Agri Gene 7 (2018) 23–33

Contents lists available at ScienceDirect

Agri Gene

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

Overexpression of mango alcohol dehydrogenase (*MiADH1*) mimics hypoxia in transgenic tomato and alters fruit flavor components

Rajesh Kumar Singh[a,1], Smriti Srivastava[a], Hemangi G. Chidley[b], Pravendra Nath[a], Vidhu A. Sane[a,*]

[a] Plant Gene Expression Laboratory, CSIR-National Botanical Research Institute, Lucknow 226001, India
[b] Plant Biochemistry and Molecular Biology,CSIR- National Chemical Laboratory, Dr. Homi Bhabha Road, Pune 411008, India

ARTICLE INFO

Keywords:
ADH
Alcohols
Fruit
Flavor
Hypoxia
Tomato

ABSTRACT

Plant alcohols and aldehydes are produced by the action of alcohol dehydrogenases (ADH) and play an important role during fruit ripening and aroma production. Alcohols are not only produced in different tissues at different stages of plant development but are also products of the fermentative pathway which gets activated by different stresses, including hypoxia. The ADH gene is a well-established marker for hypoxic response as well as plant aroma. In a previous report we have identified and characterized three ADHs (MiADH1, 2, 3) from Dashehari mango. *MiADH1* was found to be fruit specific and was upregulated by ethylene and ABA, suggesting a role in fruit aroma volatile production. In order to functionally characterize *MiADH1*, transgenic tomato plants were developed under the control of the constitutive (CaMV35S) promoter. Transgenic tomato fruit expressing *MiADH1* gene showed a change in the levels of several alcohols and aldehydes related to flavor in comparison to the control. The change in aroma and volatiles compounds was more prominent during the ripe stage as compared to unripe and mid-ripe stages of tomato fruit. The transgenic tomato plants also produced adventitious roots. Our results suggest that the overexpression of *MiADH1* in tomato plants induced the fermentative pathway in roots, and mimicked hypoxic response by development of adventitious roots from the stem as an adaptive mechanism.

1. Introduction

Plant metabolites and their production are developmentally regulated. These are influenced by several environmental factors. The same metabolites may sometimes play different and distinct roles in different developmental and metabolic processes. Alcohols are a group of compounds which play different roles in plants, from being a stress signaling molecule, to an important flavor component in several fruit bearing species. Alcohols are produced in plants with the action of enzymes known as Alcohol dehydrogenases (ADH; alcohol: NAD oxidoreductase; E.C 1.1.1.1) belonging to multigene family. Several ADH genes are involved in different aspects of plant growth and development. ADHs express in different parts of plants. These are regulated developmentally and also by other external environmental factors (Matton et al., 1990; Speirs et al., 1998; Christie et al., 1991). Some of the ADHs are fruit specific and supposed to be involved in fruit flavor (Ingersoll et al., 1994; Tesniere and Verries, 2000; Echeverría et al., 2004; Manriquez et al., 2006) while others have roles in stress responses, mainly hypoxia (Longhurst et al., 1990; Chen and Chase, 1993; Conley et al., 1999). ADH genes belonging to both these categories have been characterized from various plants. Overexpression of ADH genes in homologous and heterologous systems suggests that these prefer one role over the other (stress specific or flavor specific) depending on where and when expressed. Overexpression of a tomato ADH (*SlADH2*) under constitutive and fruit specific promoter has been shown to modify the fruit flavor components (Speirs et al., 1998), whereas overexpression of a berry specific ADH (*VvADH2*) in grapes resulted in increased levels of volatile compounds related to stress in leaves, with no significant change in fruit metabolites (Tesniere et al., 2006; Torregrosa et al., 2008). ADH has also been one of the important candidates for genetic manipulation to provide flooding tolerance to plants via increasing adaptability to hypoxic responses. In rice, ADH suppression has led to reduced viability of seeds in low oxygen while its overexpression provided better growth under waterlogging conditions

