Defence Science Journal, Vol. 59, No. 2, March 2009, pp. 147-151

SHORT COMMUNICATION

Isolation of cDNA fragment of Glycerol-3-Phosphate Acyltransferase Gene from Seabuckthorn

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ABSTRACT

The contribution of membrane lipids, particularly the level of unsaturation of fatty acids, to chilling sensitivity of plants, has been intensively discussed. The biosynthesis of phosphatidyl glycerol represents a central pathway in lipid metabolism in all organisms. Glycerol-3-phosphate acyltransferase (GPAT) catalyses the first step of glycerolipid biosynthesis and, therefore, it is a potential site for triacylglycerol synthesis regulation. The cDNA for GPAT gene has been cloned and extensively characterised from several plants excluding Seabuckthorn *Hippophae rhamnoides*. The isolation of partial cDNA (689 bp, Accession No. EU081817) for GPAT gene from Seabuckthorn plant has been reported for the first time that shows 97 per cent homology with the *Lycopersicon esculentum* at nucleotide level and 93 per cent homology with the *Capsicum annuum* at protein level. Full-length cloning and overexpression of GPAT from Seabuckthorn will modify the ability of vegetable crops to tolerate chilling temperature by protecting the photo synthetic machinery from photoinhibition under cold conditions.

Keywords: Seabuckthorn, *Hippophae rhamnoides*, glycerol-3-phosphate acyltransferase (GPAT), cold tolerance; elaeagnaceae, biosynthesis of phosphatidylglycerol

Plants being sessile, their growth and yield are strongly influenced by abiotic stress such as drought, high salt content, and temperature change. Environmental stress presents a major challenge in our quest for sustainable food production as it reduces the potential yields as high as 70 per cent in crop plants[1]. Water stress imparted by drought and temperature severity is the most prevalent abiotic stress that limits plant growth and productivity. Plants respond and adapt to these conditions with an array of biochemical and physiological alterations. Multiple signaling pathways regulate the stress responses of plant[2] and there exists an overlap between the patterns of expression of genes that are induced in response to different stress factors[3,4].

When plants are exposed to freezing temperatures, ice crystals are formed within the extracellular spaces, which causes freeze-induced cell dehydration and formation of several different lesions in the plasma membrane, most often near the chloroplast envelope in the cell. These lesions are the result of lamellar-to-hexagonal phase II transitions in the molecular structure of the plasma membrane, which disrupts both the physical continuity and semipermeable characteristics of the plasma membrane such that the cell becomes leaky and flaccid, frequently results in loss of crops. GPAT gene encoded a protein that increases the unsaturation of fatty acids present in the plasma membrane that gives resistance during cold-induced membrane injury. Chilling sensitivity of plants can be manipulated by modulating levels of unsaturation of fatty acids of membrane lipids by the actions of acyl-lipid desaturase and glycerol-3-phosphate acyltransferase[5].

The biosynthesis of phosphatidylglycerol represents a central pathway in lipid metabolism in all organisms[6]. Glycerol-3-phosphate acyltransferase (GPAT) catalyses the transfer of an acyl group from an acyl donor to the sn-1 position of glycerol 3-phosphate during *de novo* synthesis of triacylglycerol[7], and therefore, it is a potential site for triacylglycerol synthesis regulation[8]. The plant cell contains three types of GPAT, which are located in the chloroplasts, mitochondria and cytoplasm. The enzyme in chloroplasts is soluble and uses acyl-(acyl-carrier protein) as the acyl donor, whereas the enzymes in the mitochondria and the cytoplasm are bound to membranes and use acyl-CoA as the acyl donor. The cDNAs for GPAT of chloroplasts have been cloned from several plants[9-13]. The amino acid sequences deduced from the nucleotide sequences of cDNAs indicate that the product of translation is a precursor of about 460 amino acid residues, which consists of a leader sequence of about 70 amino acid residues and a mature protein of about 400 residues, with a molecular mass of about 42 kDa. Genetic engineering of the unsaturation of fatty acids has been achieved by manipulation of the cDNA for the GPAT found in chloroplasts and has allowed modification of the ability of tobacco to tolerate chilling temperatures[14,15]. It proves that unsaturation of fatty acids of phosphatidylglycerol in thylakoid membranes stabilises

Received 8 January 2008

the photosynthetic machinery against low-temperature photoinhibition by accelerating the recovery of the photosystem II protein complex[16].

