ABSCISIC ACID-STRESS-RIPENING (*ASR*) GENE MODULATES RESPONSE TO HIGH SALINITY AND WATER DEFICIT IN DURUM AND COMMON WHEAT

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BACKGROUND

In hot and dry Mediterranean regions, wheat is greatly susceptible to abiotic stresses such as extreme temperatures, drought and salinity, suffering severe yield and quality losses. Identification of genes involved in plant adaptation is crucial to design molecular tools and develop stress-tolerant varieties. Abscisic acid, stress, ripening-induced genes (*ASR*) act in the protection mechanism against salinity and water deficit in several plant species. The *TtASR1* gene from 4A chromosome of durum wheat was previously isolated for the first time in a salt-tolerant Tunisian landrace, and its involvement assessed in plant response to some developmental and environmental stimuli.

OBJECTIVES

- ✓ Focusing attention on *ASR* genes located on the homoeologous chromosome group 4 of common (4A, 4B, 4D) and durum (4A, 4B) wheat.
- ✓ *In planta* evaluating the role of *ASR* genes in the modulation of wheat adaptation to high salinity and water deficit by using for the first time a Real-Time PCR approach.
- ✓ Identifying a suitable and reliable DNA-based parameter to discriminate between stress-susceptible and stress-tolerant wheat genotypes.

METHODS

ASR gene expression was profiled by Real-Time qRT PCR (Fig.1, 2) in Tunisian genotypes with contrasting phenotype for drought and salinity tolerance. Common wheat cultivars were Ta001[T] (salt/drought tolerant) and Ta002[S] (salt/drought susceptible); durum varieties were the tolerant HmiraK1185757[T] and the susceptible HmiraK11835[S]. *ASR* expression was evaluated under different variables: stress (salt vs. drought), ploidy (durum vs. common wheat), genotype (S vs. T), tissue (roots vs. leaves), time after treatment (6, 24, 72 h), gene chromosome location (4A/4B/4D genomes). High salinity and drought were simulated by applying 200 mM NaCl, or 15% PEG to leaves and roots.

