



马铃薯 NOA 基因的克隆及序列分析

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摘要:以马铃薯(*Solanum tuberosum*)为实验材料,利用电子克隆和 RACE 技术,从马铃薯中克隆出 NOA(nitric oxide associated factor)基因,命名为 *StNOA1*,测序结果表明,其 cDNA 序列长度为 1 929 bp,此片段包含一个长为 1 632 bp 的完整编码框。氨基酸序列比对分析表明,*StNOA1* 与烟草(*Nicotiana benthamiana*),葡萄(*Vitis vinifera*),蓖麻(*Ricinus communis*),水稻(*Oryza sativa*),玉米(*Zea mays*)以及拟南芥(*Arabidopsis thaliana*)均有很高的同源性(89.44%~63.56%)。同 *AtNOA1* 一样,*StNOA1* 也具有保守的 GTP 结合区。从结构分析结果推测,*StNOA1* 和 *AtNOA1* 在功能上有一定的相关性,其也可能通过调节内源 NO 的释放参与到植物生长、发育、抗逆等过程中。

关键词:马铃薯;分子克隆;GTPase

中图分类号:Q785 文献标识码:A

Molecular Cloning and Analysis of a NOA Gene in *Solanum tuberosum*

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Abstract: The NOA (nitric oxide associated factor) gene encodes a NOA which participates in nitric oxide synthase (NOS) dependent NO synthesis. In this study, the cDNA of *StNOA1* was cloned by in silico cloning and combination with RACE from potato (*Solanum tuberosum*). The full length of *StNOA1* cDNA had 1 929 bp with an open reading frame of 1 632 bp. Phylogenetic analysis revealed that the *StNOA1* amino acid sequence shared high identity (89.44%~63.56%) with the NOA from *Nicotiana benthamiana*, *Vitis vinifera*, *Ricinus communis*, *Oryza sativa*, *Zea mays* and *Arabidopsis thaliana*. The *StNOA1* bears a centrally positioned GTPase-binding domain as well as *AtNOA1*. Its sequence conservation and structure similarity with *AtNOA1* implied the functional correlation between *StNOA1* and *AtNOA1* in plant growth, development, and responses to stresses and pathologies by regulating endogenous NO production.

Key words: *Solanum tuberosum*; molecular cloning; GTPase

Nitric oxide (NO) has been suggested to be an important signaling molecule in plants^[1-4]. NO has been shown to affect growth and development of plant

tissue^[5], induce seed germination in stead of red light^[6], affect plant maturation and senescence^[7], mediate abscisic acid (ABA) induced stomatal closure, and

收稿日期: 2009-01-13; 修改稿收到日期: 2009-08-18

基金项目: 中国科学院知识创新工程重大项目(KSCX1-YW-03); 中国科学院知识创新工程方向性项目(KSCX2-YW-N-052); 国家科技支撑计划课题(2007BAD52B08)

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play a role in the light mediated greening^[8]. Furthermore, NO has been implicated to be involved in drought, salt, and heat stresses, disease resistance and apoptosis^[9-12]. Owing to the essential role of NO in plant signaling network, its endogenous source has become very important. Increasing studies indicated that NO synthesis in plants mainly includes both nitric oxide synthase (NOS) and nitrite reductase (NR)-dependent pathways. *AtNOA1* has been proven to participate in endogenous NO production in the process of hormonal signaling and in defense responses to pathogen attack and salt stress.

In present study, a full length cDNA which was homologous with *AtNOA1* cDNA was obtained by *in silico* cloning and 3'-RACE from potato (*Solanum tuberosum*). Sequence alignments analyses and phylogenetic analysis were made. Because it was the first NOA gene isolated from potato, it was designated as *StNOA1*.

1 Material and methods

1.1 Plant material

Potato (*Solanum tuberosum*) was grown in greenhouse under conditions of 14 h photoperiod and 23°C.

1.2 Methods

1.2.1 General RNA extraction Potato leaves were used for RNA extraction using the Trizol reagent according to the manufacturer's instructions. RNA was precipitated by ethanol and suspended in 30 µL of RNase free sterile distilled water. General RNA sample was stored in -70°C. for following RT-PCR.

