A novel and integrated approach to increase multiple and combined stress tolerance in plants using tomato as a model

July 2019

WP1 – Identification of lines with improved water and nutrient use efficiency under combined abiotic stresses, and of

DELIVERABLE 1.2

Tomato lines showing arbuscular mycorrhizal fungi (AMF) responsiveness and improved root system architecture (RSA)

TOMRES has received funding by the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement N°727929

Document Information

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Work Package	WP1 – Identification of lines with improved water and nutrient use efficiency under combined abiotic stresses, and of resilience traits			
Objective of the document	Provide results of activities carried out in order to identify lines within the TOMRES collection with belowground traits that may enhance W/NUE under combined stress			
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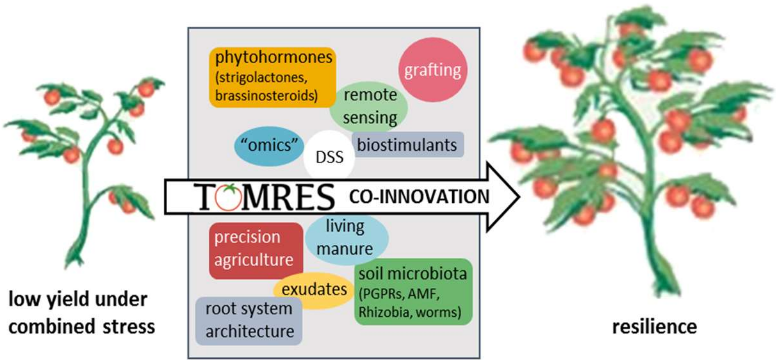
TOMRES Project Summary

Tomato is a main EU agricultural commodity, cultivated all over Europe in open and protected field and in glasshouses, representing a biological and agronomical model crop. Combined water and nutrient stress is a major problem for tomato farmers and solutions are needed to safeguard yields, while preserving the environment.

The overall goal of TOMRES is to enhance resilience to combined water and nutrient stress in tomato and to maximize water (WUE) and nutrient use efficiency (NUE) by designing and testing in the field (open and protected) novel combinations of genotypes and management practices reducing the environmental impact of agricultural activities.



To this aim, TOMRES will select, among over 10,000 available accessions, rootstocks and scions tolerating combined stress, while retaining fruit quality and yield, taking advantage of innovative screening approaches. Novel traits, in particular belowground, to be exploited in breeding, will be identified. The role of selected hormones (strigolactones and brassinosteroids) will be studied to identify further resilience traits. TOMRES will test and optimize sustainable crop management strategies such as legume intercropping, precision fertilization and irrigation techniques, manipulation of symbiotic microorganisms, and the use of rootstocks more suited to water and nutrient uptake from the soil.

Novel genotypes X management strategies will be developed with the goal of reducing N and P application by at least 20%, water input by 40%, while granting environmental sustainability and economic viability of the solutions proposed. Testing will be integrated with analysis of environmental (greenhouse emissions, water quality), and of socio-economic impact. Agronomical, environmental, and economical data will be processed to construction of models and of a Decision Support System.

Demonstration and dissemination activities will follow the whole course of the project, and will transfer the results to different environments and other cropping systems, thus ensuring the widest impact of the gained knowledge on the EU economy. Trans-disciplinary knowledge transfer among farmers, breeders, industries, associations and scientists will be granted by a solid multi-actor approach since the planning stage.

