Supplementary Material

Plant-mediated effects of water deficit on the performance of *Tetranychus evansi* on tomato drought-adapted accessions

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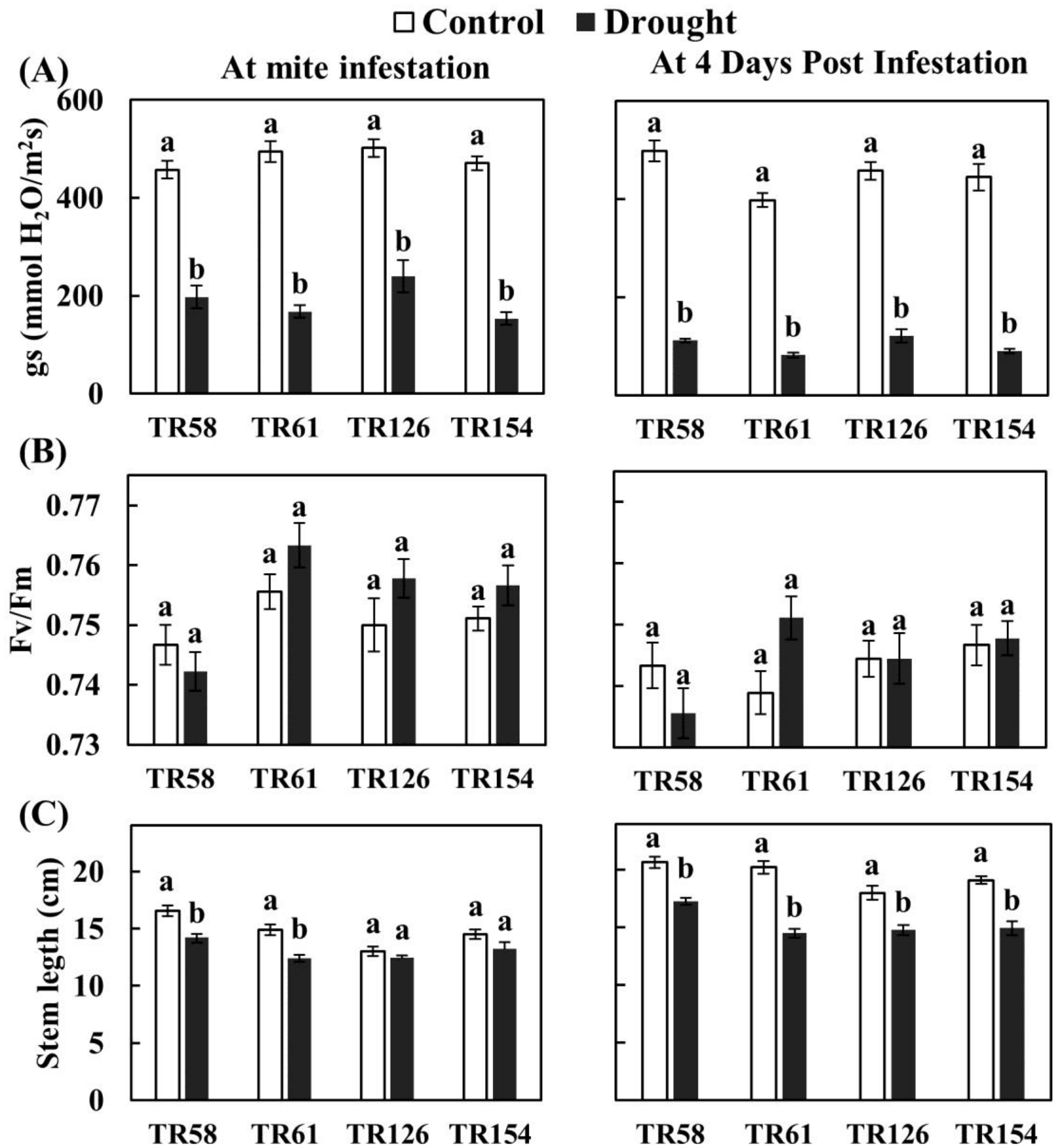
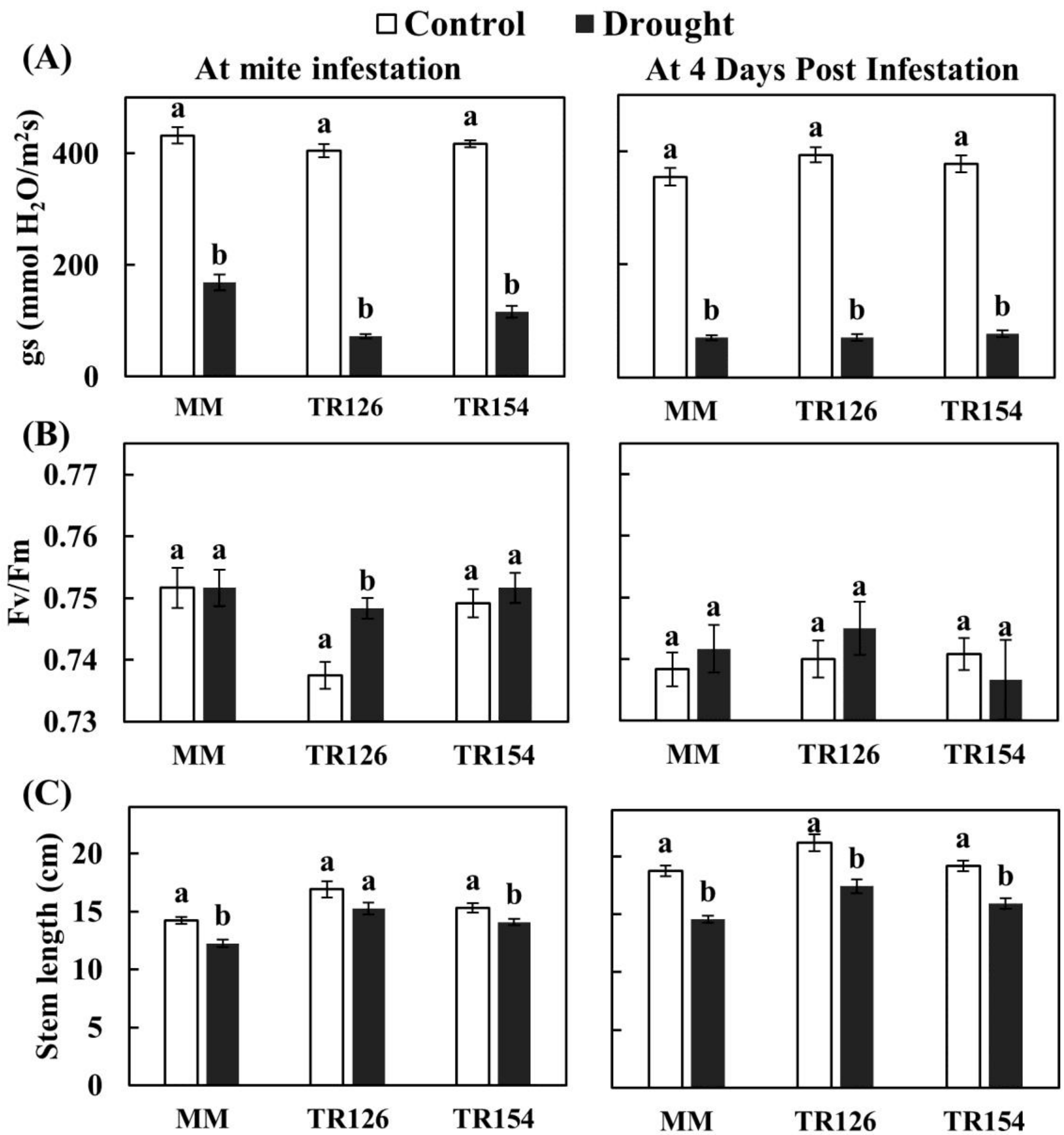
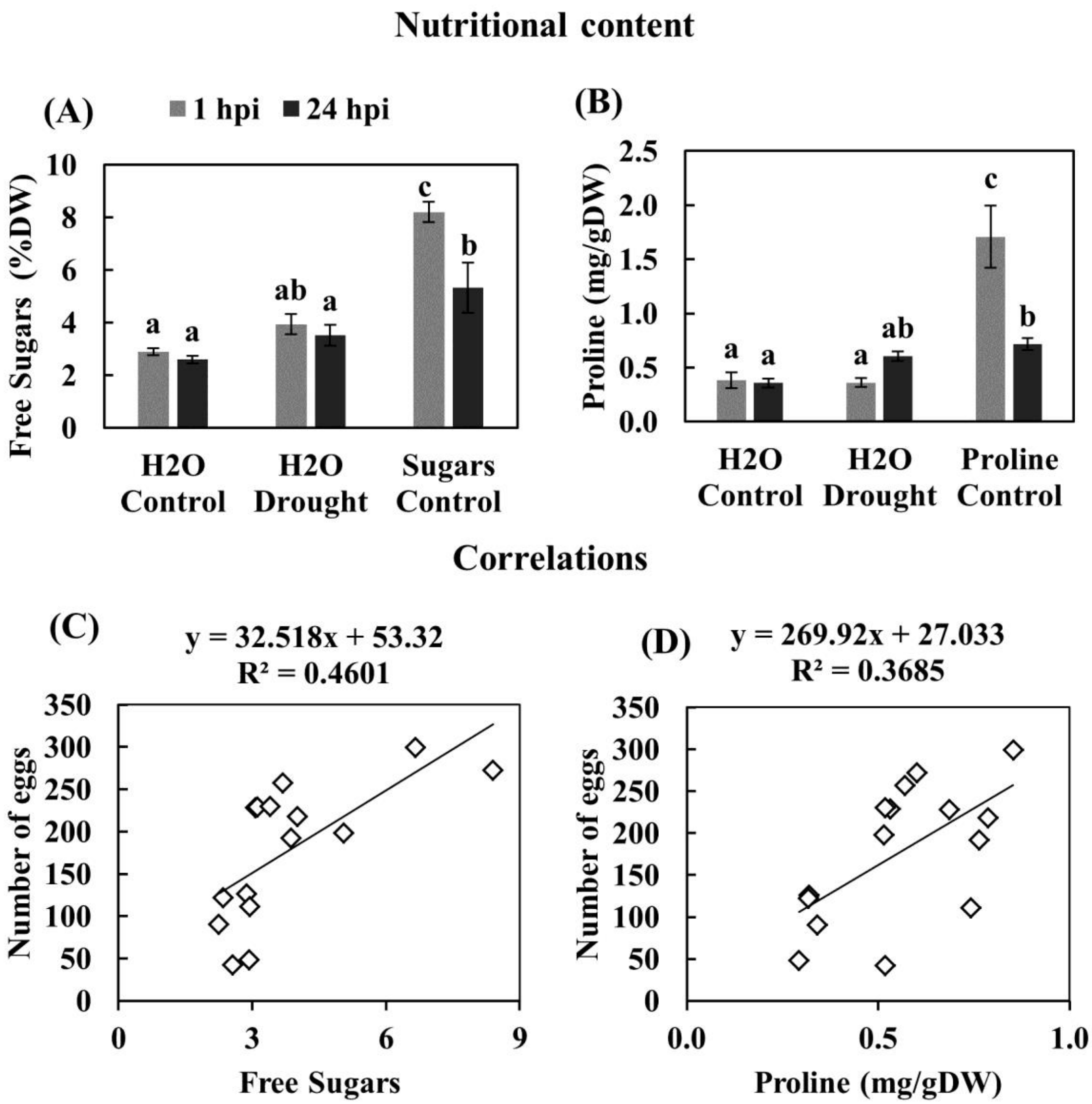
1. Supplementary Figures



