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RESEARCH ARTICLE

ALLEVIATION OF DROUGHT EFFECT ON TWO MYCORRHIZAL LEGUMINOUS PLANTS (TEPHROSIA VOGELII AND VIGNA SUBTERRANEA) AT AN EARLY GROWTH PHASE

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ABSTRACT

This experiment was conducted to evaluate tolerance of mycorrhizal or unmycorrhizal *T. vogelii* and *V. subterranea* subjected to drought stress at early growth phase, in a randomized blocks design; on sterilized substrate; with a mixture of selected fungi (AMF): *Gigaspora margarita*, *Glomus hoi*, *Glomus intraradices* and *Scutellospora gregaria*. Levels of drought stress were 90, 60, 30 and 15% of field capacity (FC) for control, mild, average and severe stress respectively with or without inoculation. Results indicated that mycorrhization performed positive effect with $r = 0.484^{**}$ and $r = 0.690^{**}$ for *T. vogelii* and *V. subterranea* respectively on improvement of plants biomass of *T. vogelii* and *V. subterranea*. This increment was 3 % significant for *T. vogelii* at 90% of FC; 40, 22, 26 and 21 % for *V. subterranea*, for: 90, 60, 30 and 15% of FC respectively. Beside, results proved that mycorrhization impaired the adverse effects of drought on growth at an early growth phase as compared to nonmycorrhizal plants. Inoculum used would be a useful, practical and effective material, for sustainable production of leguminous plants, especially herbaceous (*V. subterranea*) when water is scarce or rainfall weak.

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INTRODUCTION

Tephrosia vogelii is a significant source of rotenone and tephrosine (Elouard et al., 1982). Rotenone is not long-lasting insecticide (Gadzirayi et al., 2007; Makoshi and Arowolo, 2011) used for treatment of bovines and human dermatoses (Gaskins et al., 1972; Orowa et al., 2009) and for seed protection (Makoshi and Arowolo, 2011). In pharmacopea extracts of this plant are used amongst other things to fight against skin infections (Makoshi and Arowolo, 2011). Tephrosine is used in artisanal fishing (Makoshi and Arowolo, 2011). *T. vogelii* nitrogen content is between 3.7g and 1.2g/100g DM for plants from 2 - 3 months and 10 months respectively; P content is 0.8- 0.2g/100g DW (Orowa et al., 2009). Seeds of *T. vogelii* are sown towards the end of the farming season to enrich soil. Its seedlings settle when rains are scarce, insufficient and spend a huge part of their development cycle in dry season where drought stress is severe (Orowa et al., 2009). *V. subterranea* importance for animal, human been, in agriculture practices, enrichment of poor soils,

has been highlighted in many previous research work (Giller, 2001; Ncube et al., 2007; Hillocks et al., 2012; Touré et al., 2012; Tsoata et al., 2015). To cover their life cycle, plants needed sufficient amount of water, thus it commonly says that "life is only possible in water or with water". Water scarcity is thus detrimental to plant because it inhibits biological and physiological process taking place in plants (Pagter et al., 2005; Silva and al., 2004, 2009b, Tsoata et al., 2015), and limits plants yield worldwide (Al-Karaki et al., 2004; Valliyodan and Nguyen, 2006).

Drought stress reduces growth and yield of plants, by reducing the speed of cellular division and their expansion mainly because of the loss of turgor, leading to the decline of the components of cells water status of plant (Kiani et al., 2007). Moderate water stress leads to the closing of stomata, which limits entry of CO₂ necessary for photosynthesis (Zhu, 2001; Lawlor and Cornic, 2002; Tonon et al., 2004; Lawlor and Tezara, 2009; Chaves et al., 2009); when drought stress is severe the metabolism is affected, it is the case of sugars, proteins, amino acids and others organic compounds metabolism (Medrano et al., 2002; Sircelj et al., 2005). The water deficit below threshold value in a plant is generally characterized by changes within all structures leading to plant

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dead (Oyun *et al.*, 2010). Water deficit occurs in a plant when its requirements are higher than available quantities at roots level (Gaye and Bloc, 1992). At morphological level water deficit leads to significant reduction of stems size of *Vigna unguiculata* (Manivannan *et al.*, 2007); reduction of leaf number of *Glycine max* (Zhang *et al.*, 2004), biomass reduction of *Phaseolus vulgaris* (Specht *et al.*, 2001) and *Glycine max* (Zhang *et al.*, 2004). For *Medicago sativa* they is reduction in hypocotyls length, fresh and dry weight of stems and roots by drought stress; increase in roots length (Zeid and Shedeed, 2006). At physiological level drought stress induced: reduction in net photosynthesis for *Glycine max* (Bensari *et al.*, 1990); decrease in transpiration and lessing of CO₂ internal content for *V. unguiculata* (Scotti *et al.*, 1999).

However, in response to water deficit, in their natural environment some plants develop various means to tolerate or resist to drought stress. The taking in account of some of those means would be useful to improve their production in adverse environmental condition, such as drought stress (Al-Karaki *et al.*, 2004). Among these means of tolerance/resistances, use of bio-fertilizers in general (Nwaga *et al.*, 2011) and AMF in particular which are respectful for environment (Sadhana, 2014) became more and more necessary nowadays. The results of several studies carried out under drought condition show better growth and larger biomass (Beltrano *et al.*, 2003; Augé, 2004; Asensio *et al.*, 2012) for plants inoculated with AMF compared to uninoculated plants. Nevertheless, in better environmental conditions, it's important to note that the efficiency of one symbiosis depends on AMF specie used, on plant genotype and characteristic (herbaceous, ligneous).

It's thus important to investigate plant physiology under stressful condition coupled with research of means capable to impair negative effect of drought on plant yield, in order to optimize crop production in general and that of leguminous plant in particular. Consequently, obtaining viable information on plant material to study is needed when one knows that AMF are ubiquitous in environment. In spite of many works on drought stress and AMF (Nouaim, 1996; Guissou, 2001; Adjab, 2002; Mamoudou Dicko, 2005; Fatiha, 2009; Mouellef, 2010; Nwaga *et al.*, 2011) in sub-Saharan Africa, little of them concern the impact of drought stress on leguminous plant growth at early phase. The goal of this work is to check if the inoculation or not of selected AMF (*Gigaspora margarita*, *Glomus hoi*, *Glomus intraradices* and *Scutellospora gregaria*) can improve growth and tolerance to drought stress at early growth phase of *T. vogelii* and *V. subterranea* at various stress level.

MATERIALS AND METHODS

Microbial and plant material, growth conditions, experimental design

The microbial inoculum is a mixture of (*Glomus hoi*, *Glomus intraradices*, *Gigaspora gregaria* and *Scutellospora gregaria*) provided by the laboratory of Microbiology of soil, of the Center of Biotechnology of the University of Yaoundé I. Healthy local seeds of *T. vogelii* and *V. subterranea* were

surface-sterilized, germinated and transferred in plastic pots, in blocks randomized design according to Tsoata *et al.* (2015).