There are several species, which grow widely in cold Himalayan region such as Seabuckthorn, Ephedra, *Cornus sericea* that can survive even at –196 °C[17] and it is believed that investigations on one of the most freeze tolerant organisms will help us to identify genes that facilitate survival of many horticultural crops at low temperatures. To accomplish this objective, Seabuckthorn has been selected as a model plant for isolating GPAT gene. Seabuckthorn is a hardy, deciduous shrub of the mountainous region of China and Russia with wide but fragmented distribution in Eurasia between 27 °S and 69 °N latitude and 7 °W and 122 °E longitude. The plant grows naturally in sandy soils at an altitude of 1200 m-4500 m in cold climates. It can withstand temperatures from –43 °C to +40 °C and is considered to be drought-resistant[18,19]. It is an ideal plant for control of soil erosion, land reclamation, wildlife habitat enhancement, and farm stand protection[20]. The Seabuckthorn is highly stress-resistant as it contains vitamins C, E, beta-carotene, phytosterols, unsaturated fatty acids and flavonoids and it also serves as an antioxidant that slows the ageing process and improves memory.

The cDNA for GPAT has been cloned from several plants. However, there are no reports of this gene cloned from Seabuckthorn plant. For the first time, isolation of GPAT gene from Seabuckthorn plant has been reported. In this attempt, samples of Seabuckthorn plants were collected after giving cold stress (4 °C for 12 h) for isolating GPAT gene. Total RNA was isolated from these leave samples[21]. RT-PCR of RNA isolated from Seabuckthorn leaves resulted in the amplification of the partial cDNA of GPAT (Fig. 1) gene using degenerate primers, designed from the conserved regions of the known GPAT gene sequences present in the database. This partial cDNA of GPAT gene was cloned at *EcoRV* site of PBSSK+ vector and sequenced. Sequence analysis with BLAST (X) program of NCBI revealed that, GPAT (689 bp, Accession No. EU081817) shows 97per cent homology with the *Lycopersicon esculentum* at nucleotide level and 93 % homology with the *Capsicum annuum* at protein level. Protein sequence of partial GPAT gene of Seabuckthorn was multiple aligned with other reported GPAT proteins sequences present in the database to identify most closely related GPAT with Seabuckthorn (Fig. 2). On the basis of the partial cDNA sequence, few gene-specific primers (GSPs) were designed for 5′ RACE (rapid amplification of cDNA ends) to get the remaining 5′cDNA sequence of GPAT gene. The full-length cDNA sequence of GPAT gene will be used for cloning in suitable plant transformation vector for genetic transformation of vegetable crops. Study of the mRNA accumulation of GPAT in Seabuckthorn induced by chilling temperature was carried out by northern blot analysis. RNA isolated from 0 h, 6 h, 12 h and 24 h cold-stressed leaves samples of Seabuckthorn were used for preparation of northern blots. Northern blot was hybridised with probe prepared from cDNA fragment of GPAT as template by random primer method using á-^{32}PdCTP as the radiolabel[22]. As shown in Fig. 3, transcript of GPAT gene was highly induced by chilling temperature, suggesting its potential role in cold tolerance. These aspects are presently under study.

