# an.pdf Identification of transcriptional factors of nitrate reductase gene promoters and NRE2 cis-element in Nicotiana tabacum Huijuan Yang, Yan Zhou, Yuning Zhang, Jing Wang, Hongzhi Shi College of Tobacco Science, Henan Agricultural University, Zhengzhou, 450002, China

### Objectives

This research aimed to identify the transcriptional factors of nitrate reductase gene (NIA1 and NIA2) promoters and



1	NNNNN	NNNNN	NNNN	NNNG	GGTTG	GGGTT	TCTAC	NGGAC	GTAAA	CTAG
51	CAGAT	CTACA	GCCAT	GAATT	CAGAT	TATTC	TAAAA	AAAGA	ÅTÅTG	AATG
101	GGATT	GAAAA	*****	AGGAA	AGAAG	GAAGA	GAGAT	TTTCT	GTATT	TCTCI
151	CTCAA	AATAT	TTCTC	TCTCT	CTCTC	TCCGA	GATTT	GATTT	TCTTG	TTCT
201	TTTGG	GAACG	AAGTT	TGTGA	ATGCG	TGGGG	TGGCC	TTATA	TAGAA	ACGT
251	TCGGA	CTCTC	TCAGT	GCCTC	CATAG	CGCCA	TACTA	TACAT	AAGCA	GGAC
301	CAAAA	AAATC	GACAC	CCCAR	TTTAA	TTTTG	ATTTT	*****	ATAGT	GACA
351	CATAA	AATAA	ATAAA	ACAAT	TCTAR	CGATT	TACAC	TTTTT	ACGCC	CGTA
401	GGTCG	GGATC	AGAGG	AAGAT	GCGAT	GTTGG	GGTAA	TAGCA	AATGG	CTTA
451	ÅGÅTÅ	TGCAG	CTGTT	GGTTÅ	GCCTT	AACAG	CTGTT	TCTTA	TTCTT	GCCG
501	GGTTT	TCACC	GAGTT	ATGGT	CACAT	TARAC	ATTAT	TATGG	CTCCA	CTAR
551	TCTAA	TTTCC	TTCTC	AATTT	CTTTT	CGGAG	GAATG	GCTAG	GTAAA	ATGT
601	TAGTG	ACATC	TGATA	TTTAC	CCARG	CTARA	CATAR	TTAAA	TTTTT	AGTA
651	TCAAA	AACAG	TGGCT	AGGTA	AATGT	TTTGT	GGCAT	CTGAT	ATTTA	CCCA
701	ATTAA	ATATA	ATTAA	ATTTT	TAGTA	ATCAA	AAAGT	AAGGT	TATAT	GTAT
751	TGTAT	GTATC	ATTAR	GTACG	ATAGA	TAAAT	ATACG	TATGA	ARRAC	ACAT
801	TCCTA	TTTTC	ATTTG	TTTGG	TGTAA	ATATC	AAATT	TGAGT	ACCTA	TTTA
851	GTGAT	ATAGA	AAGTG	*****	AAATC	AGAAA	GCTAG	TGACT	ATTTA	TGAG
901	AGACC	AACTC	TCTTA	TACGT	TTTTG	AACAA	NACCA	ACTTT	TCNNA	AAGT
951	TTTCT	ATTAT	ATAGN	AGTAT	AATGT	TTTCA	GCGAG	CACGT	CCARA	TATT
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hypothetical *cis*-element NRE2 (GACCCTA-N(10)-AAG) from flue-cured tobacco.

### Materials and Methods

Based on the constructed cDNA library of *Nicotiana* tabacum K326, a K326 yeast one-hybrid system was established using the Matchmaker<sup>®</sup> Gold Yeast One-Hybrid Library Screening System from Clontech. The transcriptional factors of NIA1 and NIA2 promoters and NRE2 *cis*-elements binding proteins were screened.

## **Results and Discussion**

CRM-domain containing factor CFM3 (XP\_016503563.1) and sulfite oxidase-like protein (NP\_001312236.1) were screened from NIA 1 promoter binding proteins. CRM-domain containing factor CFM3 was annotated as chloroplastic/mitochondrial-like protein which is related to chloroplast splicing factor CRS1, and dually functions in chloroplast group II intron splicing and mitochondrial gene expression. Sulfite oxidase is the smallest eukaryotic molybdenum enzyme that utilizes a molybdopterin cofactor and a heme group.





