

Assessment of nodulation in local populations of four *Medicago* species, relationships between nodulation and plant growth

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Summary : Nitrogen is an important mineral, necessary for the life and growth of plants, but it is the main factor limiting their development. The objective of our study is the evaluation of nodulation in local populations of four annual *Medicago* species: *M. minima*, *M. laciniata* and *M. truncatula* and *M. littoralis* collected in different regions of Djelfa, and the relationships between nodulation and plant growth. The scarified seeds were sown in pots at a rate of five seeds per pot. At emergence, one plant per pot was maintained. The experimental design used is a total randomization with six replications. At the beginning of the flowering stage, plants were harvested, thus the weight of the aerial biomass, the weight of the roots, the number and weight of the nodules were measured. The results obtained showed a variability of nodulation and plant growth in populations depending on the species. Analysis of variance showed significant variation among populations for the measured traits. We also noted a close relationship nodulation between and plant growth.

Key words : *M. truncatula*, *M. minima*, *M. laciniata*, *M. littoralis*, nodulation, plant growth.

Résumé : L'azote constitue un minéral important, nécessaire à la vie et à la croissance des plantes, mais il constitue le principal facteur limitant leur développement. L'objectif de notre étude est l'évaluation de la nodulation chez des populations locales de quatre espèces de luzernes annuelles: *M. minima*, *M. laciniata* et *M. truncatula* et *M. littoralis* collectées dans différentes régions de la wilaya de Djelfa, et la relation entre la nodulation et la croissance des plantes. Les graines scarifiées ont été semées dans des pots à raison de cinq graines par pot. A la levée, nous avons effectué un démariage, en laissant seulement un plant par pot. Le dispositif expérimental utilisé est une randomisation totale avec six répétitions. Au début du stade floraison, les plants ont été déterrés, ainsi le poids humide de la biomasse aérienne, le poids des racines, le nombre et le poids des nodules

ont été mesurés. Les résultats obtenus ont montré une variabilité de la nodulation et de la croissance des plantes chez les populations selon les espèces. L'analyse de la variance a montré une variation significative entre les populations pour les caractères mesurés. Nous avons noté aussi une relation étroite entre la nodulation et la croissance de la plante.

Mots clés : *M. truncatula*, *M. minima*, *M. laciniata*, *M. littoralis*, nodulation, croissance de la plante.

INTRODUCTION

Nitrogen is abundant in the atmosphere, but bioavailable nitrogen is less abundant in soil profiles (Zubrod, 2022). Fixed nitrogen is a limiting nutrient in most environments, with the main reserve of nitrogen in the biosphere being molecular nitrogen from the atmosphere (Frache *et al.*, 2009). Plants assimilate available nitrogen from the soil through their roots in the form of ammonium (NH_4^+) and nitrate (NO_3^-) (Zaim *et al.*, 2017).

Legumes are plants that are able to fix atmospheric nitrogen through their symbiotic interaction with rhizobia. This symbiosis provides the necessary nitrogen for plant growth and contributes to the improvement of the soil nitrogen status (Farissi *et al.*, 2014). In conditions of low mineral nitrogen availability, legumes are a priori preferred since they can fix atmospheric nitrogen (Saadallah *et al.*, 2001). They are very important sources of vegetable protein for human food and animal feed and these plants do

not require nitrogen fertilization (Badri *et al.*, 2016).

Among crops reliable to promote pastoral zones that produce forage and restore destroyed pasture land especially in arid and semi-arid areas, the genus *Medicago* L. (Fabaceae) constitutes an important genetic resource (Haddioui *et al.*, 2012). Forage legumes of the genus *Medicago* L., such as *Medicago sativa* (alfalfa) and *Medicago truncatula*, play a key role in agroecosystems due to their ability to fix atmospheric nitrogen through a symbiotic association with bacteria of the genus *Rhizobium* (Zahran, 1999). They have the ability to fix 100-200 kg N per ha per year with appropriate rhizobia inocula (Zhu *et al.*, 1998). Annual *Medicago* are excellent candidates for use in sustainable agriculture systems, such as; pastures and cover crops (Dorry, 2010). They have the potential to increase forage production and extend the grazing season (Interrante *et al.*, 2011).

In this context, nodulation in *Medicago* species is crucial for selecting efficient

cultivars, characterizing symbiotic interactions and identifying limiting factors. The objective of this study is to assess nodulation in some local population of *Medicago truncatula*, *Medicago minima*, *Medicago laciniata* and *Medicago littoralis*, and the relationships between plant growth and nodulation traits.

