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Computational Analysis of Lipid Modification in Crambe

Research Article

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Abstract

Metabolic engineers are examining the non food oil seed to develop suitable crop with enhanced performance in fatty acid functionalities and properties. The growth and production of various innovative fatty acids find various application for industrial processes. Crambe species of perennial flowering plants have miniscule white and yellow flowers. These are primarily used as food plants by larvae of some Lepidoptera class. Crambe is a unique seed crop exclusive grown for its industrial manufacturing especially enhanced oleic acid content by the Crambe genes of Fatty Acid Desaturase (FAD) and Fatty Acid Elongase (FAE) via down-regulated process. The FAD 2 enzymes are expressed in different manner in various tissues of the plants and transforms the functional and vegetative features due over expression. This study involves the computational analysis of the Crambe oleate desaturase and fatty acid elongase1 genes using various web tools with the ultimate aim of providing recommendations to wet lab researchers. The oleate desaturase and fatty acid elongase1 (FAE 1) sequences from Crambe were retrieved from NCBI nucleotide database. Lipases can be tailored to mitigate the carbon chain length, extent of unsaturation, and relevant improvement in the esterification reaction. This is significantly important for molecular pathway in delta-12 desaturases FAD2. These studies will be highly beneficial in predicting the feasibility of generating amplified wax ester oils in transgenic crops, fatty acid reshaping such as oleyl-loeate for the availability of enhanced levels of fatty acid free values and confrontation towards various temperature and salt series in plants. These studies will be quite useful for the necessity of timely desirable traits in vegetable oil produce with reference to carbon economy, to meet the demand of traditional fossil fuel advancement and human health.

Keywords: Crambe; Erucic acid; FAE1 gene; FAD enzyme; Web tools

Introduction

Lipids are the heterogeneous class of organic compounds composed of long chain fatty acids. They exist as oily, waxy and greasy in nature and insoluble in aqueous media. Lipids act as a mechanical barrier in the form of cell membrane. The oils can be saturated with 12-24 carbon chain and unsaturated fatty acid having one or more carbon –carbon double bonds (Figure 1). Oleic acid (Cis -9-octadecenoic acid 18) is an example of mono saturated fatty acids. Most vegetable oils like sun flower, saff flower, corn oil are rich in linoleic acid. They are source of energy and help in signalling process. Progressive health benefits are expected with the metabolism of omega-3 fatty acids. The utilization of trans fat present in hydrogenated oil may have adverse effects on human health which are manifested in the form of various life style disease associated with heart, liver, pancreas and cancer. Fatty acids as ingredients are utilized in various industries like food, cosmetic, lubricants, plasticizers, and nano products.

Crambe is a genus of about 20 species of annual and perennial flowering plants in the cabbage family Brassicaceae, native to a variety

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of habitats in Europe, Turkey, southwest and central Asia and eastern Africa. They carry dense racemes of tiny white or yellow flowers on (mostly leafless) stems above the basal leaves [1]. Crambe species are used as food plants by the larvae of some Lepidoptera species including Lime-speck Pug. The advantages of using Crambe as an alternative source of Erucic acid are that it contains the highest level of Erucic acid in the seed oil naturally occurring within the Brassicaceae, 10-15% higher than the current HEAR (High Erucic Acid Rapeseed Oil) oils. Crambe is essentially self-pollinated and does not out-cross with any other food oilseed crops or closely related wild species [2].

Vegetable oil production with extraordinary elevated monoenoic oileic acid constitution is one of foremost areas of oil up gradation for industrial application. The high oleic oil concentration in industrial feedstock has been of concern due to its great thermal and oxidative stability during high boiling processes employed food industry. Crambe (Crambe abyssinica) is important oilseed crop, appropriate for industrial oil synthesis by FAD and FAE genes via down-regulated process. The first cloned genes of this process were the Crambe CaFAD2, CaFAD3 and CaFAE1 genes as reported in another study. In that study, numerous copies of these genes were segregated , RNAi constructs were made by exceedingly identical arrangements. These constructs were earlier tried and carried out on Arabidopsis, which controlled the elevated oleic or linoleic levels depending on the genes targeted, indicating that the RNAi constructs were efficient in regulating the expression of the selected genes in a nonidentical but closely related species. Furthermore, down-regulation of CaFAD2 and CaFAE1 in Crambe species with the FAD2-FAE1 RNAi vector resulted in even more substantial intensification in oleic acid concentration in the seed oil with up to 80% compared to 13% for wild type. The high oleic trait has been stable in subsequent five generations and the GM line grew normally in greenhouse. Crambe is indispensable for constructing high oleic oil, thus subsidizing to the category an oil crop platform for industrial oil production [3].

