# Cloning and characterization of peanut allene oxide cyclase gene involved in salt-stressed responses

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**ABSTRACT.** In this study, the full-length cDNA encoding allene oxide cyclase (*AhAOC*) was isolated from peanut (*Arachis hypogaea* L.). The deduced amino acid sequence of *AhAOC* showed high homology with other plant AOCs. The transcript of *AhAOC* was found to be abundantly expressed in roots. Expression analysis demonstrated that *AhAOC* was induced by abscisic acid, methyl-jasmonic acid, salicylic acid, salinity, polyethylene glycol, and cold stresses, particularly by high salinity. Overexpression of *AhAOC* in rice increased root elongation and plant height compared with expression in control plants and conferred tolerance against salinity. Thus, the *AhAOC* gene may play an important role in increasing the expression of transcription factors (*MYB2* and *OsONAC045*) and functional genes (*DREB1F* and *LEA3*) in transgenic rice under salt stress as well as improve stress tolerance through the

accumulation of compatible solutes (proline and soluble sugar). The *AhAOC* gene is a potential resource for enhancing salt tolerance in crop species.

**Key words:** Allene oxide cyclase gene; Gene cloning; Overexpression; Peanut; Salt stress

## INTRODUCTION

During plant adaptation to environmental stresses, such as pathogens, salinity, drought, and high light levels, various signals are produced, including jasmonic acid (JA). JA is an important endogenous plant hormone involved in various stress responses (Creelman and Mullet, 1997; Parchmann et al., 1997). In plants, JA biosynthesis occurs through the oxylipin pathway in the chloroplasts (Stumpe and Feussner, 2006). Molecular oxygen is added to linolenic acidin in a reaction catalyzed by lipoxygenase, forming (13$S$)-hydroperoxy-(9$Z$,11$E$,15$Z$)-octadecatrienoic acid. This product is then dehydrated by allene oxide synthase (AOS, EC 4.2.1.92) to an unstable allene oxide; this reaction is catalyzed by allene oxide cyclase (AOC, EC 5.3.99.6) to the stable compound 12-oxo-phytodienoic acid. After a 12-oxo-phytodienoic acid reductase reaction and 3 cycles of β-oxidation, (+)-7-iso-JA is produced. AOC is an important enzyme in JA biosynthesis as it determines the correct stereochemical structure of JA (Ziegler et al., 2000). Therefore, *AOC* genes are thought to be potential targets for controlling metabolites biosynthesis in plant species.

There are 24 *AOC* gene sequences deposited in the NCBI GenBank database, of which 6 are from soybean, 4 from *Arabidopsis thaliana*, and only 2 from common wheat and maize genomes. Characterization of *AOC* genes from various plant species has provided insight into their biochemical and physiological roles in adaptation to biotic and abiotic stresses. For example, overexpression of *JcAOC* in *Escherichia coli* improved its resistance to salt and low temperature stresses (Liu et al., 2010). Wu et al. (2011) examined the expression and functional diversity of the *AOC* gene family in soybean. They found that 6 *AOC* genes had specific and different expression patterns in multiple organs and under several stresses. Overexpression of the *GmAOC1* and *GmAOC5* genes in transgenic tobacco improved tolerance to salt and oxidative stresses, respectively. However, the precise mechanisms of *AOC* function in plant cell processes, particularly in regulating stress response, are not well-understood.

Plant *AOC* had specific and complex expression patterns in multiple organs. In soybean, *GmAOC2* shows relatively higher expression only in roots, *GmAOC5* is only strongly expressed in stems, and *GmAOC3* is expressed highly in flowers (Wu et al., 2011). In tomato, the *AOC* gene is specifically expressed in vascular bundles and surrounding parenchymatic cells (Hause et al., 2000). In *Leymus mollis*, *LmAOC* expression is highly induced by drought, heat, cold, and wounding, as well as by JA and abscisic acid (ABA) (Eltayeb Habora et al., 2013). Four *AOC* genes in *A. thaliana* are constitutively expressed in all leaf tissues (Stenzel et al., 2003). Although some plant *AOC* genes have been characterized in many plant species, the role of peanut *AOC*, particularly in stress tolerance, remains unknown.