Supplementary Figure 1. Effect of moderate drought on A) stomatal conductance (gs), B) maximum quantum yield of PSII photochemistry (Fv/Fm) and C) stem length of the tomato accessions TR58, TR61, TR126 and TR154 at mite infestation and at 4 days post infestation. Data shown are mean ± SE of 9 replicates/treatment from Experiment 1. Different lowercase letters indicate significant differences within each accession (Student's t-test, $p<0.05$). The detailed results (t and p values and degrees of freedom) are shown in Supplementary Table 3.



Supplementary Figure 2. Effect of moderate drought on A) stomatal conductance (gs), B) maximum quantum yield of PSII photochemistry (Fv/Fm) and C) stem length of the tomato cultivar Moneymaker (MM) and the accessions TR126 and TR154 at mite infestation and at 4 days post infestation. Data (mean ± SE) shown are from infested (6 replicates) plus non-infested (6 replicas) plants on Experiment 2. Different lowercase letters indicate significant differences within each cultivar/accession (Student's t-test, $p<0.05$). The detailed results (t and p values and degrees of freedom) are shown in Supplementary Table 4.



Supplementary Figure 3. Content of free sugars (A) and L-proline (B) of control tomato Moneymaker leaflets infiltrated with water, sugar or proline and of drought stressed Moneymaker leaflets infiltrated with water. Data are mean±S.E. of five replicates/treatment at 1 hour post infiltration (hpi) and 24 hpi. Different lowercase letters indicate significant differences among all combinations of treatments and time (One-way ANOVA, Newman-Keuls post hoc test, $p<0.05$. A) Free sugars: $F_{1,24}$: 19.72, $p<0.001$; B) L-Proline $F_{1,24}$: 24.76, $p<0.001$]). Correlation between the leaf content of free sugars (C) and L-proline (D) and the number of eggs laid by *T. evansi* in infiltrated plants at 24 hpi. The Pearson´s correlation coefficient (r) was significant for both free sugars (r: 0.678; p: 0.005) and L-proline (r: 0.608; p: 0.016).

