

Identification of transcriptional factors of nitrate reductase gene promoters and NRE2 cis-element in *Nicotiana tabacum*

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Objectives

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

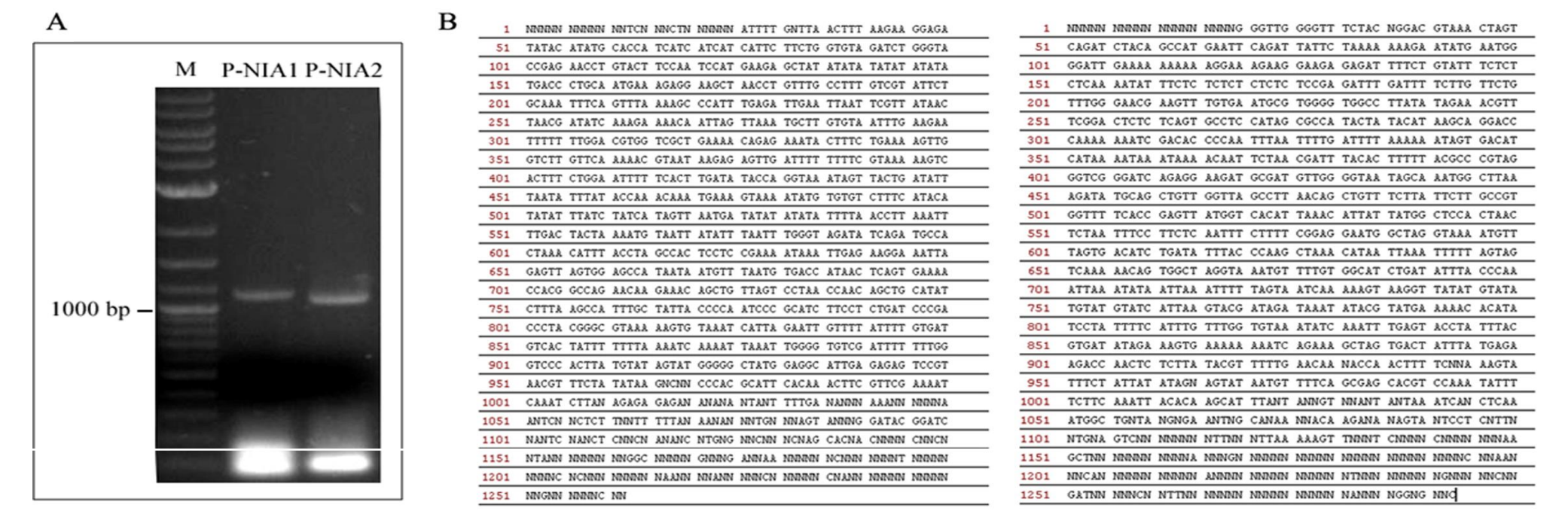
◆ CRM-domain containing factor CFM3 (XP_016503563.1) and sulfite oxidase-like protein (NP_001312236.1) were screened from *NIA1* promoter binding proteins. CRM-domain containing factor CFM3 was annotated as chloroplastic/mitochondrial-like protein which is related to chloroplast splicing factor CRS1, 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.

◆ 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.

