Plant Science xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

Isolation of an alcohol dehydrogenase cDNA from and characterization of its expression in chrysanthemum under waterlogging

Q1 Dongmei Yin, Dian Ni, Lili Song, Zhiguo Zhang*

School of Ecological Technology and Enginneering, Shanghai Institute of Technology, Shanghai 201418, China

ARTICLE INFO

Article history:
Received 27 January 2013
Received in revised form 21 May 2013
Accepted 31 May 2013
Available online xxx

Keywords:
Waterlogging
Chrysanthemum
CgADH
Ethylene
Gene expression

ABSTRACT

A PCR strategy was used to isolate a full-length *CgADH* (alcohol dehydrogenase) cDNA from chrysanthemum. The gene putatively encodes a 378 residue polypeptides, which shares 95% homology with tomato alcohol dehydrogenase class III. Endogenous ethylene generated in waterlogged *Chrysanthemum zawadskii* was enhanced by exogenous ethylene but decreased by 1-methylcyclopropene (1-MCP), an inhibitor of ethylene action. In waterlogged roots, the transcription of the gene encoding alcohol dehydrogenase (ADH, EC 1.1.1.1) increased rapidly but transiently, peaking at 7.5 fold the non-waterlogged level after 2 h of stress. Waterlogging elevated ADH activity after a prolonged episode of stress. The exogenous supply of 40 $\mu L L^{-1}$ ethylene suppressed the production of ethanol, while that of 4 $\mu L L^{-1}$ 1-MCP enhanced it. Ethylene appeared to suppress an acceleration of both *CgADH* expression and fermentation, and alleviates ethanolic fermentation probably through by as a signal to acceleration of waterlogging-induced aerenchyma formation. This supports the previously observed phenomenon that the expression level of *ADH* gene is regulated by the local level of physiologically active ethylene. The relevance of the *CgADH* gene in relation to chrysanthemum waterlogging was discussed as well.

1. Introduction

Chrysanthemum (*Chrysanthemum grandiflorum*) is an important cut flower and pot plant species [1]. A likely scenario arising from climate change includes a higher incidence of extreme rainfall events, increasing the need to improve the tolerance of plants to waterlogging [2,3]. Waterlogging can lead to the accumulation of ethylene, which in rice has been shown to trigger shoot elongation, adventitious root formation, aerenchyma development, carbohydrate consumption and ethanolic fermentation [4,5]. In the submergence tolerant species *Rumex palustris*, a 1 h episode of submergence induces a 20 fold increase in ethylene concentration [6], and this elevated level of ethylene in turn promotes out growth, which can be abolished by treatment with the ethylene inhibitor 1-methylcyclopropene (1-MCP) [7].

Hypoxia generated through waterlogging induces a switch from aerobic respiration to anaerobic fermentation via the activation of the glycolytic and fermentation pathways. The regulation of genes encoding both ADH and pyruvate decarboxylase (PDC) is at least in part related to ethylene signalling [8]. Expression of the alcohol dehydrogenase gene (*ADH*) of Arabidopsis is induced during hypoxia [9]. Because increased production and accumulation of ethylene is essential for a variety of adaptive responses to waterlogging [5], and many plants increase their ethylene production in response to hypoxic stress, we examined in this report whether ethylene is involved in the hypoxic induction of *CgADH* in chrysanthemum.

Genetic variation for tolerance has been documented both between species within a taxon and, to some extent, within species [10]. The herbaceous perennial *Chrysanthemum zawadskii*, one of the most popular plants for landscaping in China, is highly tolerant of waterlogged conditions [11] Ethylene production in the ornamental species chrysanthemum is known to be induced by waterlogging [12]. Here we report the isolation of a *CgADH* cDNA from chrysanthemum. We show that transcript levels of this gene can be modulated by the exogenous application of ethylene during waterlogging. This represents an early step towards understanding both the mechanism of plants response to waterlogging and the role of ethylene in the tolerance of chrysanthemum. Finally, this information will provide the basis for designing strategies to genetically manipulate to a short episode of waterlogging stress in this valuable ornamental species.

