Plant Science 215–216 (2014) 59–68

Contents lists available at ScienceDirect

Plant Science

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

# Fruit specific variability in capsaicinoid accumulation and transcription of structural and regulatory genes in *Capsicum* fruit

Neda Keyhaninejad, Jeanne Curry, Joslynn Romero, Mary A. O'Connell*

*Department of Plant and Environmental Sciences, New Mexico State University, Las Cruces, NM 88003, USA*

ARTICLE INFO

*Article history:*
Received 16 September 2013
Received in revised form 25 October 2013
Accepted 26 October 2013
Available online 1 November 2013

*Keywords:*
*Capsicum*
Fruit pungency
Capsaicinoids
Transcription factors

ABSTRACT

Accumulation of capsaicinoids in the placental tissue of ripening chile (*Capsicum* spp.) fruit follows the coordinated expression of multiple biosynthetic enzymes producing the substrates for capsaicin synthase. Transcription factors are likely agents to regulate expression of these biosynthetic genes. Placental RNAs from habanero fruit (*Capsicum chinense*) were screened for expression of candidate transcription factors; with two candidate genes identified, both in the ERF family of transcription factors. Characterization of these transcription factors, *Erf* and *Jerf*, in nine chile cultivars with distinct capsaicinoid contents demonstrated a correlation of expression with pungency. Amino acid variants were observed in both ERF and JERF from different chile cultivars; none of these changes involved the DNA binding domains. Little to no transcription of *Erf* was detected in non-pungent *Capsium annuum* or *C. chinense* mutants. This correlation was characterized at an individual fruit level in a set of jalapeño (*C. annuum*) lines again with distinct and variable capsaicinoid contents. Both *Erf* and *Jerf* are expressed early in fruit development, 16–20 days post-anthesis, at times prior to the accumulation of capsaicinoids in the placental tissues. These data support the hypothesis that these two members of the complex ERF family participate in regulation of the pungency phenotype in chile.



## 1. Introduction

Chile peppers (*Capsicum* spp.) are among the most important vegetables and spice crops grown worldwide. The fruit is particularly valued as a source of vitamins and of pungency, a trait unique to this crop plant [1]. With only a few exceptions, all of the species in the genus synthesize capsaicinoids, the pungent compounds in their fruit [2,3]. Capsaicin and dihydrocapsaicin are the two most abundant capsacinoids; these odorless and colorless compounds are synthesized in the epidermis of the placenta and are stored in vesicles on the surface of this tissue [4].

Pungency in *Capsicum* is both a qualitative trait and a quantitative trait. The degree of heat, or the concentration of capsaicinoids that accumulate is inherited quantitatively [5,6], while the ability to be hot or pungent is inherited simply, controlled by the dominant gene *Pun1* (also called *C*) on chromosome 2 [7]. A candidate gene for *Pun1* was identified as *At3*, an acyltransferase, which maps to the same location as *Pun1* on chromosome 2 [8,9]. Three alleles for *At3* in non-pungent varieties have been identified; $pun1^1$, in *Capsium annuum*, has a deletion of the promoter and the first exon [8]; $pun1^2$, in *Capsicum chinense*, has a smaller deletion in the first exon, also resulting in an inactive gene [9]; and $pun1^3$, in *Capsium frutescens* has a deletion in the carboxy terminal of the second exon [10]. A second locus for non-pungency was found by Votava and Bosland in *C. chinense* missing the vesicles on the placental, *Loss of Vesicle* (*Lov*) [11]; the chromosomal map location of this mutation is not known.

The capsaicinoid biosynthetic pathway is complex and requires intermediates from two distinct pathways (Fig. 1) (reviewed in [1,12]). Phenylalanine is the precursor for vanillylamine, synthesized in part by the phenylpropanoid pathway; and leucine or valine are the precursors for the synthesis of a branched chain fatty acid, e.g. 8-methyl nonenoic acid, synthesized by the fatty acid synthase complex. Finally, capsaicinoid synthase combines the vanillylamine and fatty acid chain and to make capsaicin [13]. In an earlier work, we demonstrated that the transcripts for many of the enzymes on both branches of the capsaicinoid pathway share similar patterns of accumulation; transcripts are more abundant early in fruit development and more abundant in pungent *Capsicum* varieties [9,14,15]. These results suggest an important role for transcription factors in the coordination and regulation of the expression of pungency in *Capsicum*. In this current study we used

* Corresponding author at: Department of Plant and Environmental Sciences, MSC 3Q, P.O. Box 30003, Las Cruces, NM 88003, USA. Tel.: +1 575 646 5172; fax: +1 575 646 4681.
*E-mail address:* moconnel@nmsu.edu (M.A. O'Connell).