1 Supplementary Tables

Supplementary Table 1. Summary of analytical methods used to assess the inhibitory activity of plant protein extracts[1]

Commercial enzyme [2]	Substrate [3]	Buffer [4]	Incubation	Measurement [5]
Cathepsin B from bovine spleen (EC 3.4.22.1)	Z-RR-AMC	100 mM NA phosphate, pH 6.0 (10 mM L-cysteine, 10 mM EDTA, 0.01% (v/v) Brij 35)	30 min at 28 ºC	excitation filter 350 nm emission filter 465 nm
Papain (EC 3.4.22.2)	Z-FR-AMC	100 mM Na phosphate, pH 6.0 (10 mM L-cysteine, 10 mM EDTA, 0.01% (v/v) Brij 35)	30 min at 28 ºC	excitation filter 350 nm emission filter 465 nm
Cathepsin D from bovine spleen (EC 3.4.23.5)	MocAc-GKPILFFRLK (Dnp)-D-R-NH2	100 mM sodium citrate, pH 3.5 (0.15M NaCl, 5 mM MgCl2)	20 min at 30 ºC	excitation filter 328 nm emission filter 393 nm
Trypsin from bovine pancreas (EC 3.4.21.4)	Z-LA-AMC	100 mM Tris-HCl, pH 7.5 (0.15M NaCl, 5 mM MgCl2)	1 h at 35 ºC	excitation filter 350 nm emission filter 465 nm
α-Chymotrypsin from bovine pancreas (EC 3.4.21.1)	SucAAPF-AMC	100 mM Tris-HCl, pH 7.5 (0.15M NaCl, 5 mM MgCl2)	30 min at 35 ºC	excitation filter 350 nm emission filter 465 nm
Leucine aminopeptidase from porcine pancreas (EC 3.4.11.1)	LpNa	100 mM Tris-HCl, pH 8 (0.15M NaCl, 5 mM MgCl2)	30 min at 30 ºC	absorbance at 410 nm

[1] Procedures adapted from Ximénez-Embún et al. (2016). Samples of 20 µg of plant protein extracts (40 µg in case of leucine aminopeptidase) were preincubated for 10 min with 100 ng of the commercial enzyme.

[2] All purchased from Sigma-Aldricht (St Luis, USA).

[3] The substrates were added at a final concentration of 20 µM. Z-RR-AMC (N-carbobenzoxyloxy-Arg-Arg-7-amido-4-methylcoumarin) for cathepsin B, Z-FR-AMC (N-carbobenzoxyloxy-Phe-Arg-7-amido-4-methylcoumarin) for papain, Z-LA-AMC (Z-L-Arg-7-amido-4-methylcoumarin) for trypsin, SucAAPF-AMC (Suc-Ala-Ala-Pro-Phe-7-amido-4-methylcoumarin) for chymotrypsin, all purchased from Calbiochem (MerkMilipore, Billerica, USA), MocAc-GKPILFFRLK(Dnp)-D-R-NH2 from Peptanova (Germany) for cathepsin D, and LpNa (L-leucine p-nitroanilide) from Sigma-Aldricht (St Luis, USA) for leucine aminopeptidase.

[4] Concentrations are expressed at molarity in the reaction mixture.

[5] AMC (7-amino-4-methylcoumarin) (Bachem, Swizerland) as standard for all fluorescent substrates, except MCA (MoCAC-Pro-Leu-Gly) (Peptanova GmbH, Germany) for cathepsin D. Double blanks were used to account for spontaneous breakdown of substrates and the plant protease activity, and all assays were done in duplicate.

