Supplementary material

Effect of fieldwork-friendly coffee blender-based extraction methods and leaf tissue storage on the transcriptome of non-model plants

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1.1 Nucleic acid extraction (DNA+RNA)

T (control): RNA was extracted by TRIzol reagent according to the manufacturer's manual with small modifications.

A fresh leaf was harvested from the pot and ground into powder in liquid N_2 by mortar and pestle. 0.05 g of the ground powder was added to a volume of 1 ml TRIzol solution in an RNase-free 1.5 ml tube. The mixture was vortexed briefly and incubated at room temperature for 5 minutes. 980 μ l of the mixture was transported to a new 1.5 ml tube in order to get rid of the plant debris and 200 μ l of chloroform was added to the mixture, then vortexed, and incubated at room temperature for 2 minutes. The mixture was centrifuged at 12000 g for 15 minutes at 4 °C. 500 μ l of the aqueous phase was carefully transported to a new 1.5 ml tube, 500 μ l of isopropanol was added, mixed thoroughly by vortex and incubated at room temperature for 10 minutes. The sample was centrifuged at 12000 g for 10 minutes at 4 °C. The supernatant was removed by a pipette and the pellet was washed by 800 μ l of 75% ethanol. The sample was centrifuged at 12000 g for 5 minutes at 4 °C. The supernatant was removed again and the pellet was washed again by 75 % ethanol. The sample was centrifuged again at 12000 g for 5 minutes at 4 °C. The supernatant was discarded and the pellet was dried for 3 min in a vacuum. The pellet was resuspended in 40 – 100 μ l of distilled water.

R1 (storage): 0.2 g of plant leaf was cut by sterilized scissors into smaller pieces and collected into 1.3 ml of RNA*later* in a 1.5 ml tube.

After preserving at 25 °C for 24 hours, the leaf particles were briefly dried on sterile paper tissue, and then ground in liquid nitrogen using mortar and pestle. The remaining steps were followed as in the state-of-art method. T.

R14 (storage): 0.2 g of plant leaf was cut by sterilized scissors into smaller pieces and collected into 1.3 ml of RNA*later* in a 1.5 ml tube.

After preserving at 25 °C for 14 days, the leaf particles were briefly dried on sterile paper tissue, and then ground in liquid nitrogen using mortar and pestle. The remaining steps were followed as in the state-of-art method, T.

T1 (storage): A fresh leaf was harvested from the pot and ground into powder in liquid N₂ by mortar and pestle. 0.05 g of the ground powder was preserved in 1 ml of TRIzol solution in an RNase-free 1.5 ml tube at 25 °C for 24 hours. The remaining steps were followed as in the state-of-art method, T.

T14 (storage): A fresh leaf was harvested from the pot and ground into powder in liquid N₂ by mortar and pestle.

0.05 g of the ground powder was preserved in 1 ml of TRIzol solution in an RNase-free 1.5 ml tube at 25 °C for 14 days. The remaining steps were followed as in the state-of-art method, T.

TR (on-site): RNA was extracted by TRIzol reagent according to the manufacturer's manual with few modifications to use with portable equipment.

A fresh leaf was harvested from the pot and ground into powder in liquid N_z by mortar and pestle. 0.05 g of the ground powder was added to a volume of 1 ml TRIzol solution in an RNase-free 1.5 ml tube. The mixture was vortexed briefly and incubated at room temperature for 5 minutes. 980 μ l of the mixture was transported to a new 1.5 ml tube in order to get rid of the plant debris. 200 μ l of chloroform was added to the mixture, then vortexed, and incubated at room temperature for 2 minutes. The mixture was centrifuged at 12000 g for 15 minutes at room temperature. 500 μ l of the aqueous phase was carefully transported to a new 1.5 ml tube, 500 μ l of isopropanol was added, mixed thoroughly by vortex and incubated at room temperature for 10 minutes. The sample was centrifuged at 12000 g for 10 minutes at room temperature. The supernatant was removed by a pipette and the pellet was washed by 800 μ l of 75 % ethanol. The sample was centrifuged at 12000 g for 5 minutes at room temperature. The supernatant was removed again and the pellet was washed again by 75 % ethanol. The sample was centrifuged again at 12000 g for 5 minutes at room temperature. The supernatant was discarded and the pellet was dried at 37 °C for 10 minutes. The pellet was resuspended in 40 μ l of distilled water.