0168-9452/$ – see front matter © 2013 Published by Elsevier Ireland Ltd.
http://dx.doi.org/10.1016/j.plantsci.2013.10.013

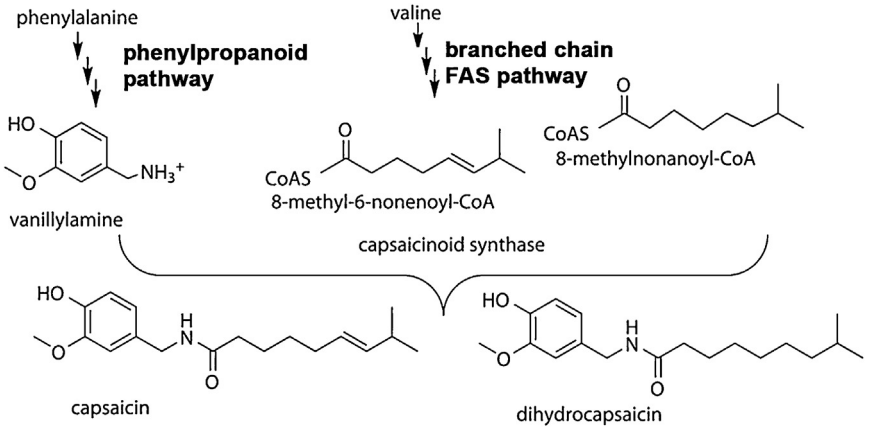


**Fig. 1.** Schema of capsaicinoid biosynthetic pathway. Phenylalanine is the precursor for vanillylamine synthesized by the phenylpropanoid pathway; valine is a precursor for the branched chain fatty acids (e.g. 8-methyl-6-nonenoyl-CoA) synthesized by the branched chain fatty acyl synthase (FAS) pathway.

four biosynthetic genes to monitor transcription activity of these biosynthetic pathways: *Pal* on the phenylpropanoid pathway [15], *Kas* and *FatA* on the FAS pathway [14], and *At3* a candidate for capsaicinoid synthase [8,9].

The key role transcription factors play in regulation of secondary metabolites has been known for decades as some of the early mutants in anthocyanin pigment accumulation were the result of mutations in transcription factors [16]. The roles of transcription factors in regulation of plant secondary metabolic pathways have been reviewed [17–19]. Members of many of the classic transcription factor families have been associated with roles in secondary metabolism: MYB [20,21], bZIP [22], bHLH and WD40 [17] and AP2/ERF [23]. Transcription factor ESTs are regularly detected in screens of transcriptomes for genes associated with secondary metabolite accumulations in Solanaceae fruits [24–26]. The expression profiles of candidate transcription factors in placental samples from pungent and non-pungent *Capsicum* fruit though have not been investigated in detail. Transcription levels of two bZIP transcription factors were included in a panel of genes but full details in support of the annotation of these genes as transcription factors was not provided [8]. More recently, transcriptome analyses identifying differentially expressed transcripts in comparisons of pericarp and placenta of pungent *Capsicum* fruit have also detected transcription factors among a number of candidate genes for the capsaicinoid pathway [27].

The current study was conducted to identify and characterize candidate genes for transcription factors likely to play a role in coordinating the transcription of the structural genes on the capsaicinoid pathway. The screening strategy selected cDNA clones with DNA sequence similarity to motifs found in known transcription factors. Transcription factor genes expressed in a placental-specific pattern and with increased expression in fruit with increased pungency were identified. Further, as we were aware that there is a great deal of fruit to fruit variability in expression of the pungency phenotype [28,29] we developed methods for fruit specific characterization of expression of these genes and direct confirmation of fruit specific capsaicinoid levels.

## 2. Materials and methods

### 2.1. Plant material

Plants were grown from seed in Metro-mix 360, in a greenhouse on the New Mexico State University campus in Las Cruces, NM USA. Plants were irrigated twice a day with a drip system and fertilized with Osmocote (14-14-14), every 2–3 months. *Capsicum* germplasm included in this study are listed in Table 1. Organs and tissues collected for RNA isolation were immediately dissected and frozen in liquid nitrogen. For the time course studies, all fruit on

**Table 1**
*Capsicum* cultivars characterized in this study.

| Species | Cultivar | Capsaicinoid SHU[a] | ERF, JERF variability | Fruit specific capsaicinoids | qRT-PCR |
|---|---|---|---|---|---|
| *C. annuum* | Canary | 0 | X | | |
| | np Jalapeño | 0 | X | | X |
| | NuMex Joe E. Parker | 1200 | X | | |
| | New Mexico 6-4 | 1200 | X | | X |
| | Early Jalapeño | 8000 | X | | X |
| | Jalapeño P105 | 0 | | X | X |
| | Jalapeño PX212 | 2000 | | X | |
| | Jalapeño PX211 | 9000 | | X | X |
| | Jalapeño PX205 | 25,000 | | X | |
| | Jalapeño PX206 | 40,000 | | X | |
| | Jalapeño PX207 | 60,000 | | X | X |
| *C. chinense* | np Habanero PI 1721 | 0 | X | | X |
| | Habanero PI 1720 | 300,000 | X | | X |
| | Bhut Jolokia | 1,000,000 | X | | X |
| *C. frutescens* | Tabasco | 40,000 | X | | X |

[a] SHU, Scoville Heat Units.