BIOLOGIJA. 2007. Vol. 53. No. 2. P. 4–7

Acyl-lipid Δ12-desaturase of the cyanobacterium increases the unsaturation degree in transgenic potato (*Solanum tuberosum* L.)

Reza Maali A.[1]*,

Irina V. Goldenkova-Pavlova[2],

Vasiliy P. Pchelkin[3] ,

Vladimir D. Tsydendambaev[3],

Dimitry A. Los[3],

Alexander M. Nosov[1]

[1] *Dept. of Agronomy and Plant Breeding, Agricultural College, University of Tehran, Iran*

[2] *Vavilov Institute of General Genetics, RAS, Moscow, Russia*

[3] *Timiryazev Institute of Plant Physiology, RAS, Moscow, Russia*

We propose a new approach to the expression of the gene of interest in plants. The approach is based on the expression hybrid gene in plant. This hybrid gene consists of the sequence of interest, fused with the reporter gene sequence in the reading frame. We constructed hybrid genes *des*A-*lic*BM3. The *DesA* gene encodes the acyl-lipid Δ12-desaturase from the cyanobacterium *Synechocystis* sp. PCC 6803, *lic*BM3 encodes a new universal reporter gene based on thermostable lichenase (LicB) of *Clostridium thermocellum*. This hybrid gene was cloned in the plant expression vector under control of the 35S RNA CaMV promoter and was introduced into *Solanum tuberosum*. Expression of the desaturase was confirmed by molecular methods. The transgenic plants grew at 22 °C to form two-month plants similar to non-transgenic plants. Lipid analysis revealed that lipid content and the unsaturation level of their fatty acid acyl moieties significantly increased in leaves of transgenic plants as compared to control plants. These results suggest that the expression of *des*A-*lic*BM3 in the membrane of leaf cells could significantly modify both total acyl lipid content and fatty acid composition.

Key words: fatty acid composition, acyl–lipid Δ12-desaturase, *Solanum tuberosum*, transgenic plants

INTRODUCTION

Many plant species are injured or killed by exposure to low non-freezing temperatures in the range of 0–15 °C. These species are classified as chilling-sensitive. They include many economically important crops [1]. Changes in unsaturation level of membrane lipids for fatty acids have been considered to be one of the factors in the metabolic adaptation of higher plants to environmental stresses, especially to temperature [2]. At a physiological temperature, polar glycerolipids that contain only saturated fatty acids cannot form the bilayer, the basic structure of every biological membrane [3]. The introduction of an appropriated number of double bonds into the fatty acid moieties of membrane glycerolipids decreases the temperature for the transition from the gel (solid) to the liquid-crystalline phase and provides membranes with the necessary fluidity [4, 5]. Fluidity of the membrane is important for maintaining the barrier properties of the lipid bilayer and for the activation and functioning of certain membrane-bound enzymes [6, 7]. The ability of cells to modulate the degree of unsaturation in their membrane lipids is mainly determined by the action of fatty acid desaturases [8, 9]. Acyl-lipid desaturases introduce double bonds into fatty acid moieties that have been esterified to glycerolipids, which are located in the endoplasmic reticulum, the chloroplast membrane in plant cells and the thylakoid membrane in cyanobacterial cells. This type desaturase is the most efficient regulator of the unsaturation level of membrane lipids in response to temperature change [8]. *Synechocystis* sp PCC 6803 has four acyl-lipid desaturases which catalyze desaturation at the Δ9-, Δ12-, Δ6-, and ω3-positions, respectively, of the fatty acyl esterified *sn*-1 position of the glycerol moiety of a glycerolipid. The Δ9-desaturase introduces the first double bond into the stearic acid moiety of stearoyl-CoA (or stearoyl-ACP) to produce oleoyl-CoA (or oleoyl-ACP). The Δ12-desaturase catalyzes the second double bond introduction at the Δ12-position of oleic acid [18 : 1 (in position 9)] moiety to produce linoleic acid [18 : 2 (in positions 9 and 12)] moiety [10]. The Δ6-and ω3-desaturases then introduce further double bonds to produce tri- and tetraunsaturated fatty acids, respectively [12].

Here we describe the introduction of the gene for the acyl-lipid Δ12-desaturase of the cyanobacterium *Synechocystis* sp. PCC 6803 to potato genome for testing whether the saturation

* Corresponding author. E-mail: Rezamaali2001@yahoo.com

level of total membrane lipid fatty acids in higher plants thus obtained might be reduced by this gene expression.