Glycerolipids form bilayers and provide the necessary background for the functioning of membrane proteins. The physical properties of glycerolipids depends on the degree of unsaturation of the fatty acids that are esterified to the glycerol backbone of the lipids, and consequently, the molecular motion of these glycerolipids is affected by alterations in the extent of unsaturation of fatty acids. Therefore, it is postulated that changes in the unsaturation of fatty acids should affect various functions of the membrane-bound proteins, such as the photochemical and electron-transport reactions in thylakoid membranes, and the import

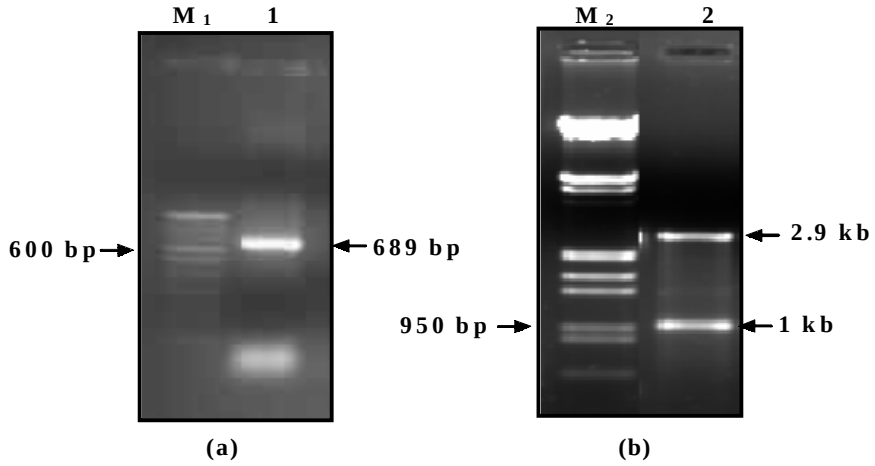

Figure 1. (a) RT-PCR amplification and (b) cloning of partial GPAT gene from Seabuckthorn leaf tissue. Lane M_1-100 bp DNA ladder. Lane M_2- *Lambda* DNA Marker (double digested with *EcoRV* / *HindIII*). Lane1- RT-PCR Amplicon (689 bp, Accession No. EU081817) of GPAT gene. Lane2- Cloned PBSSK+ Vector digested with Pvu-II restriction enzyme.

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AY318749 Capsicum          ----MLILSAASSSARISRPPLSSFSTFAASAATTSRLFPISCFGVKSTT
AY360170 Lycopersicon      -----MLSSALSSSARIPRP----------SSAATSTLFPISCFGVKSRT
EU081817 Seabuckthorn      --------------------------------------------------
AF090734 Vicia faba        ---------MTDSFAHYASHINIRPKTKTMLIFSTPCCSPSTAFFSPFRA
AB076608 Citrus            MSSLSLTFFATTAPRVLAPSSSSNPKLSPSSYSFSAITARRHSTAVSFRS
NM179407 Arabidopsis       --------MTLTFSSSAATVAVAAATVTSSARVPVYPLASSTLRGLVSFR

AY318749 Capsicum          VGNRKLQCAVFCASLKVRGMAEMIEDNKELNSSTAAAAAAIAVTASENDE
AY360170 Lycopersicon      VGIRKLRCAVFCASKVRG--------------MAEMIEDAMTVSASESHE
EU081817 Seabuckthorn      --------------------------------------------------
AF090734 Vicia faba        SNCKPLRSSTLCLRSLTSSATSITSTSNSSLAFNIVKPKEKNVVSANMTS
AB076608 Citrus            GARRTSATCPCASFSSFNVRAMAKMVQDRESAVSSSSASDEQNKKMLNIE
NM179407 Arabidopsis       LTAKKLFLPPLRSRGGVSVRAMSELVQDKESSVAASIAFNEAAG-ETPSE

AY318749 Capsicum          LPHSRAFLDARTGEDLLSAVRKAVEDKKLPLNIAEGMEELYQNYRNAVLQ
AY360170 Lycopersicon      LPQSRDFLDARTGEDLLSAVRKAVEDEKLPLNVAEGMEELYQNYQNAVLQ
EU081817 Seabuckthorn      --------------------------------------------------
AF090734 Vicia faba        SVSSRTFLNAQNEQDVLSGIKKEVEAGTLPASIAAGMQEVYLNYKSAVIK
AB076608 Citrus            VNHSRTFLDVRSEQDLLSGIGREVEAGRLPSNLANGMEELYHNYKNAVFQ
NM179407 Arabidopsis       LSHSRTFLDARSEQDLLSGIKKEAEAGRLPANVAAGMEELYWNYKNAVLS

