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Supplementary file:

Tobacco plants expressing the defensin *NaD1* enhances drought tolerance characteristics in transgenic lines

Sara Royan¹, Reza Shirzadian-Khorramabad^{2*}, Arash Zibaee³, Mohammad B. Bagherieh-Najjar⁴, Farhad Nazarian Firouzabadi⁵

¹ Ph.D. student of Agricultural Biotechnology, Faculty of Agricultural Sciences, Guilan University, Rasht, Iran

^{2*} Associate Professor, Department of Agricultural Biotechnology, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran

³ Associate Professor, Department of Plant Protection, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran

⁴ Associate Professor, Department of Biology, Faculty of Science, Golestan University, Gorgan, Iran

⁵ Professor, Production Engineering and Plant Genetic Department, Faculty of Agriculture and Natural Resources, Lorestan University, Khorramabad, Iran

Corresponding author: R.shirzadian@guilan.ac.ir

Step	Temperature (C°)	Time	Cycles
Initial denaturation	95	5 min	
Denaturation	95	45 Sec	35
Annealing	58	30 Sec	
Extension	72	30 Sec	
Final extension	72	7 min	

[illegible]

Elicitor	AT-rich sequence	element for maximal elicitor-mediated activation (2copies)	0	1	0	0	0	0	2	1	0	0	0
Hormone Responsive Elements	ABRE	involved in the abscisic acid responsiveness	3	2	3	5	6	2	3	2	1	1	4
	ABRE3a	involved in the abscisic acid responsiveness	0	1	2	2	0	0	0	0	0	1	0
	ABRE4	involved in the abscisic acid responsiveness	0	1	2	2	0	0	0	0	0	1	0
	as-1	SA and Auxin responsive element	0	2	3	1	0	1	3	1	1	2	2
	CARE	ABA response, GA response	0	0	1	0	0	0	0	0	0	0	0
	CGTCA-motif	involved in the MeJA-responsiveness	0	2	3	1	0	1	3	1	1	2	2
	ERE	Ethylene-responsive element	3	1	0	0	1	0	3	1	1	0	0
	GARE-motif	gibberellin-responsive element	0	1	0	0	1	1	0	0	0	0	0
	Myc	light, abscisic acid (ABA), and jasmonic acid (JA) signaling pathways	0	1	0	0	0	2	1	1	0	0	1
	P-box	gibberellin-responsive element	0	0	0	0	1	2	0	0	0	0	1
	TCA	salicylic acid-responsive element	2	0	0	1	0	1	1	1	3	0	0
	TCA-element	involved in salicylic acid responsiveness	1	0	1	1	1	0	0	1	1	1	0
	TGACG-motif	involved in the MeJA-responsiveness	0	2	3	1	0	1	3	1	1	2	2
	TGA-element	auxin-responsive element	0	0	2	1	0	1	0	0	0	0	2
Light Responsive Elements	3-AF1 binding site	light responsive element	0	0	0	0	0	0	1	1	2	0	0
	AE-box	part of a module for light response	0	0	1	0	0	0	0	0	0	0	0
	AT1-motif	part of a light responsive module	0	0	0	0	0	0	1	0	0	0	0
	ATCT-motif	part of a conserved DNA module involved in light responsiveness	1	0	0	0	1	0	0	0	0	0	0
	Box 4	part of a conserved DNA module involved in light responsiveness	9	4	1	1	4	1	2	1	3	0	2
	chs-CMA1a	part of a light responsive element	1	0	1	1	1	1	2	1	0	0	0
	chs-Unit 1 m1	part of a light responsive element	0	0	0	0	0	0	0	0	0	0	1
	GA-motif	part of a light responsive element	0	0	0	0	0	1	1	0	0	0	0

