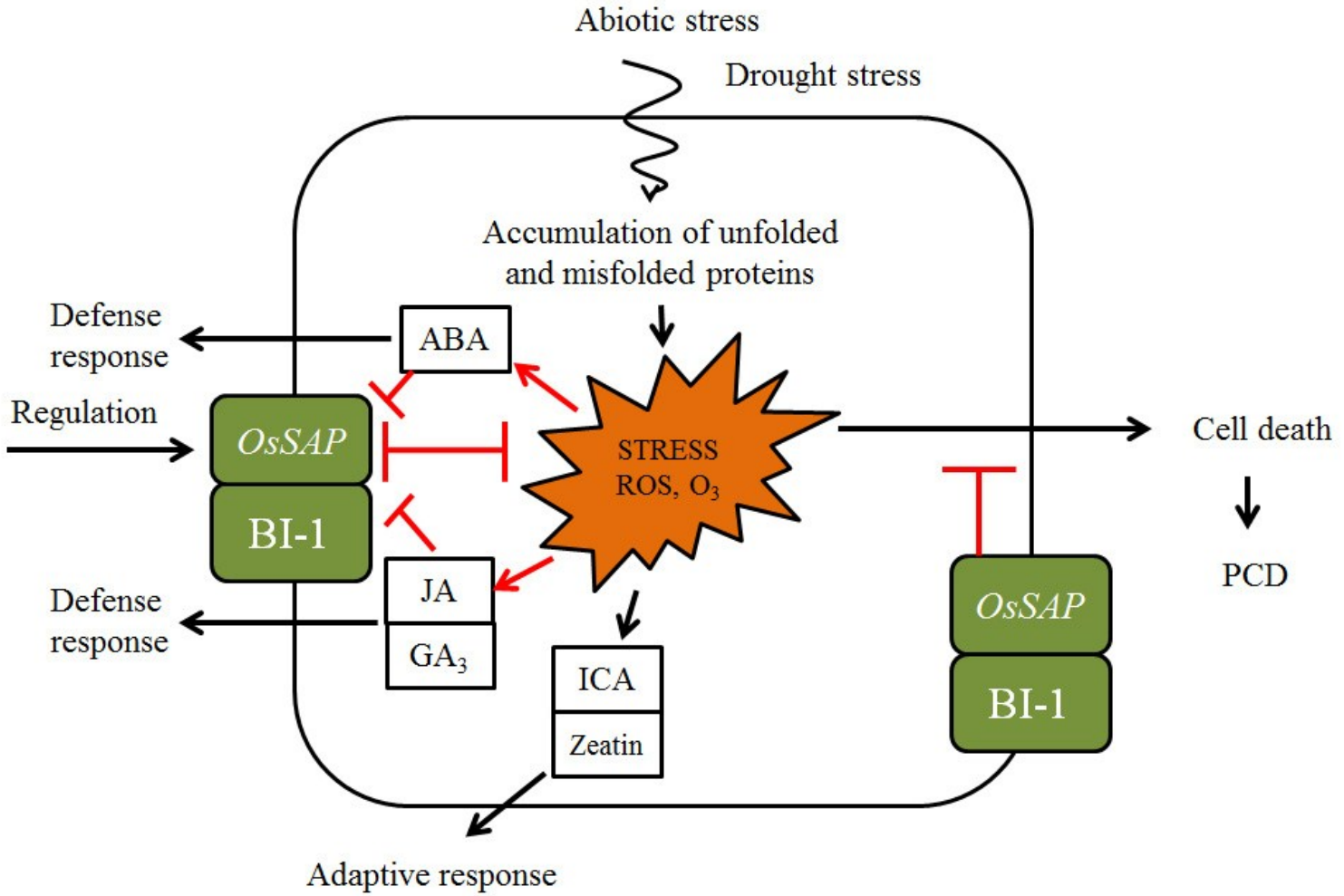


Fig. 8 Proposed model on crosstalk of abscisic acid (ABA), gibberellic acid (GA_3), and jasmonate (JA) signalling in cellular response of plants to drought, elicited by *OsSAP* and *AtBI*-1 gene

Plant hormone response of transgenic rice (*Oryza sativa* L.) over expression *CaMsrB2* to drought and salinity stress.