AY318749 Capsicum          SGVPKADEIILYNMALVLDRVFVDVKDPFEFSPYHKAIREPFDYYKFGQN
AY360170 Lycopersicon      SGVPKADEAILYNMALVFDRVFVDVKDPFEFSPYHKAIREPFDYYKFGQN
EU081817 Seabuckthorn      --------------------------------------------------
AF090734 Vicia faba        SGDPKANEIVLSNMTALLDRIFLDVKEPFVFEAHHKAKRGPFDYYMFGQN
AB076608 Citrus            SGNSRADEIVLSNMAVAFDRVLLDIEEPFTFSSYHKSMREPFDYYMFGQN
NM179407 Arabidopsis       SGASRADETVVSNMSVAFDRMLLGVEDPYTFNPYHKAVREPFDYYMFVHT

AY318749 Capsicum          YIRQLVDFRSSYVGNISVFGEMEEKLKQGDNVVLMSNHQSEADPAIIALL
AY360170 Lycopersicon      YIRQLVDFRSSYVGNMSVFSEMAEKLKQGDNVVLMSNHQSEADPAIIALL
EU081817 Seabuckthorn      --------------------------------------------------
AF090734 Vicia faba        YIRPLVDFETSYVGNMPLFIQMEEQLKQGHNIILMSNHQSEADPAIIALL
AB076608 Citrus            YIRPLVDFRSSYVGNVSLFFEMEEKLNQGHNIVLISNHQTEADPAIIALL
NM179407 Arabidopsis       YIRPLIDFKNSYVGNASIFSELEDKIRQGHNIVLISNHQSEADPAVISLL