	Gap-box	part of a light responsive element	1	0	0	1	0	1	0	0	0	0	0
	GATA-motif	part of a light responsive element	0	0	0	1	0	2	0	1	1	2	1
	G-Box	involved in light responsiveness	2	1	4	1	2	2	1	1	1	0	1
	G-box	involved in light responsiveness	1	2	0	3	5	2	2	1	0	2	2
	GT1-motif	light responsive element	1	5	2	0	1	3	0	1	0	1	0
	GTGGC-motif	part of a light responsive element	0	0	0	0	0	0	1	0	0	0	0
	I-box	part of a light responsive element	0	0	1	1	2	2	0	0	2	0	0
	LAMP-element	part of a light responsive element	0	0	1	0	0	0	0	0	0	0	0
	L-box	part of a light responsive element	1	0	0	0	0	0	0	0	0	0	0
	MRE	MYB binding site involved in light responsiveness	1	0	0	0	0	2	0	0	0	0	1
	Sp1	light responsive element	0	0	0	0	0	0	0	1	0	0	0
	TCCC-motif	part of a light responsive element	0	1	0	0	0	0	0	0	0	0	0
Site-Binding Elements	TCT-motif	part of a light responsive element	2	0	0	1	2	1	1	0	0	0	0
	AT-rich element	binding site of AT-rich DNA binding protein (ATBP-1)	0	0	0	0	0	1	0	0	0	0	0
Stress Releted Elements	CCAAT-box	MYBHv1 binding site	0	1	0	0	1	0	1	1	0	0	0
	AP-1	Cd response	0	1	0	0	0	0	0	0	0	0	0
	ARE	essential for the anaerobic induction	2	2	3	1	0	0	1	0	2	3	0
	box S	Wounding and pathogen responsievness	0	0	0	0	0	0	0	0	2	1	0
	DRE core	drought response	0	1	0	1	0	0	0	0	0	0	1
	GC-motif	enhancer-like element involved in anoxic specific inducibility	0	0	0	1	0	0	0	0	1	0	0
	LTR	involved in low-temperature responsiveness	2	0	2	1	2	1	0	0	1	1	0
	MBS	MYB binding site involved in drought-inducibility	0	1	1	0	2	0	0	1	0	0	0
	MYB	water response, drought response	1	7	4	1	4	3	1	4	2	2	4
	MYB recognition site	drought response	0	1	0	0	1	0	1	1	0	0	0
	MYC	drought response	3	1	1	5	1	4	2	3	3	5	4

	STRE	stress-response element	0	2	1	1	2	0	2	2	1	3	2
	TC-rich repeats	involved in defense and stress responsiveness	0	1	0	1	0	0	0	0	1	0	0
	W box	Wounding and pathogen responsiveness	0	0	3	3	1	0	0	1	1	0	1
	WRE3	wounding	0	0	1	4	0	0	0	1	2	0	0
	WUN-motif	Wound-responsive element	1	0	0	1	0	0	2	3	1	2	0
Transcription Regulation Time Related Elements	O2-site	involved in zein metabolism regulation	0	0	2	0	0	0	3	2	0	0	1
	A-box	involved in specific day-length	1	0	0	1	0	0	0	0	0	0	0
	circadian	involved in circadian control	0	0	1	0	0	1	0	0	0	0	0

Table S3 The frequency and function of regulatory elements identified in the promoter region of *NaDEF* genes in *Nicotiana attenuata* in Hormonal response and stress obtained from the Plant Care database.

Category	Cis-element	Function	Frequency
Hormonal response	ABRE	Involved in the abscisic acid responsiveness	138
	ABRE3a	Involved in the abscisic acid responsiveness	
	ABRE4	Involved in the abscisic acid responsiveness	
	as-1	SA and Auxin responsive element	
	CARE	ABA response, GA response	
	CGTCA-motif	Involved in the MeJA-responsiveness	
	ERE	Ethylene-responsive element	
	GARE-motif	Gibberellin-responsive element	
	MYC	Light, abscisic acid (ABA), and jasmonic acid (JA) signaling pathways	
	P-box	Gibberellin-responsive element	
	TCA	Salicylic acid-responsive element	
	TCA-element	Involved in salicylic acid responsiveness	
	TGACG-motif	Involved in the MeJA-responsiveness	
	TGA-element		

Auxin-responsive element

Stress	AP-1 ARE box S DRE core GC-motif LTR MBS MYB MYB recognition site MYC STRE TC-rich repeats W box WRE3 WUN-motif	154
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