Supplementary Table 2 Nucleotide sequence of primers used for qRT-PCR analysis

Gene	Name	Gen Model ITAG2,3	Forward Primer (5´→3´)	Reverse Primer (5´→3´)	Reference [1]
RAB18	Responsive to ABA 18	Solyc02g084850.2	CCTGGGATGCATTGAACACC	CACGGGACACCATAACACAC	Gonzalez-Guzman et al., 2014
PR-1a	Pathogenesis-related protein 1a	Solyc09g007010.1.1	TGGTGGTTCATTTCTTGCAACTAC	ATCAATCCGATCCACTTATCATTTTA	Alba et al., 2015
MYC2		Solyc08g076930.1.1	CGGTGTCATCACCTGCTTAT	TTCGGTGTCGGTAACTTCTTC	This work
PPO-F	Polyphenol-oxidase-F	Solyc08g074630.1.1	CGGAGTTTGCAGGGAGTTATAC	TTGATCTCCACACTTTCAATGG	Alba et al., 2015
CDI	Cathepsin D inhibitor protein	Solyc03g098790.1.1	ACTCGTCCTGTGCTTTGTCC	CCCAAGAGGATTTTCGTTGA	Lisón et al., 2006
PI-Ia	Protease Inhibitor Ia	Solyc09g084470	TGTACAAATGCCTGTGGTGACT	GGAGTACATGTAATTAAGCCACACT	Martel et al., 2015
Actin		Solyc03g078400.2.1	CCTCAGCACATTCCAGCAG	CCACCAAACTTCTCCATCCC	Martel et al., 2015

[1] See References list in the main text.

González-Guzmán et al. (2014). J. Exp. Bot. 65(15), 4451-4464. doi: 10.1093/jxb/eru219
Lisón et al. (2006) *Plant Physiol* 142(3), 1329–1339. doi: 10.1104/pp.106.086587
Martel et al. (2015) *Mol Plant Microbe Interact* 28(3), 343-61. doi: 10.1094/MPMI-09-14-0291-FI.

Supplementary Table 3. Results of the Student's t-test of stomatal conductance, Fv/Fm and stem length for each accession at each time point in Experiment 1.

	TR58			TR61			TR126			TR154		
	t	**df**	**p**	**t**	**df**	**p**	**t**	**df**	**p**	**t**	**df**	**p**
At mite infestation												
Stomatal conductance	7.980	16	**<0.001**	11.645	16	**<0.001**	5.650	16	**<0.001**	12.224	16	**<0.001**
Fv/Fm	0.956	16	0.353	-1.635	16	0.122	-1.419	16	0.175	-1.425	16	0.173
Stem length	4.180	16	**0.001**	4.180	16	**0.001**	1.170	16	0.259	1.785	16	0.093
At 4 days post infestation												
Stomatal conductance	26.059	16	**<0.001**	21.675	16	**<0.001**	11.194	16	**<0.001**	19.954	16	**<0.001**
Fv/Fm	1.400	16	0.181	1.400	16	0.181	0.004	16	0.997	-0.258	16	0.800
Stem length	5.921	16	**<0.001**	8.612	16	**<0.001**	4.319	16	**<0.001**	5.339	16	**<0.001**

Supplementary Table 4. Results of the Student's t-test of stomatal conductance, Fv/Fm and stem length for each cultivar/accession at each time point in Experiment 2.

	Moneymaker			TR126			TR154		
	t	**df**	**p**	**t**	**df**	**p**	**t**	**df**	**p**
At mite infestation									
Stomatal conductance	9.884	22	**<0.001**	27.143	22	**<0.001**	15.302	22	**<0.001**
Fv/Fm	-0.001	22	0.999	-3.953	22	**0.001**	-0.751	22	0.461
Stem length	4.446	22	**<0.001**	1.953	22	0.064	2.480	22	**0.021**
At 4 days post infestation									
Stomatal conductance	20.870	22	**<0.001**	18.902	22	**<0.001**	18.621	22	**<0.001**
Fv/Fm	-0.701	22	0.490	-0.935	22	0.360	0.608	22	0.549
Stem length	8.041	22	**<0.001**	4.011	22	**0.001**	4.980	22	**<0.001**

Supplementary Table 5. Results of the Student's t-test of number of eggs, mobile forms and leaf damage for each accession.

	TR58			TR61			TR126			TR154		
	t	**df**	**p**	**t**	**df**	**p**	**t**	**df**	**p**	**t**	**df**	**p**
Number of eggs	0.233	16	0.819	-2.541	16	**0.022**	0.895	16	0.384	-3.603	16	**0.002**
Mobile forms	-0.383	16	0.707	-1.237	16	0.234	0.011	16	0.991	-1.683	16	0.112
Leaf damage	-0.714	16	0.486	-2.558	16	**0.021**	0.493	16	0.629	-4.069	16	**0.001**

Supplementary Table 6 Results of the two-way ANOVA analysis of Accession TR126.