Results and discussion

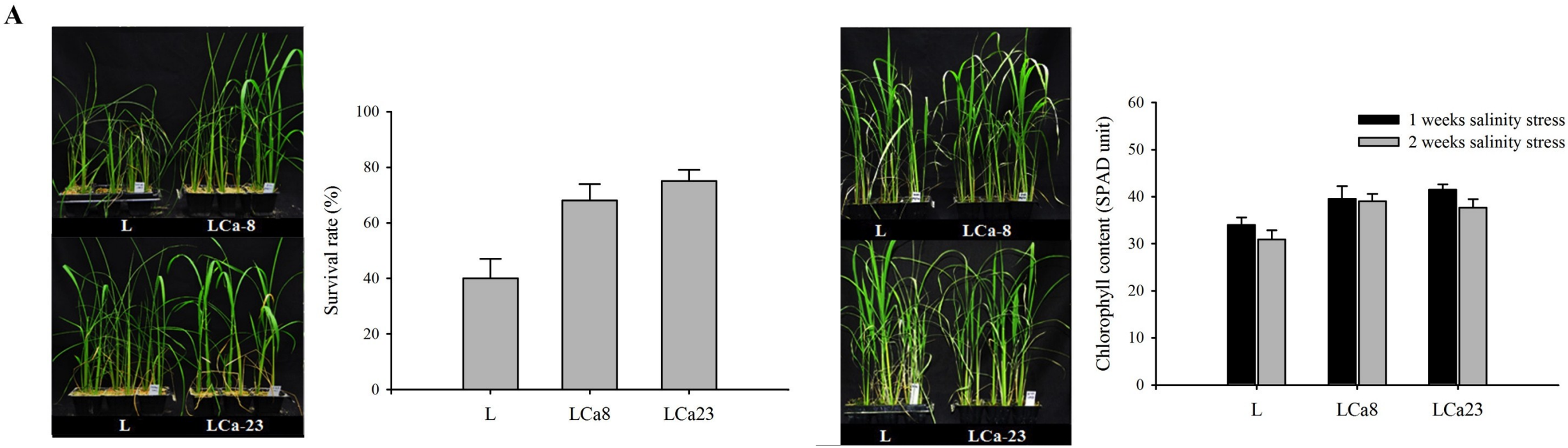


Fig. 1 Phenotype of transgenic plants compare with control rice after drought treatment and salinity stress condition. A: Rice phenotype under drought stress and survival rate, B: Rice phenotype under salinity stress and Chlorophyll content

