

Variation in tolerance to drought among Tunisian populations of *Medicago truncatula*

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Abstract

We analysed the natural variation of drought response in 11 Tunisian populations of *Medicago truncatula* sampled from environments that varied in soil composition, salinity and water availability. Plants were cultivated in a greenhouse under well-irrigated and water-deficit treatments (50% of field capacity), and a number of traits associated with drought response were measured. Variance analysis indicated that the variation of phenotypic traits was explained by the effects of population, line, treatment, and population × treatment and line × treatment interactions, with treatment being the one with the greatest effect. A large degree of phenotypic variation for most traits in the two water treatments was found within populations. Most of the measured characters showed higher broad-sense heritabilities (H^2) in well-irrigated treatment than in water-deficit treatment. Furthermore, the largest population differentiation (Q_{ST}) for most of the measured traits was observed under drought stress. Most of the correlations between measured traits under both treatments were positive. Four groups of lines differing in drought tolerance were identified, with 45 susceptible, 14 moderately susceptible, 31 tolerant and 23 most tolerant lines. The tolerant group experienced lowest reductions in the length of plagiotropic axes, length of stems, number of internodes and number of leaves. The large phenotypic variation of *M. truncatula* in response to drought stress can be used to identify genes and alleles important for the complex trait of drought tolerance.

Keywords: genetic diversity; local adaptation; population differentiation; water deficit

Introduction

Drought is a common environmental stress experienced by plants negatively affecting plant development, growth and productivity (Slama *et al.*, 2006; Tuberosa and Salvi, 2006; Verslues *et al.*, 2006). Improving drought tolerance is probably one of the most difficult tasks for

plant breeders. The difficulty comes from the diversity and unpredictability of drought conditions in the field, and from the diversity of drought tolerance strategies developed by the plants that are targeted and subjected to selection criteria (Teulat *et al.*, 2001).

Legumes are the main source of vegetable protein in human diets and livestock feed, having major impacts on agriculture, environment and health (Graham and Vance, 2003; Dita *et al.*, 2006). In the world, the limiting factors for high yield of major crop legumes are their low adaptation to the diverse local eco-geographical parameters and the slowness of genetic improvements due to their

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complex ploidy and large genomes (Cook, 1999). *Medicago truncatula*, commonly known as barrel medic, is a forage legume plant of Mediterranean origin, well adapted to semi-arid conditions, namely to alkaline clay soils (Lesins and Lesins, 1979). This species was identified as a suitable model legume because of its small genome size (500–550 Mbp), simple diploid genetics ($2n = 16$), self-fertility, short life cycle, rapid germination, excellent mutant populations and large collections of diverse ecotypes (Young and Udvardi, 2009).

Although responses and physiological mechanisms to drought stress have been extensively studied in different plant species (Yamaguchi-Shinozaki *et al.*, 2002; Leung, 2008; Badri *et al.*, 2010, 2011), research exploring natural diversity in drought tolerance is still limited in many plant species. Conventional linkage mapping can be an effective tool for identifying genes underlying natural variation. However, the genes identified by this method are restricted to the ones segregating in the cross under consideration. Genome-wide association studies (GWAS) overcome this limitation and have recently been shown to successfully reveal common variants responsible for the variation in phenotypes of *Arabidopsis thaliana* natural accessions (Atwell *et al.*, 2010). The GWAS are often complementary to QTL mapping and, when conducted together, they mitigate each other's limitations (Korte and Farlow, 2013).

Natural plant populations are often collected from a wide range of geographical locations that have a relatively large genetic diversity. Maintaining genetic diversity within natural populations can maximize their potential to withstand and adapt to biotic and abiotic environmental changes (Jump *et al.*, 2009). To date, little is known about natural variation in the drought responses of *M. truncatula* collections. Considering the heterogeneous distribution of ground water and precipitations across Tunisian areas, different natural populations are potentially subjected to different selective pressures. In these conditions, we expect variation in the response to water deficit of different genotypes. This research aims to evaluate the phenotypic diversity of drought tolerance in 11 natural Tunisian populations of *M. truncatula*. The results can be used for further investigating the molecular and genetic basis of the drought tolerance of *M. truncatula*, which may contribute to the discovery of drought tolerance mechanisms of other legumes.