	Drought			*T. evansi*			Drought**T.evansi*		
	F	**df**	**p**	**F**	**df**	**p**	**F**	**df**	**p**
Nutrients									
Water	0.034	1,20	0.857	0.286	1,20	0.599	1.338	1,20	0.261
Free sugars	3.976	1,20	0.060	0.326	1,20	0.575	1.062	1,20	0.315
Protein	0.689	1,20	0.416	0.449	1,20	0.596	0.862	1,20	0.364
Total free aa	2.780	1,20	0.111	0.140	1,20	0.712	1.395	1,20	0.251
Non-essential amino acids									
Asp	0.178	1,20	0.677	0.027	1,20	0.870	2.114	1,20	0.161
Thr	4.458	1,20	**0.048**	0.009	1,20	0.924	1.366	1,20	0.256
Ser	0.393	1,20	0.538	0.530	1,20	0.475	0.136	1,20	0.716
Glu	5.041	1,20	**0.036**	0.497	1,20	0.489	4.836	1,20	**0.040**
Gly	1.814	1,20	0.193	0.881	1,20	0.359	0.116	1,20	0.737
Ala	0.102	1,20	0.752	0.300	1,20	0.590	0.054	1,20	0.819
Cys	0.983	1,20	0.333	0.425	1,20	0.522	0.339	1,20	0.567
Pro	28.56	1,20	**<0.001**	0.224	1,20	0.641	0.092	1,20	0.765
Essential amino acids									
Val	6.656	1,20	**0.018**	1.519	1,20	0.232	0.089	1,20	0.769
Met	0.126	1,20	0.726	3.267	1,20	0.086	1.146	1,20	0.297
Ile	5.355	1,20	**0.031**	2.747	1,20	0.113	0.197	1,20	0.662
Leu	4.511	1,20	**0.046**	2.957	1,20	0.101	1.050	1,20	0.318
Tyr	3.114	1,20	0.093	3.608	1,20	0.072	0.865	1,20	0.363
Phe	4.671	1,20	**0.043**	1.803	1,20	0.194	0.088	1,20	0.769
His	2.400	1,20	0.137	3.023	1,20	0.097	0.002	1,20	0.964
Lys	1.664	1,20	0.212	2.869	1,20	0.106	0.309	1,20	0.584
Arg	2.398	1,20	0.137	1.453	1,20	0.242	0.042	1,20	0.840
Phytohormones									
ABA	1.794	1,16	0.199	0.472	1,16	0.502	0.791	1,16	0.387
JA	4.706	1,16	**0.045**	1.375	1,16	0.258	5.998	1,16	**0.026**
OPDA	12.59	1,16	**0.003**	0.055	1,16	0.818	0.042	1,16	0.840
SA	4.561	1,16	**0.049**	15.33	1,16	**0.001**	0.001	1,16	0.972
SAGE	122.8	1,16	**<0.001**	0.628	1,16	0.440	0.502	1,16	0.489
Gene expression									
RAB-18	17.32	1,20	**<0.001**	0.435	1,20	0.517	30.99	1,20	**<0.001**
PR1a	68.37	1,20	**<0.001**	1.195	1,20	0.287	4.315	1,20	0.051
MYC-2	2.045	1,20	0.168	0.082	1,20	0.778	0.348	1,20	0.562
CDI	7.920	1,20	**0.011**	38.11	1,20	**<0.001**	1.908	1,20	0.182
PPO-F	3.181	1,20	0.090	0.043	1,20	0.838	2.395	1,20	0.137
PI-Ia	0.341	1,20	0.566	5.343	1,20	**0.032**	0.032	1,20	0.859
Defense proteins									
Cathepsin B	2.823	1,20	0.108	13.68	1,20	**0.001**	0.490	1,20	0.492
Papain	3.374	1,20	0.081	1.861	1,20	0.188	0.168	1,20	0.686
Cathepsin D	7.802	1,20	**0.011**	1.584	1,20	0.223	0.390	1,20	0.539
Trypsin	11.74	1,20	**0.003**	0.481	1,20	0.496	0.259	1,20	0.616
Chymotrypsin	0.026	1,20	0.873	14.31	1,20	**0.001**	0.476	1,20	0.498
Aminopeptidase	7.612	1,20	**0.012**	5.894	1,20	**0.025**	1.701	1,20	0.207
Polyphenol oxidases	0.722	1,20	0.406	3.107	1,20	0.093	0.598	1,20	0.448
Peroxidases	0.029	1,20	0.865	0.029	1,20	0.865	0.490	1,20	0.492
	F	**df**	**p**	**F**	**df**	**p**	**F**	**df**	**p**
	Drought			***T. evansi***			**Drought*T.evansi**		

Supplementary Table 7 Results of the two-way ANOVA analysis of Accession TR154.