Results and discussion

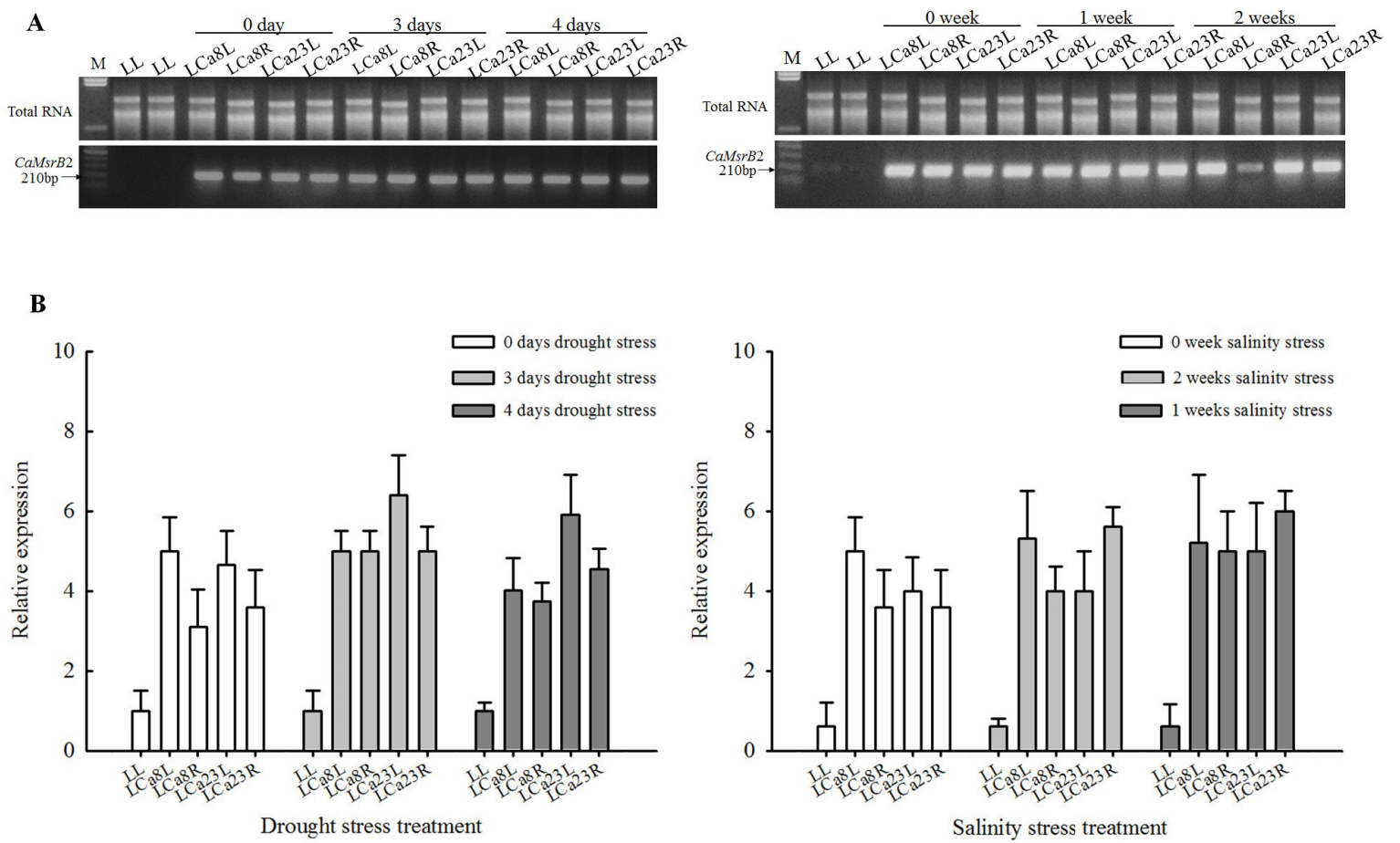


Fig. 2 Detection of 12 transgenic plant leaves and roots by Reverse Transcriptase-PCR and Quantitative Real Time PCR for *CaMsrB2* gene. A: RT-PCR assay for determining relative mRNA expression level of gene after *Left* drought stress treatment *Right* Salinity stress treatment, B: qRT-PCR analysis*Left* drought stress treatment *Right* Salinity stress treatment

