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Construction for Δ-12 Fatty Acid Desaturase (FAD2) Silencing to Improve Oil Quality of *Jatropha curcas* Linn.

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Abstract

The predominant fatty acids of jatropha oil are oleic acid (42.02 %), linoleic acid (35.38 %), palmitic acid (14.54 %), and stearic acid (6.3 %). Ratio of fatty acids content of jatropha oil, especially for oleic acid (42.02 %) compare to linoleic acid (35.38 %) is approximately equal. The oxidation stability of jatropha oil showed that methyl oleate is around 2.79 h and methyl linoleate is 0.94 h. Therefore, the presence of methyl linoleate will decrease the value oxidation stability of jatropha oil. The objective of the research was to modify fatty acid composition, especially to increase oleic acid and to reduce linoleic acid content of jatropha oil. To achieve the objective construction of transformation vectors for Δ 12 fatty acid desaturase FAD2 gene silencing was designed. In two transformation vectors, FAD2 gene fragment was constructed in inverted repeat (IR) and intron-spliced inverted repeat (ISIR) orientations to generate hair-pin RNAs (hpRNA).

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Keywords: Δ 12 *f*atty acid desaturase; gene silencing; intron-spliced inverted repeat*;* inverted repeat; *Jatropha curcas* Linn.

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Nomenclature

bp	base pair	**MUFA**	monounsaturated fatty acids
FAD2	Δ 12 fatty acid desaturase	**PUFA**	polyunsaturated fatty acids
IR	inverted repeat	**RE**	restriction enzymes
ISIR	intron-spliced inverted repeat	**h**	hour
hpRNA	hair-pin RNAs	**t**	ton = 10^3 kg

1. Introduction

Jatropha curcas L. or physic nut belongs to the family Euphorbiaceae is a drought tolerant and highly adaptable small tree up to 5 m to 7 m, originated from Central America, producing oil containing seeds and the plant was widely distributed to other countries in Africa and Asia by Portuguese pioneer [1]. The jatropha seeds are toxic and contain a-non edible oil content around 30 % to 35 %. The toxicity is due to phorbol ester (phorbol-12-myristate -13-acetate) as the main toxic agent responsible for *J. curcas* toxicity [2-4]. Annual seed production range is 0.2 kg to more than 2 kg per plant [5] depend on the agro-ecosystem and climate. In semi-arid areas and wasteland the seed production was (2 to 3) t ha^{-1} yr^{-1} dry seed, while in good soil condition with average annual rainfall of 900 mm to 1 200 mm the dry seed of 5 t ha^{-1} yr^{-1} can be achieved [6].

The predominant fatty acids of jatropha oil are oleic acid (42.02 %), linoleic acid (35.38 %), palmitic acid (14.54 %), stearic acid (6.3 %) [7]. Ratio of fatty acids content of jatropha oil, especially for oleic acid (42.02 %) compare to linoleic acid (35.38 %) is approximately equal. Oleic acid (C18:1) belongs to monounsaturated fatty acids (MUFA), while linoleic acid (C18:2) belongs to polyunsaturated fatty acids (PUFA). The increased number of double bonds in the chemical structures of the PUFA makes them susceptible to oxidation [8,9]. Oxidation rates of C18:2 is approximately 10 times higher than C18:1 [10]. The oxidation stability of jatropha oil showed that methyl oleate is around 2.79 h and methyl linoleate is 0.94 h [11], therefore, the presence of methyl linoleate will reduce oxidation stability value of jatropha oil.

Genetic engineering has become a new overview for plant improvement to raise valuable traits by producing novel plant varieties. The advance technology for oil modification has been used for rapeseed and soybean. Soybean naturally has one copy of the gene of Δ 12 fatty acid desaturase (FAD2) that control the conversion of oleic acid to linoleic acid in the seed. Genetic improvement through genetic engineering by using the silenced FAD2 in order to increase oleic acid and simultaneous to reduce linoleic acid of various plants were also reported, for examples, canola [12], groundnut [13] and cotton [14].

The objective of the research was to modify fatty acid composition, especially to increase oleic acid and to reduce linoleic acid content of jatropha oil. To achieve the objective construction of transformation vectors for FAD2 gene silencing was designed.

2. Materials and methods

2.1. Primer design, plasmid vectors and bacterial strains

Primers to amplify partial FAD2 fragment was designed from Δ 12 fatty acid desaturase (FAD2) of *Jatropha curcas* (GenBank GU353167). To amplify 500 bp of FAD2 fragment, the primer pairs used are JatFADfrontFN: 5 TAA**GGATCCCAATGG**ATGGGTGCCGGTGGCAGAAT 3 (forward primer with restriction sites of *BamH*I & *Nco*I) and JatFADfrontR: 5 GCG**GTCGAC**CAGCGGATGTTGGATTTCTT 3 (reverse primer with restriction sites of *Sal*I). To amplify 700 bp of FAD2 fragment (with antisense orientation), the primer pairs used are JatFADfrontR2: 5 GGC**CTGCAG**CAAGAACACCAGCATCAGAA 3 (reverse primer with restriction sites of *Pst*I) and JatFADbackFN: 5 GAT**AAGCTTGGTCACC**ATGGGTGCCGGTGGCAGAAT 3 (forward primer with restriction sites of *Hind*III and *BstE*II) (Figure 1).

Since Δ 12 fatty acid desaturase (FAD2) gene of *J. curcas* had no intron, the intron was isolated from lipase gen (GenBank EU650334) of *J. curcas,* the primer pairs used are JatIntF: 5