In the present work, we have used a new universal reporter system based on thermostable lichinase (LicB) of *Clostridium thermocellum*. The *lic*B gene encodes lichenase (β-1,3:1,4-D-endoglucanase, EC 3.2.1.73). Based on the properties of the lichenase reporter, this reporter may be useful for the monitoring of transgenes in agrocenoses.

MATERIALS AND METHODS

Plasmid construction. We used standard molecular cloning procedures and PCR protocols. Restriction endonucleases, T4 DNA ligase, *Pfu* DNA polymerase, alkaline phosphatase, and nuclease S1 were used according to the manufactures' recommendation (Promega, US and Fermentas, Lithuania).

As the source of the *C. thermocellum lic*BM3 gene, we used plasmid pQE-*lic*BM3-KM2-Mys25 obtained earlier [12]. To design the *lic*BM3 gene with restriction sites *Bam*HI-*Sph*I, the *lic*BM3 gene sequence was amplified using plasmid pQE30-*lic*BM3 as a template and primers (Table 1). Then, *Bam*HI-*Sph*I fragment was cloned in pGEM-Tvector, producing PGEM-Tvector-*lic*BM3.

Gene *des*A was amplified using genomic DNA of *Synechocystis* sp. PCC 6803 as a template and primers (Evrogene, Russia) (Table 1). The resultant PCR fragment was cloned in pGEM-Tvector, producing PGEM-Tvector-*des*A with sites *Xho*I and *Bgl*II. Then the *Bam*HI-*Sph*I fragment of the *lic*BM3 gene was cloned into PGEM-Tvector-*des*A, predigested at sites *Bgl*II-*Sph*I which produced the vector PGEM-Tvector-*des*A-*lic*BM3. Fragment *Sph*I-*lic*BM3-*des*A-*Xho*I was cloned into plasmid pIK, predigested at sites *Xho*I and *Sph*I producing plasmid pIK-*des*A-*lic*BM3. The fragment *Xho*I-*des*A-*lic*BM3-*XbaI* was cloned to the plant vector pBISN1-IN, predigested at sites *SalI* and *XbaI,* which produced the plant vector pBISN1-IN-*des*A-*lic*BM3 (Figure). The control potato plant was transformed with an empty vector pBISN1-IN.

The correctness of the construction of the native *des*A and hybrid *des*A-*lic*BM3 was confirmed by sequencing (Evrogen, Russia).

Plant transformation and transformant regeneration. Leaf disks of *Solanum tuberosum* L., cv. 'Desnitsa', were transformed as described by van Lijsebettens and Valvekens [13]. Regenerated plants were screened for NPTII activity. Individual NPTII-positive plants were selected for each construct and rooted in MS medium without phytohormones with 50 mg/l kanamycin. Wild-type and transgenic plants were cultured on sterile agar-solidified half-strength MS salts containing 0.8% sucrose at 22 °C and illumination of 2500 lx with a 16-h photoperiod.

Table 1. **Nucleotide sequences of specific primers for amplification genes *lic*BM3 and desA by PCR**

No.	Templates	Sequences (5'-3')
1	*Lic*BM3 sense BamHI	GGATCCGTGGTAATACGCCTTTTG
2	*Lic*BM3 anti SphI	GCATGCGTTAGGATAGTATTTTACATATTCG
3	*Des*A sense XhoI	CTCGAGATGACTGCCACGATTCC
4	*Des*A anti BglII	AGATCTTTGAACTTTTTTCAGGGAGCC

Table 2. **Efficiency of the hybrid gene expression based on the thermostable lichenase activity**

Plant lines	*Activity, U/mg total protein*
*Des*A-LicBM3	0.066
*Lic*BM3	0.038
Control, NPTII-positive	0

Preparation of protein extracts and estimation of lichenase activity followed a modified protocol [14].

Fatty acid analysis of the transgenic plants. The fatty acid composition of whole lipids extracted from leaves of transgenic potato plants was determined by gas-liquid chromatography. Lipid extraction and quantification and the estimation of their fatty acid composition were performed as described earlier [15, 16]. We used 17:0 methyl ester of a known concentration as an internal standard. All experiments were performed in six replicates with three recordings each. The results were treated statistically. Table 3 presents the mean values and their standard errors.