Materials and Methods

Plant material and growing conditions

108 lines of *M. truncatula*, from 11 Tunisian populations, collected in different eco-geographical sites (Enfidha (1), Jelma (2), Amra (3), Deguache (4), Thala (6), El Kef (7), Soliman

(TN8), Bulla Regia (TN9), Gabès (TN11), Djerba (TN12), and Tataouine (TN14) and five reference lines were used (Fig. 1) (Arraouadi *et al.*, 2009; Lazrek *et al.*, 2009). The eco-geographical variables of the collection sites of these populations are summarized in Table S1 (available online).

Seeds were surface-sterilized and scarified by immersion in concentrated H_2SO_4 for 7 min and rinsed ten times with sterile distilled water. The soaked seeds were sown in Petri dishes on 0.9% agar medium before being vernalized at 4°C for 96 h in darkness. Once the emerging root attained a length of 4 mm, seedlings were transferred to 33 cl pots (8 cm diameter and 10.5 cm deep) filled with sterilized sand that was previously washed using HCl 0.05%. Each plant was grown in a separate pot under greenhouse conditions at the Centre of Biotechnology of Borj Cedria, Tunisia. Treatments and lines were arranged in a randomized complete block design with five blocks and five replicates per experimental unit. Plants were grown in one of the two water treatments, as follows: well-irrigated treatment or mild water-deficit treatment (50% of field capacity (FC)). In order to simulate natural water-deficit conditions similar to field, water stress was applied after a growth period of 10 d. Water levels were maintained for each treatment by weighing pots every 2 d and the water lost was replaced carefully to maintain soil water content near the 100 and 50% of FC in

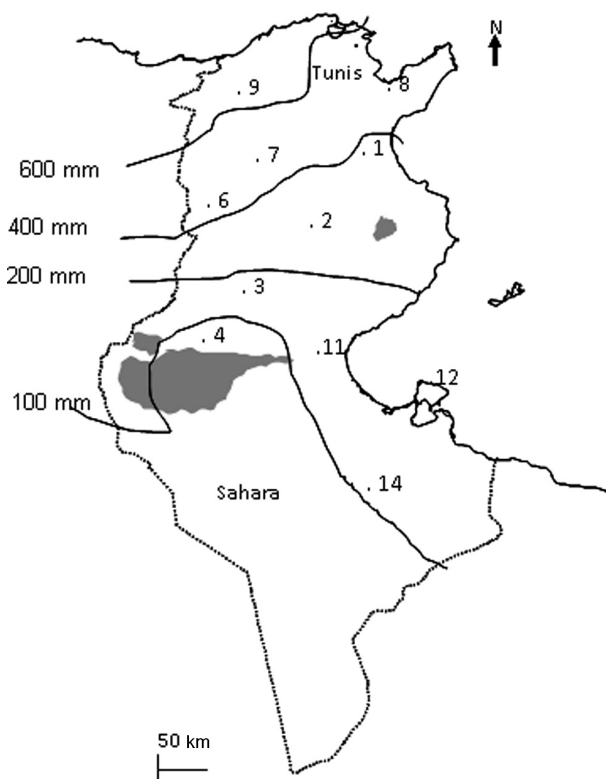


Fig. 1. Map showing the 11 Tunisian populations from which 108 lines of *M. truncatula* were obtained.

each pot. Plants were irrigated using a nutritive solution as described by Vadez *et al.* (1996), with the source of iron modified to be Fe-EDTA. For each treatment, each genotype was replicated five times, resulting in a total of 1130 planted seeds. After 2 months, for each individual plant we measured 15 quantitative traits of shoot and root growth (Table 1). For dry weight biomass, plant organs were dried at 70°C for 48 h. The aerial water content (AWC) and root water content (RWC) were estimated as follows:

$$\text{AWC} = 100(\text{AFW} - \text{ADW})/\text{AFW};$$

$$\text{RWC} = 100(\text{RFW} - \text{RDW})/\text{RFW};$$

where AFW and RFW are the aerial and root fresh weights, respectively, while ADW and RDW are the aerial and root dry weights, respectively.