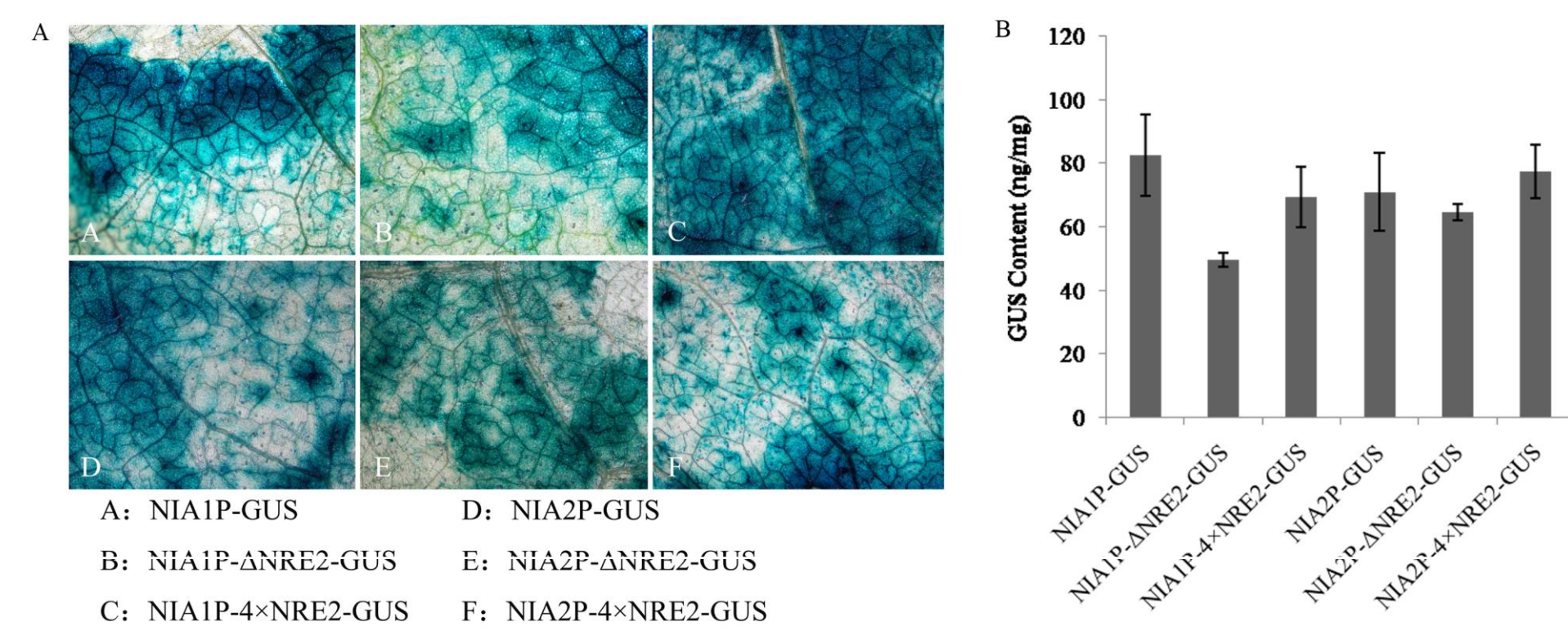
◆ RING finger protein (XM_016576899.1) which containing RING finger motifs was identified from 4 tandem NRE2 *cis*-elements. RING finger proteins contain zinc finger domains consisting of one or several Cys and His residues which can serve as DNA-binding domain, meeting the structural requirements of TFs.

Fig. 1

Cloning of *NIA1* and *NIA2* promoters.



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



(A) *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. 2

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

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

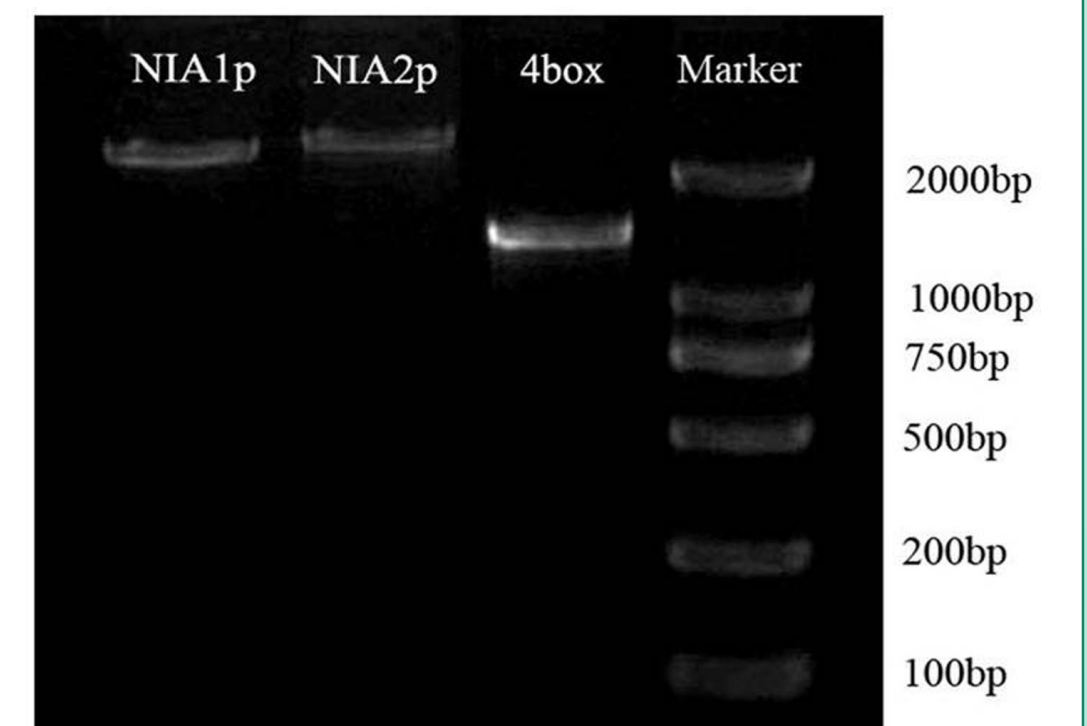


Fig. 4

Identification of the minimum inhibitory concentration of AbA.

Table 1

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

Table 2

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

Table 3

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

Fig. 5

Construction of cDNA library.

M: DNA marker; cDNA: electrophoresis of constructed cDNA library.