Measured parameters

Leaf numbers (LN) and leaf areas (LA in cm²)

Leaf number is obtained by enumeration of the new formed leaf. The third and the fourth leaf area are estimated daily according to Paul and al. (1979) method. With the following

$$\text{Formula: } LA(\text{cm}^2) = WT_p \cdot A(\text{cm}^2) / W(\text{cm}^2)$$

Where: LA= leaf area in cm²; WT_p = weight tracing paper having exactly leaf area; A(1cm²) = area exactly one square centimeter tracing paper; W(1cm²)= weight exactly one square centimeter tracing paper

Leaf specific weight (LSW in mg/cm²)

Plants leaf are weighed immediately after harvest to obtain the fresh weight (FW), it area (LA) is determined after according to Paul and al. (1979) method. The LSW is calculated using Araus *et al.*, 1998 in Mouellef (2010) formula: LSW (mg/cm²) = FW/LS.

Leaf dry weight gain (LDWG in %)

Leaf dry weight gain is evaluated according to the formula of Hetrick *et al.* (1992):

$$LDWG = (LDW_{cma} - LDW_{ncma}) / LDW_{ncma} \times 100$$

LDW_{cma}: leaf dry weight of inoculated plants; LDW_{ncma}: leaf dry weight of uninoculated plants.

Dry mass (g)

Fresh sample of leaf, shoot and root were oven-dried at 80°C until constant weight (Zerrad *et al.*, 2008). Then after leaf dry weight (LDW), shoot (SDW) and root (RDW) was measured on precision balance (Mttler). Above ground dry mass (A_gDW), dry mass per plant, ratios LDW/SDW, RDW/SDW and RDW/A_gDW are deduced.

Statistical analysis

Data was treated statistically using SPSS 18.0 software for ANOVA and correlations, comparison of difference among treatment means using the Duncan test at 5 % probability level.

RESULTS

Leaf parameters

Leaf number (LN)

Leaf number per plant (Fig. 1a) was always significantly higher for *V. subterranea* compared to *T. vogelii* whatever the treatment and water stress level. For *T. vogelii* the number of leaves for unstressed seedlings (28) was the same for control plants mycorrhizal or not (Fig. 1a).

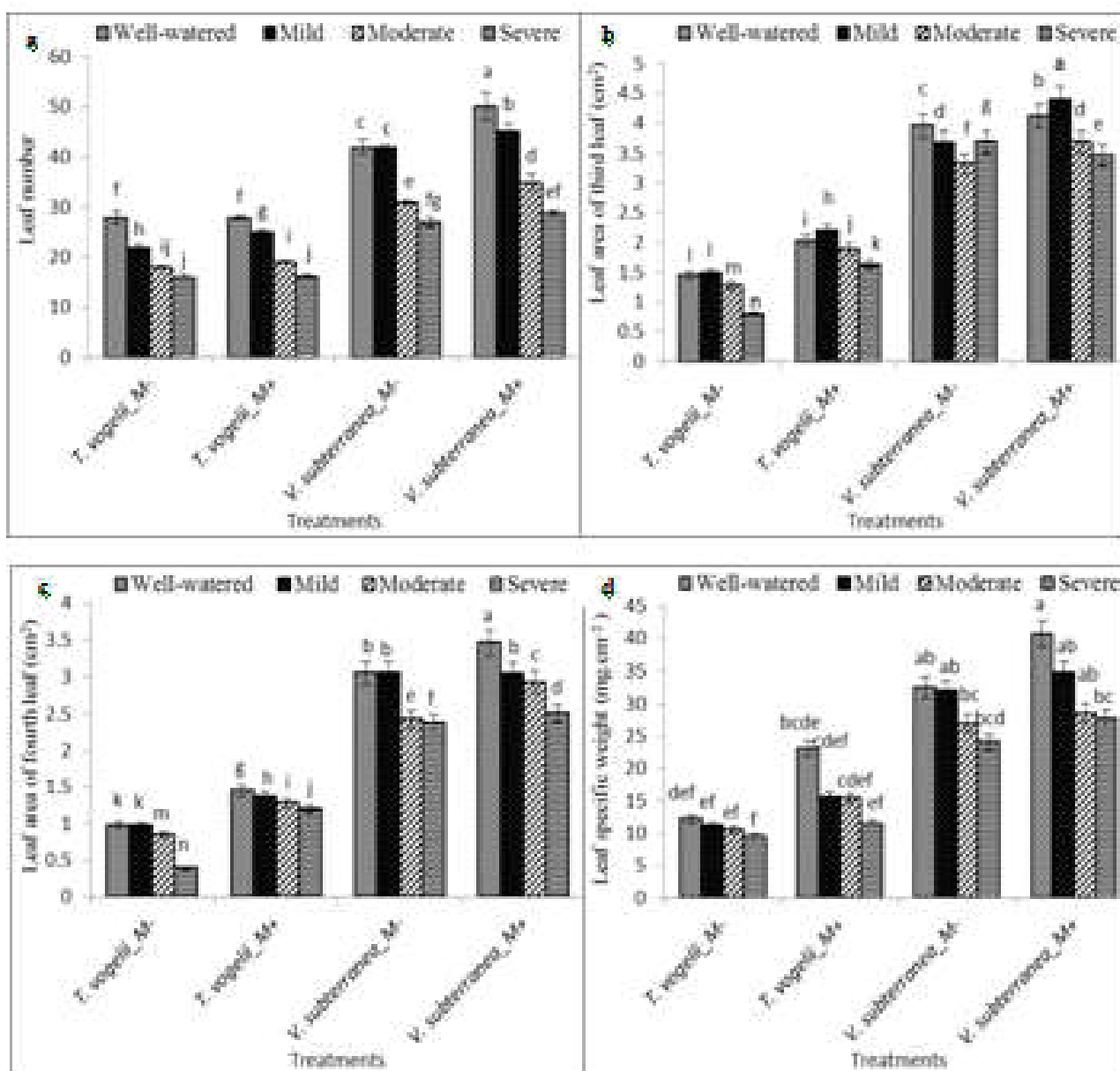


Fig. 1. Leaf number (a), leaf area of third leaf (b), leaf area of fourth leaf (c), leaf specific weight (d) for mycorrhizal (M+) and non-mycorrhizal (M-) *T. vogelii* and *V. subterranea* plants under severe (15%), moderate (30%), mild (60%) and well-watered (90%) conditions

LN decreased with the rise in stress level for all plants compared to control. The impairing of LN was 21, 36, 43% and of 11, 32, 43% for *T. vogelii* unmycorrhizal and mycorrhizal respectively, for the mild, average and severe stress. This reduction was always significantly higher for unmycorrhizal plants compared to mycorrhizal. But for severe drought stress this fall was 43% for mycorrhizal plants or not. For *V. subterranea* the LN of unstressed plants (Fig.1a) was 19% larger for mycorrhizal plants compared to unmycorrhizal. It decreased with the increase in drought stress of 0, 26, 36% for the unmycorrhizal plants and 10, 30, 42% for mycorrhizal respectively for, mild, average and severe stress. The LN number and % of fall of leaves when water stress level increases, were significantly weaker for unmycorrhizal plants compared to mycorrhizal. However, the positive coefficients of correlation between root colonization RC and LN for *T. vogelii*

($r = 0.297$) and *V. subterranea* ($r = 0.546$ **) showed that evolution of RC was correlated to LN more obvious for *V. subterranea* (Table 1 and 2).

