SUBSTITUTION OF PHE\textsuperscript{282} WITH SER IN LEA \textit{B. napus} c.v.
WESTAR RESTORES FAEI ENZYME ACTIVITY

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1. Abstract

We have isolated fatty acid elongation 1 (FAE1) genomic clones from high erucic acid (HEA) \textit{B. napus}, \textit{B. rapa} and \textit{B. oleracea}, and low erucic acid (LEA) \textit{B. napus}. Nucleotide sequences corresponding to open reading frames of 1523 bp were translated and proteins of 506 a.a. were deduced. Comparative study of FAE1 protein sequences from HEA and LEA Brassicas revealed the one crucial amino acid difference: the serine residue at position 282 of the HEA FAE1 sequences is substituted by phenylalanine in LEA \textit{B. napus}. Using site directed mutagenesis the phenylalanine\textsuperscript{282} residue was substituted with a serine residue in FAE1 polypeptide from LEA \textit{B. napus}, the mutated gene was expressed in yeast, and GC analysis showed presence of very long chain mono-unsaturated fatty acids indicating that the elongase activity was restored in the LEA FAE1 enzyme. Thus, for the first time, the low erucic acid trait in \textit{B. napus} has been attributed to a single amino acid substitution which prevents the biosynthesis of very long chain mono-unsaturated fatty acids.

2. Introduction

In high erucic acid (HEA) \textit{Brassicaceae}, a seed-specific fatty acid elongase 1 (FAE1) is the condensing enzyme (3-ketoacyl-CoA synthase; 3-KCS) that catalyzes the first of four enzymatic reactions of FAE complex, resulting in the synthesis of VLCMFAs which are the major constituents of their seed oil.

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Intense research is ongoing by several groups to elucidate the mutations involved in the loss of 3-KCS activity in LEA *B. napus* cultivars. Han et al. (2001) speculated that the presence of serine at position 282 in all functional 3-KCS proteins instead of phenylalanine in non-functional LEA *B. napus* 3-KCS could be important for the activity of the condensing enzyme. Roscoe et al. (2001) hypothesized that the LEA phenotype could be the result of one or more lesions in the genes that encode or regulate 3-KCS activity. In order to clarify this controversy, we decided to examine the role of the amino acid serine at position 282 in the 3-KCS protein sequence to determine if this apparent mutation from serine to phenylalanine led to the LEA *B. napus* phenotype. We introduced a point mutation into the LEA *B. napus* cultivar Westar FAE1 coding region to substitute phenylalanine with serine at position 282 in attempt to restore the FAE1 condensing enzyme activity.

Here we report and discuss the results of analyses of heterologous expression in yeast and site directed mutagenesis of LEA *Brassica napus* FAE1 KCSs.

3. Results

3.1. Site directed mutagenesis of the LEA *B. napus* c.v. Westar FAE1 gene: expression in yeast shows the enzyme activity is restored in a mutated condensing enzyme

In order to test the importance of serine$^{282}$ to 3-KCS function, using a site-directed mutagenesis (SDM) approach, we changed the phenylalanine$^{282}$ residue in LEA c.v. Westar FAE1 to the highly conserved serine residue. The sequence analyses of five different clones revealed that two of them (WS-SDM1 and WS-SDM18) had been successfully mutated with a Ser at position 282 (data not shown).

Due to the presence of hydrophobic phenylalanine in non-functional *B. napus* c.v. Westar wild-type 3-KCS (WS-wt) at position 282 instead of the hydrophilic serine in the mutated c.v. Westar 3-KCS (WS-SDM), the hydrophilicity values for

![Figure 1](image-url)  

**Figure 1.** Hydrophilicity (Kyte-Doolittle) plot for the WS-wt and WS-SDM protein domains. Arrows indicate position 283 with asparagine (Asn) which is the last amino acid residue in the WS-wt hydrophobic domain and the first amino acid residue in the WS-SDM hydrophilic domain. The analyses were performed using Protean, Lasergene Biocomputing Software for Windows (DNAStar, Madison, WI). WS-wt, LEA *B. napus* c.v. Westar 3-KCS; WS-SDM, Westar site directed mutated 3-KCS.