CT (on-site): CTAB TRIzol, Plant leaf homogenized in CTAB EB using a coffee-blender followed by the addition of TRIzol reagent. The remaining steps are as per the instructions in the TRIzol reagent manual, but at room temperature.

A newly harvested fresh leaf (0.5 g) in 13 ml of CTAB EB and 195 μ l of beta mercaptoethanol (1.5 %) was mixed for 40 seconds by mini-blender and incubated at room temperature for 3 minutes. 500 μ l of the mixture was transported into an RNase-free 2 ml tube and 1 ml of TRIzol solution was added to the sample and the final mixture was incubated at room temperature for 5 minutes. 200 μ l of chloroform was added to the mixture, then vortexed, and incubated at room temperature for 2 minutes. The mixture was centrifuged at 12000 g for 15 minutes at room temperature. 900 μ l of the aqueous phase was carefully transported to a new 2 ml tube, 900 μ l of isopropanol was added, mixed thoroughly by vortex and incubated at room temperature for 10 minutes. The sample was centrifuged at 12000 g for 10 minutes at room temperature. The supernatant was removed by a pipette and the pellet was washed by 800 μ l of 75 % ethanol. The sample was centrifuged at 12000 g for 5 minutes at room temperature. The supernatant was removed again and the pellet was washed again by 75 % ethanol. The sample was centrifuged again at 12000 g for 5 minutes at room temperature. The supernatant was discarded and the pellet was dried at 37 °C for 10 minutes. The pellet was resuspended in 40 μ l of distilled water.

CC (on-site): CTAB coffee-blender, Plant leaf homogenized in CTAB EB using a coffee-blender. The remaining steps were carried out as in Gambino et al. (2008), but without lithium chloride precipitation and at room temperature.

A newly harvested fresh leaf (0.5 g) in 13 ml of CTAB EB and 195 μ l of beta-mercaptoethanol (1.5 %) was mixed for 40 seconds by mini-blender and incubated at room temperature for 3 minutes. 900 μ l of the mixture was transported into an RNase-free 2 ml tube, 900 μ l of chloroform was added to the solution, and mixed thoroughly by vortex. The phase separation was done by centrifugation at 12000 g for 5 minutes at room temperature. 800 μ l of the aqueous phase was transported into a new 2 ml tube, 800 μ l of chloroform was added to the solution, and mixed thoroughly by vortex. The phase separation was done by centrifugation at 12000 g for 5 minutes at room temperature.

500 µl of the aqueous phase was transported into a new 1.5 ml tube, 500 µl of isopropanol was added, mixed thoroughly by vortex and incubated at room temperature for 10 minutes. The sample was centrifuged at 12000 g for 10 minutes at room temperature. The pellet was resuspended in 400 µl of distilled water, 16 µl of ammonium

acetate and 400 μ l of isopropanol was added to the sample and incubated at room temperature for 10 minutes. The sample was centrifuged at 12000 g for 10 minutes at room temperature. The supernatant was removed by a pipette and the pellet was washed by 800 μ l of 75 % ethanol. The sample was centrifuged at 12000 g for 5 minutes at room temperature. The supernatant was removed again and the pellet was washed again by 75 % ethanol. The sample was centrifuged again at 12000 g for 5 minutes at room temperature. The supernatant was discarded and the pellet was dried at 37 °C for 10 minutes. The pellet was resuspended in 40 μ l of distilled water.

1.2 Total RNA extraction

DNase I digestion: All samples were digested by recombinant DNase I (Takara Bio Inc.) according to the manufacturer's instructions with small modifications using Eppendorf MiniSpin® plus. Inactivation of DNase I was done by heat treatment as described in the manual. Resulting pellet was dissolved in MilliQ water.