Leaf area (LA)

LA was significantly high for *V. subterranea* compared to *T. vogelii*, regardless of treatment (Fig.1b and c), water stress level and age of leaves for unmycorrhizal plants. Mycorrhizal plants of two leguminous species had LA definitely higher than that of unmycorrhizal despite water stress level and leaf age. For *T. vogelii*, under soft water stress a 3% nonsignificant increase of 3rd LA was observed (Fig.1b) for unmycorrhizal plants, whereas 8% significant increase was recorded for mycorrhizal plants.

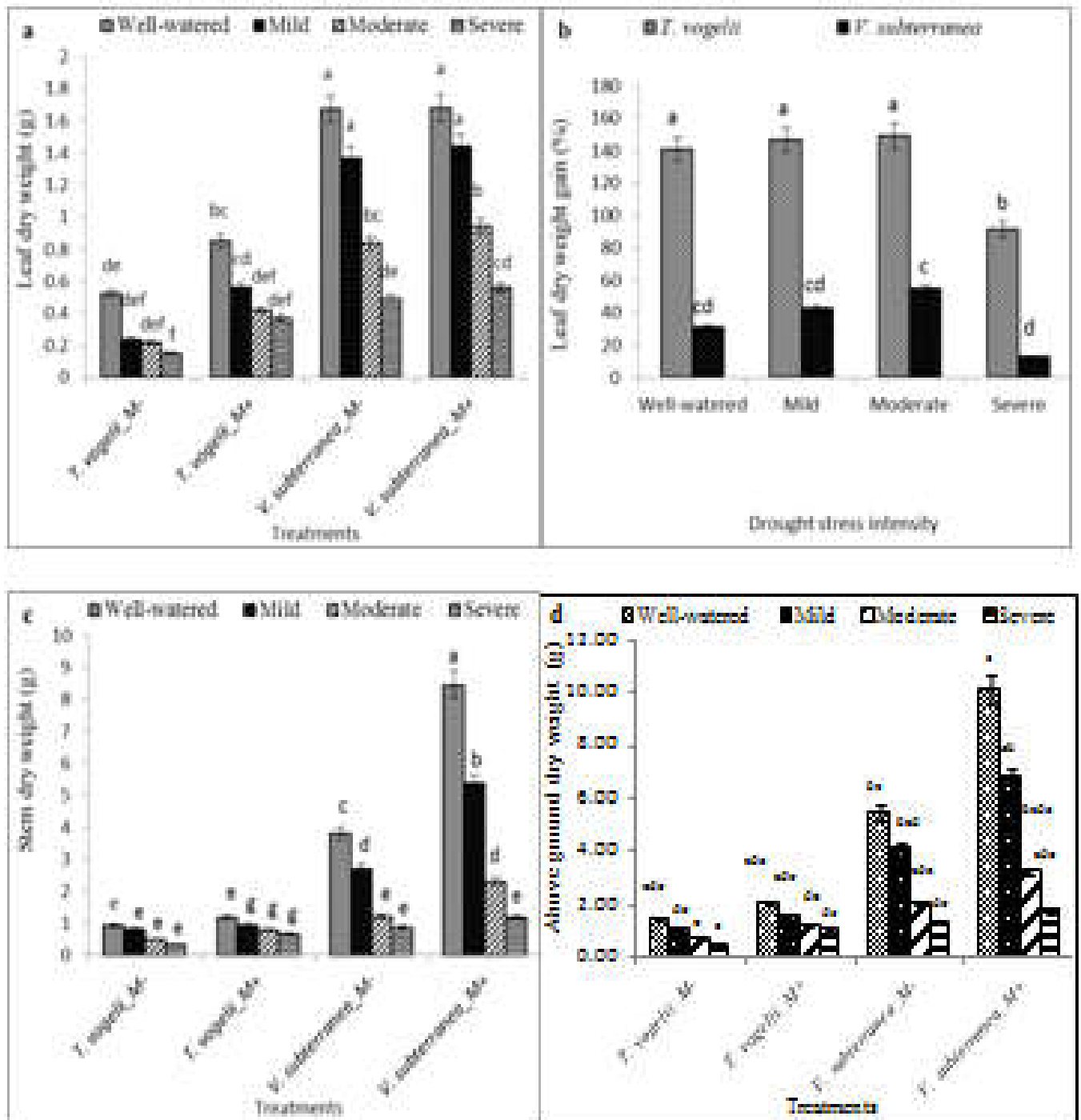


Fig. 2. Leaf dry weight (a), leaf dry weight gain (b), stem dry weight (c), Above ground dry weight (d) for mycorrhizal (M+) and non-mycorrhizal (M-) *T. vogelii* and *V. subterranea* plants under severe (15%), moderate (30%), mild (60%) and well-watered (90%) conditions

On the other hand a reduction in LA of the same leaf of 12, 46% for unmycorrhizal and 7, 20% for mycorrhizal plants was recorded for average and severe water stress respectively; this reduction was significantly weak for mycorrhizal plants compared to mycorrhizal plants. For 4th leaf (Fig.1c), LA decrease by 1, 13, 60% and 6, 12, 17% respectively for unmycorrhizal and mycorrhizal plants when level of water

stress increased; the decrement was high (60%) for unmycorrhizal plants and weak (17%) for mycorrhizal plants under severe water stress. For unmycorrhizal *T. vogelii* 3th leaf was less inhibited (46%) than 4th leaf (60%), whereas for mycorrhizal *T. vogelii* it was the reverse 20, 17% respectively for 3rd and 4th leaf. LA of the 3rd leaf of unmycorrhizal *V. subterranea* plants decreased by 7, 16, 22% for soft, average and severe water stress respectively;

