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# Comparative effects of water deficit on *Medicago laciniata* and *Medicago truncatula* lines sampled from sympatric populations

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We evaluated the responses to water deficit of twelve lines of *Medicago laciniata* and *Medicago truncatula* including eight lines from four Tunisian sympatric populations of both species, and four references lines of *M. truncatula*. They were exposed to two water treatments, well irrigated and drought-stress (33% of field capacity) for a period of 45 days. At harvest, we measured five quantitative traits including the length of stems (LS), aerial dry weight (ADW), root dry weight (RDW), RDW/ADW ratio and leaf area (LA). Analysis of variance showed that the variation of measured traits among studied lines was significantly explained by the effects of species, line, treatment, and their interactions. Treatment and species had the largest effects. We also analyzed the broad-sense heritability of the drought response index (DRI), defined as the ratio between the observed values with and without water deficit treatment. DRI of most measured traits had high broad-sense heritability ( $H^2$ ). The length of stems (LS) was the trait most affected by drought stress, while RDW/ADW ratio was not affected by drought. Generally, few significant differences were observed between DRI values of measured traits between lines within species as well as between lines of *M. laciniata* and *M. truncatula* collected from sympatric populations. Most of the correlations established between DRI values were positive. The environmental factors that most influenced variation of DRI values among populations were available phosphorus (P) and mean annual rainfall.

**Key words:** *Medicago laciniata*, *Medicago truncatula*, sympatric populations, lines, drought response index, environmental factors.

## INTRODUCTION

Abiotic stresses such as drought, high salinity and extreme temperatures, together, represent the primary cause of crop loss worldwide, reducing average yields for most major crop plants by more than 50%. In contrast, the estimated yield loss caused by pathogens is typically about 10 - 20% (Kreps et al., 2002). Water deficit is a common environmental stress experienced by plants. It affects both development and growth, and has a negative effect on productivity (Altinkut et al., 2003; Slama et al., 2006; Verslues et al., 2006; Tuberosa and Salvi, 2006). Approximately one third of the world's arable land suffers

from chronically inadequate supplies of water for agriculture, and in virtually all agricultural regions, yields of rain-fed crops are periodically reduced by drought (Boyer, 1982). Drought stress is common not only in arid and semi-arid regions, but also in places where total precipitation is high but is not evenly distributed over the growing season (Altinkut et al., 2003). Plant responses to drought occur through changes in their morphological, biochemical and metabolic processes (Li et al., 2004; Pérez-Pérez et al., 2009). Tolerance or susceptibility to drought stress is a very complex phenomenon, in part, because stress may occur at multiple stages of plant development and often more than one stress simultaneously affects the plant (Tuberosa and Salvi, 2006). Improving drought tolerance is probably one of the most difficult tasks for plant breeders. The difficulty comes

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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). Water deficit is therefore the most important abiotic stress and strategies to sustainable use of water (Kang et al., 2003) and improve plant drought resistance are urgent and should integrate both conventional breeding and biotechnological approaches (Chaves et al., 2009). Plants have evolved different adaptive strategies to alleviate the adverse effects of these abiotic stresses (Verslues et al., 2006). Legumes are the third largest family of flowering plants and contain several major agricultural and economical species (Gepts et al., 2005). They have generated much interest in the plant scientific community, not only because of the important crop plants, but also because of their interactions with microbial symbionts (Nunes et al., 2008). In Tunisia, as in the world, the limiting factors for cultivation of legumes are their low adaptation to the diverse local ecological factors and the slowness of genetic improvements due to their genomic complexity. Drought is one of the major factors that limit legumes crop productivity in Tunisia with 95% of her surface being located in semi-arid, arid and Saharan stages. Since the genus *Medicago* is endemic in Tunisia and represents an important proportion of the native flora in all bioclimatic stages (Seklani et al., 1996), most of agronomical traits of interest such as the tolerance to drought stress can be found within its annual species. *M. truncatula*, an annual forage plant and a close relative of alfalfa, was identified as being a suitable model legume because it is a diploid ( $2n = 16$ ), self-pollinating species with a small genome and the resources to create transgenic plants (Cook, 1999). Genetic and genomic tools are rapidly evolving and the scope of work performed in *M. truncatula* is expanding and diversifying (Young and Udvardi, 2009). *M. truncatula* and *M. laciniata* have two different geographical distributions both globally and in Tunisia. The first one is omni-Mediterranean and ubiquitous in Tunisia. The second is the only species of the genus *Medicago* that is limited to southern Mediterranean regions (Heyn, 1963); it is restricted in Tunisia to the inferior semi-arid, arid and Saharan stages (Badri et al., 2007).

