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Analysis of Expressed Genes in Ethylene-Treated Potato Leaves Using Expressed Sequence Tags

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To isolate useful and interesting plant genes in large quantities, random sequencing of cDNA clones from potato leaf library treated with ethylene was performed. Partial sequences of randomly selected 210 clones with the insert of longer than 500 base pair (bp) as well as poly (A) tail have been compared with sequences in GeneBank, EMBL and DDBJ nucleic acid databases and fostered 193 expressed sequence tags (ESTs). The 210 cDNA clones identified are related to various aspect of metabolic pathways such as glycolysis, amino acid synthesis, translation mechanism, ribosome synthesis, hormone response, stress response, regulation of gene expression, and signal transduction. Among the 193 ESTs, 12 ESTs (29 cDNA clones) appeared more than once and 181 ESTs appeared once regarded as a solitary group. Out of 210 clones, 29 clones (13.8%) have no similarity to the known nucleotide sequences and could serve as a potentially useful resource for plant molecular biology referring to particular genes. Nucleotide sequencing to generate more ESTs from ethylene-induced as well as non-induced potato leaf is in progress as well.

Keywords: cDNA library, ethephone, EST, *Solanum tuberosum* L.

Ethylene is an important plant growth regulator, influcing a variety of plant responses that include germination, leaf abscission, and fruit ripening, as well as responses to environmental stresses such as wounding, chilling, and pathogene infection. As the unique plant-producing hormone, ethylene induces many characteristic molecular changes in plant resistance response such as induction of PR proteins (Bol *et al.*, 1990), cell wall reinforcement (Ke and Saltveit, 1988; Esquerre-Tugaye *et al.*, 1979), local systemic resistance (Enyedi *et al.*, 1992), which prompt us to categorize the expression patterns of ethylene-specific genes.

The human and plant genes expressed during lifetime of the organisms were estimated to consist of 50,000 to 100,000 genes and 15,000 to 60,000 genes, respectively (Kim *et al.*, 1995; Flavell, 1980). However, accumulation of sequence information with respect to their structure and biological function has been deterred in plant compared with other organisms and only a few hundreds of which have been characterized until today (Aliyeva *et al.*, 1996; Hofte *et al.*, 1993). Systematic cDNA sequencing projects are currently under way for several organisms and detailed analyses of expressed sequence tags (ESTs) have been published for more than 3,000 human brain cDNA (Adams *et al.*, 1992), for *Caenorhabditis elegans* (McCombie *et al.*, 1992; Waterson *et al.*, 1992), for mouse (Hoog, 1991), for yeast (Oliver *et al.*, 1992) and for *Arabidopsis thaliana* (Hofte *et al.*, 1993). Several approaches have been adopted to isolate novel genes and the partial sequencing of annonymous cDNAs which produce EST turns to be a complementary, but extremely rapid way to identify genes through comparison with sequence database (Wei *et al.*, 1996; Yan *et al.*, 1996). Partial sequencing of cDNAs can be also applied to locate the interesting genes along the chromosomes which results in mapping large DNA fragments. ESTs can be assigned on chromosomes and applied to isolate new genes.

We have chosen the potato (*Solanum tuberosum* L.) as the experimental material, because classical genetics have developed numerous morphological as well as physiological markers and high density molecular linkage map have been constructed (Tanksley *et al.*, 1992).

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MATERIALS AND METHODS

Ethylene Treatment

Young leaves of potato (*Solanum tuberosum* L. cv. Sumi) were treated with ethylene to extract total RNA for construction of cDNA library. Ethylene treatment was performed using ethephone (Ethrel, Amchem Products, Inc.; 21.6% 2-chloroethyl phosphonic acid) which decomposes to release ethylene. The ethephon was applied as aerosols to 6 week-old leaves for 16 hours under the continuous dark condition. RNA extraction and northern blot analysis were conducted according to procedure of Sambrook *et al.* (1989).

