



Cloning and expression of a plastid-encoded subunit, beta-carboxyltransferase gene (*accD*) and a nuclear-encoded subunit, biotin carboxylase of acetyl-CoA carboxylase from oil palm (*Elaeis guineensis* Jacq.)

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ABSTRACT

Palm oil is the second largest traded oil or fat in the world market and palm is the most important crop grown mainly for its oil. Identified varieties of *Elaeis guineensis* Jacq., with a high oil content and produced through a selective breeding program, are desirable for improving the yield of oil, subsequently enhancing the economic feasibility of using oil palm in various applications, including bio-diesel. We have cloned the gene of biotin carboxylase (*accD*) from *E. guineensis* Jacq. This gene encodes a plastid-encoded subunit of heteromeric acetyl-CoA carboxylase (ACCase). The cDNA of *accD* gene (accession number DQ004687) has an open reading frame of 1479 bp that encodes a putative protein of 492 amino acid residues (AAY86362) with a predicted molecular mass of 55.47 kDa. The heteromeric form of ACCase is important as it catalyzes the first committed step of fatty acid synthesis. There is evidence that collectively suggests that the expression of *accD* in plastids is crucial to the levels of heteromeric ACCase and in turn, to the amount of seed oil in plant. Here we support the hypothesis that the expression level of *accD* is correlated with the oil palm production by using a semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) and quantitative real-time polymerase chain reaction (real-time PCR) analysis. Moreover, we observed the similar expression profile in nuclear-encoded subunit, biotin carboxylase (*accC*). This finding represents the genetic background of the expressed genes that correlate to high yield in plant and that ACCase can be used as a marker in the breeding program.

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1. Introduction

Oil palm (*Elaeis guineensis* Jacq.) is a monocotyledonous plant of the palm family (Arecaceae), with a long life cycle and no natural vegetative reproduction. Oil palm is a species of particular economic importance as it provides one of the most important sources of vegetable oil for use in a wide range of edible products. The global production of palm oil in 2003–2004 was 29.1 million tons, second only in importance to soybean [1]. Because of the oil crisis, uses of palm oil will increase in the future because palm oil has the potential for use in the production of bio-diesel. Oil palm is cultivated in the inter-tropical regions of Asia, Latin America, and Africa. The preferred plants for cultivation are *tenera* hybrids (bearing fruits with shells of intermediate thickness). These

originate from crosses between *dura* (thick shell) and *pisifera* (thin shell) types [2,3]. Due to a long selection cycle, poorly characterized genotypes and a high heterogeneity prevalent among hybrids, the use of modern breeding strategies with DNA marker-assisted breeding [4–6] is recommended for the improvement of crop quantities and qualities. There has been considerable work done to establish a genetic map for this species, notably by using RFLP, microsatellite and transposon markers [4,7–11]. In order to speed up the process of crop improvement, one possible marker looks up a key enzyme involved in fatty acid biosynthesis. Biochemical studies have indicated that the acetyl-CoA carboxylase (ACCase) gene product may be involved in the control of the lipid accumulation process [12]. The plant has two distinct forms of this enzyme: the homomeric and the heteromeric forms. In *Arabidopsis*, a homomeric form of ACCase is encoded by two genes: *ACC1* and *ACC2* [13]. *ACC1* is present in the cytosol and *ACC2* is predicted by bioinformatics tool to be present in the plastid [14]. The genome information of four subunits of heteromeric form are *accC* for a biotin carboxylase (BC), *accB* for biotin carboxyl carrier protein (BCCP), *accA* for α , and *accD* for β subunits of carboxyl