MATERIAL AND METHODS

Plant material and experimental design

Twelve local populations of *Medicago truncatula*, *Medicago minima*, *Medicago laciniata* and *Medicago littoralis* collected in different regions

of Djelfa (Algerian central steppe) were used. The latitude and longitude of collection site were given in Table I.

The study was conducted during the 2024/2025 cropping season at the experimental farm of the Research Station of National Institute of Agronomic Research of Algeria (Baraki, Algiers). The trial was conducted in a glass greenhouse. Seeds scarified mechanically using a file and sandpaper, were sown on 26 December 2024 in 5 L plastic pots containing clay-loam textured soil, at a rate of five seeds per pot. At emergence, one plant per pot was maintained. Pots were arranged in a completely randomized design with six replications.

Tableau I. List of populations and original collection sites

Species	Collection site	Symbols of populations	Altitude (m)	Latitude	Longitude
<i>Medicago truncatula</i>	Oued Touil	(MtOT)	718	35° 16' 43''	2° 33' 23''
	Mliliha	(MtMli)	806	34° 48' 89''	3° 48' 94''
	Ain Oussera	(MtAO)	758	35° 17' 08''	2° 57' 37''
	Charef	(MtCh)	960	34° 40' 31''	2° 43' 13''
<i>Medicago Minima</i>	Oued Touil	(MmOT)	718	35° 16' 43''	2° 33' 23''
	Mliliha	(MmMli)	806	34° 48' 89''	3° 48' 94''
	Ain Oussera	(MmAo)	758	35° 17' 08''	2° 57' 37''
	Charef	(MmCh)	960	34° 40' 31''	2° 43' 13''
<i>Medicago laciniata</i>	Bouiret Lahdab	(MIBL)	830	35° 12' 66''	3° 02' 88''
	Mliliha	(MIMli)	806	35° 16' 43''	3° 48' 94''
<i>Medicago littoralis</i>	Oued Touil	(MltOT)	718	35° 16' 43''	2° 33' 23''
	Messaad	(MltMd)	950	34° 15' 18''	3° 20' 12''

At the beginning of the flowering stage, plants were harvested and the following traits were measured: aerial biomass weight (**ABW**) (in g), root weight (**RW**) (in g), number of nodules (**NND**) and nodule weight (**NDW**) (in mg).

Statistical analyses

Obtained data were subjected to analysis of variance using GenStat software edition 12. The comparisons between the mean values of measured trait were made using the least significant

difference test (LSD) at 5% probability level. The relationships between traits were analyzed using linear regression. Principal Components Analysis (PCA) was performed using the XLStat software.

RESULTS AND DISCUSSION

Variance analysis showed highly significant differences between populations for all studied traits (Table II). According to Chaulet (1995), species of the genus *Medicago*

Tableau II. Mean values and coefficient of variation of measured traits for studied populations of four *Medicago* species

Populations/traits	ABW	RW	NND	NDW
MtOT	46.73 ^a	26.48 ^b	136 ^a	199.8 ^b
MtMli	44.47 ^a	17.45 ^d	51 ^e	81.0 ^d
MtAO	45.33 ^a	17.82 ^d	60 ^{de}	50.6 ^e
MtCh	44.33 ^a	29.65 ^a	89 ^c	224.0 ^a
MmOT	11.50 ^{ef}	10.12 ^f	28 ^f	24.2 ^f
MmMli	10.33 ^f	10.00 ^f	31 ^f	7.8 ^f
MmAo	15.23 ^{cd}	13.48 ^e	111 ^b	61.8 ^{de}
MmCh	13.57 ^{de}	18.43 ^d	113 ^b	64.0 ^{de}
MIBL	8.65 ^f	7.07 ^g	52 ^e	24.7 ^f
MIMli	16.82 ^c	19.48 ^d	135 ^a	71.5 ^{de}
MltOT	27.32 ^b	22.15 ^c	55 ^e	66.2 ^{de}
MltMd	34.82 ^b	27.98 ^{ab}	72 ^d	124.7 ^c
LSD	3.129	2.567	16.32	20.92
<i>p-value</i>	0.001***	0.001***	0.001***	0.001***
CV (%)	10.2	12.1	18.2	21.7

ABW: Aerial biomass weight ; **RW:** Root weight ; **NND:** Number of nodules ; **NDW:** Nodule weight ; **LSD:** Least significant difference ; **CV:** Coefficient of variation ; Significant levels: ns: not significant ; *, **, ***: significant at 5%, 1% and 0.1% respectively.

