Plant Science 181 (2011) 439–448

Contents lists available at ScienceDirect

Plant Science

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

CaMF2, an anther-specific lipid transfer protein (LTP) gene, affects pollen development in *Capsicum annuum* L.

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ARTICLE INFO

Article history:
Received 16 May 2011
Received in revised form 7 June 2011
Accepted 12 July 2011
Available online 23 July 2011

Keywords:
Anther-specific
CaMF2
Capsicum annuum L.
Lipid transfer protein
VIGS
Pollen development

ABSTRACT

Based on the gene differential expression analysis performed by cDNA-amplified fragment length polymorphism (cDNA-AFLP) in the genic male sterile–fertile line 114AB of *Capsicum annuum* L., a variety of differentially expressed cDNA fragments were detected in fertile or sterile lines. A transcript-derived fragment (TDF) specifically accumulated in the flower buds of fertile line was isolated, and the corresponding full-length cDNA and DNA were subsequently amplified. Bioinformatical analyses of this gene named *CaMF2* showed that it encodes a lipid transfer protein with 94 amino acids. Spatial and temporal expression patterns analysis indicated that *CaMF2* was an anther-specific gene and the expression of *CaMF2* was detected only in flower buds at stage 3–7 of male fertile line with a peak expression at stage 4, but not detected in the roots, tender stems, fresh leaves, flower buds, open flowers, sepals, petals, anthers or pistils of male sterile line. Further, inhibition of the *CaMF2* by virus-induced gene silencing (VIGS) method resulted in the low pollen germination ability and shriveled pollen grains. All these evidence showed that *CaMF2* had a vital role in pollen development of *C. annuum*.

1. Introduction

In flowering plants, a differentiated anther has several highly specialized cells and tissues that are responsible for carrying out non reproductive functions (e.g., support and dehiscence) and reproductive functions (e.g., spore and pollen formation). Each of these tissues and cell types carries out specialized tasks [1]. There are thousands of temporally and spatially controlled specific genes involving these complicated but important events. It is reported that there were approximately 3500 genes expressed specifically within the *Arabidopsis thaliana* anther [2], 2000–7000 pollen-specific genes in *Zea mays* [3], and approximately 10,000 anther-specific mRNAs in tobacco [4]. Some of the anther-specific mRNAs have been shown to encode lipid transfer proteins, protease inhibitors, thiol endopeptidases, glycine-rich and proline-rich polypeptides with properties of cell wall proteins, pectate lyases, polygalacturonases, and chalcone synthase isoforms [5–7]. They play important roles in pollen development and any gene mutation may affect this process or lead to male sterility [8].

A large number of lipid transfer proteins (LTPs) have been purified from various plant sources, which are known as nonspecific transporters of lipid molecules with low molecular mass [9]. In plants, LTPs are thought to play roles in many biological processes, such as cutin biosynthesis [10], β-oxidation [11], somatic embryogenesis [12], plant signaling [13,14], seed maturation [15], plant defense [16–19], anther development [7], and pollen tube tip growth [20]. LTPs are small peptides that comprise two families. LTP1 members have 90–95 amino acid residues and are approximately 10 kDa, presenting a signal peptide at the amino terminal region, which in general varies between 21 and 27 amino acids, while LTP2 members have approximately 70 amino acid residues and are 7 kDa, presenting a signal peptide at the amino terminal region, which in general varies between 27 and 35 amino acids [21–24]. Both LTP1 and LTP2 family members are basic polypeptides with p*I* values from 8.5 to 12 and have a characteristic eight-Cys motif, forming four disulfide bonds, contributing to their binding activity for different lipids and hydrophobic compounds in vitro [7,25–27]. In plants, many anther-specific LTPs, such as OsC6 (LOC_Os11g37280) in *Oryza sativa*, Protein 108 (Q43495) in *Solanum lycopersicum*, FIL1 (Q38737) in *Antirrhinum majus*, A9 (BAB10170) in *A. thaliana*, and BcA9 (AAO85389) in *Brassica rapa* subsp. *Campestris*, were identified. Recently, it was proved that OsC6 is required for postmeiotic anther development in rice [7].

Chili pepper (*Capsicum annuum* L.) is one of the most important vegetable crops in China and in other countries due to its sensory attributes of pungency, aroma and color [28,29]. In pepper, male sterility can be divided into cytoplasmic male sterility (CMS) and genic male sterility (GMS). To date, the CMS system has been considered more attractive than the GMS system in hybrid

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doi:10.1016/j.plantsci.2011.07.003

seed production of chili pepper [30]. However, CMS systems have drawbacks such as instability of sterility, the restriction of restorer and possible negative cytoplasmic effects. In contrast, recessive GMS systems have great potential in heterosis utilization, with stable and complete sterility, extensive distribution of restorers and diverse cytoplasmic sources [31]. Currently, over a dozen GMS peppers are found in nature or have been artificially produced by mutagenesis with X-rays, gamma rays, or ethylmethanesulfonate (EMS) treatment [32]. Since there are 50% male sterile plants and 50% male fertile plants in the GMS system, they have become very important materials in exploring the pollen development process. Several molecular markers linked to the genic male sterility gene have been identified in chili pepper and the selection of only heterozygous plants from every generation can be performed without the need for test crosses [30,33,34]. However, so far, the molecular mechanism of genic male sterility in pepper was still unknown. To our knowledge, there were few reports on the cloning, characterization and function analysis of genic male sterility-related genes in *capsicum* to date.