Statistical analyses

Genetic variation of drought response in *Medicago truncatula*

From each population, a single seed per pod was used to initiate lines of progeny from self-pollination. Although the Tunisian populations of *M. truncatula* we used are highly selfing in nature (Arraouadi *et al.*, 2009; Lazrek *et al.*, 2009), each line was selfed twice or three times in the greenhouse to lower residual heterozygosity. The offspring in each presumed line was considered genetically identical. Consequently, the within-line variance can be assumed as the effect of environment while the variation among the lines is assumed to be solely genetic. Drought response index (DRI) was calculated per line and for each trait as the mean of the ratios between the observed mean value under water-deficit condition and

the mean values under well-watered condition. Analysis of the effects of population, treatment, line within population, and population \times treatment and treatment \times line within population interactions on measured traits was performed using Proc GLM in SPSS version 16 (2007 Rel 1600 SPSS Inc., Chicago, IL, USA). Phenotypic mean values of each population were compared for all quantitative traits using the Duncan multiple range test at 5%.

The variance components were estimated using the procedure VARCOMP in the SPSS software. The level of population differentiation (Q_{ST}) for quantitative traits was computed as described by Neji *et al.* (2014) for a predominantly selfing species as $Q_{ST} = \sigma_p^2 / (\sigma_p^2 + \sigma_w^2)$, where σ_p^2 is the variance among populations and σ_w^2 the variance within populations. Broad-sense heritability (H^2) was calculated for each trait as $H^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_e^2)$, where σ_g^2 is the variance between lines and σ_e^2 the environmental effect. The coefficient of genetic variation (CV) was estimated as $CV = 100(\sigma_g^2)^{1/2}/m$, where m is the population phenotypic mean.

Phenotypic correlations between all trait combinations for each treatment were estimated by computing the Pearson correlation coefficient (r) using the SPSS Correlate procedure.

Clustering analysis

A standardized principal component analysis (PCA) (Data reduction-Factor procedure – SPSS) was performed on the correlation matrix of the synthetic variable based on the mean line values. To represent the relationships between studied populations and lines within populations in water-deficit conditions, cluster analysis was performed to generate phenograms based on the Euclidean distance matrix of dissimilarity, calculated using all PCA scores (Badri *et al.*, 2007), by the Ward method

Table 1. List of measured quantitative traits and their abbreviations

Trait	Abbreviation
Length of orthotropic axis (cm)	LO
Length of plagiotropic axes (cm)	LP
Total length of stems (cm)	LS = LO + LP
Length of roots (cm)	LR
Number of ramifications	NR
Number of internodes	NIN
Number of leaves	NL
Leaf area (cm ²)	LA
Aerial fresh weight (g)	AFW
Aerial dry weight (g)	ADW
Aerial water content (%)	AWC = 100(AFW – ADW)/AFW
Root fresh weight (g)	RFW
Root dry weight (g)	RDW
Root water content (%)	RWC = 100(RFW – RDW)/RFW
Root dry weight and aerial dry weight ratio	RDW:ADW ratio

using the XLSTAT software v 7.5 (Addinsoft, USA). A Discriminant Analysis (DA) was performed on DRI values for the groups of *M. truncatula* lines. Finally, the relationship between the Euclidean distance between populations based on quantitative traits and the geographical distance matrix was analysed using a Mantel test (XLSTAT software v 7.5, Addinsoft, USA). The matrix of geographical distance between populations was calculated by measuring the shortest distance between two points in the map, using geographical coordinates for each site.

Results

Genetic variation of drought response in *Medicago truncatula*

ANOVA showed that the variation of drought response was explained by the effects of population, line, treatment and interactions between population and treatment, and line and treatment. The maximum effect was observed for treatment factor (Table 2). Thus, *M. truncatula* lines differ in their response to drought treatment.

Most of the measured traits showed a significant difference between studied populations of *M. truncatula* under control treatment and drought stress (Tables S2 and S3, available online). In the control treatment, the Bulla Regia population exhibited the

largest ADW, and the highest RDW was observed for the El Kef population. The Enfidha population had the highest length of stems (LS), while the maximum number of ramifications (NR) was recorded for the Soliman population. Furthermore, the Amra and Deguache populations showed the highest RDW:ADW ratio.