1.2.2 *StNOA1* 3' cDNA end isolation and sequencing First strand of cDNA was generated from 5 µg of RNA with the Superscript Reverse Transcriptase using RT primer P_{3R} (Table 1). The *StNOA1* 3' cDNA end was obtained by cassette PCR with primer pairs (Stnoa1; Stnoa2 and Pnup; Stnoa3 and Pnup) (Table 1). Subsequently the *StNOA1* 3' cDNA fragment was ligated into Puc-T vector and sequenced. PCR were performed under the following conditions; 3 min at 94°C for full denaturalization, 3 min at 94°C, 30 min at 55°C, 1 min

at 72°C for 30 cycles of amplification, and 10 min at 72°C for additional extension.

Table 1 Sequence of primers used in this study

Primer name	Sequence(5'-3')
P _{3R}	AAGCAGTGGTAACAACGCAGAGTACTTTTTTTTTT- TTTTTTTTTTTTTTTTTTTTT(AGC)(AGCT)
Stnoa1	ATATCCTTCTGTTCGAGCTTCC
Stnoa2	TTCGAGCTTCCTTTCAATAAAC
Stnoa3	TGACCAGGAGACATACGATTTGA
Pnup	AAGCAGTGGTAACAACGCAGAGT
Stnoa-flf	CAATATCCTTCTGTTCGAGC
Stnoa-flr	CCCGTATATACCTGTGTAGCA
Stnoa5	GCGGTACCATGGCCCTAACTCCTAGTCT <i>Kpn</i> I
Stnoa6	GCGGATCCTCAGAAAAACCATTTGGGTCT <i>Bam</i> HI

1.2.3 *StNOA1* full length cDNA isolation, sub-cloning, and sequencing

The *StNOA1* full length cDNA was amplified by cassette PCR with gene specific primer pairs (Stnoa-flf and Stnoa-flr; Stnoa5 and Stnoa6) (Table 1). The gene specific primers were designed according to EST alignment sequence and 3' end sequenced results. PCR were performed under the following conditions; 3 min at 94°C for full denaturalization, 3 min at 94°C, 30 min at 55°C, 2 min at 72°C for 30 cycles of amplification, and 10 min at 72°C for additional extension. RT-PCR products of the *StNOA1* gene were purified and sub-cloned into pBluescript II SK(+) vector. The recombinant plasmid was transformed into competent cells of the *E. coli* strain DH5α and cultured in solid LB (100 mg/L) under 37°C overnight. The white bacterium spot was selected for PCR verification. Then the recombinant plasmid was verified with restriction enzyme digestion. The bacterium clone containing correct recombinant plasmid was sent to Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (China) for sequencing.

1.2.4 Sequence comparison between *StNOA1* and other plant NOA

The sequence of *StNOA1* was used for BLAST in GenBank (www.ncbi.gov.). The DNAMAN software was used for prediction of amino acid sequence and phylogenetic analysis between *StNOA1* and other plant NOA. The accession numbers of the genes used in the study are as follows: *Oryza sativa* NOA (EAY84101), *Vitis*

vinifera NOA (CAO42714); *Arabidopsis thaliana* NOA (NP_850666), *Nicotiana benthamiana* NOA (BAF93184), *Zea mays* NOA (ACN26917), *Ricinus communis* NOA (EQ973773).

2 Results and analysis

2.1 General RNA extraction and *StNOA1* 3' cDNA isolation

Potato general RNA was successfully extracted by Trizol reagent and identified by 1% agarose gel electrophoresis (Fig. 1). The 3' end was successfully amplified by cassette PCR. The amplified 3' end length was about 1 500 bp (Fig. 2).

2.2 Cloning of *StNOA1* cDNA

A 1 632 bp cDNA containing *Kpn* I and *Bam*H I enzyme cut sites was obtained by cassette PCR with gene specific primer pairs (*Stnoa*-flr and *Stnoa*-flr; *Stnoa*5 and *Stnoa*6) (Fig. 3). Enzyme digestion showed that *StNOA1* was successfully cloned to pBluescript II SK(+) vector, it was designated as pBSK-*StNOA1* (Fig. 4).