In this paper, the gene differential expression analysis was performed by cDNA-AFLP in the genic male sterile–fertile line 114AB of *C. annuum* L., and a differentially expressed cDNA fragment, named *CaMF2* (male fertile 2), was only found in fertile line. The full cDNA and DNA sequences of the *CaMF2* (GenBank ID: JF411954) were amplified. The derived protein CaMF2, which has a characteristic eight-Cys motif and contains a conservation domain of AAI_LTSS, belongs to the lipid transfer proteins family. Previously, three LTP genes, *CALTPI*, *CALTPII*, and *CALTPIII*, were isolated from a pepper (*C. annuum*) cDNA library from hypersensitive response (HR) lesions of leaves infected with *Xanthomonas campestris* pv.*vesicatoria*, and they were proved to be differentially activated by pathogens, abiotic, and environmental stresses [35]. *CaMF2* is a distinct LTP gene in *C. annuum*, because it has less than 20% identity with any of the three LTP genes. We report here that *CaMF2*, encoding a lipid transfer protein, was an anther-specific gene only detected in the fertile line of *C. annuum* L. In this study, we sought to explain and analyze the function of *CaMF2* through the virus-induced gene silencing (VIGS) approach, and then to determine its role in the process of pollen development.

2. Materials and methods

2.1. Plant materials

The genic male sterile–fertile line 114AB of *C. annuum* L. were cultivated in the experimental farm, South China Agriculture University, with 50% male sterile plants and 50% male fertile plants in the population. There are no significant size differences in flower bud, style, petal, nor difference in the degree of petal opened between male sterile plants and fertile plants. However, the dynamics of the anthers development were distinct between them, especially at anther maturity (Fig. 1). The length and diameter of the anthers in sterile plants are less than those in the fertile plants. At the stage of the sepals splaying slightly, the filaments of

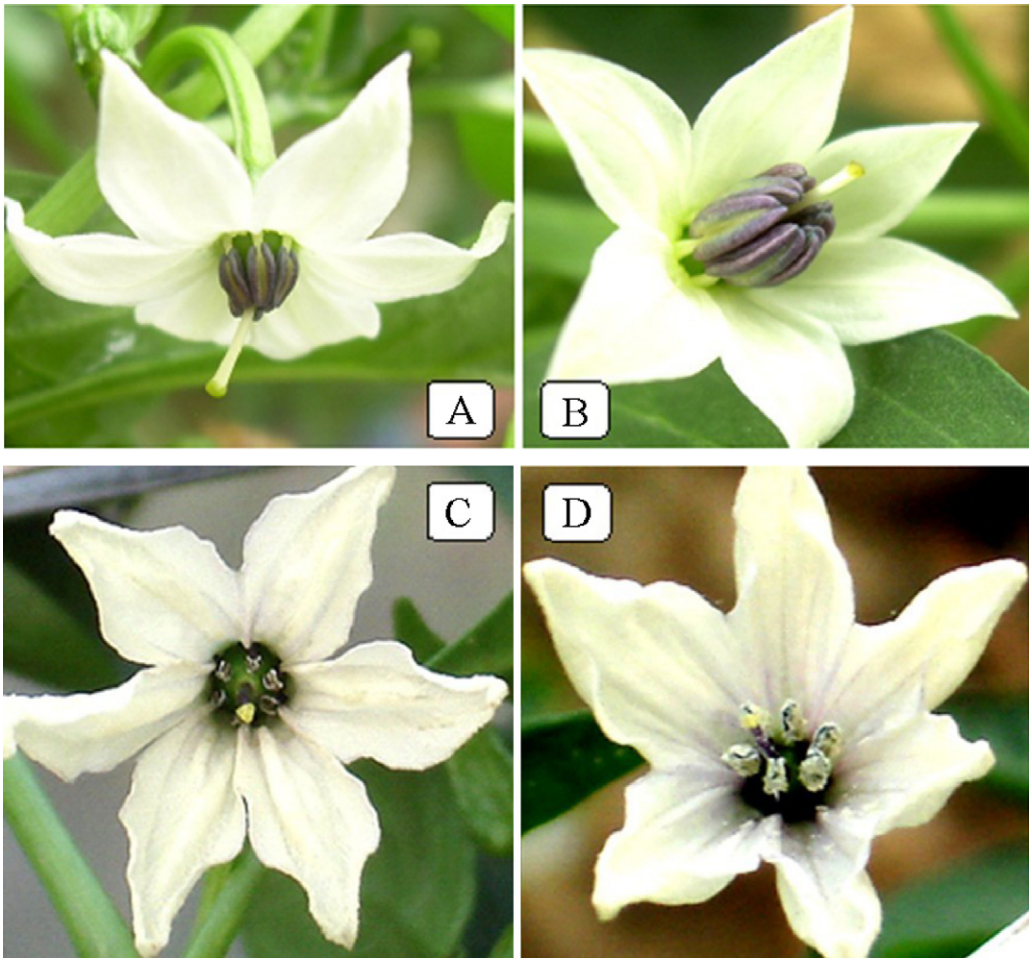

Fig. 1. Comparison of the morphology of the floral organ between male sterile(MS) and male fertile(MF) plants (A) The MS plant anther with dark purple was not only little, malnourished, but also far from the stigma. (B) The MF plant anther developed well, manifesting with the characters of plump shape, light color and short distance to stigma. (C) The MS plant anther almost had no pollen. (D) The MF plant anther was covered with large amounts of pollen.