Erucic acid is an important fatty acid in the oleochemical industry. The current major industrial source of erucic acid is high-erucic acid rapeseed oil. Erucic acid is one of the chief fatty acids existing in the oil separated from the segments of the family Brassicaceae [4]. Crambe also has other valuable by-products, such as protein meal and possible fibrous material. The acceptance of Crambe meal by the feed industry is based on its attractive price and satisfactory performance as a feed for ruminant animals. The crucifer *Crambe abysinica* has high erucic acid content (52-59%) in its seed oil, in addition to wide climatic and agronomic adaptation hence, this plant is receiving more focus as an alternative industrial crop [5].

Crambe cordifolia, syn. *Crambe glabrata* is a species of flowering plant in the family Brassicaceae, native to the Caucasus. Growing to

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2.5 m (8 ft) tall by 1.5 m (5 ft) broad, it is a substantial clump-forming herbaceous perennial with kidney-shaped dark green leaves, 35 cm (14 in) or more in length, which die down in mid- to late summer. It is widely cultivated in gardens for its foliage and spectacular multibranched inflorescences of many small, white, cruciform (crossshaped) flowers, reaching up to 2 metres (6 ft 7 in) high and appearing in early summer [6]. The phylogenetic analysis of Crambe hispanica, Crambe hispanica subsp abyssinica, Crambe filiformis and Crambe kralikii arrangements designated that the FAE1 genes of these classes might have gone through a remote gene replication phenomenon, during the process of species differentiation. Oleate desaturase FAD2 (1-acyl-2-oleoyl-sn-glycero-3-phosphocholine Delta12-desaturase) is the key enzyme responsible for the production of linoleic acid in nonphotosynthetic tissues of plants. Little is known, however, concerning the post-transcriptional mechanisms that regulate the activity of this important enzyme.

Materials and Methods

The oleate desaturase and fatty acid elongase1 sequences from Crambe were retrieved from NCBI nucleotide database (http://www. ncbi.nlm.nih.gov) (Table 1).

The protparam tool was used for analysis of Physico-chemical properties (http://web.expasy.org/protparam/)

Results

Analysis of physicochemical properties

The Physicochemical properties analysis of all protein sequences of oleate desaturase and fatty acid elongase1 of Crambe was predicted by computational method. The analysis of biochemical property has been calculated in respect of positively charged amino acid, negatively charged amino acid, molecular weight, grand average of hydropathicity (Gravy), instability index and Theoretical pI value. All 30 proteins are a class of Crambe protein family. These all sequences are native to a variety of habitats in Europe, Turkey, southwest and central Asia and eastern Africa. They carry dense racemes of tiny white or yellow flowers on (mostly leafless) stems above the basal leaves. These Crambe species are used as food plants by the larvae of some Lepidoptera species including Lime-speck Pug. The advantages of using Crambe as an alternative source of erucic acid are that it contains the highest level of erucic acid in the seed oil naturally occurring within the Brassicaceae.

The analysis of Physico-chemical properties in Oleate desaturase protein sequences from Crambe

In physico-chemical analysis of oleate desaturase class of protein shows instability index less than 40, it means they are probably stable proteins but instability index of protein oleate desaturase class of Crambe filiformis (AIA21655.1) is very less that is 33.38 and it is a very unstable protein. The quantitative value for the hydrophobicity of all proteins were measured by the GRAVY score (Grand average of hydropathicity value) has been found to be nearby similar in all oleate desaturase protein sequences of Crambe. The grand average of hydropathicity of the sequences calculates the sum of hydropathy values among all protein sequences. Very less score of GRAVY shows that less hydropathicity in that protein sequence. This principle

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Table 1: Protein Ids of crambe sequences.