Construction of a cDNA Library

Poly $(A)^+$ RNA was isolated from total RNA using oligo-dT cellulose affinity chromatography (Sambrook *et al.*, 1989). The cDNA library was constructed using the cDNA synthesis kit (Promega, Madison, WI) according to the manufacturer's manual. After treatment with T_4 DNA polymerase, the blunt-ended cDNAs were further phosphorylated using T_4 polynucleotide kinase and cloned into *Sma*I-digested pUC18 vector. *Escherichia coli* strain JM 109 was transformed by electrophoration and used to propagate recombinant plasmid.

DNA Sequencing and Database Search

DNA sequencing was carried out by the dideoxynucleotide chain termination method (Sanger *et al.*, 1977) using sequenase (version 2.0, USB, OH, USA) and [α-^{35}S]dATP (Amersham). Sequence similarities between cDNA clones and database(GenBank, EMBL, DDBJ) entries, were analyzed using the Blast program through the National Center for Biotechnology Information (NCBI, MD, USA).

RESULTS AND DISCUSSION

Construction of the cDNA Library and Sequencing

The cDNA library used in this study consisted of a total of 5×10^5 independent recombinant clones. Among the white colonies, 500 clones were randomly selected and tested for cDNA insert. Ten clones contained no inserts, 280 clones contained inserts in between 200 and 500, and 210 clones, those of which were further analyzed for DNA sequencing, contained inserts larger than 500 base pairs. Out of 210 clones, 29 (13.8%) appeared more than once (redundant group) grouped into 12 different ESTs, and 181 clones including those of which have no similarity to the known nucleotide sequences appeared only once with the consequence of 181 ESTs (Table 1). This frequency (13.8%) of redundancy was considerably lower than 31% and 26.7% of redundant clones in the other plant and fungus, *Arabidopsis thaliana* (Hofte *et al.*, 1993) and *Aspergillus nidulans* (Lee *et al.*, 1996), respectively. However, this was somewhat higher than 6.6% in human fetal liver (Kim *et al.*, 1995). This could be due to the small sample size or indicate the difference in the variety of expressed genes between induced and non-induced plant tissue. Among the redundant group, two species appeared four times, one species three times and nine species twice. The frequence of the most abundant cDNA was 1.9%, similar to the 2.2% of large scale cDNA sequencing for analysis of quantitative and qualitative aspects of gene expression (Okubo *et al.*, 1992). After sequencing of a whole EST species, a total of 29.4 kbp was analyzed and an average length of EST specie was 140bp.

Table 1. Summary of cDNA sequence analysis

	Number of cDNA clones	Percentage	EST species
Total	210	100	193
redundant group	29	13.8	12
4 times	8	3.8	2
3 times	3	1.4	1
2 times	18	8.6	9
solitary group	181	86.2	181
no matched clones	29	13.8	29

Sequence Analysis and Database Comparison

Out of 210 clones (Table 2), 27 clones (12.9%) appeared to be potato entries, 57 clones (27.1%) to other plant genes and 97 clones (46.2%) to non-plant genes. Out of 27 clones showing the homology to potato entries, 26 clones were ascribed to as identified clones based on the facts the probability was below 10^{-5} (Altschul *et al.*, 1990). In addtion, among the 57 clones showing the homology to other plant genes, 22 clones were identified to the known plant genes. Only six clones out of 97 clones from non-plant showed significant homology to the other genes. These genes were encoding minisatellite sequence of human, Fc-gamma-receptorIIB of human,

Table 2. Summaries of cDNA clones of ethylene-treated potato leaves in nucleotide match with known genes

