Available online at www.sciencedirect.com
ScienceDirect
Plant Physiology and Biochemistry 46 (2008) 174–188
Plant Physiology and Biochemistry
www.elsevier.com/locate/plaphy

Research article

The drought response of *Theobroma cacao* (cacao) and the regulation of genes involved in polyamine biosynthesis by drought and other stresses

Hanhong Bae [a], Soo-Hyung Kim [a], Moon S. Kim [b], Richard C. Sicher [a], David Lary [c], Mary D. Strem [a], Savithiry Natarajan [a], Bryan A. Bailey [a,*]

[a] *US Department of Agriculture/Agricultural Research Service, Beltsville Agricultural Research Center, Beltsville, MD 20705, USA*
[b] *College of Forest Resources, UW Botanic Gardens, University of Washington, Box 354115, Seattle, WA 98195, USA*
[c] *Goddard Earth Science and Technology Center, University of Maryland Baltimore County, Baltimore, MD 21228, USA*

Received 4 June 2007
Available online 18 October 2007

Abstract

Drought can negatively impact pod production despite the fact that cacao production usually occurs in tropical areas having high rainfall. Polyamines (PAs) have been associated with the response of plants to drought in addition to their roles in responses to many other stresses. The constitutive and drought inducible expression patterns of genes encoding enzymes involved in PA biosynthesis were determined: an ornithine decarboxylase (*TcODC*), an arginine decarboxylase (*TcADC*), an *S*-adenosylmethionine decarboxylase (*TcSAMDC*), a spermidine synthase (*TcSPDS*), and a spermine synthase (*TcSPMS*). Expression analysis using quantitative real-time reverse transcription-PCR (QPCR) results showed that the PA biosynthesis genes were expressed in all plant tissues examined. Constitutive expression of PA biosynthesis genes was generally highest in mature leaves and open flowers. Expression of *TcODC*, *TcADC*, and *TcSAMDC* was induced with the onset of drought and correlated with changes in stomatal conductance, photosynthesis, photosystem II efficiency, leaf water potential and altered emission of blue-green fluorescence from cacao leaves. Induction of *TcSAMDC* in leaves was most closely correlated with changes in water potential. The earliest measured responses to drought were enhanced expression of *TcADC* and *TcSAMDC* in roots along with decreases in stomatal conductance, photosynthesis, and photosystem II efficiency. Elevated levels of putrescine, spermidine, and spermine were detected in cacao leaves 13 days after the onset of drought. Expression of all five PA associated transcripts was enhanced (1.5–3-fold) in response to treatment with abscisic acid. *TcODC* and *TcADC*, were also responsive to mechanical wounding, infection by *Phytophthora megakarya* (a causal agent of black pod disease in cacao), the necrosis- and ethylene-inducing protein (Nep1) of *Fusarium oxysporum*, and flower abscission. *TcSAMDC* expression was responsive to all stresses except flower abscission. *TcODC*, although constitutively expressed at much lower levels than *TcADC*, *TcSAMDC*, *TcSPDS*, and *TcSPMS*, was highly inducible by the fungal protein Nep1 (135-fold) and the cacao pathogen *Phytophthora megakarya* (671-fold). The full length cDNA for ODC was cloned and characterized. Among the genes studied, *TcODC*, *TcADC*, and *TcSAMDC* were most sensitive to induction by drought in addition to other abiotic and biotic stresses. *TcODC*, *TcADC*, and *TcSAMDC* may share signal transduction pathways and/or the stress induced signal induction pathways may converge at these three genes leading to similar although not identical patterns of expression. It is possible altering PA levels in cacao will result in enhanced tolerance to multiple stresses including drought and disease as has been demonstrated in other crops.
Published by Elsevier Masson SAS.

Keywords: Polyamine; *Theobroma cacao*; Drought; Nep1; Wounding; *Phytophthora*; ODC; ADC; SAMDC; SPDS; SPMS

* Corresponding author. Tel.: +1 301 504 7985; fax: +1 301 504 1998.
E-mail address: bryan.bailey@ars.usda.gov (B.A. Bailey).

0981-9428/$ - see front matter Published by Elsevier Masson SAS.
doi:10.1016/j.plaphy.2007.10.014

1. Introduction

Theobroma cacao (cacao) is a tropical tree typically grown in areas of high annual rainfall. Although somewhat counter-intuitive, cacao production is prone to periodic drought due to seasonal rainfall patterns that often include prolonged dry cycles. *Theobroma cacao* (cacao) is intolerant of drought [1,11,48,69], although very little research has been directed towards the identification and development of drought tolerant germplasm [10]. The limited drought tolerance of cacao is a growing concern in cacao production areas due to inconsistent rainfall patterns [1,10,11,48,69]. Cacao production is often limited by other abiotic stresses, such as nutrient deficiencies, and by biotic stresses, such as disease and insects [69].