Tab. 1 TOMRES Project Partners

No	Participant organisation name (and acronym)	Country
1 (C*)	Università degli Studi di Torino (UNITO)	Italy
2	Agricultural University of Athens (AUA)	Greece
3	Agroilla SAT (AGROILLA)	Spain
4	Casella Macchine Agricole Srl (CASELLA)	Spain
5	Confederazione Generale dell'Agricoltura Italiana (CONFAGRICOLTURA)	Italy
6	Edypro Fertilisantes Srl	Spain
7	Europese Organisatie Voor Wetenschappelijk Plantenonderzoek (EPSO)	Belgium
8	Gaia Epicheirein Anonymi Etaireia Psifiakon Ypiresion (GAIA)	Greece
9	Gautier Semences SAS (GAUTIER)	France
10	Institut Jozef Stefan (JSI)	Slovenia
11	Institut National de la Recherche Agronomique (INRA)	France
12	Neurather Gärtner GbR (NEURATHER)	Germany
13	Novareckon Srl (NOVARECKON)	Italy
14	Raffaele Tamburrino (TAMBURRINO)	Italy
15	Research and Development Institute for Processing and Marketing of the Horticultural Products Horting (HORTING)	Romania
16	Rheinische Friedrich-Wilhelms-Universität Bonn (UBO)	Germany
17	STC Research Foundation (STC)	United Kingdom
18	Strigolab Srl (STRIGOLAB)	Italy
19	Technion – Israel Institute of Technology (TECHNION)	Israel
20	The Hebrew University of Jerusalem (HUJ)	Israel
21	The James Hutton Institute (JHI)	United Kingdom
22	The University of Nottingham (UNO)	United Kingdom
23	Università degli Studi di Milano (UMIL)	Italy
24	Università degli Studi di Napoli Federico II (UNA)	Italy
25	Universitat de Les Illes Balears (UIB)	Spain

*Coordinating institution

1. Scope of the document

This document aims to provide results of activities carried out in TOMRES WP1, task 1.2b. Specifically, we characterized different TOMRES accessions for responsiveness to the AM fungus (AMF) *Funneliformis mosseae* under control and combined stress (low nutrient and drought) as well as below and above ground traits under low nutrient and drought stresses, alone or in combination. The two experimental set ups allowed to identify:

- accessions with higher responsiveness to mycorrhizal fungi, in terms of growth promotion
- accessions which show insensitivity to the imposed treatments, as indicated by little to no response to selected stresses in terms of growth suppression and unchanged root/shoot ratio.

2. Chapter 2

The task aims to make a ranking of selected tomato genotypes from the TOMRES Collection (TC) for their responsiveness to the AM symbiosis under combined water and nutrient stress. The AM fungus (AMF) *Funneliformis mosseae* (BEG12), a well-known model species in tomato-AMF interaction, has been considered.

Materials and experimental set-up

At first, a miniaturized screening protocol, accounting for combined water and nutrient stress under AMF inoculation was defined and tested. The set-up was based on alveolar trays using a mono-specific inoculum (MycAgroLab, Dijon, France) 25% diluted in oven-sterilized quartz sand. A blank inoculum, holding the same characteristics of the inoculum but not containing the AMF, was used as control. After seed germination and AMF inoculation plants were grown under controlled climate-chamber conditions (16/8h light/dark, 23°C) at 100% soil water capacity (SWC) for 1 month to obtain a successful AM colonization. Moderate water stress was then applied continuously for 1 month by modulating SWC parameter. The protocol allowed to obtain a full mycorrhizal colonization in less than 8 weeks also reducing spaces in growth chambers. The method was validated in terms of induced moderate water stress (measurements of stem water potential) and effectiveness of mycorrhizal colonization using the reference tomato cultivar 'M82' (#TC162). According to this preliminary experiment, we defined a SWC threshold at 40% to have an induced moderate water stress ($-0.6<\Psi>-0.8$; data not shown). Plants were watered once a week with a modified Long-Ashton solution (Hewitt et al., 1966) at 1 mM N, 50 µM P and 1 mM K. Phosphorus concentration was kept low to promote AM colonization.

Using this set-up, different experimental modules were performed asynchronously using a selection of 4 tomato accessions from the TOMRES Collection and 2 wild relative species, *Solanum pennellii*, as it is known as a drought tolerant species, and *S. neorickii* as a non tolerant one (Table 1). Moreover, these wild relatives are considered useful genetic resources in tomato breeding strategies and no data on their response to AM colonization is currently available.

Table 1. TOMRES accessions tested for AMF responsiveness.