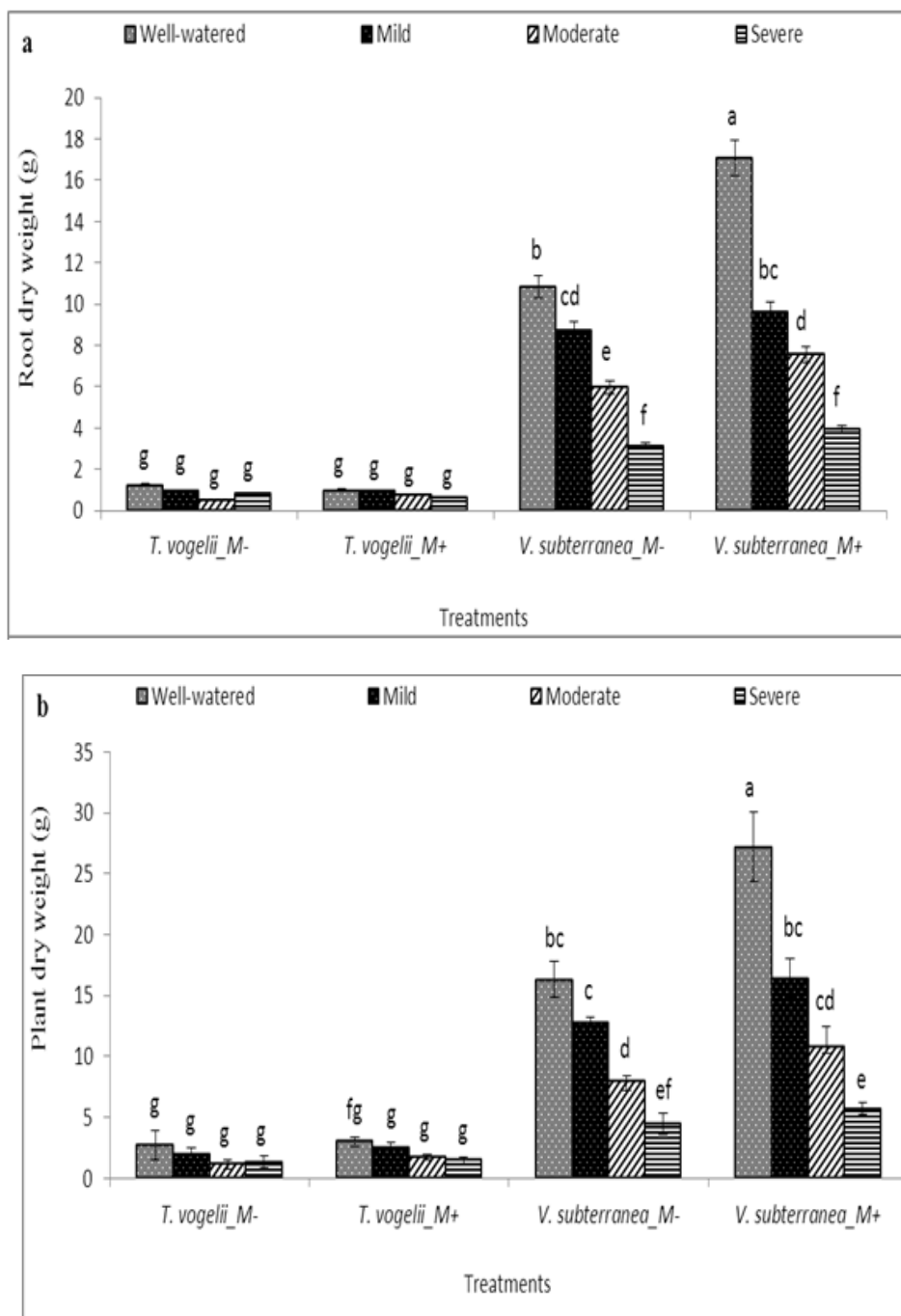


Fig. 3. Root dry weight (a), plant dry weight (b) for mycorrhizal (M+) and non-mycorrhizal (M-) *T. vogelii* and *V. subterranea* plants under severe (15%), moderate (30%), mild (60%) and well-watered (90%) conditions

whereas for mycorrhizal plants an increase in LA of 7% for soft stress, reduction of 10, 16% for average and severe stress respectively was recorded. For 4th leaves of unmycorrhizal plants of *V. subterranea* LA remains unchanged for the mild stress and dropped by 20, 22% for average and severe stress respectively. The 4th leaf of mycorrhizal *V. subterranea* plants exhibited a decrease of LA leaf of 12, 15 and 27% for mild, average and severe water stress respectively. The reduction in area of the 3rd and 4th leaf (Fig.1b and c) of unmycorrhizal *V. subterranea* was 22% for severe water stress, but for mycorrhizal *V. subterranea* for severe stress was of 16, 27% for 3rd and the 4th leaf respectively. For severe drought stress LA of 4th leaf (young leaf) of unmycorrhizal *T. vogelii*

was more inhibited compared to 3rd leaf (old leaf), but it was the reverse for mycorrhizal plants. Unmycorrhizal *V. subterranea* under severe water stress the 3rd and 4th leaf (Fig.1b and c) had the same rate of inhibition of LA, whereas for mycorrhizal plants it was the 4th (young leaf) which was inhibited compared to 3rd leaf. However, the positive correlation coefficients between RC and LA₃, between RC and LA₄ for *T. vogelii* ($r_{LA3} = 0.807^{**}$ and $r_{LA4} = 0.769^{**}$) and for *V. subterranea* ($r_{LA3} = 0.615^{**}$ and $r_{LA4} = 0.632^{**}$) showed that the evolution of RC is positively highly correlated to LA₃ and LA₄ (Table 1 and 2).

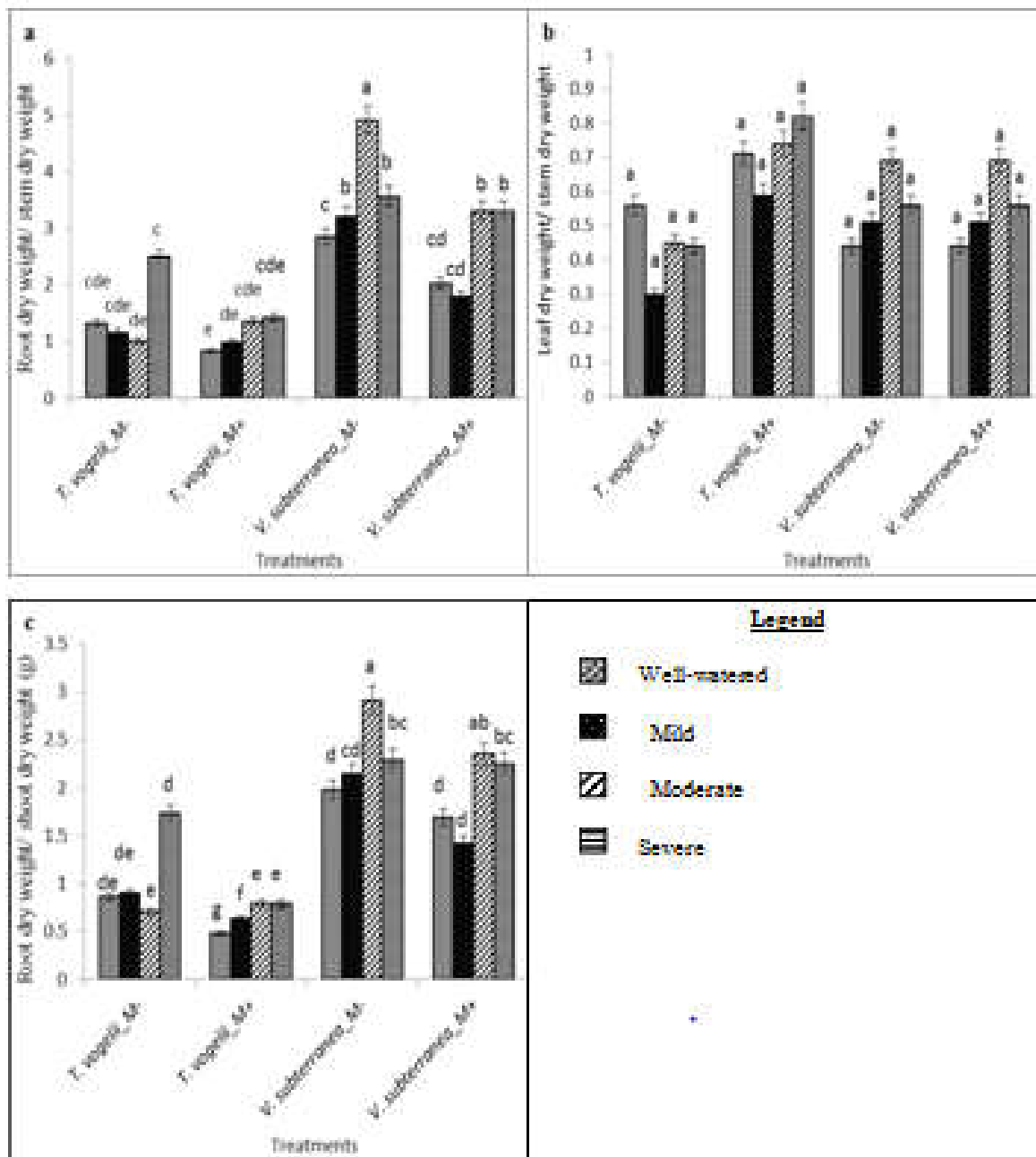


Fig. 4. Root dry weight/ stem dry weight (a), leaf dry weight/stem dry weight (b), root dry weight/above ground dry weight (c) for mycorrhizal (M+) and non-mycorrhizal (M-) *T. vogelii* and *V. subterranea* plants under severe (15%), moderate (30%), mild (60%) and well-watered (90%) conditions