Abbreviations: ADH, Alcohol dehydrogenase; PCD, Programmed cell death; PDC, Pyruvate decarboxylase; qRT-PCR, Quantitative reverse transcription-polymerase chain reaction; 1-MCP, 1-methylcyclopropene.

* Corresponding author at: Shanghai Institute of Technology, 100 Haiquan Road, Fengxian, Shanghai 201418, China. Tel.: +86 21 60873096; fax: +86 21 60873128.
E-mail address: zgzhang@sit.edu.cn (Z. Zhang).

0168-9452/$ – see front matter © 2013 Published by Elsevier Ireland Ltd.
http://dx.doi.org/10.1016/j.plantsci.2013.05.017

Table 1
The sequence of the PCR primers used in this study.

Primer name	Primer sequence (5′-3′)	Usage
oligo (dT) primer	GACTCGAGTCGACATCGATTTTTTTTTTTTTTT	3′RACE
674ADH	CGAGCAACCGATACAGCAGG	
713ADH	TGGAGGTGTTGATTACAGTTT	
dT-AP	GACTCGAGTCGACATCGA	
693fzl	CCTGCTGTATCGGTTGC	5′RACE
618-5′	TTCTGGTGTCAATATCTATCCC	
324-5′	AGCGACTCTTGCGATCATTC	
AAP	GGCCACGCGTCGACTAGTACGGGIIGGGIIGGGIIG	
AUAP	GGCCACGCGTCGACTAGTAC	
S-ADH	ATGGCTTCTACTCAAGGAAA	ORF amplification
A-ADH	TTCTACCCCATCTATGTCTGA	
Actin-F	ACATGCTATCTTGCGTTTGG	qRT-PCR
Actin-R	CTCTCACAATTTCCCGTTCA	
S-adh	AGCTGGAGACCATGTGATACCT	
A-adh	AGCGACTCTTGCGATCATTC	

2. Materials and methods

2.1. Plant materials and the exogenous application of ethylene and 1-MCP

Chrysanthemum zawadskii plants obtained from the Chrysanthemum Germplasm Resource Preserving Centre (Nanjing, China) were grown in pots containing a 2:1 mixture of garden soil and vermiculite. The materials were maintained in a greenhouse under 160 μmol m^{-2} s^{-1} photosynthetically active radiation, a 12 h photoperiod, a mean temperature of 25 °C, and a relative humidity of ~70%. Plants at the eight node stage were flooded by standing the pots in a container filled with tap water (pH 7.3, electrical conductivity 0.34 dS m^{-1}) to a level of 2.5 cm above the soil surface. For the ethylene or 1-MCP treatments, the pots were sealed in an 8.5 L chamber held at 25 °C into which either 340 μL ethylene or 34 μL 1-MCP was then introduced. A 1 M solution of NaOH was placed on the floor of the chamber to prevent the accumulation of CO_2 [13]. As a control, both waterlogged and well-watered (60% soil moisture) plants were sealed in an identical chamber without the addition of either ethylene or 1-MCP.

