International Journal of Engineering and TechnologyVol. 1, No.4 , October, 2009
ISSN: 1793-8236

Cloning and Characterization of MADS-box Gene in Oil Palm

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Abstract— **Oil palm has emerged as one of the most important source of oils and fats. The mechanism of floral organs development in this plant is still at its infancy. We describe here the cloning and characterization of a MADS-box gene in oil palm (Elaeis guineensis Jacq.) named EMADS1. It belongs to the AGAMOUS-like2 family of MADS-box gene which plays critical role in flower development as defined by the ABCDE model. EMADS1 was ubiquitously expressed in the immature male and female flower buds and its expression pattern was similar to EgAGL2 and EgMADS8 of oil palm. The EMADS1 transcript also accumulated in embryos of developing seeds. These results suggested that EMADS1 is likely to function at the initial stages of flowering in determination of the inflorescence and the identity of the flower whorls and also embryo development in seeds.**

Index Terms— **Flower, MADS-box, Oil palm, Phylogeny.**

I. INTRODUCTION

Cultivation of the oil palm (*Elaeis guineensis* Jacq.) has expanded tremendously in recent years such that it is now one of the major sources of the world supply of oils and fats. Currently, Palm oil is the second most produced vegetable oil after soya bean, amounting to almost 20% of the world's production. With a good quality planting materials and agronomic practices, oil palm begins producing the oil-bearing fruit bunches as early as two and a half years after planting.

Tissue culture of oil palm offers several advantages. It allows rapid multiplication of uniform planting materials with desired characteristics and qualities such as good oil yield and composition, slow vertical growth and disease resistance. However, tissue culture propagated oil palm produces a high incidence of flowering abnormalities called mantling. The abnormality may occur in either male or female inflorescences or in both [1]. Mantled fruits are known to be failed to sustain development of the bunch to maturity and ripeness, and thus directly affecting oil production [2].

MADS-box genes encode for a large family of transcription factors that regulate development in plants. The MADS-box is a highly conserved domain of 56-60 amino acids, named after four of the original cloned members: MCM1, AGAMOUS, DEFICIENS and SFR [3]. These genes play fundamental roles in flower development according to the quartet or ABCDE model [4]. MADS-box genes from oil palm have been reported but only a few were involved in flower development [5], [6]. We report here the identification and characterization of a novel a MADS-box gene that is likely to regulate the inflorescence development in oil palm.

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II. MATERIALS AND METHODS

A. Plant Material

Immature male and female inflorescences and young oil palm fruits of Tenera variety, a hybrid of Dura and Pisifera, were harvested from Borneo Samudera Plantation, Sabah.

B. The 3' and 5' RACE-PCR

Poly(A)+ mRNA was isolated directly from approximately 100 mg of the immature inflorescences using the Dynabeads® mRNA Direct TM Kit (Dynal Biotech). First-strand cDNA was prepared using the ReverAidTM H Minus First Strand cDNA Synthesis Kit (Fermentas) with oligo(dT) primer. MADS-box DNA fragment was amplified from this cDNA template using PCR primers: SH1 5'ATGGKIMGIGGIAARRTIGAGMTRAAG-3'and SH2 5'-GACTCGAGTCGACATCGA-dT17-VN-3' at conditions were as follows: initial denaturation at 95°C for 3 minutes; 35 cycles of 94°C for 1 minute, 50°C for 1 minute, and 72°C for 2 minutes; and with a final extension of 10 minutes at 72°C.

The 5' Race PCR was performed to obtain the complete length of the cloned MADS-box gene. It was carried out using the 5' RACE kit (Promega) with primer corresponding to the 5' RACE adaptor sequence and a specific reverse primers SH8 5'-CGGGTGCTGCTGCGATTGTTATTT-3' designed according to the least conserved portions of the MADS-box gene sequence (C-terminal) of the obtained partial 3'-end MADS-box gene sequence using programs in Lasergene software. The PCR amplification condtions were an initial initial denaturation at 94°C for 3 minutes; 35 cycles of 94°C for 30 second, 57°C for 1 minute, and 72°C for 1 minutes; and with a final extension of 7 minutes at 72°C. Amplified products were cloned and sequenced.

C. Phylogenetic Analysis

The full-length deduced amino acids sequence of *EMADS1* and 30 others plant MADS-box genes were used to construct phylogenetic tree. Phylogenetic tree was constructed using the neighbor program from the Phylip (Phylogeny inference Package version 3.63). The evolutionary distances were calculated by the "protdis" program.

D. RT-PCR Analysis

First-strand cDNAs were synthesized from 2 µg of total RNA with the ReverAidTM H Minus First Strand cDNA Synthesis Kit (Fermentas). SH1 and SH8 were used as forward and reverse primers, respectively. Beta actin gene was used as the internal control and amplified with 5'-CATGCTATCCCTCGTCTCG-3' and 5'-CGCACTTCATGCTGGAGTTG-3' primers. The cycling parameters were incubated at 95°C for 3 minutes, followed by 35 cycles of incubation at 94°C for 1 minute, 51°C for 1 minute, 72°C for 2 minutes and a final extension step of 5 minutes at 72°C.