Results and discussion

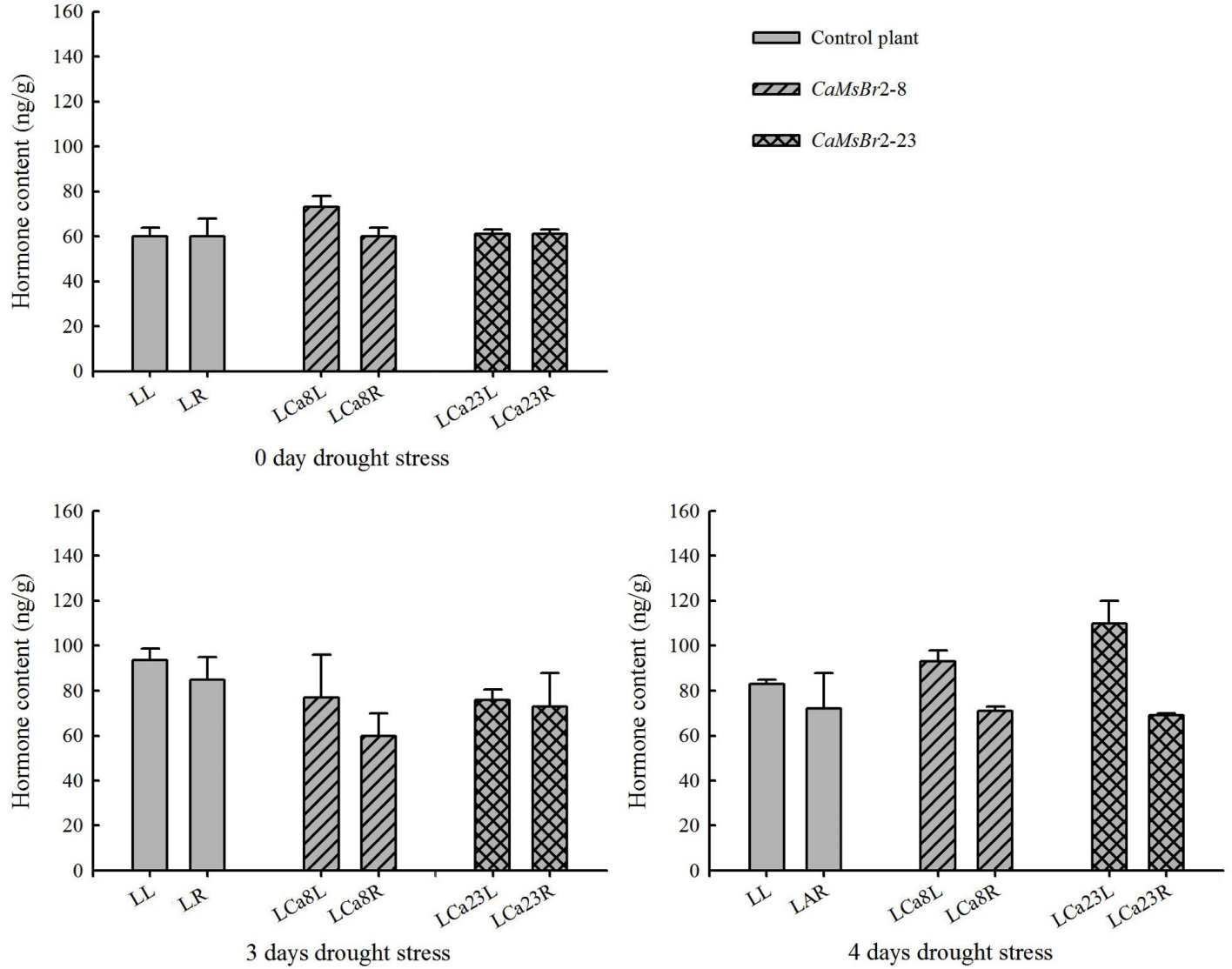


Fig. 3 Abscisic acid (ABA) level of transgenics compared with control under different drought stress conditions.