2.3 Sequence analysis of *StNOA1* and other plant NOA

From its amino acids sequence deduced by DNAMAN software, *StNOA1* also bared a GTP binding domain as well as *AtNOA1* (Fig. 5). Phylogenetic tree was constructed by DNAMAN software. The result indicated that *StNOA1* and *NbNOA1* (NOA from *Nicotiana benthamiana*) were



Fig. 1 Electrophoretic pattern of potato RNA in 1% agarose gel

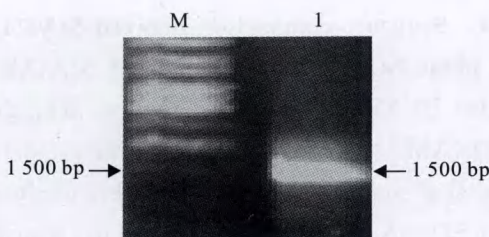


Fig. 2 *StNOA1* 3' end amplification
M. Marker; 1. PCR products

clustered into a subclass due to the high identity (89.44%). The subclass was phylogenetically near with the NOA from *Oryza sativa* and *Zea mays* NOA from *Arabidopsis thaliana* and *Ricinus communis* were clustered into the same subclass. NOA from *Vitis vinifera* was clustered into another subclass (Fig. 6).

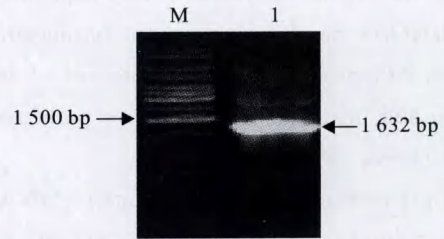


Fig. 3 PCR products of *StNOA1* gene
M. Marker; 1. PCR products

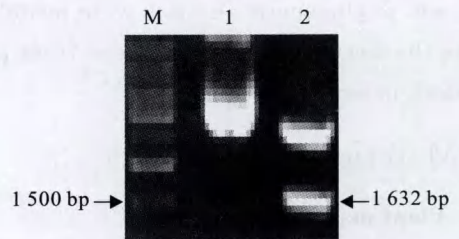


Fig. 4 Enzyme digestion of pBSK-*StNOA1*
M. Marker; 1. pBSK-*StNOA1*; 2. pBSK-*StNOA1* digested with *Kpn* I and *Bam*H I

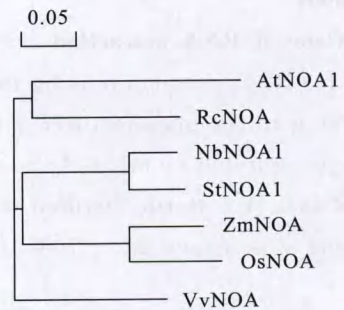


Fig. 6 Phylogenetic tree based on the amino acid sequence of NOAs from different plants
At. *Arabidopsis thaliana*; *Nb*. *Nicotiana benthamiana*; *Os*. *Oryza sativa*; *St*. *Solanum tuberosum*; *Vv*. *Vitis vinifera*; *Zm*. *Zea mays*; *Rc*. *Ricinus communis*

3 Discussion

EST (expression sequence tag) driven from mRNA. A large number of EST sequences have been obtained in the main crops and model plants. The full length cDNA information could be obtained

