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Research article

Characterization of the expression profile of a wheat aci-reductone-dioxygenase-like gene in response to stripe rust pathogen infection and abiotic stresses

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ABSTRACT

The methionine salvage pathway is conserved from prokaryotes to high eukaryotes. The reaction catalyzed by aci-reductone-dioxygenase (ARD) represents a branch point in the methionine salvage pathway. A novel aci-reductone-dioxygenase gene, designed as *TaARD*, was identified in a subtraction library constructed with RNA isolated from wheat leaves infected with the stripe rust pathogen. *TaARD* was predicted to encode a 197 amino acid protein that belongs to the cupin superfamily. In transient expression assays with onion epidermal cells, the *TaARD*-GFP fusion protein localized to the nucleus and cytoplasm. Southern blot analysis showed that the wheat genome had multiple copies of *TaARD*. Quantitative real-time RT-PCR (qRT-PCR) analyses revealed that the *TaARD* transcript was induced in wheat leaves infected with a compatible stripe rust strain. However, its expression was reduced or suppressed in incompatible interactions and by ABA, ethephon (ET), or salicylic acid (SA) treatments. With methyl jasmonate (MeJA) treatment, *TaARD* transcript level was suppressed in the first 6 h but increased afterwards. The expression of *TaARD* also was inhibited by wounding and environmental stimuli, including high salinity and low temperature. Because of the role of ARD in the methionine salvage pathway, these results suggest that *TaARD* may be involved in ethylene synthesis and ethylene signaling in response to biotic and abiotic stresses.

1. Introduction

Plants have developed a sophisticated detection and defense system to protect them against pathogen invasion. The production of ethylene is one of the earliest responses of plants to pathogen attack, and ethylene seems to be associated with defense reaction by regulating a wide range of defense-related genes, including those encoding pathogenesis-related (PR) proteins, such as chitinase and osmotin [1–4]. However, ethylene may also promote disease symptom development depending on the conditions and the plant–pathogen combination [5,6].

S-adenosylmethionine (SAM) is one of the precursors for the biosynthesis of ethylene and polyamines (PAs) [7–9]. Based on the ethylene biosynthetic pathway, the first committed step of ethylene biosynthesis is the conversion of SAM to ACC by ACC synthase (*S*-adenosyl-L-methionine methylthioadenosine-lyase, EC 4.4.14) [10]. In this reaction, ACC synthase (ACS) also produces 5′-methylthioadenosine (MTA), which can be converted back to methionine. This methionine salvage pathway ensures a high level of ethylene production even when the pool of free methionine is relatively small.

The methionine salvage cycle exists not only in plants but also in bacteria, archeae and animals. In recent years, several enzymes involved in this pathway have been well characterized at the biochemical and molecular levels [11,12]. The aci-reductone-dioxygenase (ARD) catalyzes the reaction of aci-reductone with dioxygen to produce the immediate precursor of methionine, 2-keto-4-methylthiobutyrate and formate. ARD is a unique enzyme with two different enzymatic activities, depending on the metal ion bound to it as the cofactor [13]. Fe-ARD catalyzes the methionine cycle reaction whereas Ni-ARD catalyzes an off-pathway

Abbreviations: GFP, green fluorescent protein; SA, salicylic acid; ET, ethylene; JA, jasmonate; ABA, abscisic acid; RT-PCR, reverse transcription-polymerase chain reaction; qRT-PCR, quantitative real-time-PCR; hpi, hours post inoculation; hpt, hours post treatment; SSH, suppression subtractive hybridization; BLAST, basic local alignment search tool; PEG, polyethylene glycol; RACE, rapid amplified cloning end; ACS, 1-aminocyclopropane-1-carboxylic acid synthase; PAs, polyamines.

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leading to the formation of methylthiopropionate, formate and carbon monoxide [13,14]. Methylthiopropionic acid, a blight-inducing toxin in *Xanthomonas campetris* infected-cassava leaves, was a cytotoxin derived from this ARD reaction [15]. Enzyme activities of ARD from *Klebsiella* also have been investigated [13,16].

Recently, several studies have showed that *ARD* genes are involved in plant stress responses. Two rice *ARD* genes, *OsARD1* and *OsARD2*, have been cloned [14,17]. The expression of *OsARD1* was increased in the youngest internode of deepwater rice under the submergence, which provided evidence that ethylene could regulate the methionine cycle [14]. However, similar submergence-induced expression was not observed for a barley *ARD* gene *IDI1* [18] and a Arabidopsis *ARD* gene [11]. In potato, the expression of *StARD*, along with PAs content, was increased at the wounding surface of potato tubers, suggesting that ARD and PAs may play an important role in abiotic stress such as wounding [19]. However, in wheat, ARD has not been characterized and its role in responses to biotic or abiotic stresses is not clear.

Wheat stripe rust, caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriks., is a destructive disease of wheat worldwide [20]. In a previous study, one clone namely WSRP146 was identified to be highly homologous to the barely ARD gene in a suppression subtractive hybridization (SSH) cDNA library of wheat leaves infected with an incompatible *Puccinia striiformis* strain [21]. In this study, we generated the full-length cDNA sequence for this wheat ARD gene named *TaARD*. The copy number of the *TaARD* gene and its chromosomal location in wheat were investigated. We also characterized its subcellular localization by transient expression assays in onion epidermal cells and determined *TaARD* expression in response to *P. striiformis* infection and abiotic stress. Our results suggest that *TaARD* may take a part in the ethylene synthesis pathway and utilize ethylene signals in response to biotic and abiotic stresses.

