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Invited Article

From epoxycarotenoids to ABA: The role of ABA 8′-hydroxylases in drought-stressed maize roots

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ABSTRACT

The ability of plants to withstand drought, a potentially major constraint to yield and production, is influenced by abscisic acid (ABA). ABA is synthesized in the cytosol from plastid carotenoid pathway derived precursors, and later inactivated by the action of ABA hydroxylases. Endogenous accumulation of ABA is controlled by both its synthesis and catabolism. Enzymatic activity of ABA 8′-hydroxylase (ABA8Ox), also referred to as CYP707A, is considered one of the key steps in modulating ABA levels that control numerous physiological processes. To investigate the role of this enzyme, maize *ABA8Ox* gene family members were identified. *ABA8Ox* gene expression was then analyzed in different tissues and roots during the drought-stress response in maize. These genes were found to be expressed in all tissues, with a high degree of specificity to each tissue and some degree of overlap. Maize *ABA8Ox1a* and *ABA8Ox1b* were shown to be the major transcript components for regulating ABA catabolism in drought-stressed roots. Phylogenetic and gene-structure analyses were performed to extend the implications and infer the cause of ABA catabolism in other cereal crops.

Introduction

Human survival depends on food production, and drought is a potentially major constraint to yield and production of food crops. In maize, for example, drought accounts for at least 15% of the crop loss in sub-Saharan Africa [1]. Abscisic acid (ABA)[1] is a plant hormone that influences the plant's ability to withstand abiotic stresses such as drought, cold, salt and wounding [2]. ABA has additional roles in regulating embryo and seed development, maintenance of seed dormancy and germination, seed desiccation tolerance, vegetative development, general seedling growth, and in mediating pathogen defense responses (reviewed in [3]). Transgenic plants overexpressing ABA biosynthetic genes showed improved drought tolerance [4], while overexpression of ABA 8′-hydroxylases reduced ABA levels and produced ABA-deficient phenotypes [5]. Thus, the amount of ABA is determined by the balance between biosynthesis and catabolism [6], and one strategy for achieving maximum drought tolerance in crop plants, and thereby avoiding losses, is to increase the synthesis of endogenous ABA while controlling catabolism.

ABA-biosynthesis-related genes [7,8] and their regulatory mechanisms [3,9,10] have been identified in a wide range of plants. The first committed step in ABA biosynthesis is the oxidative cleavage of a 9-*cis*-epoxycarotenoid (C_{40}) to form xanthoxin (C_{15}). Xanthoxin is oxidized to form abscisic aldehyde, and then further oxidized to ABA [7,11]. The major route to inactivation of ABA is mediated by the cytochrome P450 monooxygenase ABA 8′-hydroxylase (ABA8Ox, also referred to as CYP707A) [6]. ABAOx hydroxylates ABA to yield 8′-hydroxy ABA thus depleting the active ABA pool (Fig. 1).

Extensive studies have been conducted on the small gene families that encode the ABA8Ox catabolic enzymes from *Arabidopsis* (*CYP707A1-CYP707A4*) [6,12], barley (*HvABA8Ox1* and *HvABA8Ox2*) [13], and beans (*Pv*CYP707A1-*Pv*CYP707A3) [14]. These studies showed that ABA catabolism under stress and recovery is mainly regulated at the transcriptional level.

Although there is a wealth of knowledge on ABA catabolism in model species, ABA catabolism in staple food crops is not as well understood. Cereal crops of global importance, such as maize, sorghum, wheat and rice, are evolutionarily related as members of the Poaceae (grasses) family. The aim of this study was to characterize the *ABA8Ox* gene family in maize and to elucidate the expression of ABA-catabolism-related genes in unstressed maize tissues and in maize roots affected by drought stress. Using comparative

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[1] *Abbreviations used:* ABA, abscisic acid; CT, threshold cycle; Os, *Oryza sativa*; Sb, *Sorghum bicolor*.