Cultivated peanut (*Arachis hypogaea* L.) is one of the most important oilseed crops worldwide. It is widely cultivated in tropical, subtropical, and temperate climates. Like other crops, peanut is susceptible to many adverse environmental stresses. Therefore, understanding the molecular basis of stress responses is a key target for peanut genetic improvement

programs. In the present study, we isolated a novel *AOC* gene from peanut and transferred the gene into rice to increase salinity resistance in transgenic rice by overexpression of the protein.

## MATERIAL AND METHODS

### Plant materials, growth conditions, and treatments

Peanut cultivars (*A. hypogaea* L., 'Luhua 14') seeds were germinated at 23°C for 1 week, and then the germinated seedlings were transferred to plastic flats containing 15 L nutrient solution. The nutrient solution contained (in mM) 0.2 $KH_2PO_4$; 0.5 $MgSO_4$; 1.5 KCl; 1.5 $CaCl_2$; 1 x $10^{-3}$ $H_3BO_3$; 5 x $10^{-5}$ $(NH_4)_6Mo_7O_{24}$; 5 x $10^{-4}$ CuSO4; 1 x $10^{-3}$ $ZnSO_4$; 1x$10^{-3}$ $MnSO_4$; 1x$10^{-1}$ Fe(III)-EDTA. The seedlings were grown in a controlled environment growth chamber under the following conditions: 16 h light/8 h dark photoperiod with a light intensity of 400 $\mu$M $m^{-2}$ $s^{-1}$, 23°C day and night temperatures, and relative humidity of 60-75%. Two-week-old seedlings with similar heights were transferred to nutrient solution containing 100 $\mu$M ABA, 50 $\mu$M methyl-jasmonic acid (MeJA), 50 $\mu$M salicylic acid (SA), 120 mM NaCl, and 20% polyethylene glycol (PEG) 6000, respectively. For cold stress treatments, plants were placed in a dark chamber at 4°C. At 0, 3, 8, 12, 24, 36, and 72 h after beginning treatment, the leaves were quickly frozen in liquid nitrogen and stored at -70°C until use.

Field grown plants of Luhua14 were used to analyze tissue-specific expressions of *AhAOC*. At 15 days after flowering, leaf, stem, root, flower, and gynophore tissues were sampled for gene expression analysis.

### Isolation, sequencing, and analysis of *AhAOC*

*AhAOC* gene was obtained using an *in silico* cloning strategy. The reported nucleotide sequence of *AOC* (NCBI: EU146298) from rice was used as a query sequence to search the peanut expressed sequence tag database (http://www.ncbi.nlm. nih.gov) using the blastn program. Based on the resulting sequence of interest, primer pairs (forward primer: 5'-CACACACCCCAATAACCTTCC-3', and reverse primer: 5'-AGTCAGTGAAACCAGAAATAAC-3') were designed to amplify the cDNA sequences of *AhAOC* in Luhua 14. Polymerase chain reaction (PCR) was performed using *Pfu* DNA polymerase (Promega, Madison, WI, USA). The gene isolated was sequenced by ABI3730 DNA Analyzer (Applied Biosystems, Inc., Foster City, CA, USA).

### Gene structure and bioinformatic analysis

The DNAMAN (5.2.2) software was used for sequence analysis. The coding region was identified using the open reading frame finder (http://www.ncbi.nlm.nih.gov/gorf/gorf. html). *AhAOC* homologs were identified using the NCBI BLAST server.