In the last two decades, molecular mechanisms of drought stress response and tolerance in plants have become an active area of investigation where many genes, which are regulated by drought stress, have been reported in a variety of plants (Yamaguchi-Shinozaki et al., 2002; Leung, 2008). Numerous studies have focused on the physiological and molecular mechanisms by which the model legume *M. truncatula* responds to abiotic stresses such as flooding stress (Limami et al., 2007), drought (González-Andres et al., 2007; Badri, 2008), salt stress (Merchan et al., 2007; Mhadhbi and Aouani, 2008), and cold stress (Avia and Lejeune-Hénaut, 2007). Nevertheless, few investigations have compared the

responses of different *Medicago* annual species to water deficit by taking into account their geographical origins and the genetic variation within the studied species (Hamidi, 1991; Mefti et al., 2001; Turk, 2006).

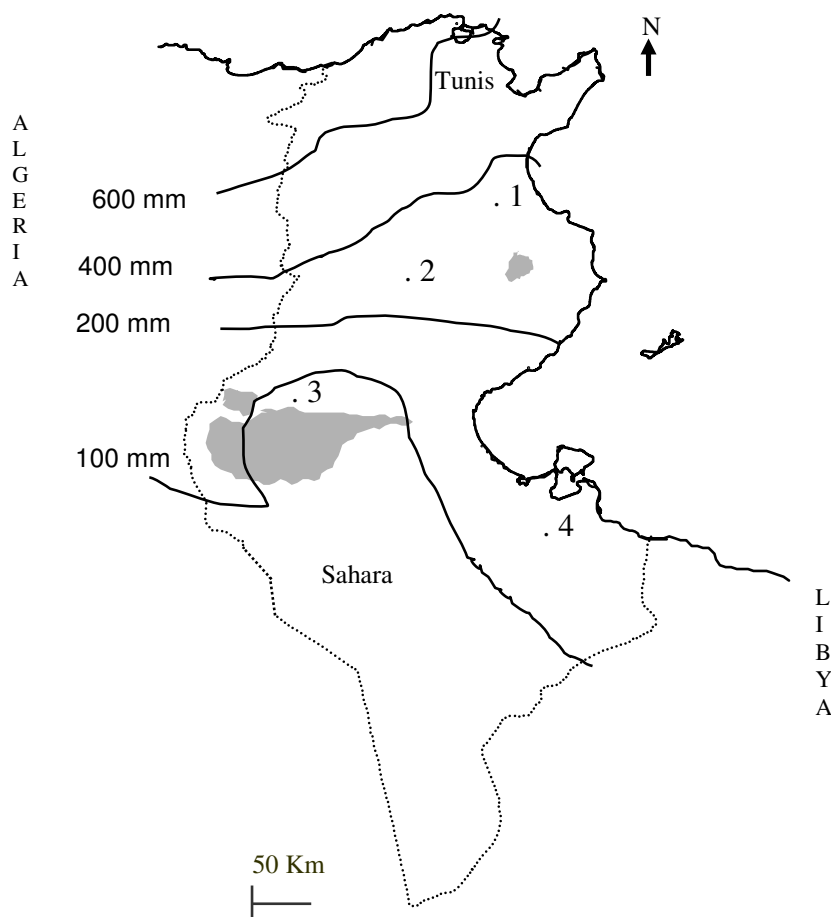
Drought response index (DRI) is the ratio between the observed values under water-stressed and well-watered conditions; it is a measurement of change in plant traits caused by drought stress (Chen et al., 2007). There is a dearth of information on the use DRI as an indicator of drought tolerance in *M. laciniata* and *M. truncatula*. The DRI value was considered as the indicator for water deficit tolerance. The aims of this study were to (i) assess and compare the effects of drought stress on *M. laciniata* and *M. truncatula* lines sampled from sympatric populations; (ii) compare the effects of water deficit on the local and references lines of *M. truncatula*; and (iii) estimate the associations of water deficit responses in local lines of both species with site-of-origin environmental factors.