derive their importance on their large variability. Aerial biomass weight values varied from a minimum of 8.65 g for Bouiret Lahdab population of *M. laciniata* to a maximum of 46.73 g for Oued Touil population of *M. truncatula*. *M. truncatula* populations produced a higher biomass than the other populations of three species. Our results are in accordance with Badri *et al.* (2007), who indicate that Tunisian populations of *M. truncatula* produce more vegetative biomass than those of *M. laciniata*. Graziano *et al.* (2010) reported large differences among Sicilian populations of *M. polymorpha* for dry matter yield and they had significantly higher biomass yields than commercial cultivars developed in Australia. Average root weight was arranged between 7.07 g for Bouiret Lahdab population of *M. laciniata* and 29.65 g for Charef population of *M. truncatula*. Miloud (2018) reported great variability in the nodulation and its effects on the weight of the aerial parts, the roots in *Medicago truncatula*.

Mean value of nodulation traits were highly variable. Oued Touil population of *M. truncatula* species displayed a high number and weight of nodules, respectively 136 nodules and 199.8 mg. In contrast, the lowest number and weight of nodules were recorded by Oued Touil population of *M. minima* and Mliliha population of *M. minima*, with

27 and 7.8 mg, respectively. Chebouti *et al.* (2022) noted that *M. truncatula* populations displayed a nodules number of 52-106 and a nodules weight of 52.0-179.8 g. Jeder *et al.* (1996) found that annual species are generally more nodulated than perennials. Hossain *et al.* (2018) reported that Nodule numbers varied significantly among the years, species and cultivars, and growth stages.

Linear regression was used to determinate the relationships level between measured traits. The root weight was highly correlated with aerial biomass weight, following a linear relationship ($r = 0.736$; $R^2 = 0.542$) (Fig. 1). There was a linear relationship between nodule weight and plant growth. This relationships was highly with a correlation coefficient of $r = 0.718$ and a determination coefficient of $R^2 = 0.516$ (Fig. 2). Allito *et al.* (2021) showed that growth in terms of shoot dry weight became more dependent on nodule dry weight in faba bean. Voisin *et al.* (2010) reported that there was a linear relationship between nodule biomass and total plant growth at the end of the vegetative period and this depended upon genotype and environmental conditions in pea. Positive and significant correlation was found by Dhan *et al.*, (2006) among nodule number and aerial dry matter in *Hedysarum coronarium* L. The weight nodule was very tightly related to weight root ($r = 0.874$; $R^2 =$

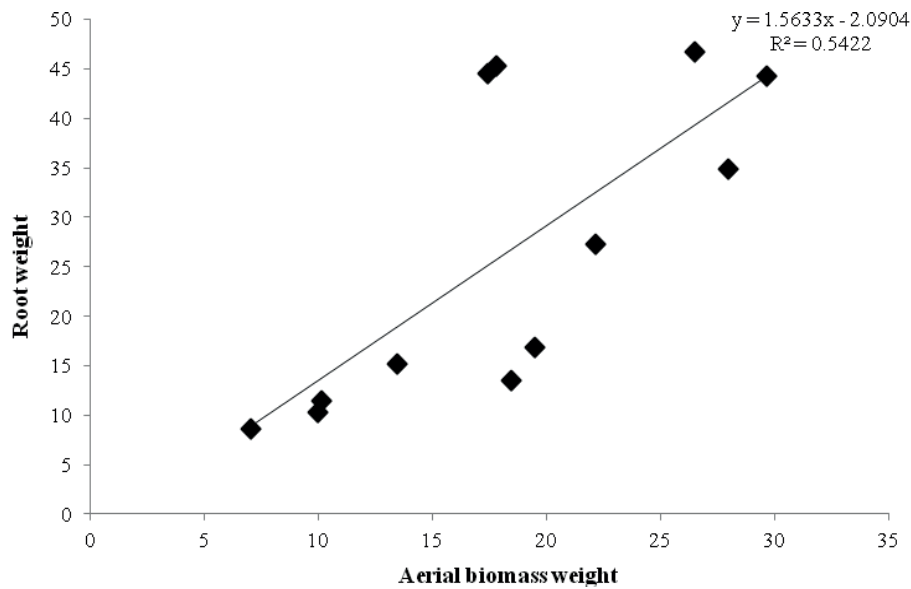


Figure 1. Relationship between aerial biomass weight and root weight.