### Real-time quantitative PCR and semi-quantitative reverse transcription (RT)-PCR analysis

Total RNA was extracted from peanut and rice using the AxyPrep™ Multisource Total RNA Miniprep Kit (Axygen, Union City, CA, USA) and treated with RNase-free DNase (Ta-

kara, Shiga, Japan). First-strand cDNA was synthesized using oligo-(dT)$_{18}$ as an anchor primer and M-MLV reverse transcriptase (Promega) according to manufacturer instructions. *AhActin* and *OsActin* were used as internal reference genes to assess expression levels in peanut and rice, respectively, and the primers for these genes were specific for amplifying a single gene. Real-time quantitative PCR was conducted by using the SYBR® Premix Ex Taq™ II (Takara) in a Mastercycler® ep realplex (Eppendorf, Hamburg, Germany). Each 20-µL sample was subjected to 40 cycles at 94°C for 5 s and 60°C for 20 s. Triplicate quantitative assays were performed for each cDNA sample. The relative expression of *AhAOC* was detected using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). For semi-quantitative RT-PCR, the following program was used: 5 min denaturation at 94°C, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, with a final extension step for 8 min at 72°C. The primer sequences used for semi-quantitative RT-PCR and real-time quantitative PCR are listed in Table1.

**Table 1.** Primer sequences used for real-time quantitative PCR and semi-quantitative RT-PCR.

| Name | Sequence (5' to 3') | Name | Sequence (5' to 3') | Function |
|---|---|---|---|---|
| *AhActin-F* | GTGGCCGTACAACTGGTATTGT | *AhActin-R* | TGGATGGCTGGAAGAGAACT | real-time quantitative PCR |
| *AhAOC-F* | GTATGAGATCAATGAGAGAGAT | *AhAOC-R* | TACCTGTCCCCTTTCTTCTCTG | semi-quantitative RT-PCR |
| *OsACTIN1-F* | CCTCGTCTCGACCTTGCTGGG | *OsACTIN1-R* | GAGAACAAGCAGGAGGACG | real-time quantitative PCR |
| *OsDREB1F-F* | GGCGGAAGAAATTCCGGGAG | *OsDREB1F-R* | GCGATGGCCGCGACGTCGTG | real-time quantitative PCR |
| *OsLEA3-F* | AGACCAAGGCCCACACCGAG | *OsLEA3-R* | CCGTCTCCGACGCCCTCTC | real-time quantitative PCR |
| *OsONAC045-F* | ATATGCCTCCACCTCCTCCT | *OsONAC045-R* | CATCTCGTAGGGCTTTCACC | real-time quantitative PCR |
| *OsMYB2-F* | GGCTGAAACGCACAGGCAAG | *OsMYB2-R* | CTGCTTG GCGTGCTTCTGC | real-time quantitative PCR |

## Vector construction and plant transformation

The full-length *AhAOC* coding sequence was amplified using the primers 5'-GG<u>GGTACC</u>ATGGCTTCCTCATCAACTACTTTTG-3' (forward, *Kpn*I site underlined) and 5'-GC<u>GAGCTC</u>TCAGTGAAACCAGAAATAACAGCATG-3' (reverse, *Sac*I site underlined). The coding region was cloned into the *Kpn*I and *Sac*I sites of the pUN1301 vector under control of the ubiquitin promoter. The pUN1301-*AhAOC* construct was transferred into *A. tumefaciens* EHA105 cells and then introduced into rice embryonic calli and T$_1$ transgenic plants were confirmed by PCR analysis using gene-specific primers of *AhAOC* as described above.