25 μ l of mixture (20 μ l of sample, 2.5 μ l of 10 x DNase I Buffer, 1 μ l of Recombinant DNase I and 1.5 μ l of distilled water) was incubated at 37 °C for 20 minutes. 2.5 μ l of 0.5 M EDTA was added to the sample and incubated at 80 °C for 2 minutes. The sample volume was increased to 100 μ l with RNase-free water, 4 μ l of 7.5 M ammonium acetate (final concentration 0.3 M) and 100 μ l of isopropanol were added and incubated at room temperature for 10 minutes. The sample was centrifuged at 12000 g for 5 minutes at room temperature. The supernatant was removed and the pellet was washed with 75 % ethanol. The sample was centrifuged at 12000 g for one minute at room temperature. The pellet was dried and re-suspended in 20 μ l of distilled water.

Supplementary tables

Table S1. RNA-Seq results of before and after quality control.

Methods	Raw reads*	Clean reads**	Read loss (%)
T	26,666,667	26,619,131	0.18
R1	26,666,667	26,617,381	0.18
R14	26,666,667	26,614,814	0.19
T	26,666,667	26,590,280	0.29
T1	26,666,667	26,612,698	0.20
T14	26,666,667	26,602,120	0.24
T	26,333,334	26,208,624	0.47
TR	26,333,334	26,205,648	0.48
CT	26,333,334	26,256,055	0.29
CC	26,333,334	26,255,060	0.29

Table S2. DEG functional annotation, GO enrichment, and fold-change values

Excel file "GO_enriched_DEGs.xlsx

^{*}The total raw data were downsampled by random selection using seqtk to approximately 8 Gb for each sample (Li 2012).

**Clean paired reads were trimmed by using Trim Galore version 0.6.7 (Phred score of Q = 20, stringency 6 bp, removal of reads below 100 bp as well as non-paired reads) (Felix et al. 2023).

Table S3. Selection of transcripts and the primers for RT-qPCR

Label	Transcript Name	GO number	GO meaning	Primers		Expected Band Size
Gene A	TRINITY_DN8584_c0_g1_i10	GO:0005576	extracellular region	Forward	GTCGTTGTTGGGGAGGTCAT	183 bp
				Reverse	GTACAGCGAAGGATACGGGG	
Gene B	TRINITY_DN22707_c0_g3_i1	GO:0003676	nucleic acid binding,	Forward	CCCCGACGTGAATTGCATTC	123 bp
		GO:0008270	zinc ion binding	Reverse	CGTGTCCATGACGGCTATGA	
Gene C	TRINITY_DN69006_c0_g1_i1	GO:0009408	response to heat,	Forward	CGACGCTGCACAGAAATACG	191 bp
		GO:0010115	regulation of abscisic acid biosynthetic	Reverse	TCTCCTTCAACAACCGGCTC	
Gene D	TRINITY DN21064_c0_g1_i2	GO:0003676	process nucleic acid binding,	Forward	TGACCTGGGGATCAAGCAAC	64 bp
Gene D	INDIVITEDINATION I COURT IN	GO:0004523	RNA-DNA hybrid ribonuclease activity	Reverse	GCGAGTGCGTCTGCTAGTAT	Стор
Gene E	TRINITY_DN42639_c0_g1_i6 TRINITY_DN42639_c0_g1_i1	GO:0004674 GO:0005524	protein serine/threonine	Forward	CGCATCGATCATTGCAGCAA	153 bp
	TRINITY_DN42639_c0_g1_i16 TRINITY_DN42639_c0_g1_i18		kinase activity, ATP binding	Reverse	AGACTCAACAGTGATGCCCG]
Gene F	TRINITY_DN13262_c0_g1_i24	GO:0005515	protein binding	Forward	GAAAGGGGTATGCTGCCCTT	167 bp
				Reverse	TAATCGCTTCGAGGGTCCAC	
Gene G	TRINITY_DN5050_c1_g2_i8	GO:0003676	nucleic acid binding	Forward	GATGCGACGACCAAATTGGG	90 bp
				Reverse	TTGGGTGTTTTTGCTGTGCC	
Gene H	TRINITY_DN1560_c0_g1_i4	GO:0000166	nucleotide binding,	Forward	TGTCCTGCATGAAGCTCAGG	188 bp
		GO:0004832	valine-tRNA ligase	Reverse	TCCCAGCCTACAAACAGCAG	
		GO:0005524	activity, ATP binding,			
		GO:0006438	valyl-tRNA			
			aminoacylation			
Gene I	TRINITY_DN78805_c0_g1_i1	GO:0005881	cytoplasmic	Forward	GGCCCTTCTGTTCCTCAGTC	98 bp
		GO:0016020	microtubule, membrane	Reverse	TTGAATGGCTGCTCACCGAT	
Gene J	TRINITY_DN87928_c0_g1_i1	GO:0003676	nucleic acid binding	Forward	TCGGAGGTCGACCAGATTCT	156 bp
				Reverse	CAGCTCTTCTGCGAAGTCCA	
Gene K	TRINITY_DN18789_c0_g1_i5	GO:0005515	protein binding	Forward Reverse	TGCAGCACCTCTTGTTCACA TGGGAGAGAGAGAGAGCC	182 bp