	Drought			*T. evansi*			Drought**T.evansi*		
	F	df	p	F	df	p	F	df	p
Nutrients									
Water	2.722	1,20	0.115	0.08	1,20	0.780	5.197	1,20	**0.034**
Free sugars	2.49	1,20	0.131	12.82	1,20	**0.002**	5.713	1,20	**0.027**
Protein	3.546	1,20	0.074	1.472	1,20	0.239	16.47	1,20	**<0.001**
Total free aa	2.174	1,20	0.156	0.735	1,20	0.401	4.023	1,20	0.059
Non-essential amino acids									
Asp	0.222	1,20	0.643	0.064	1,20	0.802	4.063	1,20	0.057
Thr	1.498	1,20	0.235	0.104	1,20	0.750	3.517	1,20	0.075
Ser	12.35	1,20	**0.002**	1.234	1,20	0.280	1.824	1,20	0.192
Glu	0.048	1,20	0.829	0.446	1,20	0.512	5.081	1,20	**0.036**
Gly	0.018	1,20	0.895	0.026	1,20	0.873	2.741	1,20	0.113
Ala	0.133	1,20	0.719	0.157	1,20	0.696	4.006	1,20	0.059
Cys	1.740	1,20	0.202	0.964	1,20	0.338	4.343	1,20	0.050
Pro	30.20	1,20	**<0.001**	1.018	1,20	0.325	3.268	1,20	0.086
Essential amino acids									
Val	10.75	1,20	**0.004**	2.043	1,20	0.168	1.415	1,20	0.248
Met	0.264	1,20	0.613	0.010	1,20	0.920	2.542	1,20	0.127
Ile	10.79	1,20	**0.004**	2.359	1,20	0.140	0.803	1,20	0.381
Leu	9.280	1,20	**0.006**	2.478	1,20	0.131	0.936	1,20	0.345
Tyr	10.10	1,20	**0.005**	2.672	1,20	0.118	0.160	1,20	0.694
Phe	2.367	1,20	0.140	1.475	1,20	0.239	2.123	1,20	0.161
His	16.22	1,20	**0.001**	4.313	1,20	0.051	0.980	1,20	0.334
Lys	17.19	1,20	**<0.001**	2.682	1,20	0.117	1.882	1,20	0.185
Arg	7.525	1,20	**0.013**	2.090	1,20	0.164	0.199	1,20	0.660
Phytohormones									
ABA	64.01	1,18	**<0.001**	0.938	1,18	0.346	4.868	1,18	**0.041**
JA	0.454	1,18	0.509	0.946	1,18	0.344	3.903	1,18	0.064
OPDA	2.968	1,18	0.102	1.855	1,18	0.190	4.578	1,18	**0.046**
SA	30.73	1,18	**<0.001**	1.972	1,18	0.177	11.36	1,18	**0.003**
SAGE	76.32	1,18	**<0.001**	37.52	1,18	**<0.001**	106.6	1,18	**<0.001**
Gene expression									
RAB-18	1.058	1,20	0.316	3.803	1,20	0.065	7.397	1,20	**0.013**
PR1a	2.457	1,20	0.133	4.346	1,20	**0.050**	0.138	1,20	0.714
MYC-2	0.212	1,20	0.651	0.763	1,20	0.394	3.112	1,20	0.094
CDI	10.91	1,20	**0.004**	18.79	1,20	**<0.001**	1.114	1,20	0.304
PPO-F	1.448	1,20	0.243	3.152	1,20	0.091	1.560	1,20	0.226
PI-Ia	9.222	1,20	**0.007**	7.486	1,20	**0.013**	5.173	1,20	**0.034**
Defense proteins									
Cathepsin B	7.347	1,20	**0.013**	25.06	1,20	**<0.001**	0.799	1,20	0.382
Papain	1.324	1,20	0.263	7.93	1,20	**0.011**	0.002	1,20	0.962
Cathepsin D	0.538	1,20	0.472	11.71	1,20	**0.003**	0.469	1,20	0.501
Trypsin	2.026	1,20	0.170	0.141	1,20	0.711	0.013	1,20	0.912
Chymotrypsin	1.182	1,20	0.290	4.228	1,20	0.053	0.265	1,20	0.613
Aminopeptidase	0.242	1,20	0.628	1.459	1,20	0.241	3.991	1,20	0.060
Polyphenol oxidases	0.028	1,20	0.868	0.315	1,20	0.581	1.390	1,20	0.252
Peroxidases	2.667	1,20	0.118	3.809	1,20	0.065	1.625	1,20	0.217
	F	df	p	F	df	p	F	df	p
	Drought			*T. evansi*			Drought**T.evansi*		

Supplementary Table 8 Results of the two-way ANOVA analysis of Moneymaker.