## Physiological analysis of plants expressing *AhAOC* under salt stress

For salt treatment, seeds from wild-type and 2 T$_3$ generation *AhAOC*-overexpressing rice lines (OE11 and OE13) were grown on MS medium containing 0, 80, and 120 mM NaCl. After 1 week, root length of 20 plants was measured. For seedling treatment with salt stress, 3-week-old plants grown in pots were treated with 120 mM NaCl for 2 weeks. Next, the leaves of wild-type and transgenic lines were collected. JA, proline, and soluble sugar content were measured as described previously by Dong et al. (2013), Bailey et al. (1958), and Shan et al. (2007), respectively.
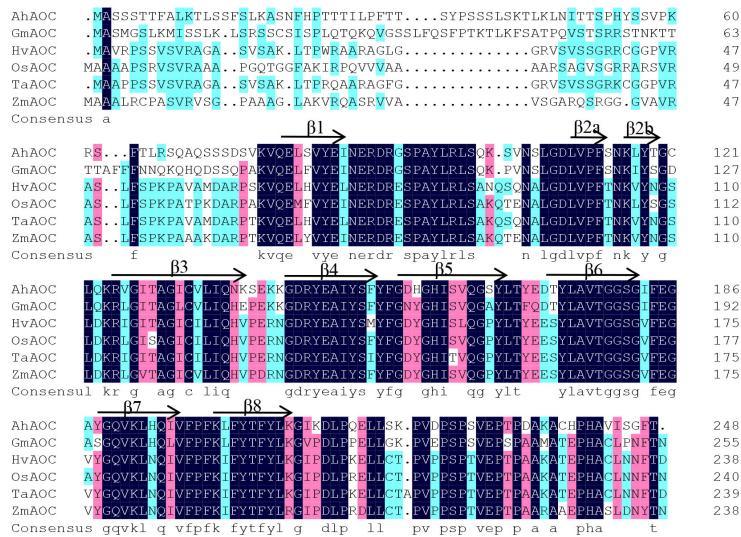
## Statistical analysis

Data was analyzed using analysis of variance.

# RESULTS

## *AhAOC* gene cloning and sequence analysis

A 852-bp cDNA fragment was amplified by RT-PCR and referred to as *AhAOC*. Present in the cDNA fragment was a 105-bp 5'-untranslated region, 746-bp coding region, and 1-bp 3'-untranslated region. The deduced peptide consisted of 248 amino acids with a molecular mass of 27,263.0 Da and pI of 9.46. Multi-alignment analysis revealed that the protein shared 70, 66, 82, and 67% amino acid identity with those in soybean (HM803106.1), *Hordeum vulgare* (CAC83766.1), *Oryza sativa* (CAD38519), wheat (AGU41846), and *Zea mays* (AAR33049) (Figure 1).

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AhAOC  .MASSSTTFALKTLSSFSLKASNFHPTTTILPFTT....SYPSSSLSKTLKLNITTSPHYSSVPK   60
GmAOC  .MASMGSLKMISSLK.LSRSSCSISPLQTQKQVGSSLFQSFPTKTLKFSATPQVSTSRRSTNKTT   63
HvAOC  .MAVRPSSVSVRAGA..SVSAK.LTPWRAARAGLG...........GRVSVSSGRRCGGPVR       47
OsAOC  MAAAAPSRVSVRAAA..PGQTGGFAKIRPQVVVAA............AARSAGVSGRRARSVR      49
TaAOC  .MAAPPSSVSVRAGA..SVSAK.LTPRQAARAGFG...........GRVSVSSGRKCGGPVR      47
ZmAOC  MAAALRCPASVRVSG..PAAAG.LAKVRQASRVVA............VSGARQSRGG.GVAVR     47
Consensus a

                       β1                          β2a     β2b
AhAOC  RS...ETLRSQAQSSSDSVKVQELSVYEINERDRGSPAYLRLSQK.SVNSLGDLVPESNKLYTGC   121
GmAOC  TTAFFENNQKQHQDSSQPAKVQELFVYEINERDRGSPAYLRLSQK.PVNSLGDLVPESNKIYSGD   127
HvAOC  AS..LFSPKPAVAMDARPSKVQELHVYEINERDRESPAYLRLSANQSQNALGDLVPETNKVYNGS   110
OsAOC  AS..LFSPKPATPKDARPAKVQEMEVYEINERDRESPAYLRLSAKQTENALGDLVPETNKIYSGS   112
TaAOC  AS..LFSPKPAVAMDARPTKVQELHVYEINERDRESPAYLRLSAKQSQNALGDLVPETNKVYNGS   110
ZmAOC  AS..LFSPKPAAAKDARPTKVQELYVYEINERDRESPAYLRLSAKQTENALGDLVPETNKVYNGS   110
Consensus      f       kvqe  vye nerdr spaylrls   n lgdlvpf nk y g