## MATERIALS AND METHODS

### Plant material and growth parameters

Eight lines from 4 sympatric populations of *M. laciniata* and *M. truncatula* and 4 references lines of *M. truncatula* were used. The sympatric populations from which studied lines were used are El Ghouilet, Jelma, Deguache and Medenine (Figure 1). The ecological factors of sampling sites of these populations are summarized in the Table 1. Soil samples were collected from three locations at these sites, and analyzed by the Laboratory of Soils at the Ministry of Agriculture, Tunisia. To distinguish between natural populations of both species, we used two symbols; TN for *M. truncatula* and TNL for *M. laciniata*. The studied lines are TNL1.9 and TN1.21, TNL2.3 and TN2.12, TNL4.7 and TN4.20, and TNL9.2 and TN13.11 for *M. laciniata* and *M. truncatula*, respectively. Natural populations of both species from which studied lines have been chosen were characterized using quantitative traits and microsatellite markers (Badri et al., 2007). From each population, a single-seed per pod was used to initiate lines of progeny from self-pollination. Inbred lines were created by two generations of spontaneous self-pollination in isolation in the greenhouse, even if we know that maternal effects could persist after two generations of self-pollination. The offspring in each presumed line was considered genetically identical. Consequently, the within-line variance can be considered as environmental, while the among-lines variance component is assumed to be solely genetic (Arraouadi et al., 2009). We used four references lines of *M. truncatula*: Jemalong A17 (JA17) from an Australian collection, two Algerian lines DZA315.16 and DZA45.5, and the French line F83005.5 from the Var region. These lines are the parents of various crosses: JA17 x DZA315.16, JA17 x F83005.5, DZA315.16 x DZA45.5, DZA45.5 x DZA315.16, and DZA45.5 x F83005.5. A number of recombinant inbred lines (RILs) populations derived from these crosses were obtained and a set of framework genetic maps were developed and others are in progress (Huguet et al., 2005).

The present work was conducted in the Centre of Biotechnology of Borj Cedria (CBBC), Tunisia. 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 agar 0.9% medium before being vernalized at 4°C for 96 h in darkness. After germination, the seedlings were transferred to 400 ml plastic pots (9.3 cm diameter and 8.5 cm deep) filled with a mixture of compost and the soil of the CBBC at a



**Figure 1.** Map of Tunisia with the location of sympatric populations of *M. laciniata* and *M. truncatula* from which studied lines were collected. 1: El Ghouilet; 2: Jelma, 3: Deguache; and 4: Medenine.