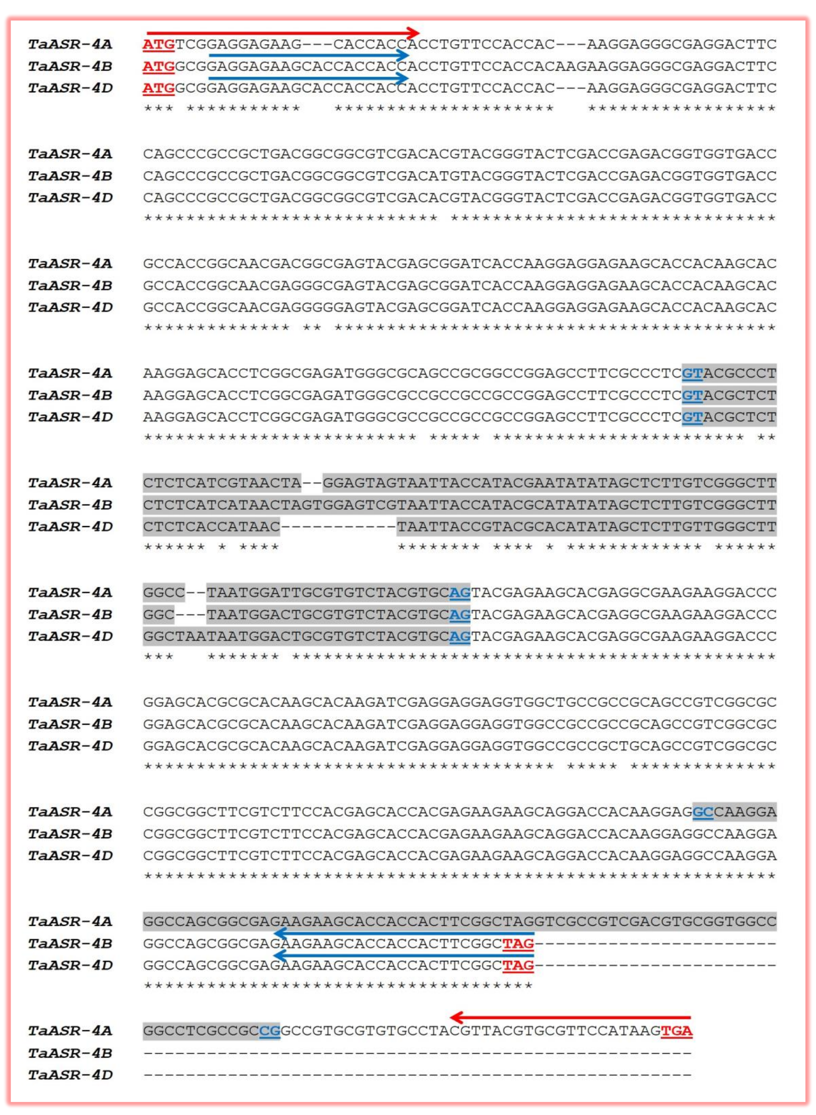

Figure 1. Multiple alignments of common wheat homoeologous *TaASR* genes on 4A, 4B, and 4D chromosomes. Grey = intron. Blue = splice junctions. Red arrow = A-genome specific primer pair for Real-Time qRT-PCR. Blu arrow = primer for Real-Time qRT-PCR of homoeologous *TaASR* genes on 4B and 4D.

RESULTS

- ➢ *ASR* response was slightly affected by ploidy level or chromosomal location, as durum and common wheat exhibited a similar gene expression following salt increase and water deficiency (Fig.3, 4).
- ➢ Gene profile was more influenced by plant tissue (roots more responsive than leaves), type of stress (NaCl more impacting than PEG), and genotype (differential transcripts accumulation in susceptible or tolerant genotypes) (Fig.3, 4).
- ➢ In durum wheat, expression variation of *TtASR-4A/4B* genes was able to discriminate between salt[S] and salt[T] genotypes (Table 1).
- ➢ In common wheat, expression levels of *TtASR-4A/4B/4D* can discriminate between SUS and TOL genotypes in both salt and water stresses (Table 1).
- ➢ *ASR* involvement was confirmed in plant adaptation to high salinity and water deficit in both *T. aestivum* and *T. durum* (Fig.5).

Durum Wheat

Stress	Tissue	Genotype	*TtASR-4A/4B*
Salt stress (200 mM NaCl)	Leaf	*HmiraK11835* [S]	↑
		HmiraK11857 [T]	↑
	Root	*HmiraK11835* [S]	↓
		HmiraK11857 [T]	↑
Water stress (15% PEG)	Leaf	*HmiraK11835* [S]	↑
		HmiraK11857 [T]	-
	Root	*HmiraK11835* [S]	↓
		HmiraK11857 [T]	↓

Common Wheat

Stress	Tissue	Genotype	*TaASR-4A*	*TaASR-4B/4D*
Salt stress (200 mM NaCl)	Leaf	*Ta002* [S]	-	↓
		Ta001 [T]	-	↓
	Root	*Ta002* [S]	↓	↓
		Ta001 [T]	↑	↑
Water stress (15% PEG)	Lea	*Ta002* [S]	↓	↓
		Ta001 [T]	↓	↓
	Root	*Ta002* [S]	↓	↓
		Ta001 [T]	↑	↑

↑ Gene induction
↓ Gene repression

Table 1. *ASR* response to salinity and drought in leaves and roots of Tunisian durum and common wheat varieties. Gene up- or down-regulation is given by expression fold-change variation relative to not-treated samples, at 72h after treatment. - = not significant variation.