Results and discussion

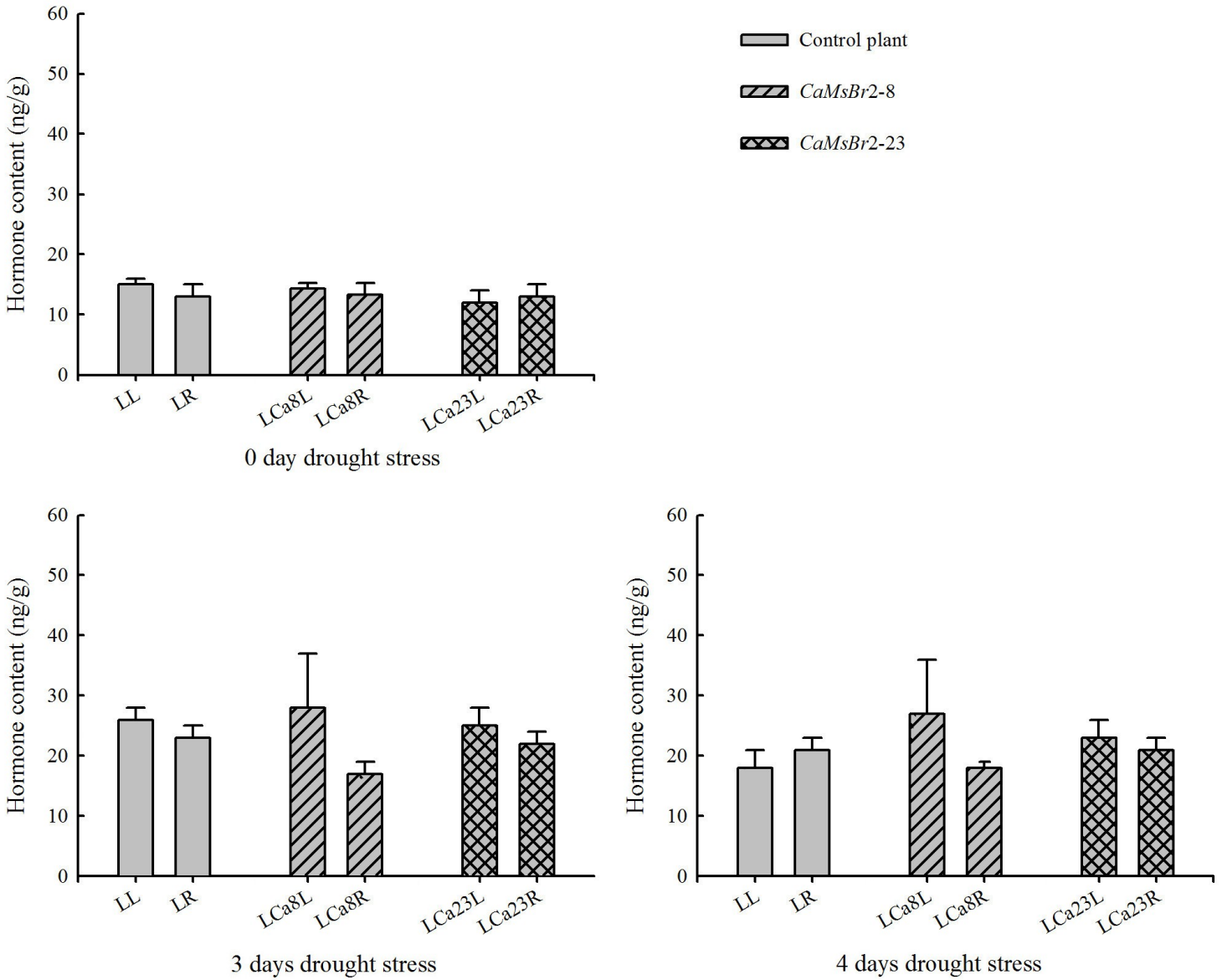


Fig. 4 Abscisic acid (ABA) level of transgenics compared with control under different salinity stress conditions

- ABA has higher determined in the transgenic leaf than control under drought stress
- It have been known ABA positively contributes toward adaptation to osmotic stress, a major component of several abiotic stresses