**Table 1.** List of the four eco-geographical factors of sampling sites of natural populations of *M. laciniata* and *M. truncatula* from which studied lines were collected.

|                           | El Ghouilet | Jelma | Deguache | Medenine |
|---------------------------|-------------|-------|----------|----------|
| Organic matter (%)        | 1.6         | 1.8   | 0.9      | 0.7      |
| Phosphorus (mg/kg)        | 26          | 18    | 22       | 18       |
| Mean annual rainfall (mm) | 350         | 250   | 50       | 175      |
| Altitude (m)              | 30          | 300   | 25       | 119      |

ratio of 3:1 having 22.5% of organic matter, pH 8, available phosphorus (P) = 224 mg/kg,  $K_2O$  = 0.36 g/kg, total carbon = 13%, active lime = 4%, electrical conductivity (ECe) = 3 mmho/cm and saturation = 43 ml/100 g. All plants were grown in individual pots in a growth chamber at temperature of 25 °C, 80% of relative humidity and a photoperiod of 16/8 h. The experimental design was completely randomized. The lines under study underwent two water treatments, well irrigated and kept under drought (33% of field capacity) as described by Badri et al. (2005). Five replicates for each line per treatment were used. Plants were harvested after a period of 45 days. At harvest, five quantitative traits of the aerial and root growth were measured including length of stems (LS), aerial dry weight (ADW), root dry weight (RDW), the RDW/ADW

ratio and the sixth leaf area (LA). Plant organs were harvested and dried at 70 °C for 48 h in order to determine the dry matter.

#### Statistical analyses

Data were subjected to an analysis of variance (ANOVA) using the Statistical Analysis System (SAS 7.02 Institute, Inc., 1998) and the means of measured traits were compared between lines with Duncan's multiple range tests. Drought response index (DRI) represents the relative change for each trait caused by drought. It was calculated using the following formula (Chen et al., 2007):

$$DRI = (\text{Value from drought treatment} / \text{Value from control}) * 100\%$$

**Table 2.** Proportions and significance levels of line, treatment, line x treatment interaction, species and species x treatment interaction effects on measured traits for *M. laciniata* and *M. truncatula*.

| Traits  | <i>M. laciniata</i> |    |                       | <i>M. truncatula</i> |    |                       | <i>M. laciniata-M. truncatula</i> |                     |    |                       |       |
|---------|---------------------|----|-----------------------|----------------------|----|-----------------------|-----------------------------------|---------------------|----|-----------------------|-------|
|         | Effects             | df | F                     | %                    | df | F                     | %                                 | Effects             | df | F                     | %     |
| LS      | Line                | 3  | 10.25 <sup>***</sup>  | 5.63                 | 3  | 3.55 <sup>*</sup>     | 1.40                              | Species             | 1  | 51.22 <sup>***</sup>  | 16.10 |
|         | Treatment           | 1  | 163.86 <sup>***</sup> | 89.92                | 1  | 251.19 <sup>***</sup> | 98.60                             | Treatment           | 1  | 255.96 <sup>***</sup> | 80.45 |
|         | Line x Treatment    | 3  | 8.12 <sup>***</sup>   | 4.46                 | 3  | 0.03 <sup>ns</sup>    | 0.01                              | Species x Treatment | 1  | 10.97 <sup>***</sup>  | 3.45  |
|         | Line                | 3  | 7.06 <sup>***</sup>   | 4.19                 | 3  | 10.60 <sup>***</sup>  | 3.87                              | Species             | 1  | 131.66 <sup>***</sup> | 34.16 |
| ADW     | Treatment           | 1  | 155.24 <sup>***</sup> | 92.24                | 1  | 259.54 <sup>***</sup> | 94.85                             | Treatment           | 1  | 206.41 <sup>***</sup> | 53.55 |
|         | Line x Treatment    | 3  | 6.01 <sup>**</sup>    | 3.57                 | 3  | 3.50 <sup>*</sup>     | 1.28                              | Species x Treatment | 1  | 47.36 <sup>***</sup>  | 12.29 |
|         | Line                | 3  | 11.28 <sup>***</sup>  | 19.76                | 3  | 5.35 <sup>**</sup>    | 6.35                              | Species             | 1  | 104.00 <sup>***</sup> | 53.26 |
| RDW     | Treatment           | 1  | 39.25 <sup>***</sup>  | 68.74                | 1  | 75.25 <sup>***</sup>  | 89.26                             | Treatment           | 1  | 62.35 <sup>***</sup>  | 31.93 |
|         | Line x Treatment    | 3  | 6.57 <sup>**</sup>    | 11.50                | 3  | 3.70 <sup>*</sup>     | 4.39                              | Species x Treatment | 1  | 28.93 <sup>***</sup>  | 14.81 |
|         | Line                | 3  | 1.06 <sup>ns</sup>    | 10.60                | 3  | 22.71 <sup>***</sup>  | 33.63                             | Species             | 1  | 21.36 <sup>***</sup>  | 52.10 |
| RDW/ADW | Treatment           | 1  | 8.74 <sup>**</sup>    | 87.57                | 1  | 36.80 <sup>***</sup>  | 54.49                             | Treatment           | 1  | 19.56 <sup>***</sup>  | 47.72 |
|         | Line x Treatment    | 3  | 0.18 <sup>ns</sup>    | 1.83                 | 3  | 8.03 <sup>***</sup>   | 11.88                             | Species x Treatment | 1  | 0.07 <sup>ns</sup>    | 0.18  |
|         | Line                | 3  | 7.23 <sup>***</sup>   | 10.38                | 3  | 148.10 <sup>***</sup> | 61.34                             | Species             | 1  | 78.79 <sup>***</sup>  | 85.04 |
| LA      | Treatment           | 1  | 61.89 <sup>***</sup>  | 88.89                | 1  | 80.41 <sup>***</sup>  | 33.30                             | Treatment           | 1  | 10.98 <sup>***</sup>  | 11.85 |
|         | Line x Treatment    | 3  | 0.51 <sup>ns</sup>    | 0.73                 | 3  | 12.93 <sup>***</sup>  | 5.36                              | Species x Treatment | 1  | 2.88 <sup>ns</sup>    | 3.11  |