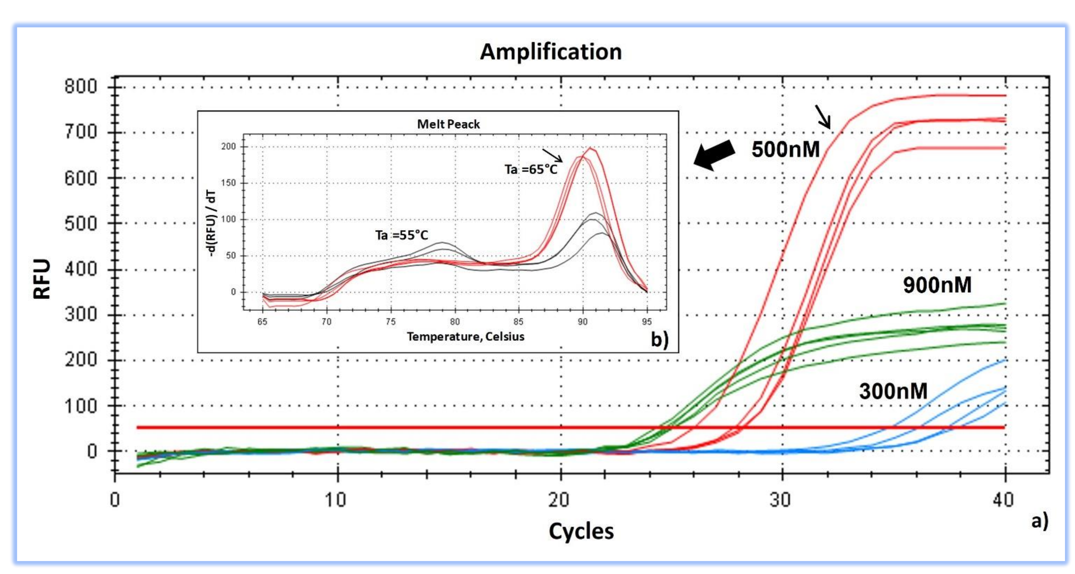

Figure 2. Preliminary qRT-PCR assay with fluorescent SYBRGreen dye of *TaASR-4B/4D* genes in common wheat cv. Ta002[S]. **a)** Testing of primer concentration in a 300-900 nM gradient. Arrow indicates optimal primer concentration **b)** Optimization of annealing temperature: primer specificity is showed by amplicons melting curves.