AtNOA1	MALRITLSTFPS. LPRRHITTR.	REPNLTVI YRNPTTSI VCKS.	I ANSEPPVSL.	ERDGF	57						
NbNOA1	MAPKLLALSSLSI SPFRLPNYHVS TPNS	TLKLN FKFHTTKNLI LCKS.	TESQTVSES.	EPDCYG	66						
RcNOA	MAPKTLVTFPS PVS LPHNLTH.	FNTKFLKFN SRKTTPI FCKSTQS	QKQPVSDTQLPSI P.	ETSCTG	65						
StNOA1	MAPKLLALSSLSI CPFPI P.	NSSPSSLNI YKFHTPKPTLI FCKS.	TEPQTI SES.	EPECYG	59						
ZmNOA	MAAPHLPLSFPKTLPLPPP.	LKTHAHRISL.	AAAPAPPPA.	PPDGAG	46					
OsNOA	MAAPLLLSLQRLFLSLSPK.	PQLAPNPSFSP.	TRAASTAPP.	PPEGAG	49					
VvNOA	MALKPLTSVFLS.	PLSLP.	YSPSNPTPKFSSFYTKPTPI SCQT.	QAHQQAAPTSDPYRPESDGLG	62						
Consensus				g							
AtNOA1	AAAPTRGERFLENQRAHEAKQVVKKEI	KKEK. KKKKEEI I ARKVVDS VS	CYCGGAPLQTS DVDS	EGFDLVLYE LKKK	136						
NbNOA1	AAAPTRGDI YLQRQQAASSTMLAIT.	KKKKKKDKI FKI SNLAPC	CYCGGAPLHIS EVDAPCYVHQB	TYDLKKK	141						
RcNOA	AAAPTRGEQFDERQAFQAKLVKEAKKKR.	RREKVRKALKVN. SIVVC	CYCGGAPLQTLDOEAPCYVQPD	TYDLKKR	143						
StNOA1	AAAPTRGDI YLQRQQAASSTMLATT.	KKKKKKKDI FKI SNLAPC	CYCGGAPLHIS EVDAPCYVQPD	TYDLKKK	134						
ZmNOA	PAAPTRGDRFLGRQLATEAARVLAPEDA	RRRRRREKRRALARKPSGLAS	CYCGGAPLQTAEEAAPCYVDP	TYDLKKR	126						
OsNOA	PAAPSRGDRFLGTQLAAEAARVLAPEDA	RRRRRREKRRALARKPS. AAA	CYCGGAPLQTAEEAAPCYVHP	TYDLKKR	128						
VvNOA	AAAPTRGDLEHHSVAASVFNAN.	KKKKVKFS GSVKAS AAS	CYCGGAPLQILETDAFCYVDP	TYDLKKK	137						
Consensus	aap g l a v		cycgap l t	pg v ty lkk							
AtNOA1	HEQLRTM CGRCQLLSHGMI TAVGGNGGY	GGKQFVSADELRKLSHLRHKALI	VKLVDI VDFNGSFLARV	RDLAGAN	216						
NbNOA1	HEQLRKVL CGRCQLLSHGMI TAVGGNGGY	SGGKQFVTAEDLRKLSHLRHKALI	VKLVDI VDFNGSFLARV	RDLAGAN	221						
RcNOA	HEQLRTVL CGRCQLLSHGMI TAVGGNGGY	SGGKQFVSADELRKLSHLRHKALI	VKLVDI VDFNGSFLARV	RDLAGAN	223						
StNOA1	HEQLRKI LCGRCQLLSHGMI TAVGGNGGY	GGKQFVTAEDLRKLSHLRHKALI	VKLVDI VDFNGSFLARV	RDLAGAN	214						
ZmNOA	HEQLRTVL CGRCQLLSHGMI TAVGGNGGY	GGKQFVSADELRKLSHLRHKALI	VKLVDI VDFNGSFLARV	RDLAGAN	206						
OsNOA	HEQLRTVL CGRCQLLSHGMI TAVGGNGGY	GGKQFVSADELRKLSHLRHKALI	VKLVDI VDFNGSFLARV	RDLAGAN	208						
VvNOA	HEQLRTVL CGRCQLLSHGMI TAVGGNGGY	GGKQFVSADELRKLSHLRHKALI	VKLVDI VDFNGSFLARV	RDLAGAN	217						
Consensus	h qlr cgrc llshg m tavgg ggy gkf a	l r kls r e l	klvdi vdfngsfla	rd gan							
AtNOA1	PII LVI TKI DILEKGTDLNCF	GDVVVEATMRKKNVLSVHLTSSKSL	LVGIVGVA	SEI QKQKGRDVI	LGANVGS	296					
NbNOA1	PII LVI TKVDLLEKGTDLNCF	GDVVVEATMRKKNVLSVHLTSSKSL	LVGIVGVA	SEI QKQKGRDVI	LGANVGS	301					
RcNOA	PII LVI TKVDLLEKGTDLNCF	GDVVVEATMRKKNVLSVHLTSSKSL	LVGIVGVA	SEI QKQKGRDVI	LGANVGS	303					
StNOA1	PII LVI TKVDLLEKGTDLNCF	GDVVVEATMRKKNVLSVHLTSSKSL	LVGIVGVA	SEI QKQKGRDVI	LGANVGS	294					
ZmNOA	PII LVI TKVDLLEKGTDLNCF	GDVVVEATMRKKNVLSVHLTSSKSL	LVGIVGVA	SEI QKQKGRDVI	LGANVGS	286					
OsNOA	PII LVI TKVDLLEKGTDLNCF	GDVVVEATMRKKNVLSVHLTSSKSL	LVGIVGVA	SEI QKQKGRDVI	LGANVGS	288					