              β3          β4          β5                  β6
AhAOC  LQKRVGITAGTCVLIQNKSEKKGDRYEAIYSFYFGDHGHISVQGSYLTYEDTYLAVTGGSGIFEG   186
GmAOC  LQKRPGITAGICVLIQHEPEKKGDRYEAIYSFYFGNYGHISVQGAYLTFQDTYLAVTGGSGIFEG   192
HvAOC  LDKRPGITAGICILIQHVPERNGDRYEAIYSMYFGDYGHISLQGPYLTYEESYLAVTGGSGVFEG   175
OsAOC  LDKRPGISAGICILIQHVPERNGDRYEAIYSFYFGDYGHISVQGPYLTYEESYLAVTGGSGVFEG   177
TaAOC  LDKRIGITAGICILIQHVPERNGDRYEAIYSIYFGDYGHITVQGPYLTYEESYLAVTGGSGVFEG   175
ZmAOC  LDKRLGVTAGTCVLIQHVPDRNGDRYEAIYSFYFGDYGHISVQGPYLTYEESYLAVTGGSGVFEG   175
Consensul kr g  ag c liq    gdryeaiys yfg  ghi  qg ylt    ylavtggsg feg

             β7          β8
AhAOC  AYGQVKLHQLVFPFKLFYTFYLKGIKDLPQSLLSK.PVDPSPSVEPTEDAKACHPHAVISGFT.   248
GmAOC  ASGQVKLHQLVFPFKLFYTFYLKGVPDLPPELIGK.PVEPSPSVEPSEAAMATEPHACLPNFTN   255
HvAOC  VYGQVKLNQIVFPFKLFYTFYLKGIPDLPKBLLCT.PVEPSPTVEPTBAAKATEPHACLNNFTD   238
OsAOC  AYGQVKLNQIVFPFKLFYTFYLKGIPDLPRNLLCT.PVEPSPTVEPTBAAKATEPHACLNNFTD   240
TaAOC  VYGQVKLNQIVFPFKLFYTFYLKGIPDLPKBLLCTAPVEPSPTVEPTBAAKATEPHACLNNFTD   239
ZmAOC  VYGQVKLNQIVFPFKLFYTFYLKGIPDLPRDLLCT.PVEPSPTVEPTBAARAABPHASLDNYTN   238
Consensus gqvkl q vfpfk fytfyl g  dlp  ll   pv psp vep p a a  pha     t
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**Figure 1.** An alignment is shown for the deduced amino acid sequence of area under the curve from peanut, soybean, barley, rice, wheat, and maize. Residues that were identical in all proteins are shown with a black background. The arrows indicate 8 β-strands. The numbers on the right indicate the amino acid position. Protein accession numbers are as follows: ZmAOC, AAR33049; HvAOC, CAC83766.1; OsAOC, CAD38519; GmAOC, HM803106.1; TaAOC, AGU41846.

## Expression pattern of *AhAOC* in different tissues and response to abiotic stresses

We first analyzed tissue-specific expression of *AhAOC* in Luhua 14 at 15 days after flowering. Transcripts of *AhAOC* under field conditions were primarily observed in the root, leaf, and root, with less expression in the stem and gynophores (Figure 2). We next examined the transcriptional responses of *AhAOC* under multiple abiotic stresses, such as MeJA, ABA, SA, cold (4°C), 120 mM NaCl, and 20% PEG6000 (Figure 3). *AhAOC* showed different transcript regulation patterns under different hormone conditions (Figure 3A-C). In the presence of MeJA, the expression of *AhAOC* increased from 0-36 h, reached a maximum at 12 h, and then decreased sharply at 72 h (Figure 3A). Treatment with ABA led to a significant increase in transcript levels within 72 h and was the highest at 36 h (Figure 3B). SA significantly improved