Significance levels; ns: not significant ( $p > 0.05$ ), \* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$ , F: Snedecor-Fisher coefficient. Length of stems (LS), aerial dry weight (ADW), root dry weight (RDW), the RDW/ADW ratio and leaf area (LA).

As described by Badri et al. (2007), total phenotypic variance ( $\sigma_T^2$ ) was the sum of two components of variance (i) arising between lines genotypes ( $\sigma_g^2$ ), and (iii) due to residual error arising between individuals within original genotype ( $\sigma_e^2$ ).

Broad-sense heritability ( $H^2$ ) was estimated for each trait as the ratio of the variance arising between lines genotypes ( $\sigma_g^2$ ) divided by the sum of ( $\sigma_g^2$ ) and ( $\sigma_e^2$ ) (Bonnin et al., 1997). Phenotypic correlations among measured traits were estimated by the SAS CORR procedure (SAS 7.02 Institute, Inc.). Correlations between DRI of measured

traits for *M. laciniata* and *M. truncatula* lines and environmental factors were estimated by computing Pearson's correlation coefficient ( $r$ ). Significance level was set to 0.05 and adjusted for multiple comparisons by Bonferroni correction (Badri et al., 2007).

## RESULTS AND DISCUSSION

Analysis of variance showed that variation of measured traits among the studied lines was significantly explained by the effects of line, treatment and their interaction (Table 2). Treatment had the largest effect on trait values,

with the exception of leaf area (LA), with effects ranging from 68.74 - 92.24% and from 54.49 - 98.60%, respectively, for *M. laciniata* and *M. truncatula*. Furthermore, analysis of variance of species and treatment effects showed that while the highest effect was observed for the treatment factor for length of stems (LS) and aerial dry weight (ADW), the highest level was found for the species effect for root dry weight (RDW), RDW/ADW ratio and leaf area (LA). Overall, species effect is largely higher than that of lines for all measured traits indicating that the variation between *M. laciniata* and *M. truncatula* is higher

**Table 3.** Means of DRI values and broad-sense heritability ( $H^2$ ) of traits measured for studied lines of *M. laciniata* (TNL) and *M. truncatula* (TN).