Protein_GI_ID	Protein_id	Protein Name
636745392	AIA21669.1	oleate desaturase [Crambe kralikii]
636745390	AIA21668.1	oleate desaturase [Crambe kralikii]
636745388	AIA21667.1	oleate desaturase [Crambe kralikii]
636745386	AIA21666.1	oleate desaturase [Crambe kralikii]
636745384	AIA21665.1	oleate desaturase [Crambe kralikii]
636745382	AIA21664.1	oleate desaturase [Crambe glabrata]
636745380	AIA21663.1	oleate desaturase [Crambe glabrata]
636745378	AIA21662.1	oleate desaturase [Crambe glabrata]
636745376	AIA21661.1	oleate desaturase [Crambe glabrata]
636745374	AIA21660.1	oleate desaturase [Crambe glabrata]
636745372	AIA21659.1	oleate desaturase [Crambe filiformis]
636745370	AIA21658.1	oleate desaturase [Crambe filiformis]
636745368	AIA21657.1	oleate desaturase [Crambe filiformis]
636745366	AIA21656.1	oleate desaturase [Crambe filiformis]
636745364	AIA21655.1	oleate desaturase [Crambe filiformis]
636745362	AIA21654.1	oleate desaturase [Crambe filiformis]
636745360	AIA21653.1	oleate desaturase [Crambe filiformis]
636745358	AIA21652.1	oleate desaturase [Crambe filiformis]
636745356	AIA21651.1	oleate desaturase [Crambe filiformis]
636745354	AIA21650.1	oleate desaturase [Crambe hispanica subsp. abyssinica]
636745352	AIA21649.1	oleate desaturase [Crambe hispanica subsp. abyssinica]
636745350	AIA21648.1	oleate desaturase [Crambe hispanica subsp. abyssinica]
636745348	AIA21647.1	oleate desaturase [Crambe hispanica subsp. abyssinica]
636745346	AIA21646.1	oleate desaturase [Crambe hispanica subsp. abyssinica]
636745344	AIA21645.1	oleate desaturase [Crambe hispanica subsp. abyssinica]
459938042	AGG87087.1	fatty acid elongase 1 [Crambe hispanica subsp. abyssinica]
459938040	AGG87086.1	fatty acid elongase 1 [Crambe hispanica subsp. abyssinica]
459938038	AGG87085.1	fatty acid elongase 1 [Crambe hispanica subsp. abyssinica]
459938036	AGG87084.1	fatty acid elongase 1 [Crambe hispanica subsp. abyssinica]
459938034	AGG87083.1	fatty acid elongase 1 [Crambe hispanica subsp. abyssinica]

Table 2 (a): The analysis of Physico-chemical properties in oleate desaturase protein sequences from Crambe.

Protein_id	Protein_name	Number of negative amino acids	Number of positive amino acids	Molecular weight	Theoretical pl	Instability Index	Gravy
AIA21669.1	oleate desaturase [Crambe kralikii]	32	35	44118.7	8.22	37.9	-0.123
AIA21668.1	oleate desaturase [Crambe kralikii]	32	35	44100.7	8.22	35.03	-0.116
AIA21667.1	oleate desaturase [Crambe kralikii]	30	37	44331.9	8.74	37.33	-0.158
AIA21666.1	oleate desaturase [Crambe kralikii]	30	35	44257.8	8.57	36.95	-0.166
AIA21665.1	oleate desaturase [Crambe kralikii]	29	34	43465.9	8.58	38.23	-0.133
AIA21664.1	oleate desaturase [Crambe glabrata]	31	36	44112.8	8.53	35.87	-0.123
AIA21663.1	oleate desaturase [Crambe glabrata]	31	36	44112.8	8.53	35.87	-0.123
AIA21662.1	oleate desaturase [Crambe glabrata]	31	36	44112.8	8.53	35.87	-0.123
AIA21661.1	oleate desaturase [Crambe glabrata]	31	36	44112.8	8.53	35.87	-0.123
AIA21660.1	oleate desaturase [Crambe glabrata]	31	36	44140.8	8.54	36.59	-0.125
AIA21659.1	oleate desaturase [Crambe filiformis]	31	33	44220.8	8.01	36.44	-0.146
AIA21658.1	oleate desaturase [Crambe filiformis]	30	35	44338	8.57	36.01	-0.16
AIA21657.1	oleate desaturase [Crambe filiformis]	31	36	44112.8	8.53	35.87	-0.123
AIA21656.1	oleate desaturase [Crambe filiformis]	29	35	44348.1	8.68	34.53	-0.136
AIA21655.1	oleate desaturase [Crambe filiformis]	31	35	44304.9	8.4	33.38	-0.14
AIA21654.1	oleate desaturase [Crambe filiformis]	29	36	44260	8.78	35.17	-0.156
AIA21653.1	oleate desaturase [Crambe filiformis]	30	34	44295	8.43	35.63	-0.13
AIA21652.1	oleate desaturase [Crambe filiformis]	31	37	44118.8	8.65	38.15	-0.121
AIA21651.1	oleate desaturase [Crambe filiformis]	30	34	44341	8.43	34.77	-0.155
AIA21650.1	oleate desaturase [Crambe hispanica subsp. abyssinica]	31	36	44034.6	8.53	35.61	-0.118
AIA21649.1	oleate desaturase [Crambe hispanica subsp. abyssinica]	31	37	44117.8	8.65	33.43	-0.126
AIA21648.1	oleate desaturase [Crambe hispanica subsp. abyssinica]	29	36	44154.9	8.74	33.52	-0.078
AIA21647.1	oleate desaturase [Crambe hispanica subsp. abyssinica]	30	36	44115.7	8.65	35.84	-0.111
AIA21646.1	oleate desaturase [Crambe hispanica subsp. abyssinica]	30	34	44115.6	8.44	36.33	-0.128
AIA21645.1	oleate desaturase [Crambe hispanica subsp. abyssinica]	31	36	44145.8	8.54	35.09	-0.109