RESULTS AND DISCUSSION

The *des*A gene for the acyl-lipid Δ12-desaturase of the cyanobacterium *Synechocystis* sp. PCC 6803 was introduces into potato plants under transcriptional control of cauliflower mosaic virus 35S RNA CaMV promoter by *Agrobacterium*-mediated transformation. A line of potato transformed with the empty vector pBISN1-IN served as a control. Here we describe one transgenic line *Des*A-*Lic*BM3. The expression of *des*A-*Lic*BM3 gene was assessed by a quantitative estimation of lichenase activity. So we used a hybrid gene that contained target gene (*des*A) and the reporter gene (*lic*BM3) in one frame reading (Table 2).

The fatty acid composition of total lipids extracted from leaves was analysed and the enzymatic activity of desaturase was

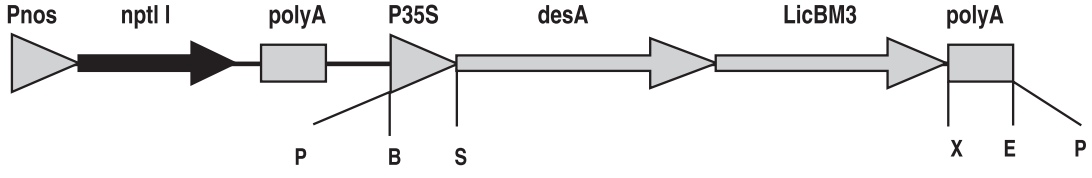

Figure. Scheme of the plant expression vector carrying the hybrid gene. *des*A – the gene for the acyl-lipid Δ12-desaturase, *lic*BM3 – reporter gene for thermostable lichenase, P35S – 35S RNA CaMV promoter, nptII – Kanamycin resistance gene. Restriction sites are shown for *Bam*HI (B), EcoR I (E), Xba I (X), PvuII (P), SalI (S)

Table 3. **Fatty acid composition of total lipids from leaves of control and transgenic potato plants grown at 22 °C**

Fatty acids	FA content, μmol/10g FW*	
	Control	Transformant
14:0	0.5	0.4
13-Me-14 : 0	0.5	0.9
15:0	0.2	0.1
iso-16 : 0	1.2	1.3
16 : 0	14.8	17.4
16 : 1ω7	3.9	5.3
16 : 2ω7	0.4	0.6
16 : 2ω5	0.2	0.1
16 : 3ω3	4	7.5
15-Me-17:0	0.1	0.1
18 : 0	1.3	1.4
18 : 1ω9	0.5	0.9
18 : 1ω7	0.3	0.4
18 : 2	15.8	21.3
19 : 0	0.1	0.2
18 : 3ω3	30.3	42.8
18-Me-19 : 0	0.0	0.7
17-Me-19 : 0	0.0	0.1
20 : 0	0.4	0.7
Mass**	**2.12**	**2.92**
IU***	**1.402**	**2.016**

* The values are in μmol/10 g FW.

** The value of mass is mg/g FW.

*** Unsaturation Index was calculated as 16 : 1*1) + (16 : 2*2) + (16 : 3*3) + + (18 : 1*1) + (18 : 2*2) + (18 : 3*3)/100.

Standard deviation was no more than 0.1%.

investigated by comparing the fatty acid profiles of control and transformed plant leaves (Table 3). One can see that significant differences in fatty acid composition were observed between transgenic and non-transgenic plant lipids. Actually, transformants that express the *des*A gene were characterized by an enhanced growth rate (not shown) and by a higher lipid content as compared with control plants (Table 3). The amount of total esterified fatty acids in transgenic and control plants was 2.92 and 2.12 mg/g FW, respectively. The result showed a 38% higher lipid content in the transgenic plant. The unsaturation index of fatty acid moieties of total lipids was by 44% higher in plants that expressed the hybrid gene. A comparison of fatty acid composition of total lipids of the control plants and transformants with the Δ12-desaturase revealed that the content of unsaturated acids (*viz.* 16 : 1, 16 : 2, 16 : 3, 18 : 1, 18 : 2, 18 : 3) significantly increased. Particularly, the amount of monounsaturated 16 : 1 and diunsaturated 16 : 2 increased by 35% and 16%, respectively and the 16 : 3 amount almost two fold. This fact may be a result of an additional desaturase gene insertion into the higher plant genome, which led to modification of fatty acid metabolism in the transgenic plant.