|         | TNL1.9   | TNL2.3   | TNL4.7  | TNL9.2  | TN1.21   | TN2.12   | TN4.20   | TN13.11  | JA17     | DZA315.16 | F83005.5 | DZA45.5  | $H^2$ (TNL) | $H^2$ (TN) |
|---------|----------|----------|---------|---------|----------|----------|----------|----------|----------|-----------|----------|----------|-------------|------------|
| LS      | 24.38abc | 16.22bc  | 11.74c  | 12.57c  | 18.28bc  | 25.56abc | 19.44bc  | 31.03ab  | 33.64ab  | 36.90a    | 17.61bc  | 24.42abc | 0.81        | 0.60       |
| ADW     | 32.74ab  | 24.49abc | 17.83bc | 13.46c  | 23.60abc | 21.64abc | 22.95abc | 27.10abc | 25.36abc | 28.75abc  | 21.20bc  | 37.29a   | 0.69        | 0.60       |
| RDW     | 123.60a  | 24.64cd  | 25.91cd | 18.41d  | 27.88cd  | 24.11cd  | 43.78bc  | 36.25cd  | 38.52bcd | 25.03cd   | 35.53cd  | 59.96b   | 0.96        | 0.82       |
| RDW/ADW | 445.52a  | 177.49b  | 155.06b | 212.57b | 120.74b  | 115.48b  | 202.02b  | 140.79b  | 149.46b  | 106.35b   | 171.01b  | 105.16b  | 0.72        | 0.86       |
| LA      | 60.38cd  | 66.96bc  | 59.45cd | 66.57bc | 65.25bcd | 65.54bcd | 43.48e   | 103.11a  | 79.56b   | 63.75bcd  | 65.46bcd | 50.27de  | 0.26        | 0.95       |

In a row, DRI means followed by the same letter(s) are not significantly different at  $P=0.05$  based on Duncan's multiple-range test. Length of stems (LS), aerial dry weight (ADW), root dry weight (RDW), the RDW/ADW ratio and leaf area (LA).

than that within either species.

#### Means comparison of DRI values and broad-sense heritability

Generally, few significant differences were observed between DRI values of measured traits in *M. laciniata*. The lowest DRI values were for TNL4.7 and TN9.2 (Table 3). Furthermore, no significant difference in DRI was observed among *M. truncatula* lines, with the exception of leaf area (LA). TN13.11 from Medenine has the largest change in LA trait between treatments. Few significant differences were observed between lines of both species collected from sympatric populations. While the highest values for RDW and LA were respectively registered for TNL1.9 and TNL4.7 lines of *M. laciniata*, the highest levels for LS and LA were for TN13.11 of *M. truncatula*. In response to water stress, the plant faces physiological changes including loss of cell turgor, closing of stomata, reduction in cell enlargement, and reduced leaf surface area (Aslam and Tahir, 2003). Altinkut et al. (2001) reported that leaf size and relative water content (RWC) tolerance are associated with enhanced tolerance to water stress in barley and wheat. On the other hand, there was no significant difference for DRI values

between local and references lines of *M. truncatula* except for DZA315.16 and TN13.11 showing the lowest and highest DRI values, respectively, for length of stems (LS) and LA traits. In contrast to the RDW/ADW ratio, for most of both species lines, the length of stems (LS) is the most seriously affected trait. The root/shoot ratio increase was due to greater inhibition of the shoot as compared to root growth (Gomes et al., 1996). Numerous studies reported that water deficit reduced the growth and productivity of plants and increased the root/shoot ratio (Slama et al., 2006). The response of *M. laciniata* and *M. truncatula* to water stress deficit would be partially related to the preferential allocation of dry matter towards roots. This parameter is considered as a criterion of adaptation to drought (Ben Naceur et al., 1999), although some findings showed no clear correlation between root trait and water extraction ability (Petrie and Hall, 1992). It has been suggested that abscisic acid is involved in the control of the root/top ratio, mainly when the plant is under water stress (Munns and Sharp, 1993). A rise in abscisic acid in drought-exposed seedlings of *Qualea grandiflora* has been reported by Sasaki et al. (1997). Understanding the physiological mechanisms that make some species tolerant and others sensitive is fundamental in identifying clearly the recognizable