Gene L	TRINITY_DN1961_c0_g1_i9	GO:0003700 GO:0006355 GO:0043565	DNA-binding transcription factor activity, regulation of DNA-templated transcription, sequence-specific DNA binding	Forward Reverse	GCTGGTAGATCAGGTGCTCC ATGGCCGTTGATCTGATGGG	102 bp
Gene M	TRINITY_DN2069_c0_g1_i1	GO:0005507 GO:0005743 GO:0006878 GO:0008535 GO:0016531	copper ion binding, mitochondrial inner membrane, intracellular copper ion homeostasis, respiratory chain complex IV assembly, copper chaperone activity	Forward Reverse	GAATTCCATCGCTGGGTCCA ATTCCGTCGAGCAAGTTCGT	193 bp
Gene N	TRINITY_DN1325_c0_g3_i1	GO:0006979 GO:0016671 GO:0030091	response to oxidative stress, oxidoreductase activity, acting on a sulfur group of donors, disulfide as acceptor, protein repair	Reverse	AGCCAGAAGAAGCCATGGTC CCTGGTTGGATTTCCGTCCA	111 bp
Gene O	TRINITY_DN13281_c0_g3_i1	GO:0016787	hydrolase activity	Forward Reverse	TCTCGGTGGTTCTTCCTTGC GCCCGAGAGAGAATAGGCAC	120 bp
Gene P	TRINITY_DN56702_c0_g2_i1	GO:0003824	catalytic activity	Forward Reverse	AGATTGCCAAGGCTCAGGTC GTCATGGAAAGCAAGGCACC	118 bp
Gene Q	TRINITY_DN126055_c0_g1_i1	GO:0010309	acireductone dioxygenase [iron(II)- requiring] activity	Forward Reverse	CTGGTATATGGACGACGCCC CAGCTTCCAGTAGAGCACCC	111 bp
Gene R	TRINITY_DN137464_c0_g1_i1	GO:0098609	cell-cell adhesion	Forward Reverse	CAGGCTGAGATTGGGCAGAA CTTCTTGGCCCCAAAGCAAC	182 bp

Supplementary figures

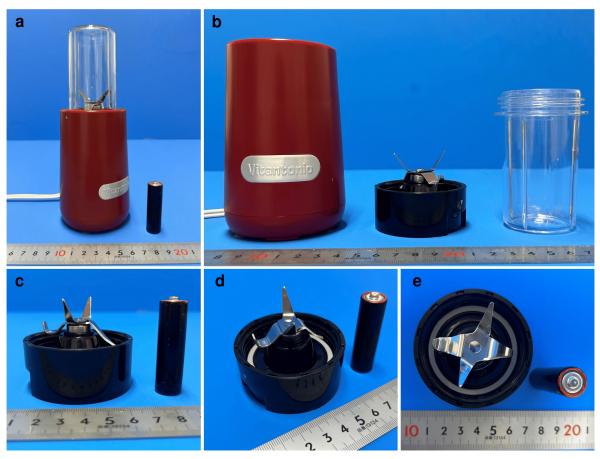


Fig. S1. Coffee blender Vitantonio Mini Bottle Blender VBL-6 used in this study. $\bf a$. Whole side view $\bf b$. Side view separated $\bf c$. Isometric view of the blade $\bf d$. Side view of the blade $\bf e$. Top view of the blade.