TC code (#TC)	TOBC code	TGRC code	Wild/Cultivated	Geographical origin	Growth habit	Notes
5	N/A	LA2133	Wild (*S. neorickii*)	South America	N/A	
111	TOBC-106	LA5240	Wild (*S. pennellii*)	South America	N/A	
162	TOBC-018	N/A	Cultivated	N/A	Determinate	'M82' (reference)
260	TOBC-121	N/A	Cultivated	Southern Italy	Indeterminate	
313	TOBC-174	N/A	Cultivated	Spain	Determinate	
327	TOBC-188	N/A	Cultivated	Spain	Indeterminate	

2.1. Accessions screening and ranking for AMF responsiveness

Different plant growth traits as well as mycorrhizal colonization in roots were measured. Mycorrhizal colonization parameters were homogeneous across analysed accessions with few and non-significant variations with the only exception of LA5240 line which displayed a very low frequency of mycorrhization (Figure 1).

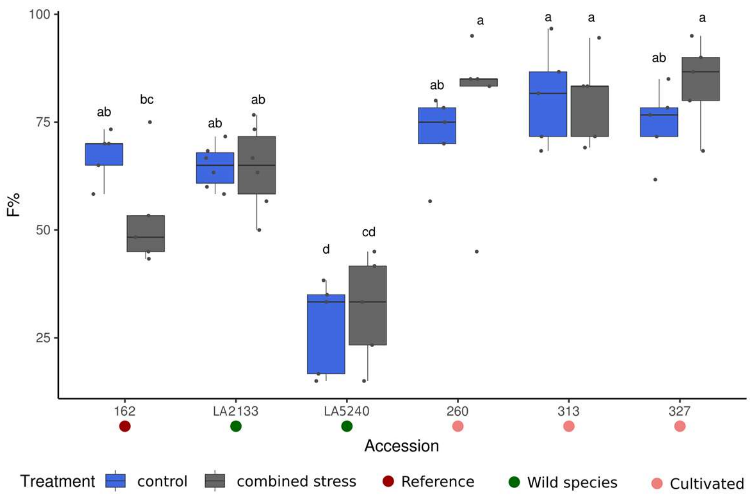


Figure 1. Frequency of mycorrhization (F%) in selected TOMRES accessions. Statistically supported differences across means are indicated with different letters according to Tukey's posthoc test ($P<0.05$) after ANOVA.

The response of plant growth to AMF inoculation is defined as mycorrhizal growth response (MGR) and this parameter is often used to measure AMF responsiveness. Here we measured MGR calculated on the vegetative stage as the ratio between total dry weight of mycorrhizal plants and that of non-mycorrhizal plants (Johnson et al. 2010). We observed a high variability across accessions (Figure 2). Independently of the water regime, the AM symbiosis induced a weak but positive (>0) MGR in the reference tomato accession 'M82' while in both wild relatives species analysed (LA2133 and LA5240) the MGR was negative (<0) (Figure 2). Interestingly, lines #TC313 and #TC327 displayed higher and positive MGR values under control (not stressed) conditions but lower values under combined stress. By contrast, line #TC260 showed no differences between control and combined stress conditions.

Due to the low variability of mycorrhizal colonization across tested genotypes we propose here the final ranking of tomato accessions based on the MGR. We selected as best-performing accessions those that showed a higher MGR value under combined stress conditions or at least no variation in MGR between control and combined stress conditions (equally responding to AMF colonization under control and combined stress treatment). Unfortunately, across accessions tested so far, none displayed higher MGR value under combined stress conditions but two of them, M82 (#TC162)(reference) and #TC260, showed no variations in control and combined stress condition. Looking at absolute MGR values, only M82 genotype displayed positive values (Figure 2).