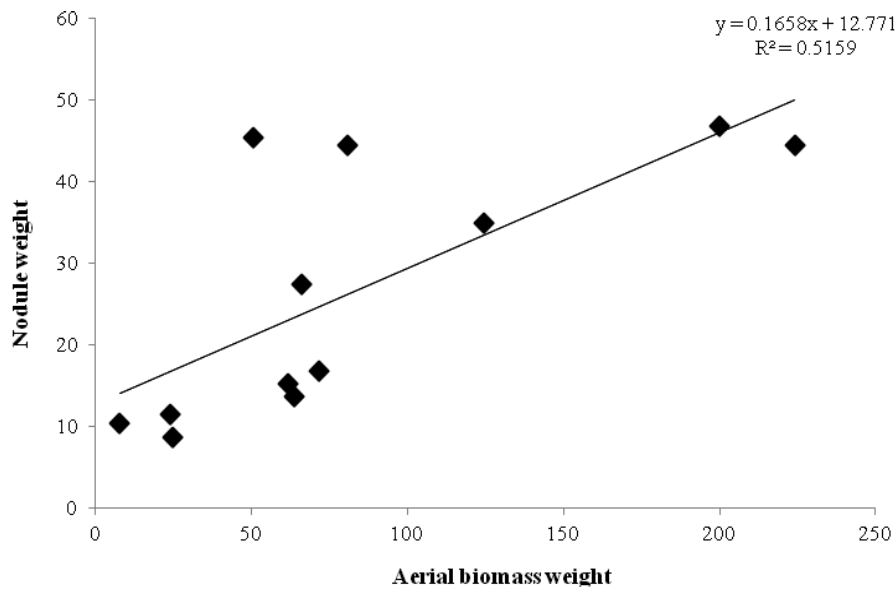


Figure 2. Relationship between aerial biomass weight and nodule weight.

0.764) (Fig. 3). Such populations with high biomass nodule are characterized by good root development. Samudin and Kuswanto (2018) reported that the relationship between nodule dry weight and root dry weight in the treatment without inoculation tended to be negative in Soybean germplasm. But the regression between nodule dry weight and root dry weight was positive with inoculation. The number of nodules was positively correlated nodule weight ($r= 0.578$; $R^2= 0.334$) (Fig. 4). Voisin *et al.* (2010) noted that nodule biomass was related to nodule number, following an exponential relationship rising to a maximum. According to Hwang *et al.* (2014), nodule number, individual

nodule weight, and size contribute to total nodule weight.

The principal component analysis was performed to visualize the main sources of variability among the populations of four *Medicago* species for the studied traits. The two principal components explained 91.74% of the total variability (Fig. 5). The first principal component, explained 70.49% of the total variation, which is strongly correlated with aerial biomass weight, root weight and nodule weight, represents a gradient of overall plant vigor associated with symbiotic efficiency. The second component, accounted for 21.25% of the variation, is related with the number of nodules may reflect differences in nodulation capacity.

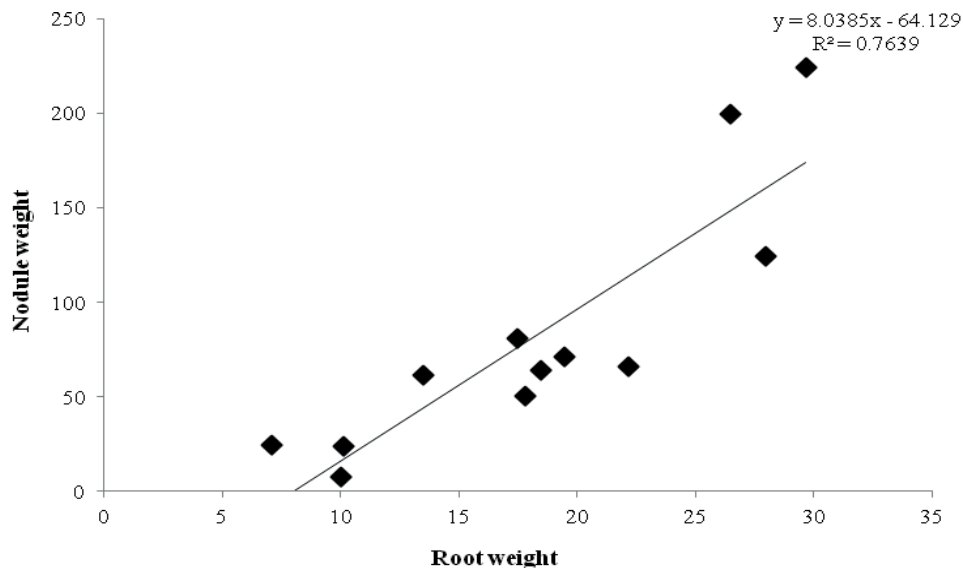


Figure 3. Relationship between root weight and nodule weight.