All measured traits, except the AFW and AWC, showed significant reductions, caused by water-deficit treatment, between studied populations of *M. truncatula* (Table S4, available online). In the drought stress, the lowest decrease in LS was recorded for the reference lines, but these lines experienced the strongest reductions in the number of leaves (NL), AFW, ADW and RDW. The highest reduction in LS was recorded for Deguache (DRI = 43.65%), Djerba (DRI = 43.36%), Amra (DRI = 46.85%) and Tataouine (DRI = 47.21%), while El Kef (DRI = 121.92%), Soliman (DRI = 114.27%) and Gabès (DRI = 108.94%) were most susceptible according to the RDW:ADW ratio. The lowest reductions were found for NR in Deguache (DRI = 79.45%) and Enfidha (DRI = 33.02%), for length of roots (LR) for Deguache (DRI = 112.38%), for NL for Thala (DRI = 71.58%), for AFW for Soliman (DRI = 53.95%), for RDW for Jelma (DRI = 87.03%) and for RDW:ADW ratio for Deguache (DRI = 167.11%). Overall, the population of Deguache allocated highest proportion of its biomass to roots in both control and water-deficit treatments. Table S4 (available online) gives the CV of each trait for studied populations in *M. truncatula*. We will arbitrarily consider as large those CV above 40%. The largest variations

Table 2. Contribution of population, line within population, treatment, and population \times treatment ($P \times T$) and line \times treatment ($L \times T$) interactions to the total variance of measured traits for studied populations of *Medicago truncatula*

	Population (P)		Line (L)		Treatment (T)		$P \times T$		$L \times T$	
	F	P	F	P	F	P	F	P	F	P
LO ^a	207,852	0.000	24,249	0.000	608,396	0.000	5573	0.000	1354	0.015
LP	13,641	0.000	6687	0.000	1,179,457	0.000	3084	0.000	2689	0.000
LS	28,832	0.000	8042	0.000	1,219,476	0.000	3975	0.000	2307	0.000
LR	3847	0.000	1198	0.099	4667	0.031	4057	0.000	1435	0.005
NR	26,262	0.000	3752	0.000	304,756	0.000	3544	0.000	1518	0.001
NIN	12,156	0.000	3216	0.000	345,651	0.000	0.63	0.800	0.77	0.952
NL	51,328	0.000	8295	0.000	1,157,475	0.000	1998	0.026	1207	0.091
LA	66,437	0.000	5665	0.000	771,579	0.000	9191	0.000	1354	0.015
AFW	38,866	0.000	3115	0.000	1,428,538	0.000	4486	0.000	1121	0.206
ADW	90,038	0.000	4790	0.000	1,485,283	0.000	7919	0.000	1126	0.198
AWC	13,718	0.000	1347	0.017	24,443	0.000	0.76	0.686	0.96	0.602
RFW	25,320	0.000	3031	0.000	953,867	0.000	7155	0.000	1313	0.026
RDW	13,000	0.000	3283	0.000	200,008	0.000	2272	0.010	1531	0.001
RWC	2958	0.001	1228	0.072	193,128	0.000	1427	0.155	1311	0.027
RDW:ADW ratio	35,004	0.000	4677	0.000	90,924	0.000	3528	0.000	1426	0.005

F_i coefficient of Snedecor–Fisher.

Significant ($P \leq 0.05$), non significant ($P > 0.05$).

^a Abbreviations of the traits are defined in Table 1.

within populations ($CV > 40\%$) were noticed for the length of orthotropic axis (LO), LS, RFW, RDW and RDW:ADW ratio.

H^2 of measured traits ranged from 0.00 to 0.74 and from 0.02 to 0.65 under control and drought stress treatments, respectively (Table 3). In the well-irrigated treatment, high heritabilities ($H^2 > 0.4$) were recorded for LO, length of plagiotropic axes (LP) and NL, moderate values ($0.2 < H^2 \leq 0.4$) were for LS, NR, leaf area (LA), ADW, RDW and RDW:ADW ratio, and lower levels ($H^2 \leq 0.2$) were registered for LR, number of internodes (NIN), AWC, RFW and RWC. Furthermore, in the drought stress treatment, high heritabilities (H^2) were observed for LO and NL, low levels were for LR, NR, AFW, ADW, AWC, RFW, RDW and RWC, and moderate values were recorded for the remaining traits. Overall, most of the measured traits showed highest heritabilities (H^2) in well-irrigated treatment (Table 3).