traits for use in breeding programs aimed at developing cultivars that would adapt to the environmental stress (Abdel-Nasser and Abdel-Aal, 2002). To our knowledge, this is the first report about the analysis of the effects of water deficit on pure lines coming from sympatric populations of two annual *Medicago* species. Previous studies comparing responses under water deficit of medics had not taken in consideration the variation within species as well as the site-of-origin eco-geographical factors of plant material (Hamidi, 1991). Broad-sense heritability ( $H^2$ ) of DRI values of measured traits ranged from 0.26 - 0.96 and from 0.60 - 0.95 for *M. laciniata* and *M. truncatula*, respectively, indicating that these traits are genetically controlled.

#### Correlation among DRI values

Among the 20 possible correlations between DRI of measured traits for both species, six and five were respectively significant for *M. laciniata* and *M. truncatula* (Table 4). Five and three of these correlations are positive, respectively, for *M. laciniata* and *M. truncatula*. These positive correlations suggest that the measured traits vary in similar ways under well-watered and water-stressed conditions in both species. Negative

**Table 4.** Estimated correlations between DRI values of measured traits for *M. laciniata* (above) and *M. truncatula* (below).

|         | LS                  | ADW                 | RDW                 | RDW/ADW             | LA                  |
|---------|---------------------|---------------------|---------------------|---------------------|---------------------|
| LS      | 1.00                | 0.78 <sup>***</sup> | 0.67 <sup>**</sup>  | 0.03 <sup>ns</sup>  | 0.16 <sup>ns</sup>  |
| ADW     | 0.75 <sup>***</sup> | 1.00                | 0.48 <sup>*</sup>   | -0.32 <sup>ns</sup> | 0.50 <sup>*</sup>   |
| RDW     | 0.34 <sup>*</sup>   | 0.69 <sup>***</sup> | 1.00                | 0.58 <sup>*</sup>   | -0.16 <sup>ns</sup> |
| RDW/ADW | -0.41 <sup>**</sup> | -0.50 <sup>**</sup> | 0.12 <sup>ns</sup>  | 1.00                | -0.58 <sup>*</sup>  |
| LA      | 0.29 <sup>ns</sup>  | 0.09 <sup>ns</sup>  | -0.16 <sup>ns</sup> | -0.19 <sup>ns</sup> | 1.00                |

Significance levels; ns: not significant ( $p > 0.05$ ), \*  $p \leq 0.05$ , \*\*  $p \leq 0.01$ , \*\*\*  $p \leq 0.001$ . Length of stems (LS), aerial dry weight (ADW), root dry weight (RDW), the RDW/ADW ratio and leaf area (LA).

**Table 5.** Estimated correlations between DRI values of measured traits for *M. laciniata* and *M. truncatula* and eco-geographical factors.

|         | <i>M. laciniata</i> |                   |                   |          | <i>M. truncatula</i> |       |                    |          |
|---------|---------------------|-------------------|-------------------|----------|----------------------|-------|--------------------|----------|
|         | O.M                 | P                 | An. rain          | Altitude | O.M                  | P     | An. rain           | Altitude |
| LS      | 0.47                | 0.51              | 0.63              | -0.13    | -0.18                | -0.43 | -0.06              | 0.26     |
| ADW     | 0.47                | 0.40              | 0.47              | -0.04    | -0.21                | -0.06 | -0.03              | -0.09    |
| RDW     | 0.39                | 0.82 <sup>*</sup> | 0.68 <sup>*</sup> | -0.45    | -0.60                | 0.01  | -0.60              | -0.44    |
| RDW/ADW | 0.21                | 0.49              | 0.48              | -0.28    | -0.56                | 0.07  | -0.76 <sup>*</sup> | -0.47    |
| LA      | 0.03                | -0.23             | 0.03              | 0.23     | -0.30                | -0.44 | 0.22               | 0.20     |