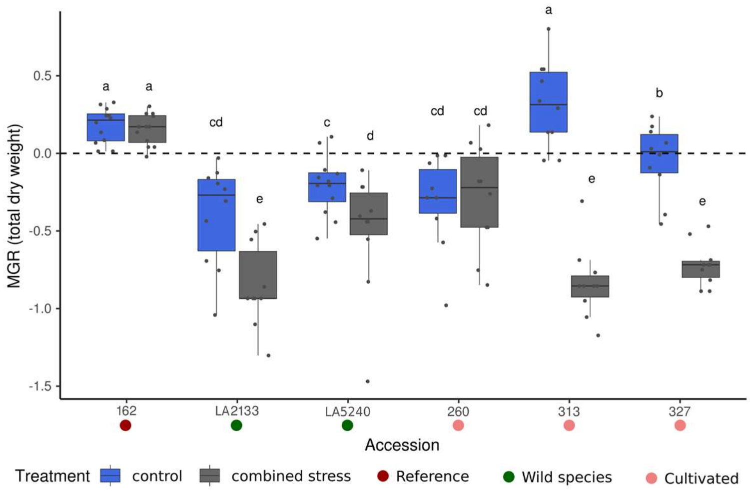


Figure 2. Mycorrhiza growth response (MGR) in selected TOMRES accessions. An MGR value above zero indicates a positive growth response to AM fungi, an MGR equal to zero no response while below zero a negative one. Statistically supported differences across medians are indicated with different letters according to pairwise Kruskal-Wallis test (P<0.05).

In conclusion, the analysis of several traits in a selected panel of genotypes considered so far revealed that, on one hand, the susceptibility to AMF colonization, measured as percentage of root AM colonization, seems to be a rather conserved trait in cultivated tomato accessions. For this reason, it seems not a useful trait for ranking. On the other hand, growth response parameter such as dry biomass, seems to be influenced (positively or negatively) by AM symbiosis also when considering water and nutrient combined stress conditions. Considering the data of this screening generated so far, a genotype of interest could be #TC260 as the one with a good responsiveness to the AMF inoculation under combined stress.

In the next months we will characterize two more TOMRES accessions for their susceptibility and responsiveness to the AM symbiosis; these new data will be integrated in this ranking.

3. Chapter 3

This task aims to characterize the root system architecture (RSA) of selected TOMRES genotypes grown in different conditions (control, low nutrient, drought stress and combined low nutrient/drought stress) and correlate below ground traits with above ground adaptation to stressful conditions.

Materials and experimental setup

A large-scale pot experimental design was set-up in order to evaluate eight replicates of ten genotypes at each time using 15 L pots filled with sand and watered with nutrient solutions in a randomized block setup. Plants were grown for five weeks under four separate treatments: Control (10.2 mM NO_3^-), Low Nitrate (2.88 mM NO_3^-), Drought (50% water) and Combined Stress (2.88 mM NO_3^- with 50% water). Nutrient stress was initiated concomitant with transfer to pots, while drought stress was imposed 7d after transplant.

Using this set-up, different experimental modules were performed asynchronously using a selection of 27 tomato accessions from the TOMRES Collection as summarized in Table 2.

Table 2. TOMRES accessions tested for RSA under stress conditions (BIL = Backcross Inbred Line with *S. pennellii*).

TC code (#TC)	TOBC code	Wild/Cultivated	Geographical origin	Growth habit	Notes
162	TOBC-018	Cultivated	N/A	Determinate	M82 Reference, Det
134	TOBC-022	Cultivated	Southern Italy	Indeterminate	
147	TOBC-B34	BIL	BIL 6325	Determinate	
149	TOBC-36	BIL	BIL 6191	Determinate	UNA04, Reference, Indet
150	TOBC-37	BIL	BIL 6649	Determinate	
151	TOBC-38	BIL	BIL 6335	Determinate	
247	TOBC-108	Cultivated	Southern Italy	Indeterminate	
249	TOBC-110	Cultivated	Southern Italy	Indeterminate	
250	TOBC-111	Cultivated	Southern Italy	Indeterminate	
260	TOBC-121	Cultivated	Southern Italy	Indeterminate	
261	TOBC-122	Cultivated	Southern Italy	Indeterminate	
263	TOBC-124	Cultivated	Southern Italy	Determinate	
265	TOBC-126	Cultivated	Southern Italy	Indeterminate	
266	TOBC-127	Cultivated	Southern Italy	Indeterminate	
267	TOBC-128	Cultivated	Southern Italy	Indeterminate	
270	TOBC-131	Cultivated	Southern Italy	Determinate	

271	TOBC-132	Cultivated	Southern Italy	Determinate	
272	TOBC-133	Cultivated	Southern Italy	Indeterminate	
273	TOBC-134	Cultivated	Southern Italy	Indeterminate	
274	TOBC-135	Cultivated	Southern Italy	N/A	
275	TOBC-136	Cultivated	Southern Italy	Indeterminate	
276	TOBC-137	Cultivated	Southern Italy	Indeterminate	
279	TOBC-140	Cultivated	N/A	Indeterminate	
292	TOBC-153	Cultivated	Southern Italy	N/A	
313	TOBC-174	Cultivated	Spain	Indeterminate	
327	TOBC-188	Cultivated	Spain	Indeterminate	
336	TOBC-197	Cultivated	Spain	Indeterminate	