Clone	Putative identification	Species	DB*	Nucleotide similarity (%)	Probability	Sources of comparison
cDNAs similarity to potato genes						
PMYPE009	trypsin inhibitor	*S.tuberosum*	gb	99/103 (96)	2.6e-26	M22145
PMYPE020	glycine hydroxymethyltransferase	*S.tuberosum*	emb	63/74 (85)	7.9e-15	Z25863
PMYPE049	wound-induced genes	*S.tuberosum*	emb	144/145 (99)	1.1e-55	X13497
PMYPE069	ribulose-(1,5)-biphosphate carboxylase	*S.tuberosum*	emb	118/133 (88)	1.1e-39	X69759
PMYPE072	amino cyclopropane carboxylate acid synthase	*S.tuberosum*	emb	93/122 (76)	1.2e-21	Z27233
PMYPE078	Potato patatin pseudogene (SB6B)	*S.tuberosum*	emb	45/74 (60)	1.0	X04077
PMYPE084	pathogenesis-related protein gene.	*S.tuberosum*	gb	128/132 (96)	1.9e-44	J03679
PMYPE087	ubiquitin	*S.tuberosum*	emb	182/187 (97)	4.4e-69	Z11673
PMYPE093	proteinase inhibitor	*S.tuberosum*	gb	109/116 (93)	2.23e-61	L06137
PMYPE100	endo-1,3-beta-D-glucanase	*S.tuberosum*	gb	95/100 (95)	1.5e-30	U01900
PMYPE101	endochitinase (EC3.2.1.14)	*S.tuberosum*	emb	58/70 (82)	6.2e-24	X07130
PMYPE108	alpha-glucan phosphorylase	*S.tuberosum*	dbj	99/126 (78)	3.8e-26	D00520
PMYPE128	light-inducible tissue-specific	*S.tuberosum*	gb	57/59 (96)	1.2e-15	X04753
PMYPE135	stolen tip protein	*S.tuberosum*	emb	39/43 (90)	6.5e-15	Z11680
PMYPE180	cathepsin D inhibitor protein	*S.tuberosum*	gb	124/132 (93)	1.1e-41	M96257
PMYPE200	endo-1,3-beta-D-glucanase	*S.tuberosum*	gb	143/148 (96)	2.1e-50	M01900
PMYPE196	alpha-glucan phosphorylase	*S.tuberosum*	dbj	165/166 (99)	4.6e-71	D00520
cDNAs similarity to other plant genes						
PMYPE001	mRNA for endochitinase (EC 3.2.1.14)	*N. tabacum*	emb	192/217 (88)	27e-61	X16939
PMYPE032	RNA-binding glycine-rich protein.	*N. tabacum*	dbj	38/42 (90)	7.6e-11	D16205
PMYPE040	GUT 8-2A cDN clone p1226.	*N. tabacum*	gb	65/81 (80)	3.9e-40	T18332
PMYPE067	thaumatin- like protein	*N. tabacum*	emb	29/31 (93)	0.069	X15224
PMYPE104	retroviral-like transposon Tnt 1-94	*N. tabacum*	emb	30/32 (93)	0.011	X13777
PMYPE116	chloroplast atpase gene	*N. tabacum*	gb	117/118 (99)	5.7e-41	K00507
PMYPE155	cystein-rich extensin-like protein-4	*N. tabacum*	gb	50/61 (81)	1.1e-08	L13442
PMYPE138	glyceraldehyde- 3-phosphophate dehy-drogenase	*N. tabacum*	gb	63/94 (67)	3.4e-07	M14419