the mRNA level within first 12 h, then reduced at 24 h, but quickly and remarkably induced transcripts again at 36 h, followed by a quick reduction at 72 h (Figure 3C). Cold did not significantly change transcription levels within 12 h, but was followed by significant increased levels, peaking at 24 h (Figure 3D). During the treatment period, NaCl stress markedly increased expression levels of the *AhAOC* gene, peaking at 8 h (Figure 3E). PEG-induced stress did not alter transcript levels during the first 3 h under treatment, but greatly increased transcription levels at 12 h, followed by a quick reduction to its basal levels at 72 h (Figure 3F).
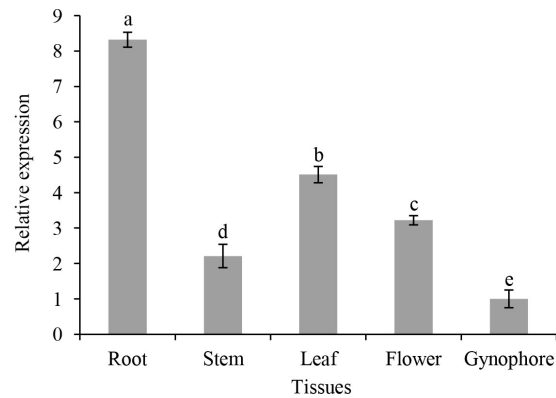


**Figure 2.** Relative expression levels of *AhAOC* in different tissues. Data are reported as means ± SE of 3 biological replicates. Each replicate contained 10 plants. Different letters above the column indicate statistical significance at the 0.05 probability level.
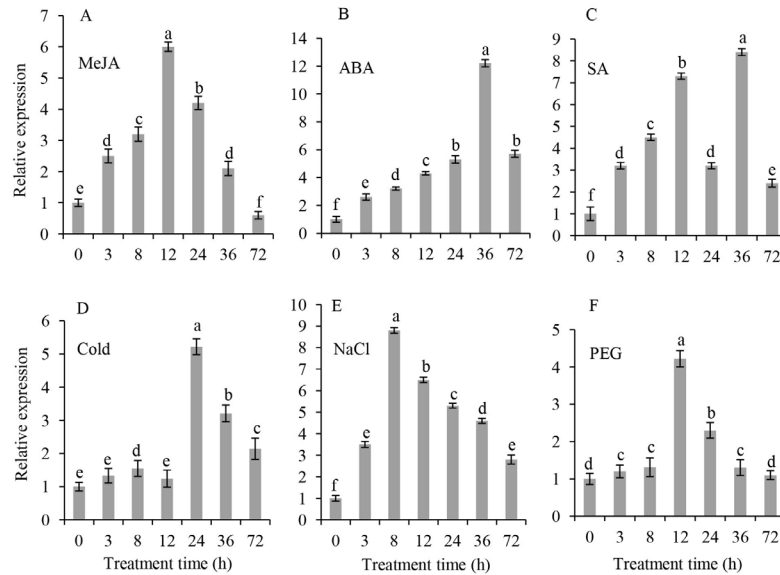


**Figure 3.** Expression profiles of *TaAOC* response to MeJA, ABA, SA, cold, NaCl, and 20% PEG6000. Each replicate contained ten plants. Different letters above the column indicate statistical significance at the 0.05 probability level.