Q_{ST} ranged from 0 to 1 and from 0 to 0.73 under control and water-deficit treatments, respectively (Table 3). In the well-watered condition, the highest Q_{ST} values were recorded for LO, NR, LA, AFW, ADW, AWC and RFW, moderate Q_{ST} were for LS, NIN, NL, RDW and RDW:ADW ratio, and lower Q_{ST} were registered for the remaining traits. In the water-deficit conditions, the highest Q_{ST} were found for LO, LR, NR, NIN, NL, LA, AFW, ADW, AWC, RFW, RWC and RDW:ADW ratio, moderate Q_{ST} values were recorded for LP and LS, and lower Q_{ST} was for RDW. Overall, the highest Q_{ST} values for most traits were recorded in drought stress. Moreover, Q_{ST} results indicated a lack of population structuring, in both treatments, such that most of the overall variation

for the majority of investigated traits was held within populations.

Among the 105 possible correlations between measured traits in control treatment, 81 were significant and 66 of them were positive (Table S5, available online). Most traits of aerial and root growths were positive. The RDW:ADW ratio was negatively correlated with the characters of aerial growth and positively with AWC and RDW. In the water-deficit condition, among the 105 correlations between measured characters, 73 were significant and 59 of them were positive. The RDW:ADW ratio was negatively correlated with the characters of aerial growth and positively with AWC, RFW and RDW.

Comparison between two matrices of intertrait correlations in inbred lines of *M. truncatula* in control and drought stress treatments (Table S5, available online) shows that (1) several correlations are specific in well-irrigated treatment such as positive correlation between LO and RWC, and (2) specific correlations in drought stress were noticed such as a positive correlation between LS and RDW.

Clustering analysis based on the DRI values

Six principal components, whose eigenvalues were higher than 0.80, were found to account for 81.93% of the total phenotypic variation. Using these six components, lines of *M. truncatula* were clustered based on Euclidean distances of dissimilarity. Four groups of lines differing in their sensitivities to drought stress were generally identified, based on the DRI values

Table 3. Variance among populations (σ_p^2), genetic variance (σ_g^2), environmental variance (σ_e^2), heritabilities (H^2) and population differentiation for quantitative traits (Q_{ST}) in measured traits for *Medicago truncatula* lines under well-watered (control) and water-stressed (drought) conditions

	Control					Drought				
	σ_p^2	σ_g^2	σ_e^2	H^2	Q_{ST}	σ_p^2	σ_g^2	σ_e^2	H^2	Q_{ST}
LO ^a	88.985	120.323	43.217	0.74	0.43	61.563	63.889	34.969	0.65	0.49
LP	15.957	174.627	212.983	0.45	0.08	8.076	24.014	53.97	0.31	0.25
LS	129.141	429.918	1278.000	0.25	0.23	34.127	65.934	129.002	0.34	0.34
LR	0.000	0.362	8.050	0.04	0.00	0.642	0.235	2.591	0.08	0.73
NR	0.628	0.839	1.900	0.31	0.43	0.448	0.548	2.23	0.20	0.45
NIN	4.177	11.918	75.598	0.14	0.26	5.967	8.862	29.237	0.23	0.40
NL	30.277	51.712	64.490	0.45	0.37	21.308	31.216	45.601	0.41	0.41
LA	0.312	0.181	0.295	0.38	0.63	0.071	0.07	0.201	0.26	0.50
AFW	0.219	0.122	0.500	0.20	0.64	0.059	0.022	0.169	0.12	0.73
ADW	0.016	0.006	0.013	0.32	0.73	0.005	0.002	0.008	0.20	0.71
AWC	4.022	0.000	25.950	0.00	1.00	5.614	6.169	175.881	0.03	0.48
RFW	0.133	0.101	0.433	0.19	0.57	0.021	0.02	0.08	0.20	0.51
RDW	0.001	0.002	0.005	0.29	0.33	0.000	0.001	0.005	0.17	0.00
RWC	1.294	5.480	35.075	0.14	0.19	1.681	1.851	107.538	0.02	0.48
RDW:ADW ratio	0.007	0.013	0.028	0.32	0.35	0.026	0.025	0.066	0.27	0.51

^a Abbreviations of the traits are defined in Table 1.