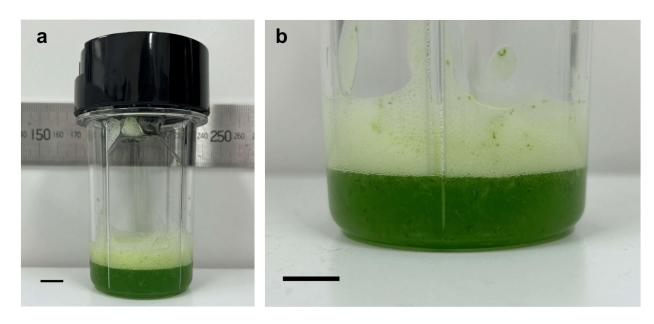


Fig. S2. Plant tissue homogenized in CTAB by coffee-bean blender for 40 seconds. $\bf a$. Whole view. $\bf b$. Zoomed in view. The scale bar is 1 cm.

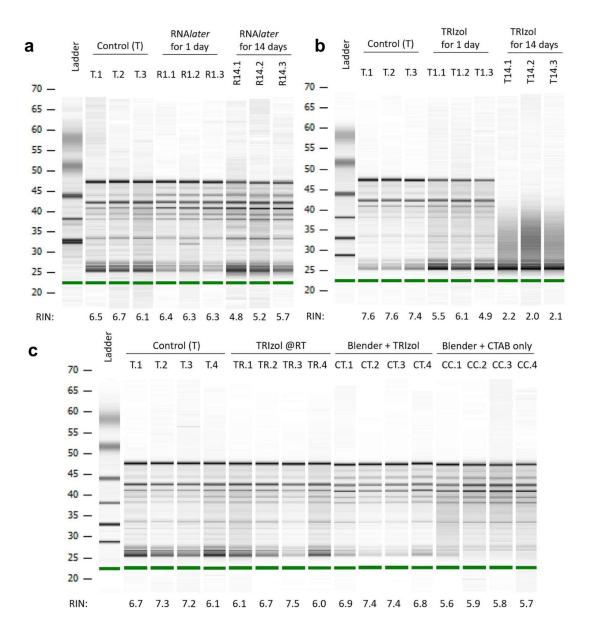


Fig. S3. Electrophoresis and RIN values for all tested conditions and replicates with *H.orientalis*. **a**. Storage in RNA*later* (n = 3). **b**. Storage in TRIzol (n = 3). c. onsite extraction protocols (n = 4) with their control (T). All samples were measured with the RNA 6000 Nano Kit on an Agilent 2100 Bioanalyzer. The numbers after dots represent the replicate number.

GENE ONTOLOGY ENRICHMENT ANALYSIS: BIOLOGICAL PROCESS

ı	<	1E-1	5					
	<	1E-1	0					
ı	·	< 1E-!	5					
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	_							•
ı	R1	R14	T1	T14	TR	СТ	cc	Biological Process
				580				photosynthesis
	10/8							photosynthetic electron
ı			200					photosynthesis, light har
ı					883			regulation of cellular res
ı		10000	100	1000	10000	1000	100	andomombrano system

P value up down

nost detialed in GO hierchary	R1 R14 T1 T14 TR CT CC Biological Process			
	reproductive process			
1 R14 T1 T14 TR CT CC Biological Process	embryo development ending in seed dormancy			
photosynthesis	carbohydrate metabolic process			
photosynthetic electron transport in photosystem II	disaccharide metabolic process			
photosynthesis, light harvesting	sucrose metabolic process			
regulation of cellular respiration	gluconeogenesis			
endomembrane system organization	transport			
acetyl-CoA metabolic process	intracellular transport			
translation	intracellular protein transport			
nucleosome organization	vesicle-mediated transport			
microtubule-based process	calcium ion transport			
carboxylic acid biosynthetic process	proton export across plasma membrane			
carboxylic acid metabolic process	protein refolding			
aromatic amino acid family biosynthetic process	tRNA aminoacylation for protein translation			
arginine catabolic process	alanyl-tRNA aminoacylation			
organonitrogen compound biosynthetic process	response to acid chemical			
spermidine biosynthetic process	response to salt			
spermine biosynthetic process	response to water			
glycine catabolic process	cellular oxidant detoxification			
serine family amino acid biosynthetic process	RNA polyadenylation			
glycine biosynthetic process from serine	cellular respiration			
carbohydrate metabolic process	energy derivation by oxidation of organic compounds			
oligosaccharide metabolic process	negative regulation of cellular biosynthetic process			
cellulose biosynthetic process	IMP salvage			
cell wall organization or biogenesis	regulation of cellular respiration			
cell wall biogenesis	protein phosphorylation			
xyloglucan metabolic process	recognition of pollen			
response to ethylene	translation			
isoprenoid biosynthetic process	cell-cell adhesion			
sulfate reduction	cellular response to lipid			
chlorophyll biosynthetic process	cellular response to alcohol			
glycolytic process	cellular response to abscisic acid stimulus			
glutamine family amino acid catabolic process	abscisic acid-activated signaling pathway			
glutamate biosynthetic process	response to heat			
L-phenylalanine catabolic process	system development			
coenzyme A metabolic process	shoot system morphogenesis			
tetrahydrofolate interconversion	guard cell differentiation			
porphyrin-containing compound biosynthetic process	post-chaperonin tubulin folding pathway			
lipid oxidation	tubulin complex assembly			
phosphatidylcholine metabolic process	maturation of LSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)			
dipeptide transport	regulation of amino acid export			
ATP biosynthetic process	actin filament-based process			
proton motive force-driven ATP synthesis	glucosylceramide catabolic process			