3.1.1. Accessions screening and identification of lines with improved root system architecture

Three experimental modules were run to screen 27 TOMRES accessions (Table 2). Each module contained at least one of the two genotypes M82 and UNA04, selected as internal reference for determinate and indeterminate accessions, respectively. During the experiment, stomatal conductance, chlorophyll-SPAD, and relative water content were monitored. Chlorophyll content and stomatal conductance were instrumental in evaluating stress progression. Impact of stress on the plant phenotype was evident after 5 weeks of culture. In low N treatment leaves appeared chlorotic, while both low N and drought inhibited growth. After 5 weeks, destructive measurements were carried out and biometric parameters were evaluated, including plant height, number of internodes, leaf area, shoot fresh/dry weight as well as fresh and dry weight of roots collected from the pots, and washed to remove residual sand. While biochemical and molecular analyses (e.g. leaf proline content, transcript quantification of stress marker and N transport/metabolism encoding genes) are still in progress and linked with WP2, biometric measurements were subjected to analysis of variance (ANOVA) and Duncan post-hoc test. This showed that above ground parameters were largely in agreement and demonstrated a significantly reduced growth in stress treated compared to control plants. Thus, shoot dry weight was selected as a representative parameter and correlated to root dry weight to identify differential behaviors. Table 3 summarizes shoot and root dry weught and root/shoot ratio in the 19 genotypes in which these parameters were statistically analysed.

Figure 3 shows shoot dry weight of #TC249 (UNA04), #TC250 and #TC270, evaluated in the first module and #TC 134,271 and 273 included in the second round of screening. As shown in Figure 3, genotypes 249 and 270, 134 and 273 had a significantly reduced shoot biomass accumulation caused by the stress treatments and this behavior is shared with virtually the several tested genotypes, with two exceptions. In #TC250, shoot biomass was significantly reduced under low N treatment, while measurements from plants subjected to drought or combined stress were not significantly different compared to controls (Figure 3). By contrast, #TC271 displayed a significant growth inhibition in low N and combined stress treatments, while drought stress alone did not cause reduced biomass accumulation (Figure 3). Interestingly, #TC250 had a less developed root system, stable across the different treatments, while #TC271 root system did not modify in response to drought stress.

Thus, root systems that maybe pre-adapted to stressful environments may contribute to stress tolerance displayed in terms of growth of above ground tissues. Indeed, #TC250 appeared to have a more efficient root system, which may assist this genotype in exploring deeper soil layers for resources. However, root dry weight accumulation was also unaltered in #TC249 irrespective of stress treatment, indicating that root dry weight cannot be used as a single parameter to predict stress tolerance. We thus calculated the root/shoot ratio and found that #TC250 and 271 did not modify biomass allocation based on the stress treatment (Figure 3), in contrast to the remaining tested genotypes, which had a significant alteration of this parameter due to stress.