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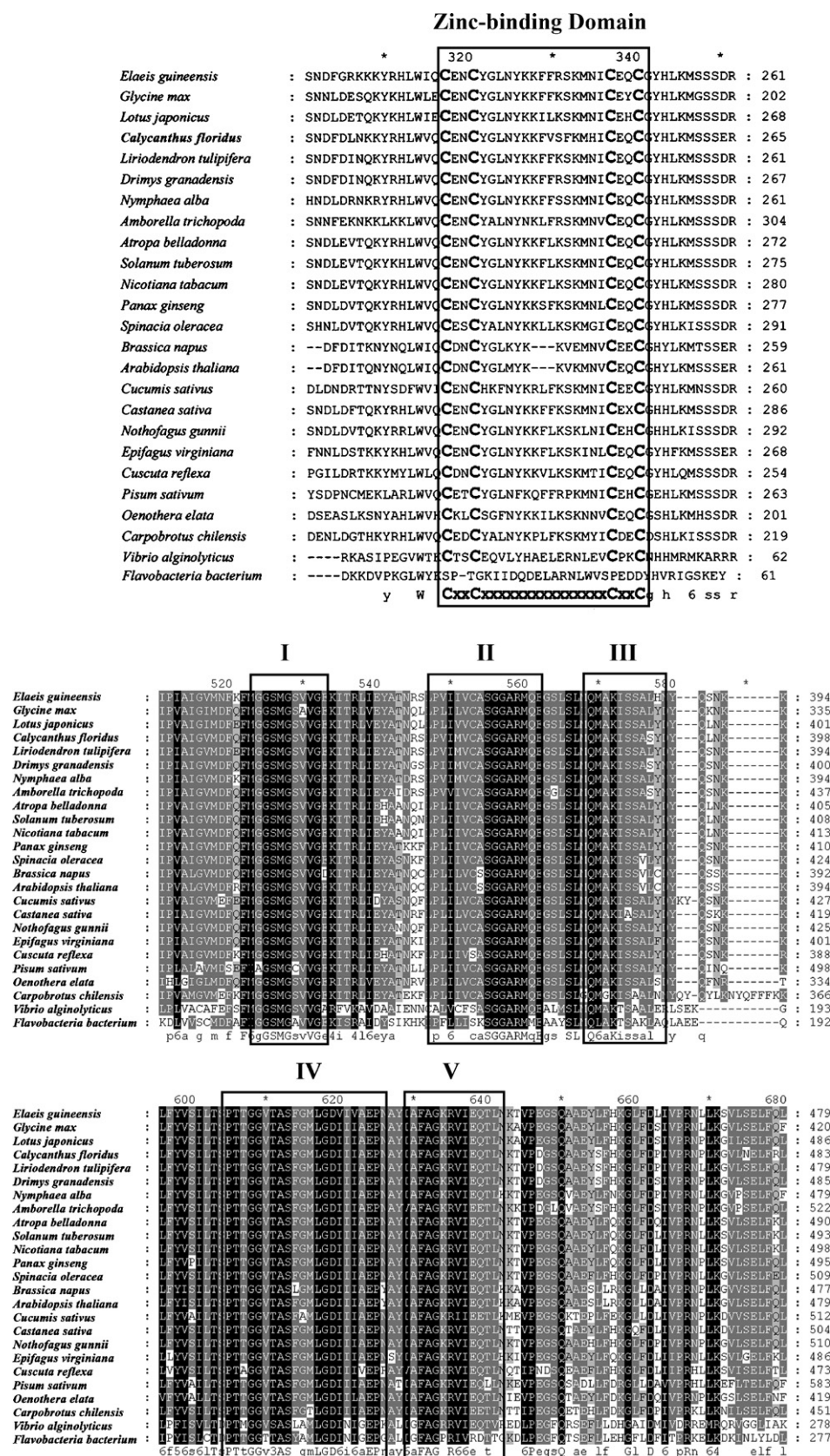


Fig. 1. Conserved zinc-binding domain (CX2CX15CX2C) and I–V motifs at the C-terminal amino acid sequence in plant accDs from oil palm (AAY86362; *Elaeis guineensis*) with that of other accD's. The species and corresponding accession number are as follows: *E. guineensis* (AAY86362), *Glycine max* (AAA80643), *Lotus japonicus* (BAB33205), *Calycanthus floridus* (NP_862763), *Liriodendron tulipifera* (YP_740211), *Drimys granadensis* (YP_784395), *Nymphaea alba* (CAF28602), *Amborella trichopoda* (CAD45116), *Atropa belladonna* (NP_783241), *Solanum tuberosum* (YP_635648), *Nicotiana tabacum* (NP_054508), *Panax ginseng* (YP_086975), *Spinacia oleracea* (CAB88738), *Brassica napus* (CAA9747), *Arabidopsis thaliana* (NP_051068), *Cucumis sativus* (ABI97426), *Castanea sativa* (AAS55872), *Nothofagus gunnii* (AAT79506), *Epifagus virginiana* (AAA65854),

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