AY318749 Capsicum          LELKHPDIAENIIYVAGDRVITDPLCKPFSMGRNLLCVYSKKHMNDDPEL
AY360170 Lycopersicon      IESKLPDIAENIIYVAGDRVITDPLCKPFSMGRNLLCVYSKKHMNDDPEL
EU081817 Seabuckthorn      ----------------------------MGRNLLCVYSKKHMNDDPEL
AF090734 Vicia faba        LEMQLPHIAENLIYVAGDRVITDPLCKPFSIGRNLICVYSKKHMLDNPEL
AB076608 Citrus            LESTNPHVAENLTYIAGDRVITDPLCKPFSMGRNLICVYSKKHMLDVPEL
NM179407 Arabidopsis       LEAQSPFIGENIKCVAGDRVITDPLCKPFSMGRNLICVYSKKHMNDDPEL
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AY318749 Capsicum          ADMKKRANTRSLKEMAMLLRGGSKLIWIAPSGGRDRPDPVTKEWSPAPFD
AY360170 Lycopersicon      AEMKKRANTRSLKEMALLLRGGSKIIWIAPSGGRDRPDPVTKEWYPAPFD
EU081817 Seabuckthorn      AEMKKRANTRSLKEMALLLRGGSKIIWIAPSGGRDRPDPVTKEWYPAPFD
AF090734 Vicia faba        IDMKRKANTRSLKEMATLLRSGSQIIWIAPSGGRDRPVANSGEWAPAPFD
AB076608 Citrus            IEMKRKSNTRSLKEMALLLRGGSQIIWIAPSGGRDRPDPVTGEWYPAPFD
NM179407 Arabidopsis       VDMKRKANTRSLKEMATMLRSGGQLIWIAPSGGRDRPNPSTGEWFPAPFD
                            :**:::********* :**.*.::************ . : ** *****
AY318749 Capsicum          ASATDNMRRLVEHAGVPGHIYPLAILCYDIMPPPAQVEKNIGEKRVVSFH
AY360170 Lycopersicon      ASATDNMRRLVQHAGVPGHIYPLAILCHDIMPPPAQVEKNIGEKRVVSFH
EU081817 Seabuckthorn      ASATDNMRRLVQHAGVPGHIYPLAILCHDIMPPPAQVEKNIGEKRVVSFH
AF090734 Vicia faba        SSSMDNMRRLVDHSGPPGHIYPLAILCHDIMPPPLKVEKEIGEKRIISYH
AB076608 Citrus            TSSIDNMRRLAEHSGIPGHIYPLALLCHDIMPPPPQVEREVGEKRVISFH
NM179407 Arabidopsis       ASSVDNMRRLVEHSGAPGHIYPMSLLCYDIMPPPPQVEKEIGEKRLVGFH
                           :*: ******.:*:* ******:::**:****** :**:::****::.:*
AY318749 Capsicum          GAGVSVAPKIDFREVAGTLEDPE-AKMVYTKALYDSVSQQYNVLNSAIHG
AY360170 Lycopersicon      GAGISVAPKIDFHEVAGALEDPE-AKMVYTKAIYDSVSQQYNVLNSAIHG
EU081817 Seabuckthorn      GAGISVAPKIDFHEVAGALEDPE-AKMVYTKAIYDSVSQQYNVLNSAIHG
AF090734 Vicia faba        GTGISTAPEISFSSTTAACENPETAKDAYTKALYDSVTEQYDVLKSAIHG
AB076608 Citrus            GAGLSVAPEISFADIITASKNPEEAKEVYTQAFYNSVTEQYNVLKSAIHG
NM179407 Arabidopsis       GTGLSIAPEINFSDVTADCESPNEAKEAYSQALYKSVNEQYEILNSAIKH
                           *:*:* **:*.* .     :.*: ** .*::*:*.**.:**::*:***:
AY318749 Capsicum          KQGLEASIPSVSLSQPWQ-
AY360170 Lycopersicon      KQGLEASIPSVSLSQPWQ-
EU081817 Seabuckthorn      KQGLEASTPSVSLSQPWQ-
AF090734 Vicia faba        KKGLQASTPVVSLSQPWK-
AB076608 Citrus            KQGLKASIPSVSLSQPWGD
NM179407 Arabidopsis       RRGVEASTSRVSLSQPWN-
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Figure 2. CLUSTAL multiple alignment of the deduced amino acid sequences of GPAT gene.

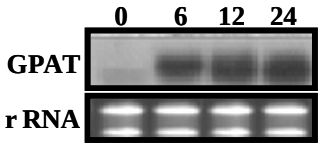

Figure 3. Northern blot analysis of cDNA fragment of GPAT gene isolated from Seabuckthorn plant. 30 μg total RNA isolated from 0, 6, 12 and 24 h cold stressed leaves samples of Seabuckthorn were used for preparation of northern blot. The gene used for probing is shown on the left of the picture.

and export of metabolites and proteins across the plasma membrane. However, the contribution of the unsaturation of fatty acids to cold tolerance has not been obvious, since acclimatisation to low temperature induces not only desaturation of fatty acids of membrane lipids but also a number of other metabolic modifications. To determine whether the unsaturation of fatty acids contributes to the ability to tolerate low temperatures, it is necessary to alter the extent of unsaturation of fatty acids of glycerolipids exclusively by manipulation of genes for fatty-acids desaturase, thereby minimising effects on any other metabolic processes[23].

Vegetable crop transformed with GPAT gene will involve one time investment and help arrangement of the availability of nutrient-rich fresh food even during the winter months through local production when it is not possible to get fresh vegetables due to road breaches. In addition, genetic engineering of the unsaturation of fatty acids achieved by manipulation of the cDNA for the GPAT will modify the ability of vegetable crops to tolerate chilling temperatures by protecting the photosynthetic machinery from photoinhibition under cold conditions. This study is likely to provide newer biotechnological approaches to prevent the damage to crops due to unexpected / expected cold snaps.

ACKNOWLEDGEMENT

The Authors wish to thank DRDO, Ministry of Defence, Government of India for financial support for this project work.

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