PMYPE022	DNA sequence, complete transposon.	*A. thaliana*	gb	44/69 (63)	0.75	L12220
PMYPE034	transcribed sequence; clone VBVAA07.	*A. thaliana*	emb	44/71 (61)	1.0	Z30733
PMYPE077	transcribed sequence; clone YAP043T.	*A. thaliana*	dbj	38/49 (77)	0.0061	Z17609
PMYPE096	transciribed sequence; clone GBGe280.	*A. thaliana*	gb	43/53 (81)	2.0e-05	Z34064
PMYPE099	calcium-dependent protein kinase.	*A. thaliana*	emb	36/52 (69)	0.99	D28582
PMYPE131	ATAF1 mRNA	*A. thaliana*	emb	31/36 (86)	0.045	X74755
PMYPE133	ribulose bisphosphate carboxylase	*A. thaliana*	gb	32/44 (72)	1.0	M86720
PMYPE177	transcribed sequence; clone FAI214.	*A. thaliana*	gb	25/31 (80)	0.95	Z34946
PMYPE179	HAT22 homebox protein gene,	*A. thaliana*	gb	25/29 (86)	1.0	U09337
PMYPE004	mRNA Transcribed Sequence 1087.	*Z. mays*	gb	31/42 (73)	1.0	X78029
PMYPE046	mitDNA for tRNA-Trp, tRNA-Pro and ORF1	*Z. mays*	emb	33/48 (68)	1.0	X13704
PMYPE199	ribosomal protein S2	*Z. mays*	emb	45/71 (63)	0.77	X17318
PMYPE007	25S ribosomal RNA	*L. esculentum*	emb	37/39 (94)	1.0e-05	X13557
PMYPE052	TAP1 gene for anionic peroxidase	*L. esculentum*	dbj	56/82 (68)	2.7e-05	X15853
PMYPE073	Ca^{2+} ATPase gene, complete cds.	*L. esculentum*	gb	39/49 (79)	0.0021	M96324
PMYPE105	wound-repressed gene, partial cds	*L. esculentum*	gb	54/69 (78)	1.6e-10	X59883
PMYPE136	25S ribosomal RNA gene	*L. esculentum*	emb	85/85 (100)	5.8e-28	X13557
PMYPE137	25S ribosomal RNA gene	*L. esculentum*	gb	67/69 (97)	8.3e-20	X13557
PMYPE156	mRNA for cell wall protein.	*L. esculentum*	emb	39/42 (92)	3.4e31	X77373
PMYPE157	cyclophilin (CyP) mRNA.	*L. esculentum*	gb	79/116 (68)	5.8e-12	M55109
PMYPE159	1-aminocyclpropane-1-carboxylic acid synthase	*L. esculentum*	emb	30/40 (75)	1.0	X59139
PMYPE163	superoxide dismutase	*L. esculentum*	gb	62/78 (79)	2.2e-24	M37150
PMYPE188	TAS14 mRNA inducible by abscisic acid and environmental stress	*L. esculentum*	emb	23/24 (95)	0.00095	X51904
PMYPE008	cDNA, partial sequence (C1101_12).	*Oryza sativa*	dbj	70/92 (76)	1.9e-14	D22731
PMYPE010	cDNA, partial sequence (C3084_1A).	*Oryza sativa*	dbj	24/25 (96)	0.75	D23608
PMYPE064	cDNA, partial sequence (C0583A).	*Oryza sativa*	dbj	48/73 (65)	0.023	D15401
PMYPE075	putative polyprotein.	*Oryza sativa*	dbj	29/37 (78)	1.0	D32136