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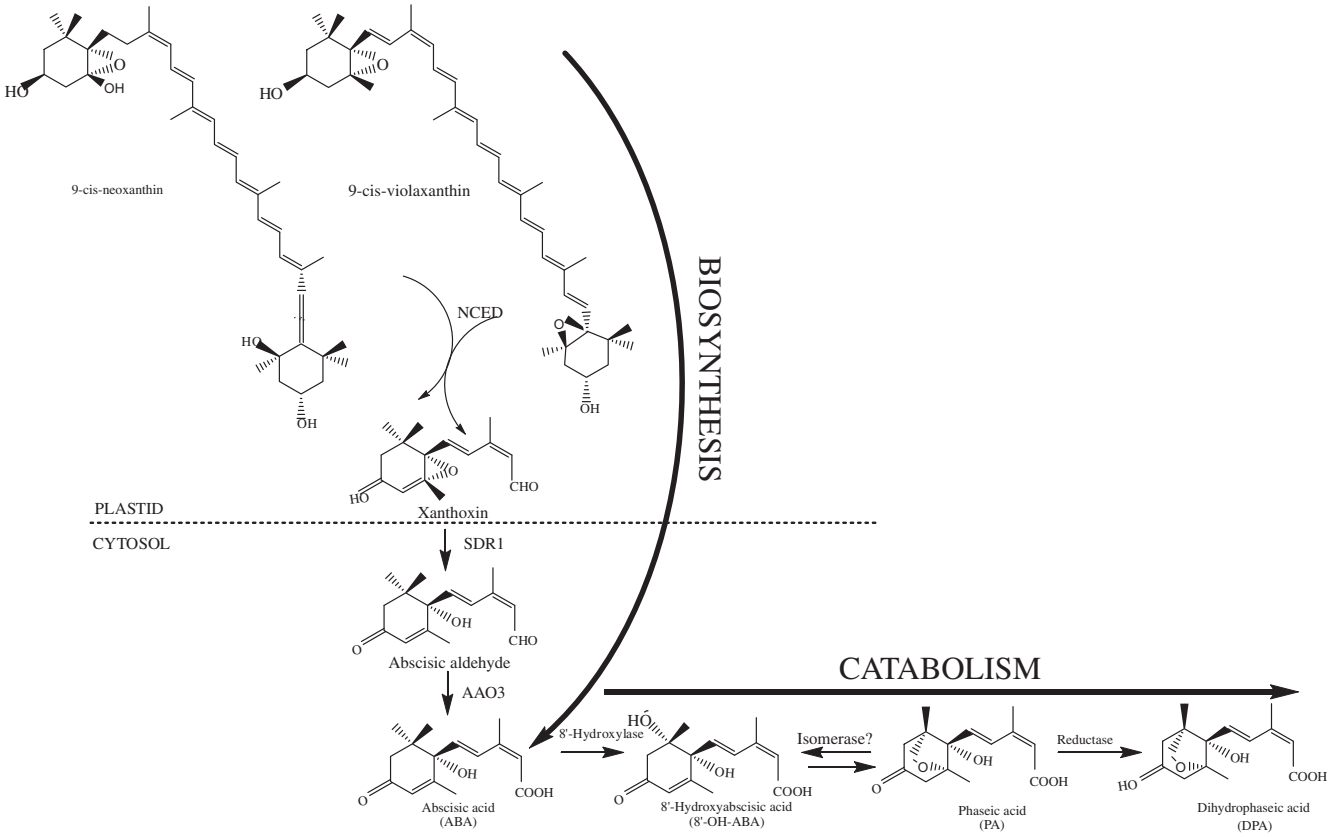

Fig. 1. ABA biosynthesis and catabolism in higher plants. Carotenoid precursors of ABA are synthesized in the plastid. The *cis*-xanthophylls are cleaved by a family of 9-*cis*-epoxycarotenoid dioxygenases (NCED) to form the first 15-carbon precursor, xanthoxin. Xanthoxin moves to the cytosol and is converted to abscisic aldehyde by a short-chain dehydrogenase reductase (SDR1), which is then oxidized to ABA by an abscisic aldehyde oxidase (AAO3). ABA is hydroxylated to 8′-hydroxy ABA in the presence of 8′-hydroxylase (ABA8Ox), and subsequently converted to phaseic acid and dihydrophaseic acid. The genes involved in these downstream steps are still unknown.

genomics, orthologs were also identified in other grass genomes, creating the possibility of breeding these plants for drought tolerance.

Materials and methods

Plant materials and stress treatments

Maize (*Zea mays* L.) inbred line B73 plants were field grown in the Bronx, New York, sibling-pollinated, and endosperm and embryo tissues were dissected 20 days after pollination (DAP). Non-stressed leaf and root tissues and drought-stressed root samples were collected at the six-leaf stage. B73 was grown in a greenhouse as described previously [8]. Dissected tissues were stored at −80 °C until analysis.

Sequence analyses

The genomic DNA sequences of ABA hydroxylases of *Arabidopsis thaliana*, *Oryza sativa*, *Sorghum bicolor* and *Zea mays* were obtained from NCBI GenBank, Gramene, Phytozome and PlantGDB, respectively (Table 1). The sequences were analyzed using Vector NTI Suite, Version 9.0 (InforMax, North Bethesda, MD), and processed for gene-structure analysis, while deducing cDNA and protein se-

Table 1
Summary of ABA8Ox enzymes and genes in grasses (maize, rice, and sorghum) and Arabidopsis. CYP, cytochrome P450.

Enzyme	*Arabidopsis thaliana*	*Oryza sativa*	*Zea mays*		*Sorghum bicolor*
		Gramene	BAC clones/ESTs	MAP	Phytozome
ABA8Ox1	At4g19230 (CYP707A1)	LOC_Os02g47470	AC194862 (*ABA8Ox1a)* DR806072; EC902187; DY688015; CO520018; EB706067; DR803102; EE157729	5.06	Sb04g030660
	At5g45340 (CYP707A3)		AC182107 (*ABA8Ox1b)* CD433445; EE190691; EE169703; EE036846; EE023371; EC902781; EE036847; EC894886; EE169704; EC884504; EC898571; EC884505	4.06	
ABA8Ox2	At2g29090 (CYP707A2)	LOC_Os08g36860	AC212409 (*ABA8Ox2)* CO460095; CO456959; CO459318; DV494257; EB701816; AI670285; EE040561; CO456726; DV529214; CA398898; DY623142; DY239249; EB701815	4.04	Sb07g022990
	At3g19270 (CYP707A4)				
ABA8Ox3		LOC_Os09g28390	AC190490 (*ABA8Ox3a)* CD941324; CD941122; CD955590	2.06	Sb02g026600
			AC195926 (*ABA8Ox3b)* EC904849; DV527897; EE043845; DR785156; EE175998; EE036989 DV514713; DR962771; DV519081	7.02	