Significant after using Bonferroni correction at  $\alpha = (0.05/20=0.0025)$ . Organic matter (O.M), available phosphorus (P), and mean annual rainfall (An. rain). Length of stems (LS), aerial dry weight (ADW), root dry weight (RDW), the RDW/ADW ratio and leaf area (LA).

correlation between DRI of RDW/ADW ratio and leaf area (LA) for *M. laciniata*, indicates that plants showing the highest reduction percentage for LA were the most tolerant ones. Nevertheless, no consistent pattern of association was found for *M. truncatula* between RDW/ADW ratio and LA.

#### Associations of DRI values with environmental factors

Among the 40 possible correlations between DRI of measured traits and environmental factors, only 2 and 1 out of them were significant, respectively for *M. laciniata* and *M. truncatula* (Table 5). Root dry weight (RDW) is positively correlated with assimilated  $P_2O_5$  and mean annual rainfall in *M. laciniata*. A negative correlation was detected between RDW/ADW ratio and mean annual rainfall for *M. truncatula* lines. Correlations between DRI of measured traits and environmental factors suggest that these particular characters have adapted in response to the regional differences in eco-geographical factors. This is similar to Kloss and McBride (2002), who reported significant correlations between variables of growth measured for California's blue oak (*Quercus douglasii*), cultivated in greenhouse under three different water regimes, and mean annual rainfall and altitude factors.

DRI of most measured traits had high broad heritability indicating that these characters can be used as good descriptors in the genetic analysis of tolerance to drought in both species. Most of the correlations between DRI of the measured traits were positive, suggesting that behaviors of lines in drought follow the same general pattern. Correlations between DRI of scored traits and environmental factors suggest that the response to drought is partially dependent upon local adaptation of these lines in their original sites.

#### Conclusions

The significant variation in drought tolerance – as measured by the DRI - found between the studied lines from *M. laciniata* and *M. truncatula* suggests that a rich genetic resource for drought tolerance exists in different genotypes, and that it is feasible to select water deficit tolerant lines in both species. Furthermore, breeding schemes should be based upon heritability of traits. It will be relatively easier to select lines for these traits and pass them onto the offspring. The correlation analyses performed in this study indicate that different drought tolerance traits are inter-correlated; it is thus more

appropriate to evaluate a breeding strategy comprehensively based on multiple traits.

Further study is needed to analyze the water deficit responses in sympatric populations of *M. laciniata* and *M. truncatula*. Numerous traits related to water deficit tolerance should be taken into account including leaves, stems and roots ions content of Na<sup>+</sup>, Cl<sup>-</sup> and K<sup>+</sup>, accumulation of proline, chlorophyll quantity and relative water content (RWC). In spite of the fact that a large number of drought-induced genes have been identified in a wide range of plant species (Verslues et al., 2006; Shinozaki and Yamaguchi-Shinozaki, 2007), a molecular basis for plant tolerance to water stress still remains far from being completely understood. Thus, lines from *M. laciniata* and *M. truncatula* with opposite behaviors under water deficit will be inter-crossed to obtain recombinant inbred lines (RILs) populations. The long term purpose of this study is to identify quantitative trait loci (QTLs) and/or genes of the tolerance to drought stress. Indeed, identifying genomic regions (QTLs) that contribute to drought resistance will help to develop *M. truncatula* lines suitable for water-limiting environments through marker-assisted breeding. Detailed characterization of these genomic regions through the development and evaluation of near-isogenic lines will lead to an improved understanding of drought tolerance and might set the stage for the positional cloning of drought tolerance genes.

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