Table 2. Continue.

Clone	Putative identification	Species	DB*	Nucleotide similarity (%)	Probability	Sources of comparison
PMYPE031	lectin (Blec4) gene, complete cds.	*P. sativum*	gb	40/56 (71)	0.094	L11745
PMYPE050	retrotransposon	*P. sativum*	emb	28/34 (82)	1.0	X66399
PMYPE178	legumin beta-polypeptide	*P. sativum*	gb	42/66 (63)	0.99	M1689
PMYPE019	26S ribosomal RNA gene 3' region	*C. limon*	emb	102/110 (92)	4.1e-33	X03641
PMYPE024	right telomeric region and ATP6 gene	*C. parapsilosos*	emb	33/43 (76)	0.47	X76197
PMYPE038	26S rRNA	*M. sativa*	emb	106/108 (98)	7.9e-58	Z11498
PMYPE051	tRNA-Val. 15S rRNA and 21S rRNA	*W. saturnus*	emb	38/55 (69)	0.0059	X71392
PMYPE057	dihydroflavonol-4-reductase	*P. hybrida*	emb	34/48 (70)	1.0	X15537
PMYPE085	mRNA for cinnamate 4-hydroxylase	*C. roseus*	emb	57/68 (83)	2.0e-28	Z32563
PMYPE091	chalcone synthetase protein	*G. max*	emb	49/79 (62)	0.48	X53958
PMYPE102	chloroplast complete genome	*E. gracilis*	emb	43/66 (65)	0.056	X70810
PMYPE118	beta-ketoacyl-ACP sytase I isozyme	*H. vulgare*	gb	56/91 (61)	0.014	M95172
PMYPE122	NADP-dependent malic enzyme.	*P. vulgaris*	gb	87/110 (79)	3.7e-21	J03825
PMYPE140	polymorpha chloroplast genome DNA	*L. Marchantia*	gb	44/70 (62)	0.92	X04465
PMYPE153	NR ORF, 3' end	*P. tetragonolobus*	gb	55/96 (57)	1.0	L16780
PMYPE154	ATP synthase	*S. oleracea*	emb	51/81 (62)	0.060	X05916
PMYPE167	photosystem I subunit N	*H. vulgare*	emb	51/76 (67)	0.00040	X66428
PMYPE183	chloroplast genome DNA	*M. polymorpha*	emb	31/46 (67)	0.061	X04465
PMYPE184	peroxidase mRNA, complete eds.	*G. hirsutum*	gb	30/38 (78)	0.53	L08199
PMYPE190	rbcL, rps14, trnM, trnG, trnD, trnS, rps4, atpE, atpB genes, complete cds.	*C. ellipsoidea*	dbj	45/64 (70)	0.0032	D10997
cDNAs similarity to non-plant genes						
PMYPE002	tyrosine phosphatase mRNA,	*Styela plicata*	gb	38/58 (65)	1.0	M37998
PMYPE003	HOX-8 protein, 3' terminal sequence.	*H. sapiens*	dbj	46/73 (63)	0.7	D14970
PMYPE011	insulin receptor (hINSR) gene.	*H. sapiens*	gb	42/68 (61)	1.0	M32830
PMYPE018	interleukin 5	*H. sapiens*	gb	28/34 (82)	0.99	J02971
PMYPE027	Homo sapiens ala gene.	*H. sapiens*	gb	22/25 (88)	0.35	M58318