Fig. S4. Simplified result of Biological Process by Gene Ontology Enrichment Analysis using the BiNGO tool in Cytoscape, showing the overrepresentation of upregulated (orange) and downregulated (blue) genes. The statistical significance of the enrichment is indicated as a color gradient (cf. top-left legend).

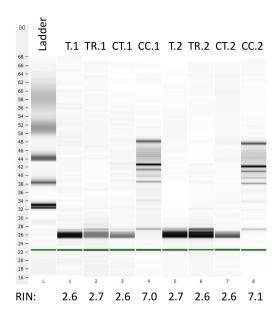


Fig. S5. Electrophoresis and RIN values for for all tested conditions with $Fragaria\ vesca$. T: control, onsite TR: TRIzol at room temperature, CT: CTAB in coffee bean blender followed by TRIzol, CC: CTAB in coffee bean blender. All samples were measured using the RNA 6000 Nano Kit on an Agilent 2100 Bioanalyzer. These conditions were replicated (n = 4), of which only two were checked by Bioanalyzer. Due to the high level of degradation when using TRIzol, the experiment was stopped at this stage. The numbers after dots represent the replicate number.

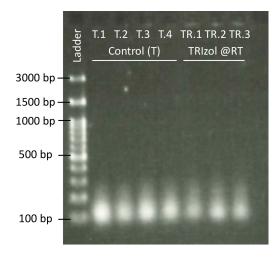


Fig. S6. Electrophoresis image of control (T) (n = 4), onsite TR: TRIzol at room temperature protocols (n = 3) of *Fragaria vesca* leaves, on a 1.5 % TBE gel stained with ethidium bromide.

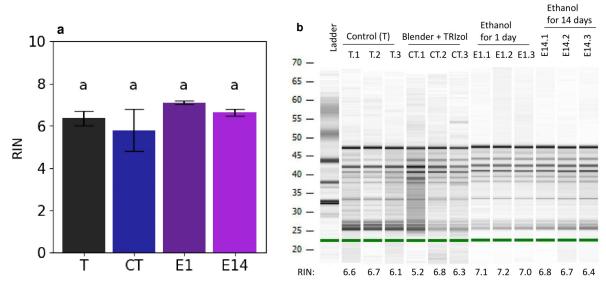


Fig. S7. RNA quality of RNA extracted from H. orientalis leaves measured by Agilent 2100 Bioanalyzer using the Agilent RNA 6000 Nano Kit **a**. Bar graph of the RNA quality (RIN). T: TRIzol control, CT: CTAB in coffee bean blender followed by TRIzol onsite extraction method, E1: Extraction by CT method, followed by storage of the pellet in the 75 % ethanol of the last wash step for 1 day at 25 °C, E14: the same as for E1 but stored for 14 days at 25 °C. All samples were subjected to DNase digestion before running on the Bioanalyzer. The results were compared using the ANOVA post hoc Tukey Honestly Significant Difference Test (P < 0.05). Different letters show significant differences, therefore there were no significant differences between any of the protocols. **b**. Electrophoresis by Bioanalyzer of all samples. All extractions were replicated three times (n = 3).