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Protein_id	Protein_name	Number of negative amino acids	Number of positive amino acids	Molecular weight	Theoretical pl	Instability Index	Gravy
AGG87087.1	fatty acid elongase 1 [Crambe hispanica subsp. abyssinica]	46	63	56416.9	9.31	37.32	-0.128
AGG87086.1	fatty acid elongase 1 [Crambe hispanica subsp. abyssinica]	46	63	56402.9	9.31	36.76	-0.128
AGG87085.1	fatty acid elongase 1 [Crambe hispanica subsp. abyssinica]	45	63	56515.1	9.36	38.82	-0.111
AGG87084.1	fatty acid elongase 1 [Crambe hispanica subsp. abyssinica]	45	64	56528.2	9.39	36.8	-0.116
AGG87083.1	fatty acid elongase 1 [Crambe hispanica subsp. abyssinica]	46	63	56444.9	9.31	36.73	-0.156

Table 2 (b): The analysis of Physico-chemical properties in fatty acid elongase1 protein sequences from Crambe.

is based on the basis of increasing negative score of GRAVY shows that smaller hydropathicity. In this result the protein oleate desaturase (Crambe hispanica subsp. abyssinica) shows that larger hydropathicity (AIA21648.1) with GRAVY score -0.078 and the protein oleate desaturase (Crambe kralikii) (AIA21666.1) showing smaller hydropathicity with GRAVY score -0.166. The protein sequence of oleate desaturase (Crambe filiformis-AIA21659.1) has less positive value of isoelectric point. 8.0, which means that have less solubility (Table 2(a)).

The analysis of Physico-chemical properties in fatty acid elongase1 protein sequences from Crambe

Physico-chemical property analysis of fatty acid elongase 1 of Crambe family sequences shows instability index less than 40 which means they are probably stable proteins as well as oleate desaturase class of protein family. Instability index of protein fatty acid elongase 1 Crambe hispanica subsp. abyssinica (AGG87083.1) is very less that is 36.73 and this protein is very unstable. The score of GRAVY shows the hydropathicity character in particular protein. The grand average of hydropathicity of the sequences calculates the sum of hydropathy values among all protein sequences. Very less score of GRAVY shows that less hydropathicity in that protein sequence. This principle is based on the basis of increasing negative score of GRAVY shows that smaller hydropathicity. In this result the fatty acid elongase 1 of Crambe protein family sequences shows that the GRAVY score is nearby in all these sequences and they have similar hydropathicity character. The protein sequence of these family shows strong positive value of isoelectric point in range of 9, which means they have strong solubility (Table 2(b)). The Instability index is smaller than 40 which show it is stable in test tube.

The conventional approach for lipidomic investigation deals

in enzymatic assays and use of Gas Chromatography (GC), Mass Spectrometry (MS) and Nuclear Magnetic Resonance (NMR) for the evaluation and analysis of lipids. These methods have their pros and cons and can be overcome by combining with bioinformatics tools. The results obtained in this study can be utilized for recommendations for the wet lab experiments. The amino acid conformation can predicts the basic properties of the enzymes, the isoelectric point can be utilized for the purification techniques using various buffers. The instability index value can give indication of stability of protein. This can help in altering the lipid composition, enzymatic activity and characterisation. Thus the present investigation can aid in formulating the various lipids and proteins for various industrial application with reference to oil enrichment in less time and economical preposition.

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