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ABA 9′-hydroxylation is catalyzed by CYP707A in Arabidopsis

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ABSTRACT

Abscisic acid (ABA) catabolism is important for regulating endogenous ABA levels. To date, most effort has focused on catabolism of ABA to phaseic acid (PA), which is generated spontaneously after 8′-hydroxylation of ABA by cytochrome P450s in the CYP707A subfamily. Neophaseic acid (neoPA) is another well-documented ABA catabolite that is produced via ABA 9′-hydroxylation, but the 9′-hydroxylase has not yet been defined. Here, we show that endogenous neoPA levels are reduced in loss-of-function mutants defective in CYP707A genes. In addition, *in planta* levels of both neoPA and PA are reduced after treatment of plants with uniconazole-P, a P450 inhibitor. These lines of evidence suggest that CYP707A genes also encode the 9′-hydroxylase required for neoPA synthesis. To test this, *in vitro* enzyme assays using microsomal fractions from CYP707A-expressing yeast strains were conducted and these showed that all four Arabidopsis CYP707As are 9′-hydroxylases, although this activity is minor. Collectively, our results demonstrate that ABA 9′-hydroxylation is catalyzed by CYP707As as a side reaction.

1. Introduction

Abscisic acid (ABA) (**1**) is a sesquiterpene plant hormone that regulates numerous processes during plant life cycle including seed maturation, induction and maintenance of seed dormancy and stress response including stomatal closure (Zeevaart and Creelman, 1988; Nambara and Marion-Poll, 2005). Endogenous ABA (**1**) levels increase during seed maturation and during drought stress, whereas levels are reduced prior to germination and during rehydration of plants after drought. ABA (**1**) levels are maintained by the balance between biosynthesis and catabolism (Zeevaart, 1980) which are regulated as part of a plant's response to developmental and environmental stimuli (Yamaguchi-Shinozaki and Shinozaki, 2006; Nambara et al., 2010).

ABA (**1**) can be inactivated by oxidation or conjugation reactions, and several ABA catabolic pathways exist in plants (Fig. 1). Among them, ABA (**1**) 8′-hydroxylation is a key step in the major ABA (**1**) catabolic route (Nambara and Marion-Poll, 2005). Hydroxylation at C-8′ of ABA is catalyzed by 8′-hydroxylases to form 8′-hydroxy-ABA (**2**). In the subsequent step, 8′-hydroxy-ABA (**2**) spontaneously isomerizes to (−)-phaseic acid (PA) (**3**), and is subsequently reduced by an unidentified reductase to dihydrophaseic acid (DPA) (**4**). ABA 8′-hydoxylase is encoded by the CYP707A subfamily of cytochrome P450 monooxygenases (Kushiro et al., 2004; Saito et al., 2004). ABA (**1**) is also hydroxylated at its C-7′ and -9′ methyl groups to form 7′- and 9′-hydroxy-ABAs, respectively (Hampson et al., 1992; Zhou et al., 2004). (−)-*R*-ABA, an unnatural ABA (**1**) analog, is metabolized into (+)-phaseic acid in corn cell culture possibly by ABA 7′-hydroxylase (Balsevich et al., 1994). Neophaseic acid (neoPA) (**6**) is formed from 9′-hydroxy-ABA (**5**) by isomerization like as for PA (**3**) and its occurrence has been documented in a wide variety of plant species including Arabidopsis (Zhou et al., 2004). However, genes encoding 7′- and 9′-hydroxylases have not been identified.

In contrast to the oxidative catabolic pathways described above, the conjugation of ABA (**1**) to form its glucose ester (ABA-GE) (**7**) is readily reversible and is thought to create a releasable form of ABA (**1**) that may function in transport and storage (Sauter et al., 2002). Glucosylation at the carboxyl group of ABA (**1**) is catalyzed by ABA

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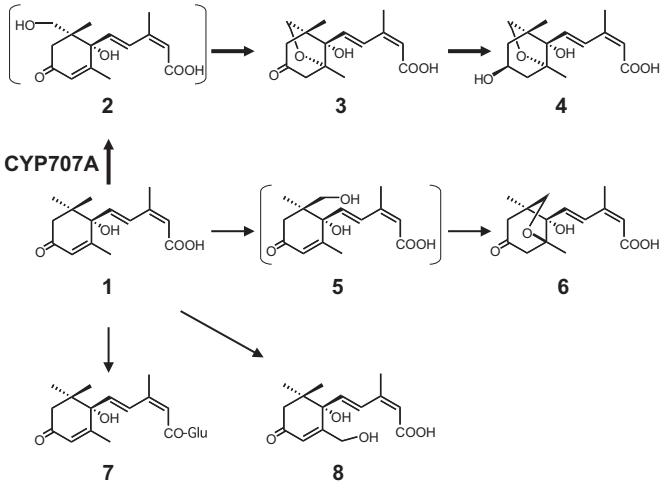

Fig. 1. ABA catabolic pathways in higher plants. ABA (**1**), 8′-hydroxy-ABA (**2**), phaseic acid (**3**), dihydrophaseic acid (**4**), 9′-hydroxy-ABA (**5**), neophaseic acid (**6**), ABA glucose ester (**7**) and 7′-hydroxy-ABA (**8**).

glucosyl transferase (Xu et al., 2002; Lim et al., 2005). Although eight ABA glucosyl transferases are reported only one (UGT71B6) shows specificity for the naturally occurring (+)-ABA enantiomer (Priest et al., 2005).