Polyamines (PAs) are ubiquitous in both eukaryotes and prokaryotes (reviewed in [16,35]). The most common PAs in higher plants are putrescine (Put), spermidine (Spd) and spermine (Spm). PAs have important roles in plant physiological and developmental processes, such as cell division, regulation of morphogenesis, embryogenesis, floral initiation and development, flower and fruit development and ripening, leaf senescence, root growth, and tuberization. In plants, PAs are commonly associated with responses to biotic and abiotic stresses and have been shown to function in drought and chilling tolerance in some situations (reviewed in [12,30]).

In higher plants, there are two main pathways for PA biosynthesis (Fig. 1, modified from [27]). In eukaryotic cells, Put is synthesized directly from ornithine, through the activity of ornithine decarboxylase (ODC). In plants, there is an alternative pathway for Put synthesis, via the activity of arginine decarboxylase (ADC) and *Arabidopsis* lacks a sequence for ODC [21]. Spd and Spm are synthesized by spermidine synthase (SPDS) and spermine synthase (SPMS), respectively.

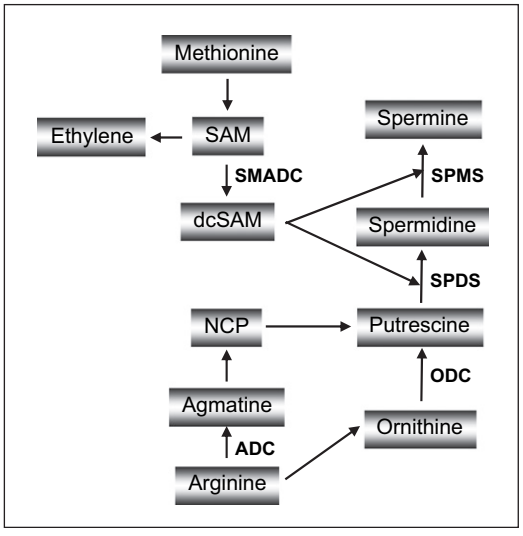

Fig. 1. Polyamine biosynthetic pathway in plants (modified from [27]). SAM, *S*-adenosylmethionine; SAMDC, *S*-adenosylmethionine decarboxylase; dcSAM, decarboxylated *S*-adenosylmethionine; NCP, *N*-carbamoylputrescine; ADC, arginine decarboxylase; SPMS, spermine synthase; SPDS, spermidine synthase; ODC, ornithine decarboxylase.

Spd and Spm are also synthesized by the successive addition of aminopropyl groups derived from decarboxylated *S*-adenosylmethionine (dcSAM) that is generated by *S*-adenosylmethionine decarboxylase (SAMDC).

In some cases the genes involved in PA biosynthesis are regulated developmentally and may show tissue specificity [17,24,25,46,51,59]. In addition, both ODC and ADC are known to be induced by various stresses, such as disease [18,71], chilling [24], osmotic stress [43,47,59], acidic pH [43,50], and nutrient deficiency [43]. SAMDC also plays an important role in plant developmental and physiological processes, as well as in plant responses to environmental stresses [25,54,64].

The study of PAs and the associated genes involved in their biosynthesis is limited in *Theobroma cacao*. Understanding the regulation of PA biosynthesis may aid in the development of stress management strategies and the selection of stress tolerant cacao. We have identified ESTs encoding ODC (*TcODC*), ADC (*TcADC*), SAMDC (*TcSAMDC*), SPDS (*TcSPDS*), and SPMS (*TcSPMS*) in cacao. The primary objectives of this research were to determine the tissue specific and developmental expression patterns of these five ESTs encoding enzymes involved in polyamine biosynthesis and to determine the relationship between their expression and physiological measures of the drought response in cacao. In addition, their inducibility by ABA and their responsiveness to multiple stresses were studied.

2. Methods

2.1. Plant materials and drought treatment

Open pollinated cacao seeds (*Theobroma cacao* variety comun, Lower Amazon Amelonado type) were harvested by Alan Pomella from the Almirante Cacau, Inc. farm (Itabuna, Bahia, Brazil) and shipped to Beltsville, MD. After removing the seed coat, seeds were surface sterilized in 14% sodium hypochlorite for 3 min followed by 3 washes in sterile distilled water. Three sterile seeds were placed on 1.5% water agar plate (100 mm in diameter) and the plates were sealed with parafilm. The plates were incubated under fluorescent lights at 22 °C for 3 days until germinated.

For the drought experiments, germinated seeds were planted 3-cm deep in sterile soilless mix (2:2:1, sand/perlite/promix), in double Magenta boxes (77 × 77 × 194 mm; Chicago, IL), in which the sterile soilless mix was added up to 9 cm. Four holes (0.5 cm in diameter) were made on the bottom of the magenta boxes and the holes were taped. Sterile distilled water (20 ml) was added to the soilless mix after planting and seedlings were grown in a controlled environment chamber (model M-2, EGC Corp., Chagrin Falls, OH) for 3 weeks with 12-h light/12-h dark photoperiod at 25 °C. The irradiance was 50 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR). Relative humidity was not controlled in this experiment, but relative humidity was always above 50%. After 14- or 16-day growth in the double magenta box system, the upper boxes and tape on the box bottoms were removed.