Leaf specific weight (LSW)

The LSW (fig.1d) was high for *V. subterranea* plants compared with those of *T. vogelii* for all treatments and water stress levels. It decreased when level of stress increased for mycorrhizal and unmycorrhizal plants. This reduction of LSW for the two leguminous plants was relatively weak for unmycorrhizal plants compared to mycorrhizal plants. This decrement was 9, 14, 31 and 32, 33, 50% for unmycorrhizal and mycorrhizal *T. vogelii* respectively for mild, average and severe water stress; 2, 17, 26 and 15, 30, 32% respectively for unmycorrhizal and mycorrhizal *V. subterranea* when water

stress level increased. The positive correlation coefficients between RC and LSW for *T. vogelii* ($r = 0.543^{**}$) and *V. subterranea* ($r = 0.359^*$) showed that the evolution of LSW was highly positively correlated to that of RC and more obvious for *T. vogelii* (Table 1 and 2).

Growth parameters

Leaf dry weight (LDW) and leaf dry weight gain (LDWG) LDW

Dry matter produced by plant (Fig.2a) was increasingly higher for *V. subterranea* compared to *T. vogelii* regardless of treatment and drought stress level.

Table 1. Pearson correlation of evaluated parameters for *T. vogelii*: leaf dry weight (LDW), stem dry weight (SDW), root dry weight (RDW), ratio LDW/SDW, RDW/SDW; above ground dry weight (AgDW) and ratio RDW/ AgDW; plant dry weight (PDW) ; leaf number (LN) ; leaf area of third leaf (LA3), leaf area of fourth leaf (LA4) ; leaf specific weight (LSW) ; leaf dry weight gain (LDWG)

	LDW	SDW	RDW	LDW/SDW	RDW/SDW	AgDW	RDW/AgDW	PDW	LN	LA ₃	LA ₄	LSW	LDWG
LDW	1	0.665**	0.178	0.400	0.106	0.340*	0.400	0.738**	0.512**	0.609**	0.623**	0.348*	0.407
SDW		1	0.389*	0.110	0.120	0.550*	0.230	0.883**	0.616**	0.487**	0.458**	0.489**	0.105
RDW			1	0.015	0.020	0.400	0.500	0.709**	0.440**	0.119	0.066	0.005	0.024
LDW/SDW				1	0.304	0.040	0.034	0.074	0.010	0.020	0.012	0.054	0.018
RDW/SDW					1	0.022	0.434*	0.007	0.064	0.012	0.023	0.044	0.019
AgDW						1	0.100	0.285*	0.290*	0.300*	0.414*	0.210	0.167
RDW/AgDW							1	0.010	0.004	0.006	0.001	-0.080	0.008
PDW								1	0.672**	0.493**	0.459**	0.354*	0.098
LN									1	0.503**	0.443**	0.288	0.101
LA ₃										1	0.954**	0.378*	0.075
LA ₄											1	0.364*	0.080
LSW												1	0.100
LDWG													1

Note: * Significant effect at 5 %; ** significant effect at 1 %.

Table 2. Pearson correlation of evaluated parameters for *V. subterranea*: leaf dry weight (LDW), stem dry weight (SDW), root dry weight (RDW); ratio LDW/SDW, RDW/SDW; above ground dry weight (AgDW) and ratio RDW/ AgDW; plant dry weight (PDW) ; leaf number (LN) ; leaf area of third leaf (LA3), leaf area of fourth leaf (LA4) ; leaf specific weight (LSW) ; leaf dry weight gain (LDWG)

	LDW	SDW	RDW	LDW/SDW	RDW/SDW	AgDW	RDW/AgDW	PDW	LN	LA ₃	LA ₄	LSW	LDWG
LDW	1	0.699**	0.787**	0.320	0.010	0.680*	0.300	0.794**	0.821**	0.826**	0.814**	0.400*	0.345
SDW		1	0.936**	0.109	0.090	0.350*	0.300	0.970**	0.820**	0.749**	0.798**	0.423**	0.199
RDW			1	0.010	0.201	0.040	0.310	0.992**	0.850**	0.779**	0.839**	0.470**	0.010
LDW/SDW				1	0.304	0.050	0.044	0.084	0.022	0.032	0.022	0.064	0.028
RDW/SDW					1	0.032	0.532*	0.009	0.054	0.022	0.023	0.054	0.019
AgDW						1	0.105	0.700**	0.554**	0.185	0.680	0.199	0.186
RDW/AgDW							1	0.120	0.010	0.063	0.017	-0.122	0.086
PDW								1	0.912**	0.854**	0.888**	0.472**	0.100
LN									1	0.849**	0.929**	0.477**	0.204
LA ₃										1	0.836**	0.425**	0.068
LA ₄											1	0.469**	0.059
LSW												1	
LDWG													1

Note: * Significant effect at 5 % ; ** significant effect at 1 %.

This LDW decreased significantly for all plants mycorrhizal or not when the level of stress increased. The inhibition for unmycorrhizal plants was always higher than that of the mycorrhizal plants. For *T. vogelii* the decrement was 54, 58, 71% for unmycorrhizal plants and 34, 51, 56% for mycorrhizal plants respectively for mild, average and severe water stress. For *V. subterranea* LDW decreases of 18, 50, 71% for the unmycorrhizal plants and 14, 44, 67% for the mycorrhizal plants respectively for mild, average and severe water stress. The LDW of mycorrhizal plants was always higher than those of unmycorrhizal plants. The increment of LDW was 65, 133, 91, 147% for *T.vogelii* and 1, 5, 12, 14% for *V. subterranea* respectively for control, mild, average and severe water stress.

In drought stress condition and regardless of stress level for mycorrhizal plants, LDW production was more stimulated for *T. vogelii* compared to *V. subterranea*.

LDWG

The increment of leaf dry weight (Fig.2b) was significantly higher for *T. vogelii* compared to *V. subterranea* regardless of water stress level. For *T. vogelii* it didn't change significantly compared to blank for soft and average water stress, but dropped by 50% for severe stress. For *V. subterranea* it didn't vary significantly compared to control for soft stress, but increased by 24% and dropped by 18% for average and severe water stress respectively.