Fig. 2

A, Cloned NIA1 promoter and NIA2 promoter. B, Sequencing of (a) NIA1 promoter and (b) NIA2 promoter.



**GUS** histochemical staining results of tobacco leaves transferred by NRE2 cis-element deleted promoters.

NIA1 promoter, (B) NIA1 promoter without NRE2 sequence; (C) NIA1 promoter containing 4 tandem NRE2 elements;

(D) *NIA2* promoter; (E) *NIA2* promoter without NRE2 sequence; (F) *NIA2* promoter containing 4 tandem NRE2 elements.



Fig. 3 Identification of bait-yeast strains

#### by yeast colony PCR.

NIA1p: PCR product of colony transferred by pAbAi-NIA1p plasmid;

NIA2p: PCR product of colony transferred by pAbAi-NIA2p



• For the NIA2 promoter one binding protein was found to have a pepsin-like aspartic protease domain (XP\_016510250.1). Eukaryotic pepsin-like proteases have C- and N-terminal domains with similar topological characteristics. The active site motif (Asp-Thr/Ser-Gly-Ser) is conserved between retroviruses and eukaryotic proteases, as well as eukaryotic N- and C-terminal pepsin-like proteins. finger protein (XM\_016576899.1) RING which containing RING finger motifs was identified from 4 tandem NRE2 *cis*-elements. RING finger proteins contain zinc finger domains consisting of one or several Cys and His residues which can serve as DNA-binding domain, meeting the structural requirements of TFs.

#### plasmid;

4box: PCR product of colony transferred by pAbAi-(4×NRE2) plasmid; Marker: DL2000 DNA marker.



Fig. 4 Identification of the minimum

inhibitory concentration of AbA.

# Table 1 Screening of NIA1 promotor transcriptional factors

Screen Number	Fragment Length (bp)	Blastx /Blastn	NCBI Access Number	<b>Biological Annotation</b>	Species
71	314/95	blastx	XP_016503563.1	CRM-domain containing factor CFM3, chloroplastic/mitochondrial-like	Nicotiana tabacum
126	1149/591	blastx	NP_001312236.1	Sulfite oxidase-like	Nicotiana tabacum
95	377/185	blastn	XM_016598923.1	Predicted protein LOC107778635	Nicotiana tabacum
66	333/129	blastn	XR_001647849.1	Predicted protein LOC107785360	Nicotiana tabacum
94	317/115	blastx	YP_173415.1	Hypothetical protein NitaMp073	Nicotiana tabacum
135	437/236	blastx	XP_016455866.1	Predicted protein LOC107779879	Nicotiana tabacum
153	403/196	blastn	XM_016643438.1	Predicted protein LOC107817585	Nicotiana tabacum
51a	449/269	blastx	XP_009771766.1	Predicted protein At2g39795	Nicotiana sylvestris
68	329/127	blastx	XP_009790808.1	Predicted protein LOC104238210	Nicotiana sylvestris

#### M cDNA



### Table 2 Screening of NIA1 promotor transcriptional factors

Screen Number	Fragment Length (bp)	Blastx /Blastn	NCBI Access Number	<b>Biological Annotation</b>	Species	
7	272/66	blastx	XP_006380094.1	Hypothetical protein POPTR_0008s21830g	Populus trichocarpa	
25	249/41	blastn	CP023132.1	chromosome LG-20	Lupinus angustifolius	
72	412/62	blastx	XP_016510250.1	Conserved pepsin-like aspartic protease domain	Nicotiana tabacum	

### Table 3 Screening of 4×NRE2 transcriptional factor

Screen Number	Fragment Length (bp)	Blastx /Blastn	NCBI Access Number	<b>Biological Annotation</b>	Species
34	247/42	blastn	XM_017753776.1	Predicted protein LOC108455177	Gossypium arboreum
46	321/130	blastx	KDB15619.1	Glycoside hydrolase family 16	Ustilaginoidea virens
48	242/37	blastn	XM_016576899.1	RING-H2 finger protein ATL16-like	Nicotiana tabacum

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