	Drought			***T. evansi***			**Drought*T.evansi**		
	F	**df**	**p**	**F**	**df**	**p**	**F**	**df**	**p**
Nutrients									
Water	1.150	1,20	0.296	2.957	1,20	0.101	4.065	1,20	0.057
Free sugars	12.37	1,20	**0.002**	0.278	1,20	0.604	0.057	1,20	0.814
Protein	0.968	1,20	0.337	0.143	1,20	0.709	0.036	1,20	0.861
Total free aa	11.66	1,20	**0.003**	1.308	1,20	0.266	1.154	1,20	0.296
Non-essential amino acids									
Asp	3.539	1,20	0.075	0.199	1,20	0.660	0.570	1,20	0.459
Thr	9.003	1,20	**0.007**	0.391	1,20	0.539	0.213	1,20	0.649
Ser	10.17	1,20	**0.005**	0.717	1,20	0.407	0.668	1,20	0.423
Glu	4.194	1,20	**0.054**	6.272	1,20	**0.021**	5.116	1,20	**0.035**
Gly	3.403	1,20	0.080	0.044	1,20	0.835	0.030	1,20	0.865
Ala	2.631	1,20	0.120	0.018	1,20	0.894	0.002	1,20	0.962
Cys	4.890	1,20	**0.039**	0.034	1,20	0.855	0.022	1,20	0.885
Pro	16.38	1,20	**0.001**	0.865	1,20	0.364	0.850	1,20	0.368
Essential amino acids									
Val	7.496	1,20	**0.013**	0.362	1,20	0.554	0.150	1,20	0.703
Met	3.811	1,20	0.065	0.326	1,20	0.574	0.217	1,20	0.647
Ile	7.675	1,20	**0.012**	0.556	1,20	0.465	0.235	1,20	0.633
Leu	6.009	1,20	**0.024**	0.147	1,20	0.706	0.019	1,20	0.891
Tyr	7.087	1,20	**0.015**	0.641	1,20	0.433	0.246	1,20	0.625
Phe	5.322	1,20	**0.032**	0.155	1,20	0.698	0.062	1,20	0.806
His	10.03	1,20	**0.005**	1.428	1,20	0.246	0.636	1,20	0.434
Lys	6.754	1,20	**0.017**	0.114	1,20	0.739	0.016	1,20	0.899
Arg	9.306	1,20	**0.006**	0.778	1,20	0.388	0.517	1,20	0.480
Phytohormones									
ABA	37.75	1,19	**<0.001**	2.483	1,19	0.132	3.340	1,19	0.083
JA	2.571	1,19	0.125	0.004	1,19	0.953	0.015	1,19	0.905
OPDA	10.50	1,19	**0.004**	1.905	1,19	0.184	4.666	1,19	**0.044**
SA	0.263	1,19	0.614	0.309	1,19	0.585	0.069	1,19	0.795
SAGE	0.410	1,19	0.530	0.415	1,19	0.527	1.003	1,19	0.329
Gene expression									
RAB-18	28.79	1,18	**<0.001**	0.265	1,18	0.613	13.41	1,18	**0.002**
PR1a	0.484	1,18	0.496	0.154	1,18	0.699	0.162	1,18	0.692
MYC-2	1.601	1,17	0.223	0.383	1,17	0.544	0.057	1,17	0.814
CDI	20.79	1,17	**<0.001**	39.37	1,17	**<0.001**	0.029	1,17	0.867
PPO-F	2.243	1,18	0.152	4.018	1,18	0.060	3.088	1,18	0.096
PI-Ia	11.28	1,18	**0.003**	6.979	1,18	**0.017**	2.745	1,18	0.115
Defense proteins									
Cathepsin B	1.759	1,17	0.202	4.255	1,17	0.055	0.296	1,17	0.593
Papain	1.003	1,17	0.331	7.899	1,17	**0.012**	0.115	1,17	0.739
Cathepsin D	0.105	1,17	0.750	8.090	1,17	**0.011**	2.630	1,17	0.123
Trypsin	1.078	1,17	0.314	0.307	1,17	0.587	0.356	1,17	0.559
Chymotrypsin	4.866	1,17	0.041	8.000	1,17	**0.012**	0.426	1,17	0.523
Aminopeptidase	0.171	1,17	0.684	2.467	1,17	0.135	0.019	1,17	0.893
Polyphenol oxidases	0.982	1,20	0.334	0.265	1,20	0.612	2.904	1,20	0.104
Peroxidases	4.121	1,20	0.056	0.119	1,20	0.734	4.438	1,20	**0.048**
	F	**df**	**p**	**F**	**df**	**p**	**F**	**df**	**p**
	Drought			***T. evansi***			**Drought*T.evansi**		