Fatty Acid Analysis of the Transgenic Tobacco Expressing A Delta 6-Desaturase Gene from *Microula sikkimensis*

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Introduction

γ-Linolenic acid (GLA, $18:3^{\Delta 6, 9, 12}$) and octadecatetraenoic acid (OTA, $18:4^{\Delta 6, 9, 12, 15}$) are important polyunsaturated fatty acids (PUFAs), which have been proved to be benefit for human health (Fan and Chapkin 1998; Whelan 2009). Currently, fish are the predominant source of PUFAs. However, with the growth of world's population and the more nutrition requirements, fishery resources are shrinking. Alternative sources of PUFAs are being investigated (Truksa *et al.* 2009). The major oil crops do not contain GLA and OTA, only several plant species contain these important fatty acids in their leaf lipids and seed oils (Zhou *et al.* 2006). Genetic modification of oil crops may be an effective approach to produce these fatty acids. This process requires an enzyme–delta 6-desaturase, which can introduce a double bond at the delta 6 position (Meesapyodsuk and Qiu 2012). *Microula sikkimensis* is a kind of rare wild oil plant, which is widely distributed in Bhutan, Sikkim, Nepal and the northwest region of China (Cao and Suo 2010). Fu *et al.* (1997) reported that unsaturated fatty acids made up 86.5% of total fatty acids, and that GLA accounted for 6.4% of fatty acids in *M. sikkimensis* seeds that is known as a new source of GLA.

In this research, the delta 6-desaturase gene of *M. sikkimensis* was introduced into tobacco and the fatty acid composition of transgenic tobacco was analysed.

Methods

Transformation of tobacco

The plant expression plasmid designated pCAM1301-MsD6D was introduced into *Agrobacterium tumefaciens* GV3101 by freeze–thaw transformation method (Chen *et al.* 1994). Tobacco (NC89) was transformed by the leaf disk method according to Horsch *et al.* (1985). Initial transformants were selected by 20 μg/ml hygromycin B.

Fatty acid analysis

Extraction and methylation of fatty acid from tobacco leaves were carried out according to the method of Mao *et al.* (2012). Gas chromatography-mass spectrometry (GC-MS) 6890N /5975C (Agilent Technologies) was used to analyze the fatty acid methyl esters. Gas chromatography was as follows: 70°C for 3 min, followed by a gradient of 15°C/min from 70°C to 190°C, 2 min at 190°C, and a gradient of 5°C/min from 190°C to 230°C and 12 min at 230°C. The fatty acid methyl esters were identified using the NIST 08 mass spectrum libraries by comparison of retention times with standard compounds (Sigma, United States).

Results

To determine the function of the *MsD6DES* gene in plants, the gene was introduced into tobacco under the control of CaMV 35S promoter via *Agrobacterium*-mediated transformation. Wild-type tobacco had no GLA and OTA but significant amount of linoleic acid (LA, $18:2^{\Delta 9, 12}$) and α-linolenic acid (ALA, $18:3^{\Delta 9, 12, 15}$), which are substrates for biosynthesis of GLA and OTA, respectively. The fatty acid composition of transgenic tobaccos containing only the empty plastid was similar to that of wild-type plants, while, two novel fatty acids GLA and OTA were present in transgenic tobaccos with pCAM1301-MsD6D. The content of GLA was 1.2% of total fatty acids in leaf lipids, which was slightly less than that of OTA (1.6% of total fatty acids). The combined delta 6-desaturaed fatty acids reached 2.8% of total fatty acids. The conversion rate of LA to GLA and ALA to OTA were 8.4 and 3.4, respectively (Table 1).