Stem dry weight (SDW)

The SDW per plant (Fig.2c) was always significantly higher for *V. subterranea* compared to *T. vogelii* regardless of treatment and level of water stress; that of mycorrhizal plants being always higher than that of unmycorrhizal. It decreased for all plants when the level of stress increases. The reduction was 13, 47, 63% for unmycorrhizal *T. vogelii* and 21, 53, 63% for mycorrhizal *T. vogelii* respectively for mild, average and severe water stress. For unmycorrhizal *V. subterranea* the decrement was 29, 68, 77% and 36, 73, 86% for mycorrhizal *V. subterranea* respectively for mild, average and severe drought stress. Mycorrhization allowed insignificant increase for *T. vogelii*, but significant increment ($P = 5\%$): 122, 91, 89, 37% respectively for blank, mild, average and severe water stress.

Above ground dry weight (A_gDW)

For two species studied in stress less condition A_gDW (Fig.2d) was always significantly higher for the mycorrhizal plants compared to unmycorrhizal, that of *V. subterranea* been always higher compared that of *T. vogelii*. Under drought stress A_gDW decreased with increase in stress level for both species, that of mycorrhizal plants remains always higher than that of unmycorrhizal regardless of water stress level.

Root dry weight (RDW)

The RDW (Fig.3a) was always significantly weaker for *T. vogelii* compared to that of *V. subterranea* regardless of treatment and drought stress level. It decreased significantly for all plants when drought stress level increased. The inhibition was 24, 60% for unmycorrhizal *T. vogelii* and 4, 21% for mycorrhizal *T. vogelii* respectively for mild and average water stress, whereas for severe stress, they were very close 31 and 34% respectively for unmycorrhizal and mycorrhizal plants. For *V. subterranea* the decrement was 20, 45, 71% for unmycorrhizal plants and 44, 56, 77% for mycorrhizal plants respectively for mild, average and severe water stress. Inhibitory effect being significantly low for mycorrhizal plants of *T. vogelii* except for severe stress, compared with unmycorrhizal, whereas for *V. subterranea* inhibition was strong for mycorrhizal plants compared to unmycorrhizal. Mycorrhization allowed an increase in RDW of 56% for *T. vogelii* subjected to average water stress; for *V. subterranea* this increase was 58, 11, 27, 27% respectively for blank, mild, average and severe water stress.

Dry weight plant (PDW)

The PDW (Fig.3b) was significantly weaker for *T. vogelii* compared to *V. subterranea* despite treatment and water stress level. It was increasingly larger for mycorrhizal plants compared to unmycorrhizal, and decreased when level of stress increases. For *T. vogelii* decrement was 26, 55, 50% for unmycorrhizal plants and 19, 41, 52% for mycorrhizal plants respectively for mild, average and severe water stress. The inhibitory effect on PDW production for *V. subterranea* was 22, 51, 73% for unmycorrhizal plants, 40, 60 and 79% for mycorrhizal plants. The inhibition was significantly higher for the mycorrhizal plants of *V. subterranea* compared to

unmycorrhizal; whereas for *T. vogelii* it was reverse, except for severe stress where the inhibition was very close, 50% for unmycorrhizal plants and 52% for mycorrhizal plants.

Ratio: LDW/SDW; RDW/SDW; RDW/ A_gDW LDW/SDW

This ratio (Fig.4b) was lower than one and is practically not modified by the mycorrhization and the drought stress. These values indicated that the two leguminous plants produced more biomass at the level of stems than at leaves level. The values of this ratio were high for *T. vogelii* compared to *V. subterranea*. Mycorrhisation improved LDW/SDW for *T. vogelii*, but for *V. subterranea* it was the reverse. For the two leguminous species significant improvement of this ratio were recorded with increased in the water stress level.

RDW/SDW

The ratio (Fig.4a) was higher than one for unmycorrhizal control plants of two leguminous plants, with higher values for *V. subterranea* compared to *T. vogelii*. The higher values of this ratio indicated a production of high root dry biomass, than that of stems; those values were maintained above one for mycorrhizal or unmycorrhizal plants and at various level of water stress. Mycorrhization didn't improve RDW/SDW significantly for two leguminous plants. A significant increase in this ratio was recorded with rise in water stress level for the two species, except unmycorrhizal *T. vogelii* where the increment was insignificant for mild and average water stress.

RDW/ A_gDW

The RDW/ A_gDW (Fig.4c) for control plants was lower than one for *T. vogelii*, but higher than one for *V. Subterranea*; with significantly higher values for unmycorrhizal plants compared to mycorrhizal plants. Thus *T. vogelii* produced more dry matter on the level of shoot compared to root; whereas for *V. subterranea* it was the reverse. In drought stress condition RDW/ A_gDW remained lower than one for *T. vogelii* except for severe stress where it reached 1,73; whereas for *V. subterranea* it was always higher than one regardless of treatment applied for the mycorrhizal or unmycorrhizal plants. It increased with water stress level for all plants mycorrhizal or not and was significantly low for mycorrhizal plants compared to unmycorrhizal plants. The positive correlation coefficients between RC and PDW for *T. vogelii* ($r = 0.484^{**}$) and *V. subterranea* ($r = 0.690^{**}$) showed that the evolution of the PDW was proportional to that of RC (Table 5 and 6). RC thus induced a PDW definitely more significant for *V. subterranea*. The results obtained for ratios RDW/SDW and LDW/SDW showed that root biomass was more significantly high than that of stems, the latter being higher than that of leaves. These leguminous plants would develop root prior to other organs.

DISCUSSION

Leaf parameters

Leaf number

It's thanks to cell divisions, their elongation and differentiation that plants growth is carried out.

It implies genetic, physiological, ecological and morphological phenomena with their complex interactions (Farooq *et al.*, 2009). Qualitative and quantitative growth depends on these phenomena, which are affected by drought stress (Farooq *et al.*, 2009; Atti *et al.*, 2013). Cell multiplication is physiological process most sensitive to the dryness, because of fall in cell turgor pressure (Taiz and Zeiger, 2006). The number of leaves formed by *V. subterranea* is higher than that of *T. vogelii* regardless of treatment and level of water stress. Results of previous works showed that dryness effects on plants depend on several factors like genetic resistance, stage of growth and exposure time to water stress (Echave *et al.*, 2005; Song *et al.*, 2011; Abdelmoneim *et al.*, 2014). Significant inter specific differences were observed between two species of genus *Populus* for total number of leaves, total leaf area and total leaf biomass in water stress condition (Yin *et al.*, 2005). Difference of number of leaves formed in this experimentation could be due to genotypic differences between two studied leguminous plants. It is generally allowed that the mycorrhization affects the growth of plants (Zhu *et al.*, 2010).

For control plants, mycorrhization doesn't improve significantly the number of leaves emitted for *T. vogelii*, whereas for *V. subterranea* a significant increase of 19% is recorded for mycorrhizal control compared to unmycorrhizal. This kind of AMF effect was already observed for pot experiments and could be due to the fact that mycorrhization would quickly reach its full functioning for *V. subterranea* compared to *T. vogelii*. *V. subterranea* would quickly take profit of positive effects of mycorrhization on water and mineral nutrition (Nonami, 1998; Almagrabi and Abdelmoneim, 2012; Smith and Smith, 2012) whereas *T. vogelii* would still translocating many photosynthetats to fungi for full establishment of the symbiosis, this could explain the delay shown by mycorrhiza in the improvement of leaves number formed by *T. vogelii*; because mycorrhiza is not yet able to provide to its plant host P and other types of nutrients, probably due to short time of experimentation in this experimentation (Nonami, 1998; Kaschuk *et al.*, 2009; Almagrabi and Abdelmoneim, 2012; Smith, 2012). A moderate water stress causes reduction of leaves number, their development, consequently reduction in their size and in the case of severe stress, their elongation speed decreased and their growth can stop (Jayakumar *et al.*, 2007; Prasad *et al.*, 2008) because of interruption of circulation of xylemic sap towards cells in elongation phase (Nonami, 1998).