Results and discussion

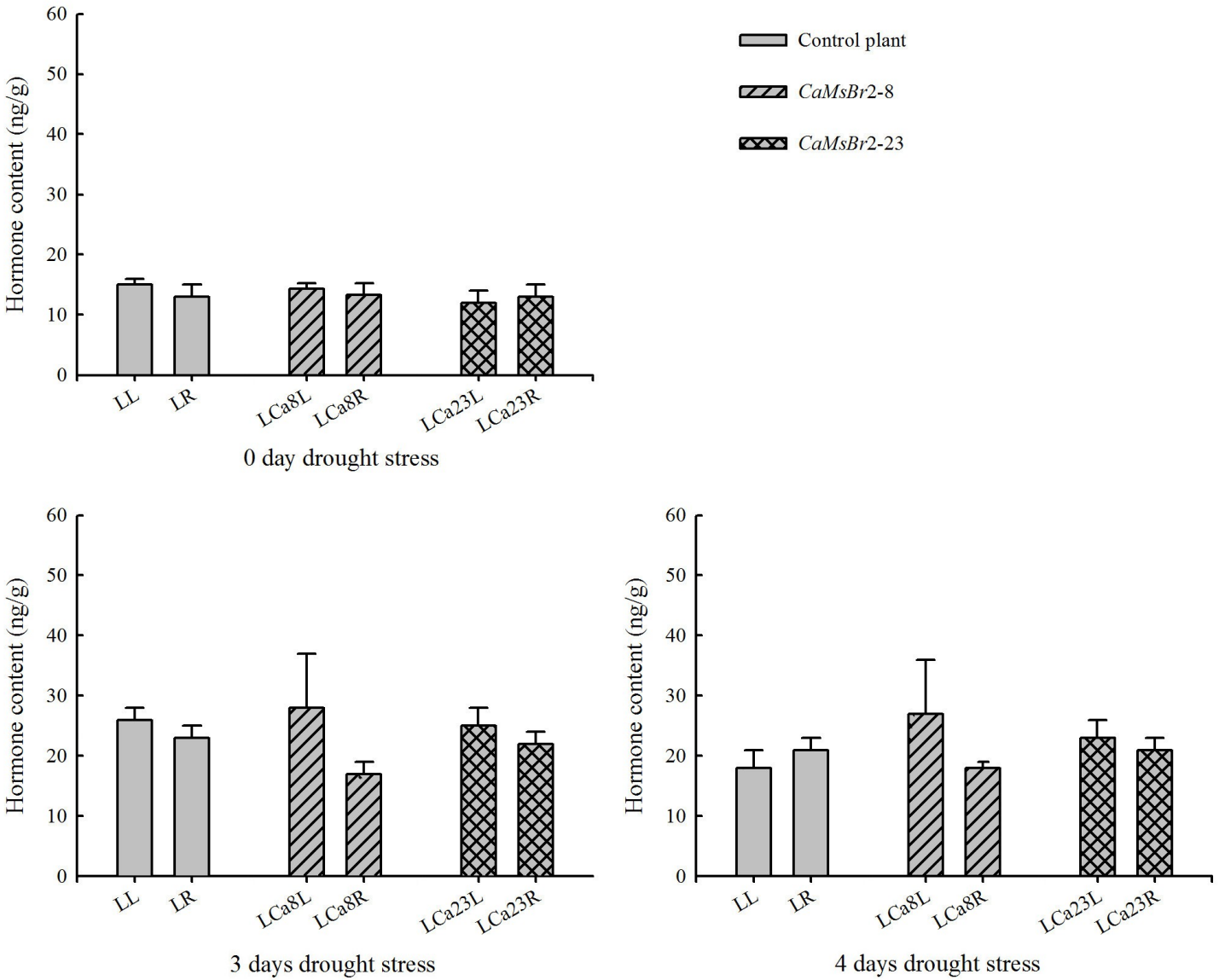


Fig. 5 Jasmonic acid (JA) level of transgenics compared with control under different drought stress conditions