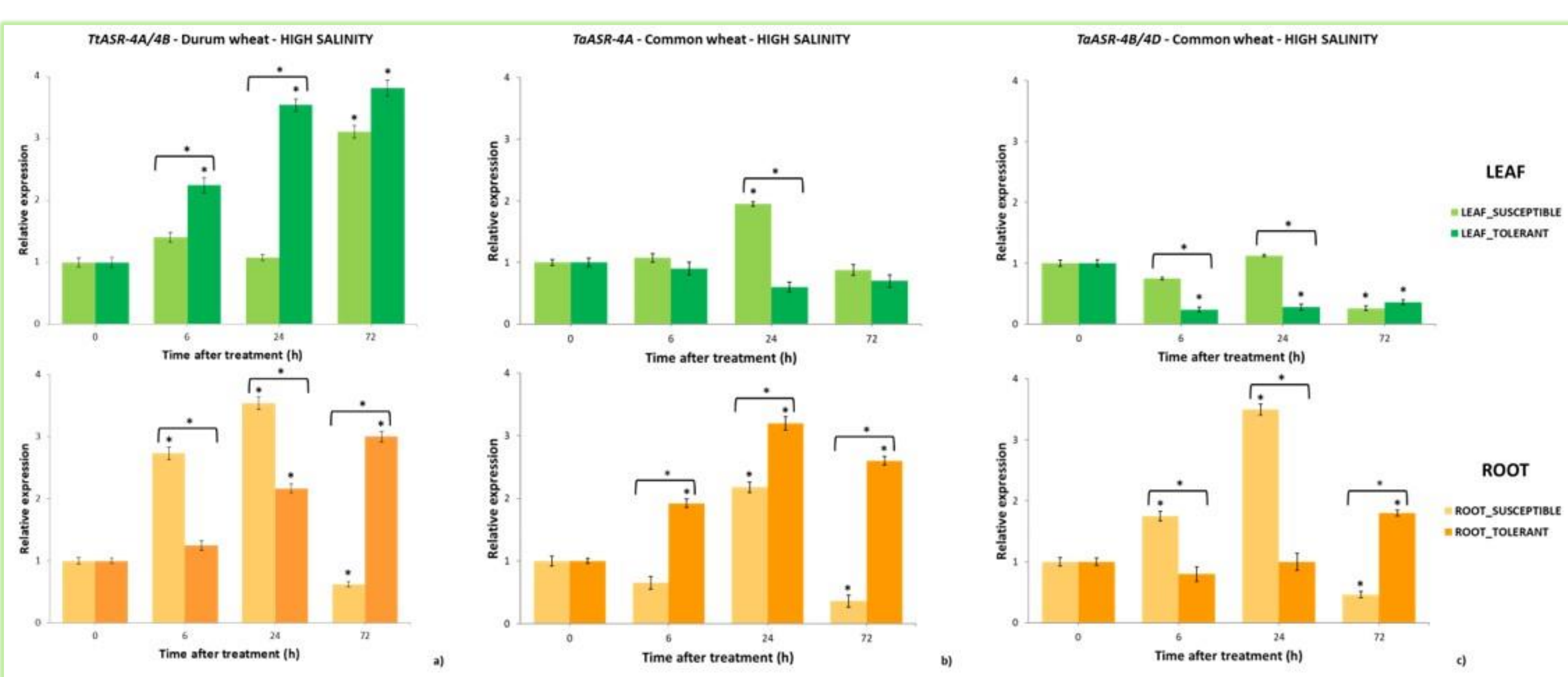

Figure 3. qRT-PCR time-course expression of *ASR* genes in leaves and roots of salt-susceptible and salt-tolerant Tunisian genotypes after 200 mM NaCl application. Values are reported as fold-changes relative to control. Asterisks indicate data significantly different between samples and control (* on bars) or between susceptible and tolerant genotypes (* over two bars), according to Student's t-test (*$p < 0.05$).
(a) Expression profile of *TtASR* genes from 4A and 4B chromosomes of tetraploid genotypes.
(b), (c) Expression profile of *TaASR* genes from 4A and 4B/4D chromosomes of hexaploid genotypes.

Leaf-Susceptible / Leaf-Tolerant

		Gene	Fold change vs Control - 6 H	Fold change vs Control - 24 H	Fold change vs Control - 72 H	Mean	Fold change vs Control - 6 H	Fold change vs Control - 24 H	Fold change vs Control - 72 H	Mean
Durum wheat	NaCl	*TtASR-4A/4B*	1.41	1.08	3.12		2.25	3.54	3.81	
	PEG	*TtASR-4A/4B*	0.41	1.50	1.11		0.40	1.05	0.87	
Common wheat	NaCl	*TaASR-4A*	1.08	1.95	0.88	1.30	0.90	0.60	0.70	0.70
	NaCl	*TaASR-4B/4D*	0.75	1.13	0.26	0.71	0.24	0.28	0.36	0.29
	PEG	*TaASR-4A*	1.20	0.84	0.20	0.75	0.32	0.50	0.25	0.36
	PEG	*TaASR-4B/4D*	0.68	0.45	0.33	0.49	0.38	0.30	0.47	0.38

Root-Susceptible / Root-Tolerant

		Gene	Fold change vs Control - 6 H	Fold change vs Control - 24 H	Fold change vs Control - 72 H	Mean	Fold change vs Control - 6 H	Fold change vs Control - 24 H	Fold change vs Control - 72 H	Mean
Durum wheat	NaCl	*TtASR-4A/4B*	2.73	3.55	0.62		1.25	2.17	3.00	
	PEG	*TtASR-4A/4B*	1.67	0.89	0.76		3.45	2.45	0.48	
Common wheat	NaCl	*TaASR-4A*	0.65	2.18	0.36	1.06	1.92	3.20	2.60	2.57
	NaCl	*TaASR-4B/4D*	1.75	3.50	0.46	1.90	0.80	1.00	1.80	1.20
	PEG	*TaASR-4A*	1.86	4.30	0.45	2.20	2.46	2.56	4.88	3.30
	PEG	*TaASR-4B/4D*	1.50	1.65	0.33	1.16	2.30	3.30	4.97	3.52

Legend: 0 - 0.5; 0.51 - 1; 1.01 - 1.5; 1.51 - 2; 2.01 - 2.5; 2.51 - 3; 3.01 - 4; > 4

Figure 5. Heat map of *ASR* gene expression levels in leaves and roots of salt-draught susceptible/tolerant Tunisian durum and common wheat genotypes under high salinity and water deficit. Colors represent magnitude of gene expression variation as fold-changes relative to control (0 h, set to 1) at 6, 24, and 72 h post stress application.