VvNOA	PII LVI TKVDLLEKGTDLNCF	GDVVVEATMRKKNVLSVHLTSSKSL	LVGIVGVA	SEI QKQKGRDVI	LGANVGS	297					
Consensus	piilv tk dl l p t d nc	gdvvve kknvlsvhl tssksl	g gv sei e	kgr vyilg anvgsafi							
AtNOA1	NALNTMIAERDVAASAAQKRPPI	QSAVPGTTLGPI QI NAFVCGE	RLYDTPGVHLHHRQAAVVHSD	DLPLAPQNR	376						
NbNOA1	NALNTMSYNDVAATAARKYKPI	QSAVPGTTLGPI PI DAFLGCG	VYDTPGVHLHHRQAAVI HTE	DLPLAPQSR	381						
RcNOA	NALLKMAQRDVAASAAQKRPPI	QSAVPGTTLGPI QI DAFLGCG	GLFDTPGVHLHHRQAAVVQ	SEDLP LAPRS	383						
StNOA1	NALNTMPPKDPVAASAAQKRPPI	QSAVPGTTLGPI PI DAFLGCG	ELYDTPGVHLHHRQAAVI H	VEDLPLAPQSR	374						
ZmNOA	SAMURTMAYRDPVAASAAQKRPPI	QSAVPGTTLGPI QI BAFLGCG	ELYDTPGVHLHHRQAAVI H	ADDLPLAPQSR	366						
OsNOA	SAMURTMAYRDPVAASAAQKRPPI	QSAVPGTTLGPI QI BAFLGCG	ELYDTPGVHLHHRQAAVI H	ADDLPLAPQSR	368						
VvNOA	NALLKMAQRDVAASAAQKRPPI	QSAVPGTTLGPI QI DAFLGCG	ELYDTPGVHLHHRQAAVVH	SEDLP LAPRS	377						
Consensus	a l m dp aa a y pi	qsavpgt tlg i i af g k	dtpgvhl hhrqaav	dl p lap rl							
AtNOA1	FPD. I STLPTQSSSSPKGES	LNGYTFVGGLVRI DI LKALPET	CFTFYGPKALEI HAVPTK	TATAFYEA. .	KLGVLLTPP	452					
NbNOA1	FPSSGLKLESQI ADRVRS	GLNGLSI FVGGLVRI DVLK	VLPECLTFYGPKALQ	LMVPTTEADEFYQK. .	ELGLLLTPP	459					
RcNOA	FP. VCSLQTDKFS. . .	NLNGFSI FVGGLVRI DVLK	VLPECLTFYGPKALQ	FHI VPTDEADEFYQAS	KELGVLLTPP	458					
StNOA1	FPSSGQNLDSQI ANRVRS	GLSGLSI FVGGLVRI DVLK	VLPECLTFYGPKALQI	HVPTTEADEFYQK. .	ELGVLLTPP	452					
ZmNOA	FP.	MLN			371						
OsNOA	FP.	ANDTDVGLSGNSL	FVGGLVRI DVVKAL	PRTRTFYGPK	KLKI NNVPTTEADEFYER. .	EVGVLLTPP	435				
VvNOA	FP.	VLAFDDSTLSRI	KSNGLNGFSI FVGGLVRI	DI VKVLPQ	TRTRTFYGPKALNI HNVPTDKADEFYQK. .	ELGVLLTPP	453				
Consensus	f										
AtNOA1	SGKNQVQEVKGLQSHRLQI	EI NDAKRPASDVAI SGLGVI	SI EPI RKTRGTEPRDL	NEA. .	EHEI HI CVS	VPKPVEVFL	529				
NbNOA1	TGKEKADGVGLET	KRQLQI KYEDI ERPTCDVAI	SGLGWSI PVNKS	VGTS DPVSEVT. .	AGELTFVHVHPK	PVEI FV	536				
RcNOA	TGI EGARDVGLGI I RQLQI	KFEDAERPASDVAI SGLGVI	AVEPVS	KSRKPVNL	LEET. .	AKELHFAHVHPN	PVEI FV	535			
StNOA1	TGKEKADDVVGLKTRQLQI	KYEDI ERPTCDVAI SGLGWSI	VVPVNS	SAGI FNPVSEV	K. .	AGELTFI	VHVHPK	PVEI FV	529		
ZmNOA											
OsNOA	AGKEKAEGVGLQGVRELI	KYEESSDRPACDI AI SGLGVA	VEPLGVPSSNPDES	AEEDNES	GELHLR	VHVHPK	PVEI FV	515			
VvNOA	TGQRAEDVGLGLET	ERLQI KFEDSDRPACDLAI	SGLGVI AVEPI	GRSLR	TS	DS	DLEET. .	AEQLQSI	QVPK	PVEI FV	530
Consensus											
AtNOA1	RPPLPI	GTS	GTEVYQYREL	TDKEE	EV	RP	KWY			560	
NbNOA1	RPPLPVGKAGGQVYD	REL	TEES	EV	RP	KWF				567	
RcNOA	RPPLPVGKGS	EVYQYREL	TEKEE	EV	RP	KWH				566	
StNOA1	RS	PVPVKGAGRVYD	REL	TEEE	EV	RP	KWF			560	
ZmNOA											
OsNOA	RPPLPVGKAAS	QVYRQEL	TEEE	EV	RP	KWH				546	
VvNOA	RPPI	PVGGGGEVYQYREL	TEKEE	EV	RP	QWY				561	
Consensus											