Table 1. Fatty acid composition (% w/w) in transgenic tobacco leaves

Plant	Fatty acid (%)							Conversion rate (%)[d]	
	16:0	18:0	18:1	LA	GLA	ALA	OTA	LA → GLA	ALA → OTA
WT[a]	16.3±0.2	4.1±0.1	3.0±0.1	15.6±0.3	_	51.6±1.1	_	_	_
pCAMBIA[b]	15.7±0.1	4.6±0.1	3.7±0.1	14.8±0.5	_	52.8±0.3	_	_	_
MsD6D[c]	18.8±0.4	3.5±0.2	2.7±0.2	13.1±0.2	1.2±0.1	45.2±0.8	1.6±0.1	8.4	3.4

[a] Wild type tobacco; [b] Transgenic tobacco plants containing the empty pCAMBIA1301 vector; [c] Transgenic tobacco plants expressing the *MsD6DES* gene; [d] Conversion rate of the total available substrate to the desaturated product

Discussion

In this research, two novel fatty acids GLA and OTA were detected in transgenic plants. Delta 6-desaturases from some plants exhibited different substrate selectivity. In this study, the content of OTA in transgenic tobacco leaves was slightly higher than that of GLA. It may be due to the amount of ω3 substrate α-linolenic acid (ALA, $18{:}3^{\Delta 9, 12, 15}$) was higher than that of ω6 substrate linoleic acid (LA, $18{:}2^{\Delta 9, 12}$). However, the conversion rate of LA to GLA was 2.5 times higher than that of ALA to OTA. It appears that the *MsD6DES* gene prefers the ω3 substrate ALA to the ω6 substrate LA (Table 1). Similar results were found in *Echium gentianoides* and *Echium plantagineum* (García-Maroto *et al.*, 2006; Zhou *et al.*, 2006). However, the delta 6-desaturase gene from *Primula vialii* showed a preference for the substrate ALA (Sayanova *et al.*, 2003). Further work will be continued to characterize the other functions of the MsD6DES and the PUFA metabolic pathway. Understanding the PUFA biosynthesis may be benefit for engineering the oilseed plants to produce these useful fatty acids.

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References

Cao Y, Suo YR (2010) Extraction of *Microula sikkimensis* seed oil and simultaneous analysis of saturated and unsaturated fatty acids by fluorescence detection with reversed-phase HPLC. *Journal of Food Composition and Analysis* **23**, 100-106.

Chen H, Nelson RS, Sherwood JL (1994) Enhanced recovery of transformants of *Agrobacterium tumefaciens* after freeze-thaw transformation and drug selection. *Biotechniques* **16**, 664-670.

Fan YY, Chapkin RS (1998) Importance of dietary γ-linolenic acid in human health and nutrition. *Journal of Nutrition* **128**, 1411-1414.

Fu H, Wang Q, Zhou ZY (1997) Analysis of fatty acids of seed oil of *Microula sikkimensis* Hemsl in Tianzhu by GC/MS. *Acta Agrestia Sinica* **5**, 205-209 (in Chinese)

García-Maroto F, Mañas-Fernández A, Garrido-Cárdenas JA, López Alonso D (2006) Substrate specificity of acyl-Δ^6-desaturase from Continental versus Macaronesian *Echium* species. *Phytochemistry* **67**, 540-544.

Horsch RB, Fry JE, Hoffman NL, Eichholz D, Rogers SG, Fraley RT (1985) A simple and general method for transferring genes into plants. *Science* **227**, 1229-1231.

Mao ZX, Fu H, Nan ZB, Wang J, Wan CG (2012) Fatty acid content of common vetch (*Vicia sativa* L.) in different regions of Northwest China. *Biochemical Systematics and Ecology* **44,** 347-351.

Meesapyodsuk D, Qiu X (2012) The front-end desaturase: structure, function, evolution and biotechnological use. *Lipids* **47**, 227-237.

Sayanova OV, Beaudoin F, Michaelson LV, Shewry PR, Napier JA (2003) Identification of *Primula* fatty acid Δ^6-desaturases with n-3 substrate preferences. *FEBS Letters* **542**, 100-104.

Truksa M, Vrinten P, Qiu X (2009) Metabolic engineering of plants for polyunsaturated fatty acid production. *Molecular Breeding* **23**, 1–11.

Whelan J (2009) Dietary stearidonic acid is a long chain (n-3) polyunsaturated fatty acid with potential health benefits. *Journal of Nutrition* **139**, 5-10.

Zhou XR, Robert S, Singh S, Green A (2006) Heterologous production of GLA and SDA by expression of an *Echium plantagineum* Δ6-desaturase gene. *Plant Science* **170**, 665-673.