Abbreviations: ADH, Alcholo dehydrogenase; CA, Controlled atmosphere; NADH, Nicotinamide adenine dinucleotide; NADPH, Nicotinamide adenine dinucleotide phosphate; EDTA, Ethylenediaminetetraacetic; ARs, Adventitious roots; PDC, Pyruvate Decarboxylase; AAT, Alanine aminotransferase; Sl, *Solanum lycopersicum*; Mi, *Mangifera indica*

* Corresponding author.
E-mail address: va.sane@nbri.res.in (V.A. Sane).
[1] Present Address-Dept. of Forest Genetics and Plant Physiology, SLU, S-901 83, Umeå, Sweden.

https://doi.org/10.1016/j.aggene.2017.10.003
Received 19 July 2017; Received in revised form 31 October 2017; Accepted 31 October 2017

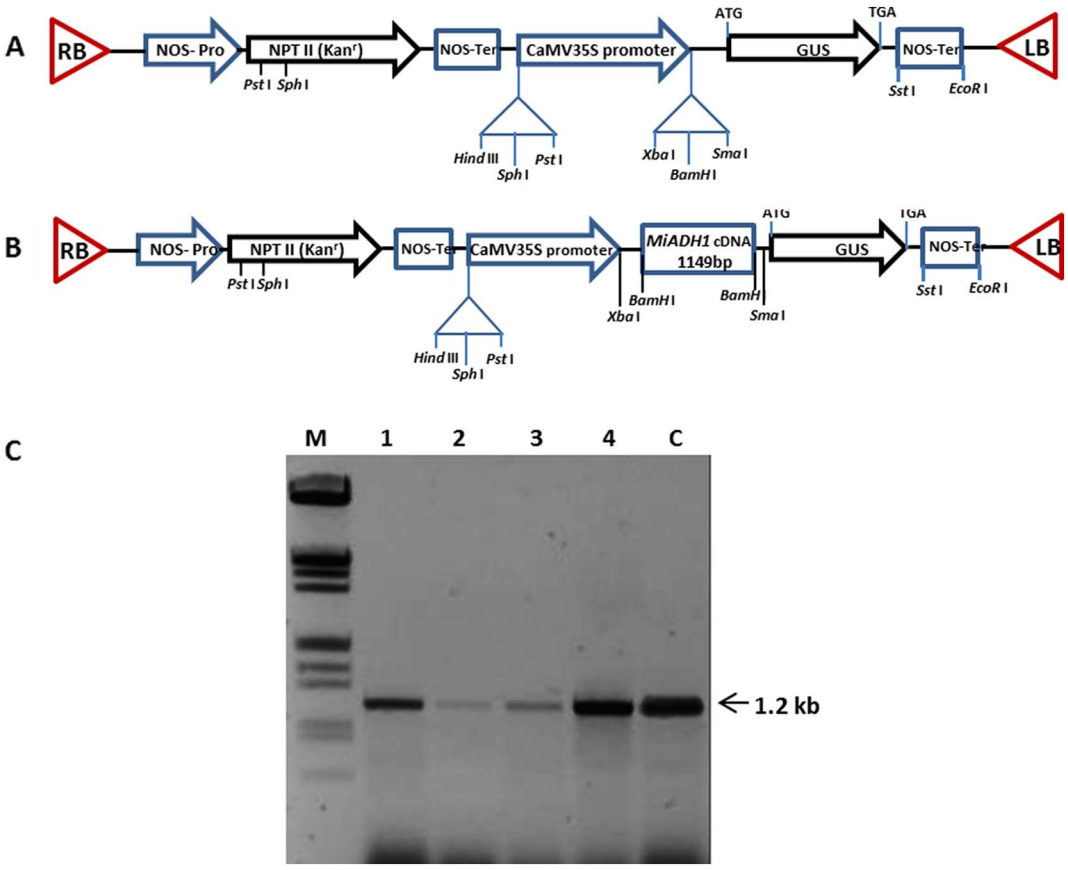

Fig. 1. Construction and confirmation of *MiADH1* transformation cassette. (A) General map of the T-DNA region of the binary vector pBI121 showing the NPTII-selectable marker, CaMV35S promoter and the polylinker site. (B) pBIMiADH1 construct.*MiADH1* ORF (1149 bp) was ligated at *BamHI* site of the *pBI121* vector. (C) Confirmation of *MiADH1* transgene in transformed tomato plants by PCR. Lane M, λHE marker, Lane 1–4, amplification using genomic DNA from four independent transgenic lines, C-Positive control, amplification using *pBIMiADH1*plasmid DNA.

(Ellis et al., 1999). The role of ADH in fruit aroma and hypoxia together was studied in fruits stored in controlled atmosphere (CA). CA usually has low oxygen and induces hypoxic response in tomato fruit which in turn activate ADH gene and it affects/alters fruit aroma during CA storage (Yahia, 1994; Nanos et al., 1992).

Three ADH genes were identified and characterized from Dashehari mango (MiADHs) which were differentially expressed during mango fruit ripening. These MiADHs also exhibited specific preference towards cofactors (NADH and/or NADPH) (Singh et al., 2010). Out of three *MiADHs*, *MiADH1* showed fruit specific, ripening related and ethylene induced expression suggesting a possible role in mango fruit aroma biosynthesis. *MiADH1* showed specific preference towards conversion of aldehyde to alcohols using NADPH as a cofactor. In order to functionally evaluate whether *MiADH1* plays a role in fruit aroma, and with its expression in tomato increase or produce new mango specific alcohols, *MiADH1* was expressed