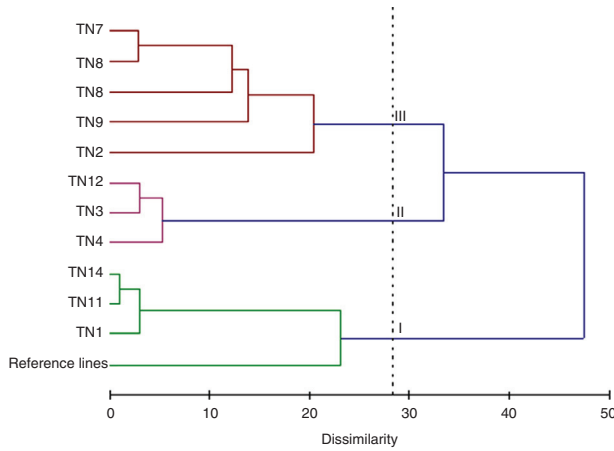


Fig. 2. Clustering of populations of *M. truncatula* based on Euclidean distances of dissimilarity into three groups with different water-deficit responses.

(Fig. S1, available online). A first group contained 31 lines with one line per population from Amra and Deguache, one reference line, two lines from Gabès, three lines per population from Enfidha, El Kef, Djerba and Tataouine, four lines from Soliman and five lines per population from Thala and Bulla Regia. A second group included 45 lines with one line of Thala, three reference lines, three lines per population from Jelma, Deguache, Soliman and Bulla Regia, four lines per population from El Kef, Djerba and Tataouine, five lines per population from Amra and Gabès, and seven lines from Enfidha. A third group constituted by 23 lines with one reference line, one line per population from Amra, Bulla Regia,

Djerba and Tataouine, two lines from El Kef, three lines per population from Deguache, Thala, Soliman and Gabès, and four lines from Jelma. A fourth group contained 14 lines with one line per population from Thala, El Kef and Bulla Regia, two lines per population from Amra, Deguache, Djerba and Tataouine, and three lines from Jelma.

Five principal components, whose eigenvalues were higher than 0.80, were found to account for 91.92% of the total variation. Using these five components, populations of *M. truncatula* were clustered based on Euclidean distances of dissimilarity into three groups differing in their water-deficit response (Fig. 2). A first group contained reference lines, Enfidha (TN1), Gabès (TN11) and Tataouine (TN14) populations. A second group comprised Deguache (TN4), Amra (TN3) and Djerba (TN12). A third group included Jelma (TN2), Bulla Regia (TN9), Soliman (TN8), Thala (TN6) and El Kef (TN7).

Based on the DA, LP, LS, NIN, NL, RFW, RWC and RDW:ADW ratio are the main determinant traits for the classification of studied lines of *M. truncatula* into the four clusters (Table 4). Groups 1 and 3 were less affected by drought for LP, LS, NIN and NL, group 3 was least affected for RFW and RWC and group 4 had lowest effect for RDW:ADW ratio. Group 2 was, on average, the most affected group by drought.

On the other hand, DA showed that the LO ($\lambda = 0.52$, $F = 4.17$, $P = 0.05$), NIN ($\lambda = 0.4$, $F = 6.88$, $P = 0.015$) and RWC ($\lambda = 0.25$, $F = 13.26$, $P = 0.002$) are the main determinant characters for the classification of the studied populations. The first class contained the populations

Table 4. Means of drought response index (DRI) for classes of *Medicago truncatula* lines

Class\variable	DRI				λ	F	P
	1	2	3	4			
LO ^a	77.19	68.82	84.89	66.21			
LP	36.58b	15.07c	48.23a	22.13c	0.55	29.75	<0.0001
LS	55.31b	43.26c	65.42a	44.99c	0.55	30.19	<0.0001
LR	98.17	97.95	103.09	100.85			
NR	72.55	43.71	82.87	55.44			
NIN	72.82b	59.56c	80.73a	64.18c	0.57	27.40	<0.0001
NL	68.37b	58.95c	77.84a	60.83c	0.48	39.05	<0.0001
LA	73.57	73.94	85.20	71.25			
AFW	50.35	42.78	57.92	42.81			
ADW	54.62	47.66	63.76	47.01			
AWC	98.64	95.83	97.31	98.49			
RFW	39.43bc	37.29c	59.76a	46.06b	0.66	18.83	<0.0001
RDW	67.17	54.93	81.58	98.78			
RWC	90.67bc	93.15ab	95.39a	89.09c	0.89	4.58	0.005
RDW:ADW ratio	125.04b	117.12b	131.65b	222.95a	0.43	48.24	<0.0001

λ , Lambda of Wilks; F, coefficient of Snedecor–Fisher.

Means of each trait followed by different letters are significantly different between the four lines' groups.