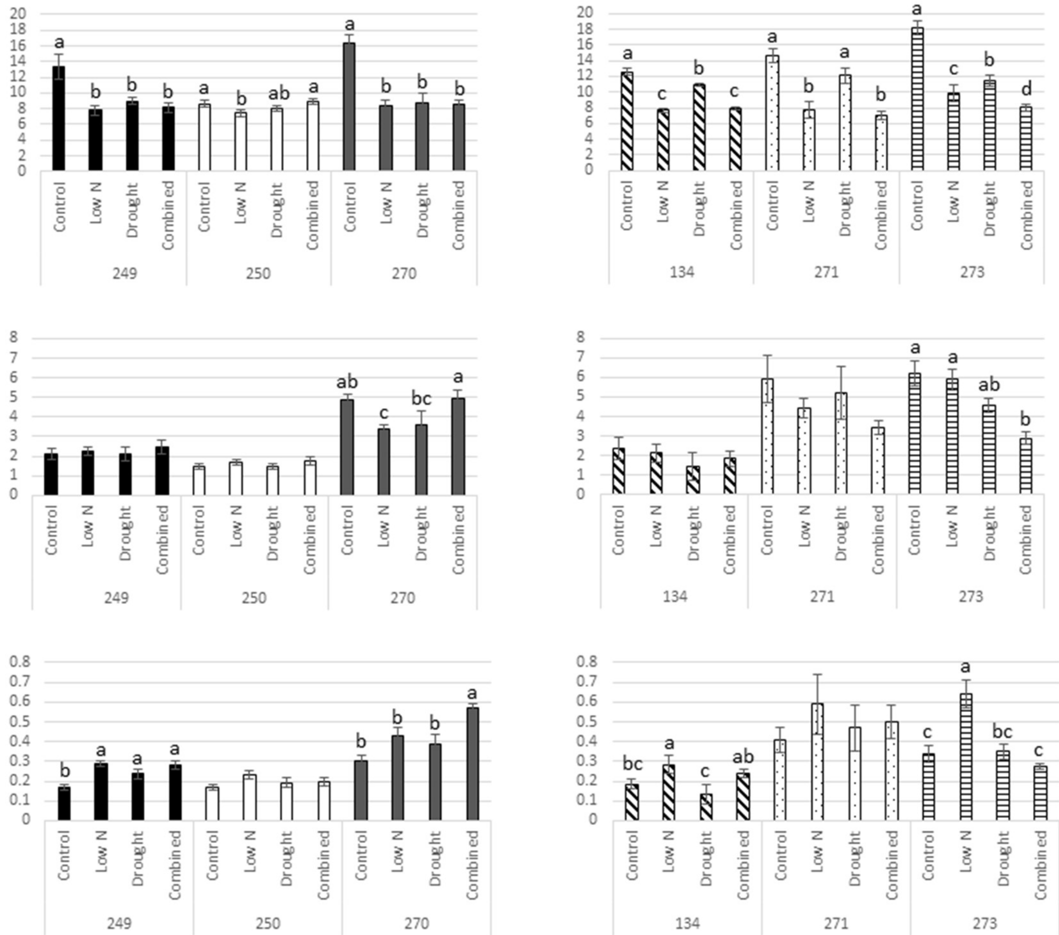


Figure 3. Biometric parameters in selected TC genotypes grown in sand and subjected to the indicated treatments. A) Shoot Dry Weight; B) Root Dry Weight; C) Root/Shoot ratio. Different letters indicate significant difference at $P < 0.05$ according to Duncan post hoc test; Error bars indicate SE of eight replicates.

In conclusion, we have characterized above and below ground responses to stresses in 27 TOMRES accessions and identified thus far genotypes #TC250 and #TC271, which show tolerance to drought stress in terms of growth and biomass allocation in above and below ground tissues. Statistical analysis is in progress for final 8 genotypes and will be integrated to data gathered thus far. Molecular analyse on roots and leaf samples of selected genotypes will allow dissection of mechanisms contributing to stress tolerance.

Table 3. Shoot dry weight (Shoot DW), Root dry weight (Root DW) and Root to Shoot Ratio (R/S, Root DW/ Shoot DW) measured in selected TOMRES accessions. Different letters indicate significant difference at $P < 0.05$ according to Duncan post hoc test.

TOMRES code (#TC)	Treatment	Shoot DW		Root DW		R/S	
134	Control	12.60	a	2.38	*ns*	0.18	bc
	Low N	7.66	c	2.16	*ns*	0.28	a
	Drought	10.94	b	1.45	*ns*	0.13	c
	Combined	7.91	c	1.86	*ns*	0.24	ab
162	Control	12.74	a	3.74	*ns*	0.29	b
	Low N	9.08	b	4.22	*ns*	0.46	a
	Drought	8.41	b	3.36	*ns*	0.41	ab
	Combined	8.24	b	3.59	*ns*	0.48	a
247	Control	14.84	a	4.53	a	0.30	b
	Low N	8.84	b	3.09	b	0.35	b
	Drought	8.98	b	3.42	ab	0.38	b
	Combined	7.97	b	4.60	a	0.58	a
249	Control	14.42	a	2.42	*ns*	0.17	b
	Low N	7.86	b	2.26	*ns*	0.29	a
	Drought	9.73	b	2.25	*ns*	0.24	a
	Combined	7.49	b	2.08	*ns*	0.28	a
250	Control	8.63	a	1.47	*ns*	0.17	*ns*
	Low N	7.42	b	1.69	*ns*	0.23	*ns*
	Drought	7.99	ab	1.50	*ns*	0.19	*ns*
	Combined	8.91	a	1.75	*ns*	0.20	*ns*
260	Control	14.35	a	5.37	*ns*	0.36	b
	Low N	7.30	c	3.90	*ns*	0.54	a
	Drought	10.33	b	3.32	*ns*	0.32	b
	Combined	7.14	c	4.16	*ns*	0.56	a