2. Materials and methods

2.1. Plant materials, infection assays, and abiotic stress treatments

Two wheat (*Triticum aestivum* L.) cultivars (Suwon 11 and Chinese Spring) and two *P. striiformis* races (CYR23 and CYR31) were used in this work. The Chinese Spring nulli-tetrasomic lines were used for the chromosomal localization analysis of *TaARD*. Suwon11 contains the stripe rust resistant gene *YrSu*. It displays typical HR to CYR23 but is susceptible to CYR31 [22,23]. Wheat plants were grown and maintained following the procedures described by Kang and Li [24]. Freshly collected urediniospores of *P. striiformis* were inoculated onto the surface of the primary leaves of 2-week-old wheat seedlings with a paintbrush. Control plants were inoculated with sterile water as mock inoculations. After inoculation, all plants were kept in a humid chamber (100% humidity) for 24 h and then transferred to a growth chamber. After incubating at 15 °C with a regular day–night cycle, infected wheat leaves were excised at the indicated times post inoculation, rapidly frozen in liquid nitrogen, and stored at −80 °C before RNA extraction.

For tissue-specific expression analyses of *TaARD*, intact root, stem, and leaf tissues of 4-week-old wheat seedlings were collected. For chemical treatments, 4-week-old seedlings were sprayed with 0.2 mM and 2 mM salicylic acid (SA), 10 μM and 100 μM methyl jasmonate (MeJA), 10 μM and 100 μM ethephon (ET) or 10 μM and 100 μM abscisic acid (ABA) that were dissolved in 0.1% (v/v) ethanol [25]. For the mock control, wheat plants were treated with 0.1% (v/v) ethanol. Wheat seedlings were removed from soil and their roots were soaked in 200 mM NaCl for high salinity treatment, and in 15% PEG for drought stress. For low temperature stress, the seedlings were transferred to an incubator in which the temperature was set at 4 °C. Wounding was applied by cutting wheat leaves with a pair of sterilized scissors. Wheat seedlings treated with various chemicals and stress elicitors along with control plants were sampled at 0 h, 0.5 h, 2 h, 6 h, 12 h and 24 h post treatment (hpt). All leaf samples were rapidly frozen in liquid nitrogen and stored at −80 °C prior to RNA extraction. Three independent biological replications were performed for each experiment.

2.2. Total RNA isolation and first strand cDNA synthesis

Total RNA was isolated with the RNeasy Plant Mini kit (Qiagen, Germany) following the manufacture's instructions. DNaseI treatment was applied to remove contaminating genomic DNA. First strand cDNA was synthesized with 2 μg of purified RNA using the RevertAid™ First Strand cDNA Synthesis Kit (MBI, Fermentas) with the pd(N)6 random primer according to the manufacturer's protocol.

2.3. Isolation of full-length cDNA of TaARD

One expression sequence tag (EST) representing a gene with homology to the barely aci-reductone-dioxygenase (ARD) gene was identified in the SSH library [21]. In order to obtain the full-length cDNA of the *TaARD* gene, a rapid amplification of the 3′ cDNA end (3′ RACE) and 5′ cDNA end (5′ RACE) were performed with the SMART RACE-PCR kit (Clontech, Palo Alto, CA, USA). Gene-specific primers GSP1 for 5′ RACE and GSP2 for 3′ RACE are listed in (Table 1). The nucleotide and deduced amino acid

Table 1
A list of polymerase chain reaction primers used in this work.

Primer name	Primer sequence[a]	Usage
GSP1	5′-ATGGATGACAGTGAGGAGGACCAGAGGC-3′	Amplification of *TaARD* for 3′ cDNA end (3′ RACE) and 5′ cDNA end (5′ RACE)
GSP2	5′-GGAAGCCTCTGGTCCTCCTCACTGTCAT-3′	by rapid amplified cloning end (RACE)
Sfc146-1	5′-ATAAGAAACGCACATCCG-3′	Amplification of *TaARD* full-length coding sequences by RT-PCR
Afc146-1	5′-GCCGAACACTGTAGAAGC-3′	
GFP-P1	5′-CCCAAGCTT ATGGAGAACGAGTTCCAG-3′	P1 and P2 for construction of *TaARD* (WT)-GFP
GFP-P2	5′-CGGGATCCACGAGCTTCAACAGTTTG-3′	
F-P1	5′-ACTGCCCTCAAGACAATAG-3′	Quantitative real-time RT-PCR of *TaARD* transcripts
F-P2	5′-TGGGAGAAAGACGAGAATC-3′	
F-P3	5′-ACGGGATACTTGCCTTGA-3′	Quantitative real-time RT-PCR of *TaACS* (Accession No. U35779) transcripts
F-P4	5′-CGTCGCACCAGATGTTAT-3′	
Srt146-1	5′-CAGGATGGCAAGGAGGAGGT-3′	For chromosomal location of *TaARD* in Chinese spring derived aneuploid nulli-tetrasomic (NT)
Art 146-1	5′-TCATGGGGACGGTTGTATGG-3′	line and semiquantitative RT-PCR of *TaARD* transcripts
18S-F	5′-TTTGACTCAACACGGGGAAA-3′	Quantitative real-time RT-PCR and semiquantitative RT-PCR of 18S rRNA transcripts
18S-R	5′-CAGACAAATCGCTCCACCAA-3′	

[a] The underlined nucleotides form *Hin*dIII (AAGCTT), *Bam*HI (GGATCC), *Eco*I (GAATTC) or *Sac*I (GAGCTC) restriction sites.