PMYPE028	minisatellite sequence	*H. sapiens*	emb	45/56 (80)	1.9e-07	A08920
PMYPE029	EST640 cDNA clone 30A7.	*H. sapiens*	gb	53/84 (63)	0.011	T25065
PMYPE036	glucose-regulated protein (GRP75)	*H. sapiens*	gb	31/41 (75)	0.90	L15189
PMYPE060	NIB1267 cDNA 3' end	*H. sapiens*	gb	46/76 (60)	0.99	T16415
PMYPE081	cystathionine gamma-lyase	*H. sapiens*	gb	31/38 (81)	0.0052	S52028
PMYPE086	ISG-54K gene.	*H. sapiens*	gb	40/58 (68)	0.27	M14660
PMYPE089	Homo sapiens cDNA clone HFBDY87.	*H. sapiens*	gb	31/40 (77)	0.88	T06793
PMYPE090	5' nucleotidase (EC 3.1.3.5)	*H. sapiens*	emb	52/84 (61)	0.18	X55740
PMYPE095	putatively transcribed partial sequence.	*H. sapiens*	emb	29/38 (76)	1.0	Z21192
PMYPE111	Human STS UT1010.	*H. sapiens*	gb	37/56 (66)	1.0	L17745
PMYPE125	Fc-gamma-receptorIIB (FCGR2B)	*H. sapiens*	gb	108/109 (99)	1.6e-37	M90734
PMYPE127	cytochrome P450 (Cyp1A2) gene.	*H. sapiens*	gb	34/45 (75)	0.29	U02993
PMYPE160	Human interleukin 2 (IL-2) gene.	*H. sapiens*	gb	32/43 (74)	0.95	K02056
PMYPE169	Wison disease gene.	*H. sapiens*	gb	34/48 (70)	0.87	M97499
PMYPE174	EST01013 cDNA clone HHCPA05.	*H. sapiens*	gb	26/32 (81)	1.0	M78865
PMYPE181	H. sapiens biliary glycoprotein (BGPa) gene, partial cds.	*H. sapiens*	gb	24/28 (85)	0.96	M76741
PMYPE185	Human platelet glycoprotein IIIa.	*H. sapiens*	gb	22/22 (100)	1.0	M32684
PMYPE191	Human GPI-H mRNA, complete cds.	*H. sapiens*	gb	34/49 (69)	0.99	L19783
PMYPE005	Caenorhabditis elegans cosmid M106.	*C. elegans*	emb	51/83 (61)	0.39	Z46935
PMYPE014	MAD homolog 1 (cem-1) mRNA.	*C. elegans*	gb	28/33 (84)	0.69	U10327
PMYPE048	early switch (xol-1) gene exone 1-7.	*C. elegans*	gb	34/48 (70)	0.51	L35129
PMYPE071	Caenorhabditis elegans cosmid ZK675	*C. elegans*	embl	41/61 (67)	0.32	Z46812
PMYPE106	Caenorhabditis elegans cosmid C16C10.	*C. elegans*	emb	45/74 (60)	1.0	Z46787
PMYPE110	Caenorhabditis elegans cosmid R107	*C. elegans*	gb	26/32 (81)	1.0	Z14092
PMPYE123	cDNA clone CEMSF78	*C. elegans*	gb	26/30 (86)	0.87	M79944
PMYPE144	Caenorhabditis elegans cosmid ZK757	*C. elegans*	gb	36/52 (69)	0.83	Z29121
PMYPE193	Caenorhabditis elegans cosmid K04C2.	*C. elegans*	gb	46/72 (63)	0.40	U00044