## *AhAOC* overexpression in rice results in enhanced tolerance to salt stress

The mRNA level of *AhAOC* was strongly increased in the 2 $T_3$ transgenic lines OE11 and OE13 (Figure 4A). To determine the salt tolerance of transgenic rice, *AhAOC*-overexpressing and wild-type seeds were germinated on MS plates supplemented with 0, 80, or 120 mM NaCl. The high concentrations of NaCl inhibited root growth, but the transgenic lines showed longer root lengths (Figure 4B and C). We next examined the salt tolerance of rice seedlings from the 2 overexpression lines. The seedlings were cultured in pots for 3 weeks and were subsequently treated with 120 mM NaCl. After salt treatment for 2 weeks, most of the wild-type seedlings were wilting and yellow. Under control conditions (0 mM NaCl), there was no significant difference in plant heights between the wild-type and transgenic plants. After the plants were treated with NaCl for 2 weeks, plant heights reduced significantly. Moreover, this reduction was greater in wild-type than in the transgenic plants (Figure 4E). In addition, the contents of JA, soluble sugar, and proline were higher in *AhAOC*-overexpressing plants under salt stress (Figure 4F-H).
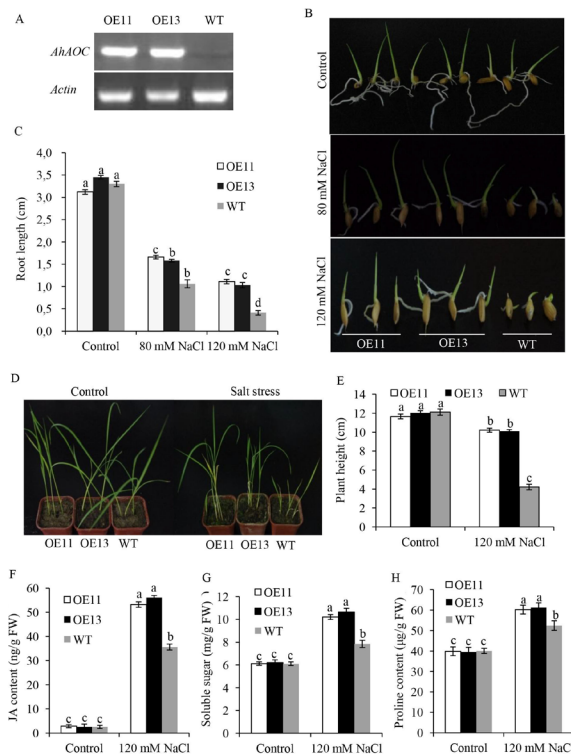


**Figure 4.** Analysis of salt tolerance in *AhAOC*-overexpressing rice. **A.** Semi-quantitative RT-PCR analysis of the *AhAOC* expression in wild-type (WT) and transgenic plants. **B.** Germination phenotypes of WT and transgenic plants on medium containing 0, 80, or 120 mM NaCl for 1 week. **C.** Root lengths of WT and *AhAOC*-overexpressing plants on medium containing 0, 80, or 120 mM NaCl for 1 week. **D.** Seedling growth performance of WT and *AhAOC*-overexpressing plants after 3 weeks with 120 mM NaCl. **E.** Plant heights, JA, soluble sugar, and proline content in transgenic and WT plants exposed to 120 mM NaCl for 3 weeks. Different letters above the column indicate statistical significance at the 0.05 probability level.

## Expression of stress-associated genes in transgenic rice

The relative expression levels of several known stress-associated marker genes *Os-DREB1F*, *OsLEA3*, *OsMYB2*, and *OsONAC045*(Moons et al., 1995; Wang et al., 2008; Zheng et al., 2009; Yang et al., 2012) were examined in 2 $T_3$ generation *AhAOC*-overexpressing rice lines using real-time quantitative PCR. All genes exhibited increased expression levels in 2 transgenic lines with salt treatment compared with control plants (Figure 5).
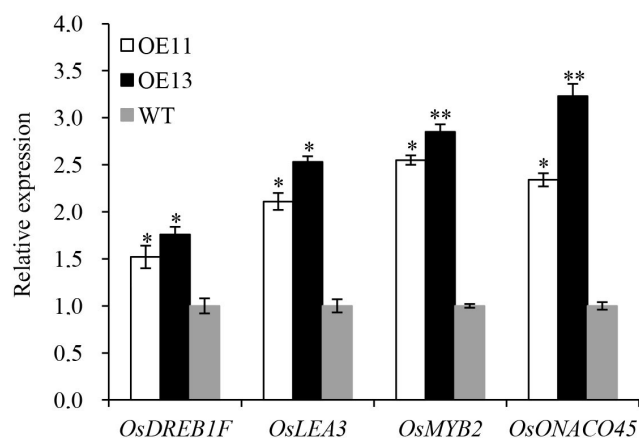


**Figure 5.** Expression patterns of 4 stress-responsive genes in response to salt treatment. Each column represents an average of 3 replicates. ** and * indicate significant differences compared with WT at $P < 0.01$ and 0.05, respectively.