2.2. Isolation of genes encoding CgADH from chrysanthemum

Total RNA was isolated from frozen roots (0.3 g fresh weight equivalent) using the TRIzol reagent (Invitrogen). Contaminating DNA was removed by a DNase I treatment, and the integrity of the RNA preparations was checked by agarose gel electrophoresis. The cDNA first strand from 500 ng total RNA was synthesized using a M-MLV RTase cDNA Synthesis kit (TaKaRa, Japan) in the presence of 5 μM oligo dT following the manufacturer's protocol. For sequences, see Table 1, designed from relevant cDNA sequences available at http://blocks.fhcrc.org/blocks/codehop.html. Each 50 μL PCR contained 10 mM Tris–HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 10 pmol of each primer, 1.25 U Ex Taq (TaKaRa) and 50 ng first strand cDNA. The cycling regime began with a denaturation step (95 °C/5 min), followed by 35 cycles of 94 °C/60 s, 60 °C/60 s and 72 °C/60 s, and completed with an extension step (72 °C, 10 min). Amplicons were separated by 2% agarose gel electrophoresis, the relevant fragments were recovered from the gel and purified using an Agarose Gel DNA Purification Kit v2.0 (TaKaRa), after which they were introduced into the pGEM-T easy vector (Promega, Madison, WI, USA) for the purpose of sequencing. A full-length ADH cDNA sequence was obtained via RT-PCR supplemented with RACE-PCR, using primers designed from a chrysanthemum *CgADH* cDNA sequence (GenBank accession AB206874). For the 3′ RACE, the first-strand cDNA was synthesized from poly (A+) RNA using oligo (dT) primer with the adaptor primer sequence at the 5′ end. Subsequently, nested PCR was applied using the gene-specific primers 674ADH and 713ADH (sequences given in Table 1) and the adaptor primer (dT-AP). For the 5′ RACE, the first-strand cDNA was synthesized from poly (A+) RNA using the gene-specific primer 693fzl (sequence shown in Table 1) in combination with a 5′ RACE System kit v2.0 (Invitrogen), and subjected to nested PCR using the 5′ RACE adaptor primer (abridged anchor primer, AAP) and the abridged universal amplification primer (AUAP) provided with the kit, along with the gene-specific primers 618-5′ and 324-5′ (sequences given in Table 1). All amplicons were introduced into the pGEM-T easy plasmid for the purpose of sequencing. The open reading frame of *CgADH* was isolated by PCR using primer S-ADH and A-ADH (sequences given in Table 1) from a template of pooled cDNA.

2.3. Quantitative reverse transcription-PCR (qRT-PCR)

The amplification signal in a Light Cycler Instrument machine (Bio-Rad iQ5, USA) was measured using SYBR GreenER™ qPCR SuperMix Universal (Invitrogen). The amplification regime consisted of a denaturation step of 95 °C/3 min, followed by 40 cycles of 95 °C/15 s, 55 °C/15 s and 72 °C/45 s, using the primer pairs A-adh/S-adh or A-pdc/S-pdc (sequences given in Table 1). The chrysanthemum gene encoding actin (AB205087) was used as a reference, and was amplified by the primer pair Actin-F and Actin-R (sequences given in Table 1). Each qRT-PCR consisted of three technical and three biological replicates, and the level of gene expression was calculated following Yang et al. [14]

2.4. Ethylene production

Ethylene was detected following the method described by Yin et al. [12]. Stem segments were harvested at 0, 2, 6, 12, 24, 48 and 72 h after first being exposed to either waterlogging, ethylene treatment and waterlogging, 1-MCP treatment and waterlogging, or control (no treatment). The material was washed and blotted dry. Ethylene release was monitored using a gas chromatograph equipped with TRB-5 capillary column (0.32 mm id, 30 m length, 0.25 μm df) and a flame ionization detector. The column, injection and detector temperatures were, respectively, 50 °C, 150 °C and 150 °C. Nitrogen was employed as the carrier gas.

2.5. Enzyme and metabolite extraction and assay

ADH activity was assessed spectrophotometrically at 340 nm, following the methods described by Yin et al. (2009b). Fresh root sections (3.0–5.0 cm long, weight ~0.5 g) were harvested at 0, 6, 12, 24, 48 and 72 h after first being exposed to either waterlogging,

Please cite this article in press as: D. Yin, et al., Isolation of an alcohol dehydrogenase cDNA from and characterization of its expression in chrysanthemum under waterlogging, Plant Sci. (2013), http://dx.doi.org/10.1016/j.plantsci.2013.05.017