III. RESULTS AND DISCUSSION

A. Primary structure of EMADS1

The PCR product obtained was named EMADS1. Nucleotide sequence of the *EMADS1* cDNA was 996 bp long, including a 75 bp 5' untranslated region, a 192 bp 3' UTR, and a poly(A)+ tail as shown in Fig.1. The *EMADS1* cDNA contained a 729 bp coding region encoding 242 amino acids. The start codon ATG was at bp77-79 and termination codon TAA was present at bp 802-804 followed by the 3' UTR. The deduced amino acid sequence of *EMADS1* contained the conserved MADS domain and K domain as indicated in Fig.1. In addition, I domain and C-terminal domain were also revealed.

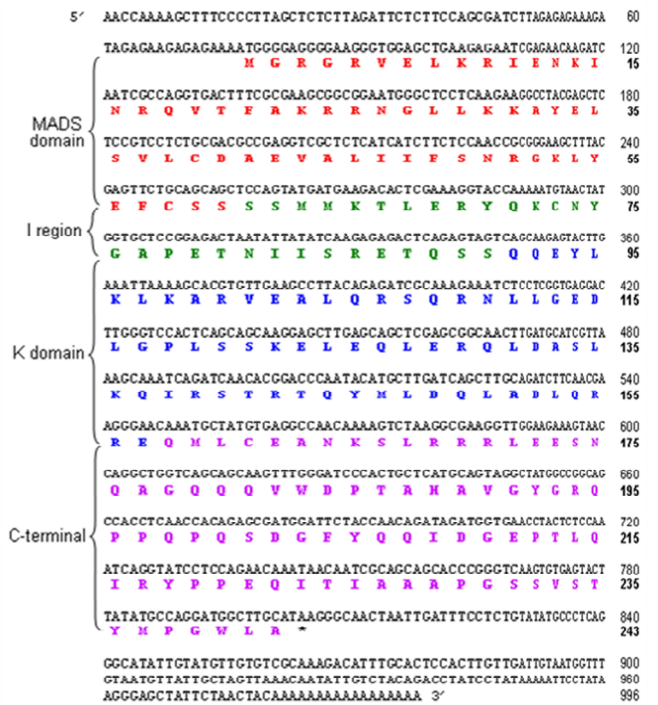

Fig. 1: Nucleotide and amino acid sequences of EMADS1. The MADS, K, I and C-terminal domain are as indicated. Asterik indicates stop codon.

Further analysis of the amino acids sequence of EMADS1 using BLASTp against the GenBank database revealed significant homology with MADS-box proteins from other higher plants especially the conserved domains. The MADS domain of EMADS1 showed 100% similarity to AOM1 of Asparagus, PaAGL9.2 of avocado, VvMADS4 of grapevine, FBP2 of petunia as well as SEP1and SEP3 of Arabidopsis (Table 1).

Table 1. Percentage of identity in MADS and K domains among EMADS1 and higher plants.

Plant Species	Gene	% of Identity	
		MADS domain	K domain
Asparagus officinalis	*AOM1*	100	96
Liriodendron tulipifera	*LtAGL9*	98	90
Aranda Deborah (orchid)	*OM1*	95	93
Persea (avocado)	*PaAGL9.2*	100	93
Vitis vinifera (grapevine)	*VvMADS4*	100	90
Arabidopsis thaliana	*SEP1*	100	79
	SEP2	95	78
	SEP3	100	81
	SEP4	95	66
Petunia hybrida	*FBP2*	100	90
Lycopersicon esculentum (tomato)	*TM5*	98	88

B. Phylogenetic Analysis

EMADS1 sequence was compared mainly to those from Arabidopsis, as many of the MADS-box genes family have been well studied in this plant. Phylogenetic relationships of the full-length amino acids sequences were performed using Phylip (version 3.63). The neighbor-joining analysis and statistical significance of nodes were checked by computing the corresponding bootstrap values. Fig. 2 showed the phylogenetic relation of the MADS-box genes. The phenogram contains five groups of plants MADS-box genes. The first group, SQUA/AP1-like gene group, includes Arabidopsis AP1 (GenBank assesion number Z16421), Antirrhinum SQUA (X63701) and tomato TDR4 (X60757). The second group, the DEF/AP3-like gene group, includes Antirrhinum DEFA (X62810), Arabidopsis AP3 (D21125) and tomato TDR6 (X60759). The third group, the GLO/PI-like gene group, includes Arabidopsis PI (D30807), Antirrhinum GLO (X68831), and petunia FBP1 (M91190) and FBP3 (X71417). The fourth group, the AG-like gene group, includes Arabidopsis AG (X53579), Antirrhinum PLE (S53900) and petunia FBP6 (X68675). The fifth group, the AGL2-like gene group, includes Arabidopsis SEP1 (AY727593), SEP2 (M55552), SEP3 (AF015552), and SEP4 (NM201682), petunia FBP2 (M91666), tomato TM5 (X60480) and TM29 (AJ302015), Vitis vinefera VvMADS4 (AF373603), asparagus AOM1 (AY382400), rice OsMADS7 (U78891) and OsMADS8 (U78892), orchid OM1 (X69108), Liriodendro tulipifera LtAGL9 (AY850182), avocado PaAGL9.2 (AY850186), Silene latifolia SlSEP1 (AB162019) and SlSEP3 (AB162020) and apple MdMADS4 (U78950).