^a Abbreviations of the traits are defined in Table 1.

and the reference lines, which are most affected for RWC, the second group included the populations less affected for NIN and the third group had the populations with highest reductions in LO.

Discussion

The significant genotype \times treatment interaction observed for most of the traits suggests that lines of *M. truncatula* may have developed an adaptive response to drought that can be exploited to further determine the genetic basis of this physiological adaptation.

The LP was the most seriously affected trait (DRI = 28.59%) for populations while the RDW:ADW ratio had lowest reductions (DRI = 135.36%). The importance of root systems in acquiring water has long been recognized (Jaleel *et al.*, 2009). A prolific root system can confer the advantage to support accelerated plant growth during the early crop growth stage and extract water from shallow soil layers that is otherwise easily lost by evaporation in legumes (Johansen *et al.*, 1992). An increased root growth due to water stress was reported in sunflower (Tahir *et al.*, 2002), *Catharanthus roseus* (Jaleel *et al.*, 2008), and *M. truncatula* and *M. laciniata* (Badri *et al.*, 2011). The root dry weight was decreased under mild and severe water stress in *Populus* species (Wullschleger *et al.*, 2005), and in maize and wheat (Sacks *et al.*, 1997).

Moderate levels of genotypic variance and heritability estimates were found for most traits under control treatment and drought stress. It will be relatively easy to select for these traits and pass them onto offspring. H^2 of traits in this study was dependent on treatment effect with higher values generally recorded in well-irrigated treatment. Gomez-Mestre and Tejedo (2004) showed that the heritability of the trait in the natterjack toad (*Bufo calamita*) populations increased as the osmotic stress increased, as differences among full-sibs became accentuated. However, the more informative analysis of half-sibs revealed that narrow-sense heritability might be decreasing as salinity increases (Tejedo M *et al.*, unpublished data). This apparent contradiction is most probably caused by the presence of non-additive components (dominance and epistasis) and maternal effects that may be sensitive to the level of stress.

Our results indicate that differences in water availability can result in population differentiation (Q_{ST}) and local adaptation in natural populations of *M. truncatula*. Drought stress should exert strong selection on plant physiology, morphology and growth, and population differentiation in response to variation in water availability has been documented in several species and at several geographical scales (Nienhuis

et al., 1994; Eckhart *et al.*, 2004; Sambatti and Rice, 2007; Wu *et al.*, 2010). Accordingly, Gomez-Mestre and Tejedo (2004) showed that most estimates of quantitative genetic distances between population pairs (Q_{ST}) increased as salinity increased, indicating that differences in salinity tolerance among populations broadened with increased osmotic stress.

Most of the correlations between measured traits under control and water-deficit treatments were positive; they were dependent on the treatment effect. This finding is in accordance with that reported by Teulat *et al.* (1997), demonstrating that genotypic correlations between growth characters were dependent on the water treatment in recombinant inbred lines of barley (*Hordeum vulgare* L.). In the current study, traits of *M. truncatula* are positively correlated, probably because they are developmentally or structurally related. Furthermore, reduced genetic correlations between investigated characters can speed adaptation to novel habitats (e.g. Badyaev *et al.*, 2005).

Using the five components of PCA that explain 91.92% of the total variation, populations of *M. truncatula* were clustered into three groups differing in their drought response. Some of the populations selected for this study varied with geographic information such as altitude, electro-conductivity, annual rainfall, mean temperature and mean relative humidity. However, no consistent association ($r = 0.001$; $P = 0.99$) was found between matrices of drought responses among populations and geographical distances. Similar results were recorded for natural populations of *M. truncatula* in salt stress (Arraouadi *et al.*, 2011).

On the other hand, the clustering illustrated in Fig. S1 (available online) of studied lines could be a useful guide for breeding programmes.

Overall, natural populations of *M. truncatula* showed a large variation in drought tolerance. Four groups of lines differing in their sensitivities to drought stress were identified, with 23 tolerant, 31 moderately tolerant, 14 susceptible and 45 highly susceptible lines. The tolerant group was less affected for LP, LS, NIN and NL than the susceptible group. The results can be used for selecting materials for further investigation of the genes and alleles important for drought tolerance in *M. truncatula*.

Supplementary material

To view supplementary material for this article, please visit <http://dx.doi.org/10.1017/S1479262115000052>

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