Table 2. Continue.

Clone	Putative identification	Species	DB*	Nucleotide similarity (%)	Probability	Sources of comparison
PMYPE006	vertebrate U2 small nuclear RNA.	*S. cerevisiae*	gb	47/69 (68)	0.003	M14625
PMYPE015	put. cytochrome P450 involved in spore wall maturation	*S. cerevisiae*	emb	35/51 (68)	0.80	X55713
PMYPE021	gene for TBF1 protein	*S. cerevisiae*	emb	47/77 (61)	1.0	X69394
PMYPE025	Yeast mitochondrial gene	*S. cerevisiae*	emb	42/61 (68)	0.16	V00686
PMYPE043	mitochon-drion oxi3 gene. aapl gene.	*S. cerevisiae*	gb	39/62 (62)	1.0	L36897
PMYPE044	DNA polymerase III catalytic subunit	*S. cerevisiae*	emb	29/40 (72)	0.23	X61920
PMYPE035	leucine zipper domain protein homolog	*S. cerevisiae*	gb	20/20 (100)	0.16	S47695
PMYPE053	carbamyl phosphaste synthetase	*S. cerevisiae*	gb	63/103 (61)	0.0017	M27174
PMYPE074	S. cervisiae SDB25 gene	*S. cerevisiae*	emb	33/47 (70)	1.0	X78309
PMYPE092	DNA for right arm of chromosome IV	*S. cerevisiae*	emb	32/44 (72)	1.0	X82086
PMYPE124	mitochondrion DNA, complete genome.	*S. cerevisiae*	gb	31/39 (79)	0.45	M62622
PMYPE182	Leu-and Gln-tRNA genes	*S. cerevisiae*	gb	42/62 (67)	0.096	K00538
PMYPE012	3-hydroxy-3-methylglutaryl coenzyme A synthase	*Syrian hamster*	gb	32/39 (82)	0.044	L00334
PMYPE013	plasminogenactivator-inhibitor type 2	*Unidentified*	emb	34/46 (73)	0.80	A10352
PMYPE016	glutamic acid-rich protein gene	*P. falciparum*	gb	36/51 (70)	0.61	J03998
PMYPE041	ribosomal RNA from the inverted repeat within the 35-Kb circular DNA	*P. falciparum*	emb	35/48 (72)	0.72	X61660
PMYPE079	rign-infected erythrocyte surface antige	*P. falciparum*	emb	42/60 (70)	0.058	X04572
PMYPE023	mitochondrial cytochrome b gene	*N. aurispinosus*	gb	34/46 (73)	0.78	L19728
PMYPE026	transferrin binding proteins 1 and 2	*N. meningitidis*	emb	27/30 (90)	0.76	Z15130
PMYPE055	clathrin heavy chain (chcA) mRNA.	*D. discodieum*	gb	30/41 (73)	1.0	M83660
PMYPE061	adenylyl cyclase germination protein	*D. discodieum*	gb	32/43 (74)	0.29	M87278
PMYPE165	Slime mould dutA mRNA	*D. discodieum*	dbj	62/100 (62)	4.2e-07	D16417
PMYPE192	cyclic nucleotide phosphodiesterase	*D. discodieum*	gb	43/44 (77)	0.047	M23449
PMYPE168	adh gene for alcohol dehydrogenase	*D. lebanonensis*	emb	19/23 (82)	0.86	X53429
PMYPE141	spore germination- specific protein	*D. discoideum*	gb	45/69 (65)	0.13	M33862
PMYPE033	solube NSF attachment protein	*D. melanogaster*	gb	30/38 (78)	0.94	U09374
PMYPE097	bxd gene	*D. melanogaster*	gb	49/78 (62)	0.35	L32205
PMYPE143	zinc-binding protein (trithorax)	*D. melanogaster*	gb	36/51 (70)	0.67	M31617
PMYPE166	hedgehog gene DNA	*D. melanogaster*	emb	39/59 (66)	0.86	Z11840
PMYPE045	snRNA U3-1	*T. thermophila*	emb	37/46 (80)	0.0012	X63788
PMYPE054	glycerol kinase (glpK) and	*B. subtilis*	gb	31/42 (73)	1.0	M34393
PMYPE059	beta-D-galactosidase (lacZ) mRAN	*Brugia malayi*	gb	40/59 (67)	0.64	M63098
PMYPE063	18S rRNA gene	*Tritoxa flexa*	gb	16/17 (94)	0.012	U01274
PMYPE065	S-adenosylmethionine decarboxylase	*R. norvegicus*	emb	27/32 (84)	0.052	Z15122
PMYPE066	nitrogenase iron protein genes (nifH1).	*C. pasteurianum*	gb	31/40 (77)	0.88	M21537
PMYPE068	cytochrome P3-450	*M. musculus*	emb	36/52 (69)	1.0	X01682
PMYPE088	immunoglobulin heavy chain	*M. musculus*	gb	27/31 (87)	0.82	L09584
PMYPE094	ribonucleotide synthetase mRNA	*M. musculus*	gb	28/35 (80)	1.0	U01023
PMYPE161	Moue 28S rRNA gene, 5' end	*M. musculus*	gb	27/28 (96)	0.12	M57720
PMYPE164	Mouse protein tyrosine kinase (jak3)	*M. musculus*	gb	30/40 (75)	1.0	L32955
PMYPE070	enkephalinase	*R. norvegicus*	gb	32/42 (76)	0.89	M15944
PMYPE082	mRNA for Ran/TC4, complete cds.	*T. pyriformis*	dbj	27/33 (81)	1.0	D21825
PMYPE098	phospho-transbutyrylase	*C. cetobutylicum*	gb	40/57 (70)	0.13	L14744
PMYPE107	mitochondrial NADH dehydrogenase	*R. culicivorax*	gb	36/54 (70)	0.99	L08174
PMYPE109	mitochondrial epsilon-sen DNA	*P. anserina*	gb	38/54 (70)	0.15	X03127
PMYPE115	serine hydroxymethyltransferase	*S. typhimurium*	gb	28/36 (77)	1.0	X15816
PMYPE117	outer membrane protein (fasD) gene	*E. coli*	gb	34/48 (70)	0.68	L22659
PMYPE119	DNA polymerase III (polC) gene	*M. pulmonis*	gb	55/90 (61)	0.038	U06833
PMYPE129	benomyl/methotrexate resistance gene	*C. albicans*	gb	28/33 (84)	0.54	X53823
PMYPE132	sigma factor gene (fliA)	*S. typhimurium*	dbj	34/47 (72)	0.76	D00497
PMYPE134	C-reactive protein (CRP)	*O. cuniculus*	gb	42/67 (62)	1.0	M13497
PMYPE139	soluble angiotesin-binding protein	*S. sclomestica*	gb	29/35 (82)	0.42	D11336