This reduction represents one of plants responses to dehydration; it contributes to conservation of water resources, which would allow the survival of plant (Lebon *et al.*, 2004). Vegetative development when water is scarce is strongly disturbed (Ferryra *et al.*, 2004), mitosis, cell elongation and expansion are inhibited (Nonami, 1998; Kaya *et al.*, 2006; Hussain *et al.*, 2008), thus leading to reduction of size and plant growth, as well as a strong reduction in leaf area: for *Abelmoschus esculentum* (Bhatt and Srinivasa Rao, 2005); *Asteriscus maritimus* (Rodriguez *et al.*, 2005); reduction of size and number of cell formed by leaf meristem (Tardieu *et al.*, 2000). The decrement of leaf number and leaf area of mycorrhizal and unmycorrhizal plants of *T. vogelii* and *V. subterranea* with severity of water stress would be due to

dysfunction related to progressive rarefaction of water, associated to loss of turgor, inhibition of mitosis (Farooq *et al.*, 2009).

Leaf area (LA)

When water is scarce and out of root reach, plant loses part of its internal water and water potential of cells drops, leading to reduction of turgid pressure, that is involved in cell multiplication. Cells and leaves are then of small size, leaf area being reduced, its capacity to intercept the drops with its photosynthetic potential; plant closes its stomata to limit transpiration (Sigarbieux et Feller, 2011). By so doing assimilation decreases (Boschma *et al.*, 2009; Volaire *et al.*, 2009), tissue density increases as well as the dry biomass (Meisser *et al.*, 2013) plant switches from a strategy of growth to that known as of conservation of resources (Grime 1997; Lavorel et Garnier, 2002). LA strongly determines transpiration and much of plants subjected to drought stress react initially by reducing LA (Lebon *et al.*, 2004; Yin *et al.*, 2005). It determines the amount of carbon fixed by photosynthetic way as well as resistance to dryness, considering that a high surface will lose more water than a weak surface (Belkharchouche *et al.*, 2009). The plasticity of LA plays a significant role in the control of water use of plants (Shao *et al.*, 2009). The plants subjected to water deficit reaches usually smaller apparent final leaf sizes compared to control (Granier *et al.*, 2000).

The increase in LA for plants subjected to water stress can be regarded as an adaptation character (Adjab, 2002). However, LN and the LA of two studied mycorrhizal leguminous plants was higher than for unmycorrhizal plants for each water stress level; this result would be explained by the capacity of AMF to reduce the desiccation of plant by an improvement of water supply, leading consequently to reduction of negative impact of stress on leaf apparatus and to the improvement of their capacity to be adapted to water stress. Mycorrhization reduces leaf senescence frequency of stressed plants. This senescence of leaves in water deficit period is often regarded as a mechanism of tolerance to water stress (Prasad *et al.*, 2008). The results of this study are similar to those of Mirzaei et Fazeli (2013) and are not in agreement with those of Habibzadeh and Abedi (2014) which respectively studied LA of *Acacia albida* and LN of *Vigna radiata* exposed to level of water stress corresponding to 100, 75, 50 and 25 % of field capacity. These results showed that for 8 month plants of *A. albida* inoculated or not with *Glomus mosseae*, LA decreases significantly when water stress increased, the decrement was 43-76 % and 29-66 % for unmycorrhizal and mycorrhizal plants respectively; in addition, mycorrhization increases significantly LA of 30, 44, and 30 % respectively for 100, 75 and 25 % of field capacity. For *V. radiata* plants, inoculated or not with *Glomus intraradices* and *Glomus mosseae* after 4 months of growth, LN increases proportionally with severity of water stress of 60, 66 and 72 % respectively for 75, 50 and 25 % of field capacity compared to control (100 % of field capacity); however mycorrhization with *Glomus intraradices* and *Glomus mosseae* within a general framework respectively allowed an increase of 23 and 17 % of LN. Other work completed by Wu et Xia (2006) on *Citrus tangerine* seedlings,

mycorrhizal or not with *Glomus versiforme* and exposed during 80 days to 2 watering levels (75 % of field capacity for control and 55 % of field capacity for drought stress) showed that, water stress reduces LA of 37 and 21 % and LN of 27 and 25 % respectively for unmycorrhizal and mycorrhizal seedlings. In addition, mycorrhization improved LA and LN of 18 and 34 %, as well as 25 and 27 % respectively for control and stressed treatment.

In this experimentation, reduction of LA was highly significant for average and severe water stress for the two species. This reduction in LA could be regarded as a mean used to reduce plant water requirement in drought condition (Darera et al., 1969), compensated by long lasting leaves (Atti, 2013). Reduction of LA can be beneficial for plants subjected to water stress, because it reduces transpiration surface and area intercepting solar radiations. A specie or variety having a weak LA highly uses luminous energy per unit of LA to produce good yield (in Atti, 2013).

Leaf specific weight (LSW)

LSW, significant marker in the response of plants to water constraint, can be regarded as a simple criterion of selection of genotypes having high effectiveness of water use in drought condition (Ykhlef, 2001). Its increase for some species or varieties under water stress is highly correlated with reduction of LA (Blum, 1989 in Ykhlef, 2001). The process of reduction of LA and increase in LSW makes allows plants to face lack of water through reduction of transpiration (Ykhlef, 2001). A high LSW is an indicator of a better photosynthetic capacity, a less sensitivity to the photo inhibition and consistency of the photosynthetic apparatus (Araus et al., 1998). Thus *V. subterranea* which has high LSW compared to that of *T. vogelii* would be able to preserve the integrity of its photosynthetic apparatus under water stress, in order to have better photosynthesis. In the case of this experimentation, the significant improvement of LSW of inoculated plants, with increasing level of water stress could be justified by the maintenance of high carbon metabolism making it possible to synthesize more carbon compound in leaves. But also by the fact that water deficit can generate thickening of leave (Kramer, 1969).

Indeed, Dubey (1994) thinks that the increase in LSW for plants under water stress is due to the contracting of cells which lead to reduction in its volume and even intracellular juice, leading to more concentrated cell sap. In a study on rice cultivars, Cabuslay et al. (2002) showed that drought stress acts positively on LSW and the correlation of DW with yield is positive. Similar results were obtained on before last leave for barley (Bort et al., 1998), corn (Araus et al., 1997). The fall of LSW obtained according to severity of water stress is similar to that obtained with Gorom variety of *V. unguiculata* by Mamoudou Dicko (2005).