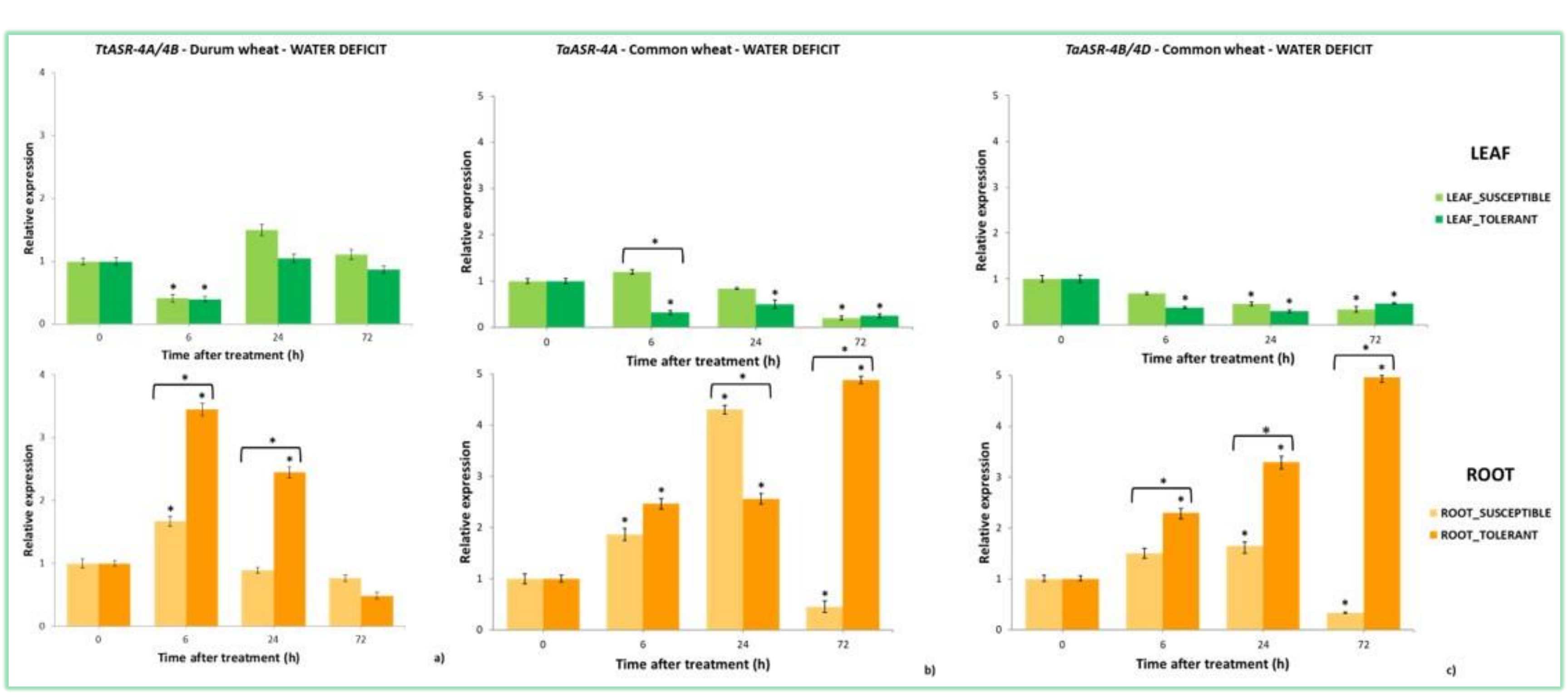

Figure 4. qRT-PCR time-course expression of *ASR* genes in leaves and roots of drought-susceptible and drought-tolerant Tunisian genotypes after 15% PEG application. Values are reported as fold-changes relative to control. Asterisks indicate data significantly different between samples and control (* on bars) or between susceptible and tolerant genotypes (* over two bars), according to Student's t-test (*$p < 0.05$).
(a) Expression profile of *TtASR* genes from 4A and 4B chromosomes of tetraploid genotypes.
(b), (c) Expression profile of *TaASR* genes from 4A and 4B/4D chromosomes of hexaploid genotypes.

CONCLUSIONS

- ✓ *ASR* genes were confirmed key factors influencing wheat adaptability to high salinity and drought.
- ✓ Quantification of *ASR* expression after long salt exposure (72 h) was a reliable parameter to discriminate between salt-tolerant and salt-susceptible genotypes in roots of both *T. aestivum* and *T. durum* (Table 1).
- ✓ *ASR* was a robust candidate gene for abiotic stress resistance, and a tool for the development of functional markers to support breeding programs for more tolerant and productive varieties via marker-assisted selection.