Table 2. Continue.

Clone	Putative identification	Species	DB*	Nucleotide similarity (%)	Probability	Sources of comparison
PMYPE146	random genomic clone hsb12	*M. genitalium*	gb	50/62 (80)	3.8e-08	U01771
PMYPE150	Microtus epiroticus element sequence	*M. epiroticus*	gb	34/41 (82)	0.079	M94696
PMYPE152	amylopullulanase (apu) gene	*T. saccharolyticum*	gb	38/39 (97)	1.5e-19	L07762
PMYPE170	ribosomal DNA	*C. thummi*	emb	39/55 (70)	0.10	X01842
PMYPE175	B. mori 19G1 gene for 30 K protein	*B. mori*	emb	36/54 (66)	1.0	X54736
PMYPE187	rfbP, orf17.4 and gnd genes	*S. enterica*	emb	37/53 (69)	0.56	X60666
PMYPE189	kinetoplast maxicircle variable region	*T. brucei*	emb	58/88 (65)	6.0e-05	Z15118
PMYPE195	Simian immunodeficiency virus	*S. sciureus*	emb	43/64 (67)	0.19	X14307

*gb; GenBank; emb, EMBL; dbj, DDBJ

random genomic clone hsb12 of *M. genitalium*, amylopullulanase gene of *T. saccharolyticum*, kinetoplast maxicircle variable region of *T. brucei* and slime mould dutA gene of *D. discoideum*. A total of 54 clones (25.7%) showed the significant homology to the known gene which were 3.2 times higher than the known EST analysis for the rice (8%) (Uchimiya *et al.*, 1992) and similar to human (22.5%) (Okubo *et al.*, 1992). 29 clones (13.8%) have no similarity to the known nucleotide sequences, which can be applied as a particular marker for ethylene treatment or for isolation of novel genes.

The plant stress hormone, ethylene, is known to be associated with induction of characteristic chitinase and β-1,3-glucanase (Boller, 1985). In the addition, molecular and biochemical studies of plant defense response have identified ethylene as one of the potential signals in systemic acquired resistance to pathogens and pests (Chang 1996; Ross, 1961). Our studies clearly show the high frequencies of 6 out of 210 clones and 4 out of 210 clones for both characteristic β-1,3-glucanase and chitinase, respectively (Table 2). Furthermore, 17 clones out of 27 potato entries consisting of PMYPE009, 049, 084, 093, 100, 101, 180, and 200, were identifed as genes which were related to the stress as well as pathogens (Table 2). Although the number of each clone in this study was not propotional to the number of each transcript, the relative abundancy of both chitinase and β-1,3-glucanase gene as well as other inducible genes implys that these gene are induced by ethylene, suggesting that the cDNA library in this study indeed represents the detailed profile of gene expression patterns in an ethylene-treated potato leaf (Fig. 1).

Fig. 1. Northern blot analysis of β-1,3-glucanase. Lanes 1, 2, 3 and 4 contain 15 μg of RNAs from leaves before as well as 6, 12 and 24 hours after the ethylene treatment, respectively. Lane 5 contains the plasmid, PMYPE100, of which the probe was made.

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