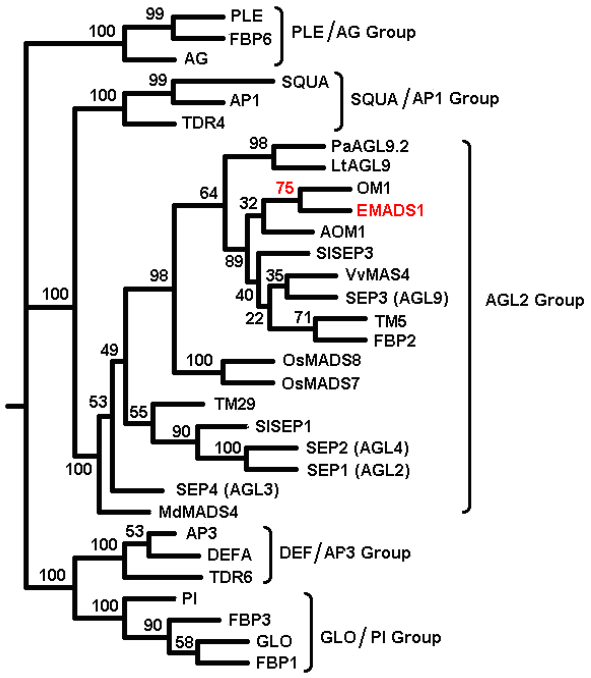

Fig. 2. Phylogenetic relationship of MADS-box gene and 12 representative plants. The bootstrap values are expressed as a percentage (100 replicates) at each node and the branch lengths are drawn to scale.

The phylogenetic tree showed that *EMADS1* belong to the *AGL2* group. All the *AGL2*-like genes have in common that their expression patterns do not fit a function as a typical floral meristem identity gene or an A-, B-, or C-class floral organ identity genes. Furthermore, these *AGL2*-like genes have a preference for simultaneous expression in the second, third and fourth whorl, or even more ubiquitous expression. In fact, *AGL2*-like genes represent a new functional class of MADS-box genes, which is known as the Class-E genes that are required for petal, stamen, carpel, and ovule development and for determining the proliferation of the floral meristem [7].

C. RT-PCR Analysis

The relative steady-state of *EMADS1* transcripts was study as a means to investigate its function in different floral organs of oil palm. In Fig. 3, *EMADS1* was expressed in both immature male and female flower buds. However, *EMADS1* expression was significantly higher in immature flower buds compared to the male flower buds. Similarly, the MADS-box gene AGL2 in *Arabidopsis* was also reported to be abundant throughout floral development in sepals, petals, stamens and carpels. It was also highly expressed in developing ovules and in developing embryos and seed coats, abating as seeds mature [8]. As the subfamily members share highly related sequence similarity, expression patterns and functions, this suggested that EMADS1 is likely to function at the initial stages of flowering in determination of the inflorescence and the identity of the flower whorls. In addition, *EMADS1* expression was also detected in seed embryo although the expression was relatively low.

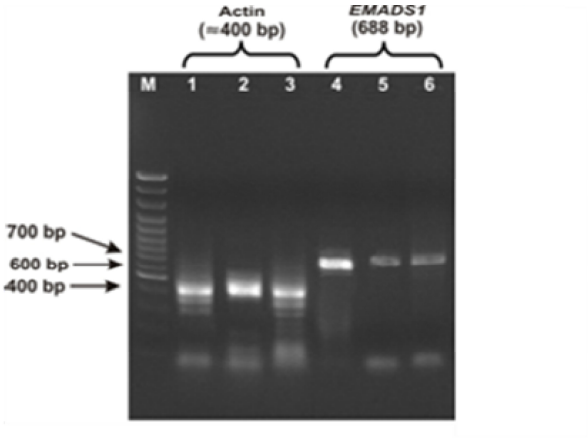

Fig. 3. RT-PCR analysis of *EMADS1* in floral organs of oil palm. M: GeneRuler™ 100 bp DNA Ladder. Lanes 1, 4 and 7: immature female flower bud; Lanes 2, 5 and 8: immature male flower bud; and Lanes 3, 6 and 9: seed embryo.

IV. CONCLUSION

A MADS-box gene was cloned and charactierized, designated as *EMADS1*. The deduced amino acids sequence of *EMADS1* consists of the typical domain structure, namely the MADS-box, K and I domains of higher plant. Phylogenetic analysis of this MADs-box gene grouped it into the AGL2-like subfamily. *EMADS1* expressions were detected in both immature male and female flower buds indicating this gene is likely to be involved in floral development. *EMADS1* transcript was also detected in seed embryo suggesting a wider role including seed development. Further studies will now be required in order to obtain a deeper understanding of the diverse roles played by *EMADS1*.

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