Fig. 5 Comparison of amino acid sequences between *Solanum tuberosum* NOA and other NOA. Black lines show the crucial GTPase-specific motifs of StNOA1 and its orthologous proteins; [NT]KxD (the GTP specificity motif), GxxxGKS (Walker A), DxxG (Walker B). At, *Arabidopsis thaliana*; Nb, *Nicotiana benthamiana*; Os, *Oryza sativa*; St, *Solanum tuberosum*; Vv, *Vitis vinifera*; Zm, *Zea mays*; Rc, *Ricinus communis*

by analysis and alignment of reported ESTs. On the basis of the alignment cDNA information, the full length cDNA could be quickly and efficiently isolated by RACE in combination with PCR. In the present study, *AtNOA1* was used as the template to search potato EST sequence in GenBank. The highly homogenous EST sequences were obtained. The cDNA sequence information with complete ORF(open reading frame) was obtained. The cDNA of *StNOA1* was efficiently isolated by the RACE-PCR.

The phylogenetic analysis of *StNOA1* with the other NOA family proteins indicates that *StNOA1* and *NbNOA* are in the same clade, they are from Solanaceae. *OsNOA* and *ZmNOA* are in the same clade, which are from Gramineae (Fig. 6). *AtNOA1* as well as *NbNOA1* have been identified as putative regulators of NOS activity and participates in regulating endogenous NO production in plants^[4,13,14].

AtNOA1 is the first gene that was found to participate in NO synthesis in plant species. It has been recently reported to be a member of the circu-

larly permuted GTPase family (cGTPase) and *AtNOA1* specifically binds GTP and hydrolyzes it^[15]. However, complementation experiments of *Atnoa1* mutant plants with different constructs of *AtNOA1* show that the C-terminal domain of the *AtNOA1* may play a crucial role in plant endogenous NO production^[15]. The deduced *StNOA1* also bared a centrally positioned GTPase-binding domain as well as *AtNOA1*(Fig. 5). Its sequence conservation and structure similarity with *AtNOA1* implied the functional correlation between *StNOA1* and *AtNOA1* in plant growth, development, and responses to stresses and pathologies by regulating endogenous NO production.

The present study is the basis for elucidating molecular information and biochemical characteristics of *StNOA1*, and is very significant for elucidating the nature of NOA and its action mechanisms in NO synthesis in plant as well as its functions in plant development and defense responses, and applying it in crop species molecular breeding ultimately.

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