in tomato using the constitutive CaMV35S promoter. It is already known that ADH is induced by hypoxia, but whether an adaptive hypoxic response can be triggered by overproduction of alcohols was not studied. Here in this paper we report that metabolite (probably alcohols and aldehydes) and transcriptome changes due to overexpression of MiADH1 in tomato could trigger an adaptive response period.

2. Material and methods

2.1. Materials

Solanum lycopersicum (Var. NBR UDAY) was used for developing transgenics and further study. Tomato plants were grown in a glass house at 22 °C ± 2 °C and 16 h/8 h light-dark photoperiods. Fruit and root samples were frozen in liquid nitrogen and kept in − 80 °C until further use. The experiments were performed by using three independent biological and three technical replicates.

2.2. Methods

2.2.1. Construction of ADH overexpression cassette and genetic transformation of tomato

The full length coding region (1149 bp) of *MiADH1* (Accession no. GU233766) was used to prepare the transformation cassette. *MiADH1* cDNA was inserted at the *BamH*I site of the binary vector pBI121 under CaMV35S promoter. The positive clone pBIMiADH1, selected through PCR was used for subsequent transformation of tomato through Agroinfection. The construct was introduced into *Agrobacterium tumefaciens* strain GV3101 by a freeze-thaw method as described by Höfgen and Willmitzer 1988, prior to tomato transformation. Tomato transformations was carried out as described by Batra et al., 2010 with some modifications using sterilized leaf explants (grown for two weeks on MS medium). Agro infected explants were first cultured on callus medium (MS medium supplemented with 0.2 mg/l IAA, 1.0 mg/l Zeatin riboside, Kanamycin 50 mg/l and 500 mg/l cefotaxime). After selection, calli were transferred on shoot enhancing medium (0.2 mg/l IAA, 0.1 mg/l Zeatin riboside) and elongation of shoots were initiated by adding GA_3 (1 mg/l) to the medium. Elongated shoots were transferred to rooting medium having 0.5 mg/l IBA, 25 mg/l kanamycin and cefotaxime for 12–15 days. Rooted plants were then hardened in KNOPS solution for 1 week and transferred to the soil and finally shifted to glass house for optimum growth. The integration of the transgene was