261	Control	15.92	a	7.00	*ns*	0.45	*ns*
	Low N	7.99	c	4.79	*ns*	0.59	*ns*
	Drought	11.22	b	3.97	*ns*	0.35	*ns*
	Combined	7.44	c	4.05	*ns*	0.54	*ns*
263	Control	14.83	a	5.01	a	0.34	*ns*
	Low N	8.58	b	3.42	b	0.39	*ns*
	Drought	9.29	b	3.40	b	0.39	*ns*
	Combined	8.95	b	3.39	b	0.38	*ns*
265	Control	15.15	a	3.55	a	0.24	*ns*
	Low N	7.83	b	2.01	b	0.25	*ns*
	Drought	8.44	b	3.03	ab	0.37	*ns*
	Combined	8.66	b	3.15	a	0.38	*ns*
266	Control	13.13	a	3.41	*ns*	0.22	c
	Low N	8.51	c	4.40	*ns*	0.52	a
	Drought	10.50	b	3.48	*ns*	0.33	bc
	Combined	7.93	c	3.19	*ns*	0.40	ab
267	Control	13.39	a	3.21	*ns*	0.24	*ns*
	Low N	8.72	b	3.02	*ns*	0.35	*ns*
	Drought	9.95	b	3.00	*ns*	0.31	*ns*
	Combined	8.36	b	3.05	*ns*	0.37	*ns*
270	Control	16.43	a	4.87	ab	0.30	b
	Low N	8.33	b	3.40	c	0.43	b
	Drought	8.66	b	3.59	bc	0.39	b
	Combined	8.64	b	4.94	a	0.57	a
271	Control	14.59	a	5.91	*ns*	0.41	*ns*
	Low N	7.78	b	4.41	*ns*	0.59	*ns*
	Drought	12.11	a	5.23	*ns*	0.47	*ns*
	Combined	7.04	b	3.45	*ns*	0.50	*ns*
272	Control	14.85	a	4.79	*ns*	0.33	*ns*
	Low N	8.03	c	4.58	*ns*	0.57	*ns*
	Drought	10.84	b	4.13	*ns*	0.40	*ns*
	Combined	8.15	c	2.98	*ns*	0.37	*ns*
273	Control	18.16	a	6.17	a	0.34	*ns*
	Low N	9.92	c	5.90	a	0.64	*ns*

	Drought	11.42	b	4.56	ab	0.35	*ns*
	Combined	8.05	d	2.89	b	0.27	*ns*
274	Control	17.34	a	3.25	a	0.16	b
	Low N	9.78	c	1.51	b	0.16	b
	Drought	13.11	b	2.52	ab	0.19	b
	Combined	8.22	c	3.02	a	0.39	a
275	Control	13.85	a	2.54	b	0.20	b
	Low N	8.98	b	1.74	c	0.20	b
	Drought	10.13	b	2.10	bc	0.21	b
	Combined	7.60	b	3.33	a	0.46	a
276	Control	14.00	a	4.42	*ns*	0.30	b
	Low N	9.59	b	4.65	*ns*	0.47	ab
	Drought	9.74	b	3.29	*ns*	0.34	b
	Combined	7.20	b	5.35	*ns*	0.62	a
279	Control	11.52	a	2.45	*ns*	0.20	b
	Low N	7.97	b	2.26	*ns*	0.28	b
	Drought	8.05	b	2.41	*ns*	0.24	b
	Combined	8.34	b	3.97	*ns*	0.47	a

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