The content of diunsaturated fatty acid 18 : 2 in the transgenic plant lipids increased by 34.8%. Δ12-desaturase introduced the second unsaturated bond into oleic acid moiety to produce linoleic acid moiety, and this may be considered as the basic well known effect of this gene expression [11, 17, 18, 19]. When the gene encoding Δ12-desaturase from *Synechocyctis* PCC6803 was expressed in a cyanobacterium that normally contains monounsaturated fatty acids (*Synechococcus* PCC7942), the membrane lipids of this organism became enriched with up to 25% polyunsaturated fatty acids [20, 21]. This large increase in membrane unsaturation was shown to reduce the low-temperature damage to the photosynthetic machinery. Expression of this gene in yeast cells resulted in the accumulation of 16 : 2 and 18 : 2 acids [22].

In the transgenic potato leaf lipids, linolenic acid (18 : 3) content increased by 41.3% as compared with control ones. These results suggest that the expression of *des*A in leaf cells could significantly modify the total fatty acid composition of membrane lipids.

The high unsaturation level of membrane lipid fatty acid moieties is considered to be a key factor of the adaptation to low temperatures in higher plants. This parameter is implicated by the functionality of biomembranes at chilling temperatures, as it is the primary determinant of the phase transition point of membrane lipids [23]. In consistence with this phenomenon, now we are examining the chilling tolerance of transgenic potato plants. If insertion of an additional desaturase gene (*des*A gene) into the genome of a higher plant (potato) results in an increase of its cold tolerance, this approach may be considered as a novel method of production of transgenic plants with enhanced cold resistance. On the other hand, as plant lipids have a high variety of industrial and nutritional uses, the modification of fatty acid composition of these lipids through genetic manipulation may contribute to the improvement of oil quality [24].

In general, this work demonstrates that thermophilic acyl-lipid Δ12-desaturase is active in transgenic potato and increases the content of linoleic acid, thus producing a substrate for the further synthesis of trienoic FAs.

This work was supported by RFBR grants No. 05-04-49186 and No. 06-04-81009.

Received 15 November 2006
Accepted 14 January 2007

References

1. Ishizaki-Nishizawa O, Fujii T, Azuma M, Sekiguchi K, Murata N, Ohtani T, Toguri T. Nature Biotechnology 1996; 14: 1003–6.
2. Somerville C, Browse J. Science 1991; 252: 80–7.
3. Stubbs CD, Smith AD. Biochim Biophys Acta 1984; 779: 89–137.
4. Russel NJ. Trends Biochem Sci 1984; 9: 108–12.
5. Hazel JR. Ann Rev Physiol 1995; 57: 19–42.
6. Houslay MD, Gordon LM. Curr Top Membr Transp 1984; 18: 179–231.
7. Thompson JA. J Bioenerg Biomembr 1989; 21: 43–60.
8. Murata N, Wada H. Biochem J 1995; 308: 1–8.
9. Los DA, Murata N. Biochim Biophys Acta 1998; 1394: 3–15.
10. Panpoom S, Los DA, Murata N. Biochimica et Biophysica Acta 1998; 1390: 323–32.
11. Los DA, Murata N. J Mol Microbiol Biotechnol 1999; 1(2): 221–30.

12. Мусычук КА, Голденкова ИВ, Абдеев РМ и др. Биохимия 2000; 65(12): 1659–65.
13. Van Lijsebettens M, Valvekense D. EMBO Practical Course on Plant Molecular Biology. Belgium, Gent, 1987: 15–8.
14. Movsesyan NR, Alizadeh KH, Musiychuk KA et al. Rus J Genet 2001; 37(6): 610–6.
15. Багдасарьян СГ, Кораблёва НП, Тсыдендамбаев ВД. Прикладная биохимия и микробиология 1983; 19(4): 459–65.
16. Tsydendambaev VD, Christie WW, Brechany EY, Vereshchagin AG. Phytochemistry 2004; 65: 2695–703.
17. Wada H, Avelange-Macherel M, Murata N. J Bacteriology 1993; 175(18): 6056–8.
18. Murata N, Wada H, Gombos Z. Plant Cell Physiol 1992; 33: 933–41.
19. Higashi S, Murata N. Plant Physiol 1993; 102: 1275–8.
20. Wada H, Gombos Z, Murata N. Nature 1990; 347: 200–3.
21. Wada H, Gombos Z, Murata N. Proc Natl Acad Sci 1994; 91: 4273–7.
22. Peyou-Ndi MM, Watts JL, Browse J. Archives of Biochemistry and Biophysics 2000; 376(2): 339–408.
23. Nishida I, Murata N. Annu Rev Plant Mol Biol 1996; 47: 541–68.
24. Shen JB, Kinney AJ, Hitz WD. Plant Biotechnology and *in vitro* Biology in the 21st Century. Dordrecht: Kluwer, 1999: 605–8.