## DISCUSSION

In the present study, we cloned the full-length cDNA of *AhAOC* containing an open reading frame of 746 bp and encoding a protein of 248 amino acids from the cultivated peanut Luhua 14. Analysis of tissue-specific expression revealed *AhAOC* expression in 5 different tissues, with the highest relative expression in the roots (Figure 2A). A previous study in soybean also found that *GmAOC2* was predominantly expressed in the roots (Wu et al., 2011). This is reasonable because roots are buried in soil and can sense the presence of salt. The dynamic expression patterns of *AhAOC* were affected by cold (4°C), NaCl, and 20% PEG6000 (Figure 3), indicating that *AhAOC* may be involved in the cross-talk of the multi-environmental stress response in peanut. ABA, MeJA, and SA are important cellular regulators in the response to various environmental stresses in plants (Grill and Himmelbach, 1998; Wasternack and Hause, 2002; Horváth et al., 2007). *AhAOC* was temporally regulated by ABA, SA, and MeJA treatments (Figure 3), suggesting that upregulation of *AhAOC* in the presence of NaCl, cold, and 20% PEG6000 may be associated with ABA-, SA-, and MeJA-mediated signaling pathways. Further studies should be conducted to examine the molecular mechanism through which the *AhAOC* protein interacts with the signal transduction systems of these 3 hormones.

In the current study, we found that overexpression of *AhOC* in rice significantly improved salt stress tolerance. This tolerance was supported by phenotypic performance and

physiological indices changes. Root elongation was significantly higher in transgenic lines compared to in wild-type plants (Figure 4B). Plants may enhance stress tolerance by accumulating osmolytes, such as soluble sugars and free proline, to protect cell structures (Szabados and Savouré, 2010; Cui et al., 2011). The increased soluble sugars and proline content under environmental stresses significantly enhanced plant stress tolerance (Abrahám et al., 2003; Verbruggen and Hermans, 2008; Cui et al., 2011; Cvikrová et al., 2012). To investigate the potential mechanisms underlying the improved stress tolerance of *AhAOC* in overexpressed plants, soluble sugars and free proline levels were measured under salt stress conditions. The results indicated that the content of soluble sugars and free proline in the transgenic plants were significantly higher than those in wild-type plants under salt stress (Figure 5). Therefore, higher tolerance against salt stresses in transgenic rice may result from osmolytes accumulation. To analyze the improved salt tolerance at the molecular level, expression of 4 stress-responsive genes (*MYB2*, *ONAC045*, *DREB1F*, and *LEA3*) were examined between transgenic plants and controls. Overexpression of *AhAOC* in rice led to higher expression of all 4 stress-related genes (Figure 6). It has been found that the transcription factors *MYB2* and *OsONAC045* were involved in ABA-inducible gene expression, *OsDREB1F* and *OsLEA3* maybe induced by different stresses through an ABA-dependent pathway, and they also play positive roles in salt tolerance (Moons et al., 1995; Wang et al., 2008; Zheng et al., 2009; Yang et al., 2012). *AhAOC* may act as an activator to increase the expression levels of transcription factors (*OsMYB2* and *OsONAC045*) and functional genes (*OsDREB1F* and *OsLEA3*) under salt stress condition.

In conclusion, we identified and characterized a novel *AhAOC* gene from cultivated peanut. *AhAOC* was differentially expressed in response to diverse stresses and hormones, and overexpression of *AhAOC* led to enhanced tolerance to salt stress in transgenic rice. *AhAOC* may be useful for molecular breeding of salt tolerant crops, such as peanut, wheat, maize, and other gramineous plants.

## ACKNOWLEDGMENTS

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