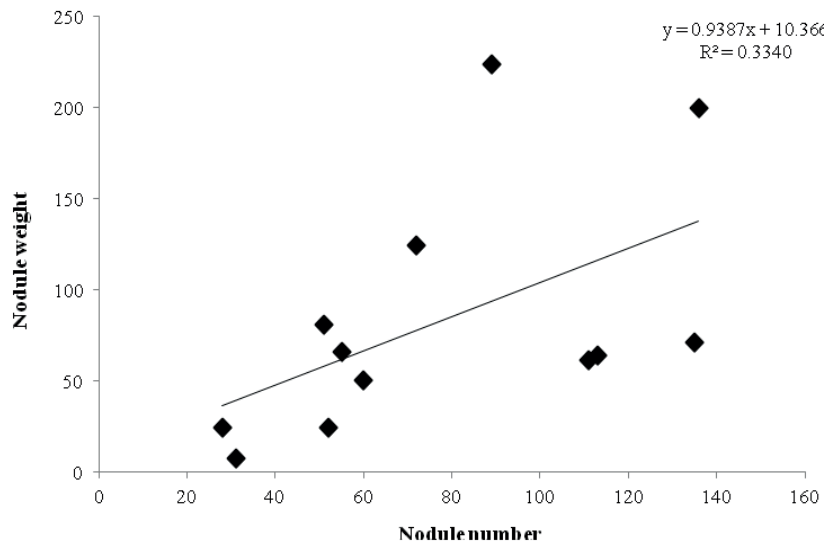


Figure 4. Relationship between number and weight of nodules.

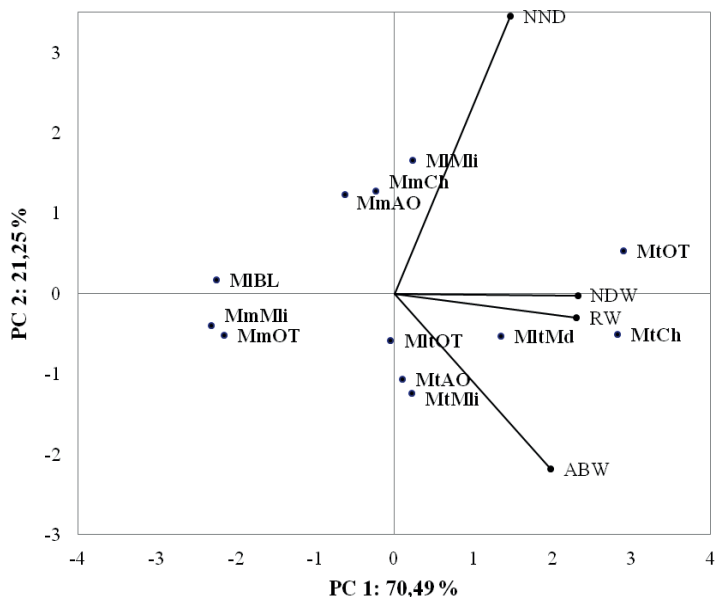


Figure 5. Biplot of Principal Components Analysis performed on traits populations of *M. truncatula*, *M. minima*, *M. laciniata* and *M. littoralis*.

The principal component analysis distinguished clearly the populations according to their growth and nodulation performance. PC1 differentiates clearly two groups of populations. The group consisted of population of *M. truncatula* from Charef and Oued Touil regions and *M. littoralis* population from Messaad, projected on the positive side of the first axis, was distinguished by high weights of aerial biomass, root and nodules, unlike the group grouped populations of *M. minima* from Mliliha and Oued Touil and population of *M. laciniata* from Bouiret Lahdab. On the other hand, the group comprised populations of *M. minima* from Charef and Ain Oussera regions and *M. laciniata* population from Mliliha, plotted on the positive side of the second axis, was characterized by high number of nodules. Contrarily to group formed by populations of *M. truncatula* from Mliliha and Ain Oussera regions and *M. littoralis* population from Oued Touil region, plotted on the negative side of the second axis.

CONCLUSION

In conclusion, we noted a large differentiation among local populations of *M. truncatula*, *M. minima*, *M. laciniata* and *M. littoralis* for plant growth and nodulation traits. From the results of this study, the highest aerial biomass, better nodulation and

good root development were shown by population of *M. truncatula* from Charef and Oued Touil regions and *M. littoralis* population from Messaad region. Regarding the relationship between the measured parameters, the results showed that there is a close relationship between nodule weight and plant growth and root development. Further future research should be conducted with the aim to select populations with good nodulation for better atmospheric nitrogen fixation and better resilience of annual *Medicago* crops under abiotic stress conditions.

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