Supplementary file

Validation of transcriptome experiments using qRT-PCR

Methods

1 µg of RNA (samples, used for RNA-SEQ) was used for cDNA synthesis using RevertAid reverse transcriptase (Thermo Scientific) according to manufacturer's instructions. Two µl of 5-fold-diluted cDNA were used as the template for qPCR. Real-time polymerase chain reaction (qPCR) was carried out on a CFX96 Touch RT-PCR Detection System (Bio-Rad, USA). The efficiency of the primers was calculated by performing RT-PCR on several dilutions of first-strand cDNAs. Efficiencies of the different primer sets were similar. A cDNA dilution of 2.5 µl (1/15) was used for amplification. The PCR mixture (10 µl) was constituted by 0.4 µM of each forward and reverse gene-specific primer, 0.2 mM dNTPs, 1× SYBR Green (Sigma, USA) and 0.1 µl of 5 U µl−1 heat-stable Taq Polymerase (Evrogen, Russia). The thermal cycling conditions were 95°C for 3min, 40 cycles each at 95°C for 15 s and 60°C for 1 min. A 60-to-95°C melting curve was constructed to confirm specificity of the products. For each of the two biologically independent cDNA samples, two independent technical replications were performed and averaged for further calculations. Gene-specific primers for the analyzed genes were designed using the Universal ProbeLibrary Assay Design Centre (http://lifescience.roche.com/). Expression of *FesTIR1*, *FesARF19*, *FesEXP1*, *FesSAND* and *FesCACS* genes characterized in the table below was evaluated by qPCR. Three housekeeping genes were used [1]. The level of expression of each housekeeping gene was calculated as well (by normalization relatively to two remaining housekeeping genes). The relative standard curve method was used for calculation of expression (https://www.gu.se/digitalAssets/1125/1125331_ABI_-_Guide_Relative_Quantification_using_realtime_PCR.pdf). Relative expression levels were determined as the ratios between the quantities of cDNA corresponding to the target genes and values of housekeeping genes, then ratio between treated samples and control samples was calculated (Figure).

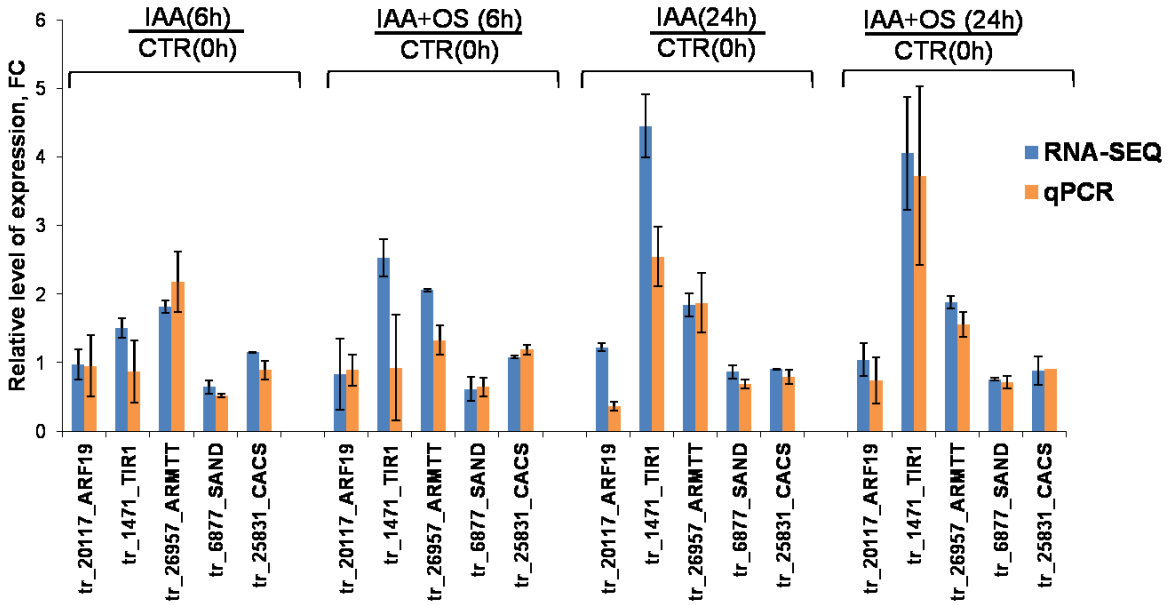

Figure. Comparison of gene expression values obtained by qRT-PCR and RNA-SEQ analyses.

Primer sequences

name (f - forward, r- reverse)	**tr**	**Primer sequences**	**Arabidopsis ortholog**	**Ref**
FesTIR1_f	tr_1471 tr_11620 (paralogs)	GATGCTATCCGTGGCTTTTG	AT3G62980, transport inhibitor response 1	
FesTIR1_r		CAGCCAGATAGCACATGACAG		
FesARF19_f	tr_20117	GAGATGCATTGACGTGAACC	AT1G19220, auxin response factor 19	
FesARF19_r		CCTTCAATCCCAAACATACGA		
FesARMTT_f (Exp1_f [1])	tr_26957	AGGCCAGTTCCTGCTGAATGTAATGC	AT4G33380, dimethylallyl, adenosine tRNA methylthiotransferase	[1]
FesARMTT_r (Exp1_r [1])		TAGCCTGATCCAAACAAGCCTGGCAA		
FesCACS_f	tr_25831	AAGACAGTCAGTTTCGTGCCACCTGA	AT5G46630, clathrin adaptor complexes medium subunit family protein	[1]
FesCACS_r		TCCATGCGTGTTCTACCCAACTCCTT		
FesSAND_f	tr_6877	GACCCCCTTGCAGACAAAGCATTGGCA	AT2G28390, SAND family protein	[1]
FesSAND_r		TCTCGTTCTCAACGTCTTTTACCCACTGG		

CDS sequences http://fagopyrum.org/ (yellow – forward primer, blue – reverse primer)

tr_1471

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