A body of evidence has shown that ABA (**1**) is catabolized via ABA 8′-hydroxylation in response to developmental and environmental signals such as drought stress, rehydration after drought stress, submergence or high humidity responses, seed maturation and during germination (Okamoto et al., 2006, 2009; Umezawa et al., 2006; Saika et al., 2007; Matakiadis et al., 2009). It is unclear how other catabolic pathways are targeted for the regulation of ABA (**1**) levels, however there are reports showing the differential accumulation of other catabolites. For example, ABA-GE (**7**) levels are increased in *Xanthium strumarium* plants during long-term drought stress (Zeevaart, 1983), in moist-chilled Arabidopsis seeds (Chiwocha et al., 2005) and 7′-OH-ABA (**8**) levels are high in western pine seeds (Feurtado et al., 2004). Nonetheless, the physiological roles of these catabolic pathways need to be evaluated by genetic, molecular and pharmacological analyses to fully appreciate their biological roles and importance. A key step towards this for neoPA (**6**) is the identification of its catabolic enzymes so that proper genetic experiments can be conducted. Towards this goal, we report here that the CYP707A enzymes produce neoPA (**6**) as a minor product during ABA (**1**) catabolism.

2. Results

2.1. ABA (*1*) catabolite profiles in the cyp707a double mutant

ABA (**1**) catabolites accumulate abundantly when ABA (**1**) levels increase and reach a plateau under drought stress conditions. To examine the accumulation pattern of ABA (**1**) catabolites during drought, LC–MS/MS was used to measure the levels of ABA (**1**), ABA-GE (**7**), PA (**3**), DPA (**4**), neoPA (**6**) and 7′-OH-ABA (**8**) in 6-h-dehydrated wild-type and *cyp707a1a3* double mutant plants (Fig. 2A). Dehydrated wild-type plants accumulated higher amount of catabolites derived from the 8′-hydroxylation pathway (PA (**3**) and DPA (**4**)) than other catabolites (Fig. 2A). ABA-GE (**7**) was less abundant than PA (**3**) and DPA (**4**), but accumulated substantially in the dehydrated wild type. NeoPA (**6**) was detected in wild-type plants as a minor catabolite, whereas 7′-OH-ABA (**8**) levels could not determined due to its low abundance (Fig. 2A). The *cyp707a1a3* double mutant accumulated twice as much ABA (**1**) than wild type in 6-h-dehydrated plants (Fig. 2A). As expected, levels of PA (**3**) and DPA (**4**) were severely reduced in the *cyp707a1a3* double mutant compared with those of wild type (Fig. 2A). The *cyp707a1a3* double mutant accumulated more ABA-GE (**7**) than the wild-type controls. Interestingly, neoPA (**6**) levels were 10-fold lower in *cyp707a1a3* double mutant than those in wild type. As observed for wild type, 7′-OH-ABA (**8**) was not detectable.

Levels of ABA catabolites in the siliques during mid-maturation were next investigated, during which ABA (**1**) levels are high. In siliques, while ABA 8′-hydroxylation was predominant, the composition of ABA catabolites in siliques was different from that observed in 6-h-drought-stressed plants (Fig. 2B). A large amount

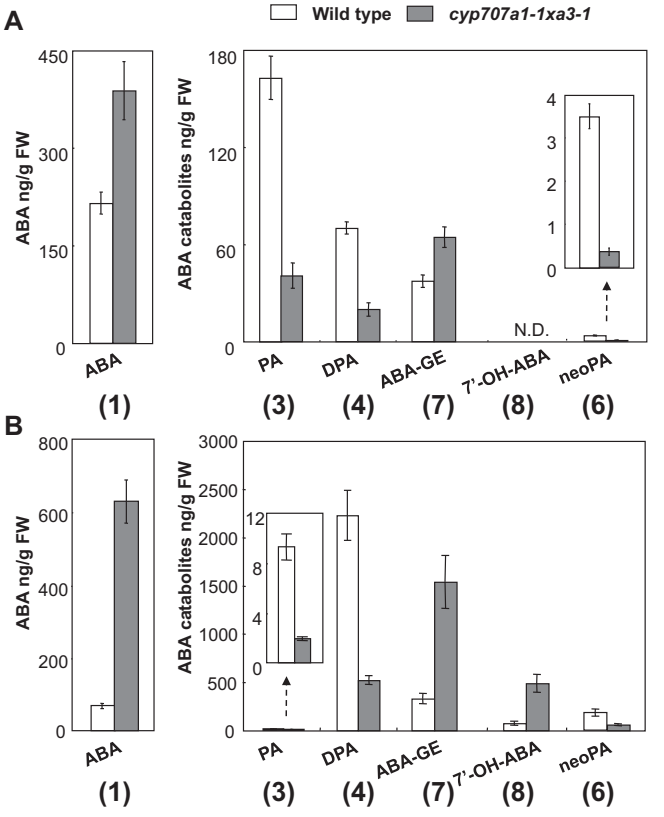

Fig. 2. Profiles of ABA (**1**) and its catabolites in wild type and *cyp707a* double mutant. Endogenous ABA (**1**) and its catabolite levels in wilted plants (A) and siliques at 10–12 DAF (B). Experiments were performed four times using independent biological samples, and averages are shown with standard errors. N.D., not detected due to low levels.