Results and discussion

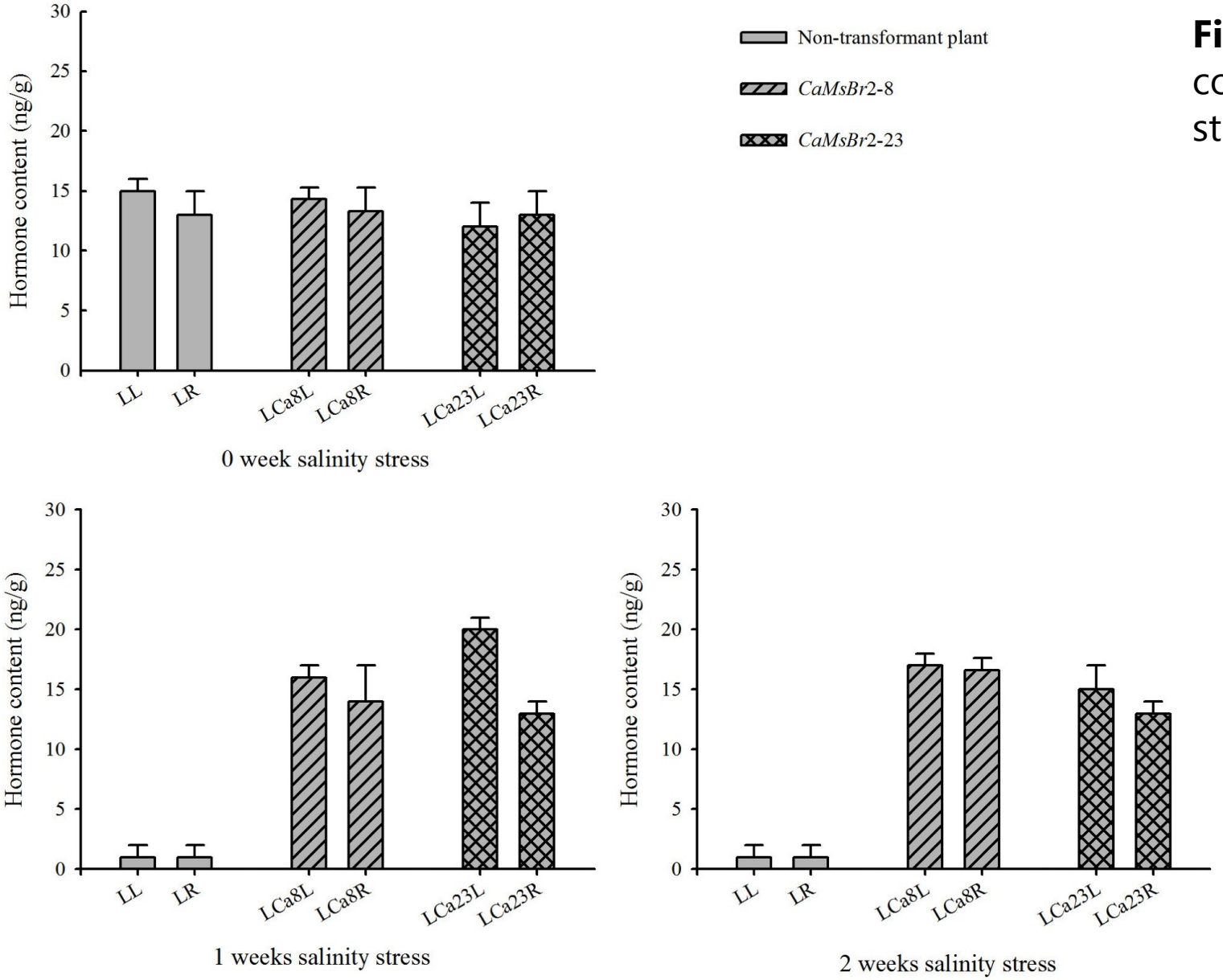


Fig. 6 Jasmonic acid (JA) level of transgenics compared with control under different salinity stress conditions

Results and discussion

- The precise mechanisms by which JA signalling improved plant defence to *CaMsrB2* remain to be elucidated. Under drought and salinity stresses, plants have developed complex mechanisms to perceive external signals, allowing them optimal response to the environment.
- In the investigation of JA levels, the accumulation of JA in transgenic lines was higher than non-transgenic under both stresses. However, significant increasing JA hormone can be showed under salinity stress in this present study.

Conclusion

- While many components of the pathways act in a similar manner, and many genes are induced by abiotic stresses, it should be noted that the expression pattern of any specific gene varies in its timing and level of expression within specific tissues and under different experimental conditions.

- While the variation may occur because the treatments vary in their severity and duration, the hormonal status of the plants under different stress conditions may also affect cross-talk and the complex feedback regulatory mechanisms associated with the interacting pathways.

- However, the complex regulatory network of phytohormone signaling in plants subjected to abiotic stress needs to be explored, and the stress-related genes involved in the network of hormone signaling await identification

Thank you very much