Growth parameters

Dry weight of organs

Leaf dry weight gain (LDWG): the increase in LDWG of mycorrhizal plants studied in water stress condition is in

agreement with the results of several authors who showed that the mycorrhization stimulates growth of plant host in condition of drought stress (Faber et al., 1991; Sylvia et al., 1993). This stimulation of growth and production of biomass could be due to synergy between several positive effects of mycorrhization like: increase in resistance of plant to dryness (Al-Karaki and Clark, 1998). It makes it possible to host plant to continue to absorb water and minerals nutrients (Ghazi Al-Karaki et al., 2004), thanks to prolific and extensive root system (Turner et al., 2001; Kavar et al., 2007), allowing him to avoid stress and to continue its normal metabolism. This behavior could be correlated with degree of tolerance to water stress of studied species, more the specie is tolerant, less leaf damage are perceptible (Da Matta, 2004). The significant fall of LDWG obtained for severe water stress (15 % of field capacity) for *T. vogelii* could be due to a significant translocation of assimilats towards roots to satisfy needs of the symbiosis. For *V. subterranea*, the highest LDWG is recorded for average water stress (30 % of field capacity); this increase would result from the weak translocation of assimilats towards stems and especially roots; symbiosis would be certainly well established between plant and AMF, thus reducing requirements in carbohydrates for AMF, requirements which are higher during symbiosis establishment processes.

Dry biomass of various organ and whole plant is always high for *V. subterranea* compared to *T. vogelii* regardless of treatment and water stress level. It was already observed for two species of the genus *Populus* (Wullschlegel et al., 2005) and it would be due to genotypic differences. The reduction in dry biomass of organs and whole plant simultaneously with increase in water stress level for mycorrhizal or unmycorrhizal plants was already observed by several authors: Chartzoulakis et al. (2002) on cultivar of Avocado; Shubhra et al. (2003) for *Cyamopsis tetragonoloba*; Rodriguez et al. (2005) on *Astericus maritimus*; Yin et al. (2005) for *Populus cathayana*; Wu et Xia (2006) for *Poncirus trifoliata*. It would be due to a noticeable reduction in plants growth (Bhatt et Scrinivasa Rao, 2005) and photosynthesis (Bhatt et Scrinivasa Rao, 2005; Waraich et al., 2011) by closing of stomata and destruction of chlorophyll as well as photosynthetic apparatus (Waraich et al., 2011). Reduction in dry biomass was relatively weak for unmycorrhizal plants; it would be explained by the fact that part of photosynthesis products is translocated to AMF which are heterotrophic (Smith et Read, 1997).

But it seems that the installation and full functioning of mycorrhiza for *T. vogelii* and *V. subterranea* are not synchronous, this would explain differences observed on level of biomass of root and that of whole plant of two studied species. Thus *V. subterranea* for which dry biomass of roots and whole plant for mycorrhizal plants is always higher than that of unmycorrhizal, would have faster mycorrhization compared to that of *T. vogelii* where dry root biomass and whole plants dry biomass are always weak for mycorrhizal plants compared to unmycorrhizal. For *T. vogelii* mycorrhizal plants subjected to severe water stress have dry root biomass and higher dry biomass of whole plant compared to unmycorrhizal plants; thing occurs as if increase water stress level would stimulate for this specie the installation and full functioning of mycorrhiza. The substrate level of water deficit

would influence installation of mycorrhiza for some species. Results of a former study (Ghazi Al-Karaki *et al.*, 2004) emphasize the fact that the increase in biomass due to the inoculation with AMF is always high for Wheat cultivated in drought condition compared to that cultivated under optimum conditions of irrigation. This result could be due to increase in the dependence of plant on mycorrhiza for its mineral nutrition and absorption of water. On this basis, Michelsen *et al.* Rosendahl (1990) suggest that mycorrhiza is relatively more significant for growth of plant in dry soil, then in well moisten soil. AMF would improve absorption of nutrients by increasing mycelia network exploring substrate (Sylvia *et al.*, 1993). Considering dry biomass production as criterion of tolerance in water stress environment, *V. subterranea* which produces more dry matter under drought stress condition would be more tolerant than *T. vogelii*.

Ratios: LDW/SDW, RDW/SDW and RDW/A_gDW

The two leguminous plants studied significantly produce more dry matter on the level of stems than for leaves in water stress condition. This result is not in agreement with that of Mohammadian *et al.* (2005), indeed these authors observed a significant reduction of dry matter of stems, caused by drought stress for sugar beet genotypes. The increase in biomass of stems could be due to the degree of tolerance of the two species or to genotypic differences. The fact that mycorrhization improves ratio LDW/SDW could be due to improvement of plant degree of tolerance to drought stress by mycorrhiza (Al-Karaki *et al.* Clark, 1998). The significant improvement of this ratio with increase in water stress level could be due to the fact that in water stress condition, plant reacts in a dynamic way to the new environment conditions until a certain threshold value (Tardieu, 2005).

RDW/SDW

The contribution of stems and roots biomass appeared by high values of ratio RDW/SDW for unmycorrhizal control plants of *T. vogelii* and *V. subterranea* with definitely higher values for *V. subterranea* compared to *T. vogelii*. The development of root system which depends on stems in particular and in general on above ground apparatus could be useful like a criterion of resistance to dryness (Van Hess, 1997) allowing better use water available. *V. subterranea* having more raised values compared to *T. vogelii* would be more resistant than *T. vogelii*. The importance of this ratio could be explained either by the increase in dry root biomass or drops in vegetative biomass of stems (Trought, 1980) under water stress. The high values of RDW/SDW under drought stress regardless of treatment, level of water stress and independently of the mycorrhization, would be conditioned by the genotype of each species (Wullschleger *et al.*, 2005).

The significant increase in RDW/SDW ratio with the level of water stress and for the two species would be due to a dynamic reaction of the plant enabling him to restore balance between the climatic demand and water supply of the soil, utilizing mechanisms which are specific to him for example development of root system, stomatic regulation and/or osmotic adjustment (Tardieu, 2005).

RDW/A_gDW

In well watering condition *T. vogelii* which is a woody shrubby species produced more above ground biomass than underground biomass compared to *V. subterranea*, creeping herbaceous species, it would be due to genotypic differences being expressed in water stress condition or not, for plants mycorrhizal or not. This result could be due to the high degree of tolerance of this specie to water stress or the fact that it would optimize absorption of water, by elongation of his tap root in order to better explore deep horizons of soil. This dynamics of rooting of stressed plants was highlighted by many authors and constitutes one mechanisms of dryness avoidance often used by some plants. *V. subterranea* accumulates more biomass on the level roots. This behavior was already observed by several authors: Van Hess (1997); Monroy *et al.* (1988).

The development of roots which depends on caulinary system was announced like criterion of resistance to dryness (Van Hess, 1997) allowing a better use of water available. The results obtained here corroborate those of Fatiha (2009) on *Phaseolus vulgaris* and seems to indicate that water deficit increases ratio RDW/A_gDW (Fatiha, 2009). Trought *et al.* (1980) explain this increment by the increase in root dry biomass by ramification or drop of above ground biomass under drought stress. Thus *T. vogelii* and *V. subterranea* confronted to water deficit would use different strategies to tolerate the drought stress; *T. vogelii*, woody specie would lengthen its tap root for better exploring deep layers of soil, whereas *V. subterranea* which is herbaceous creeping plant, would increase ramification of its root system in order to better absorb available water of soil (Trought *et al.*, 1980).

The ratio RDW/A_gDW is significantly low for mycorrhizal plants compared to unmycorrhizal, it would indicate an attenuation of negative effects of drought stress on growth of both above ground system and root system by mycorrhiza. Several authors already highlighted the positive effects of mycorrhization on plants growing on area victim of dryness: Faber *et al.* (1991); Sylvia *et al.* (1993). This attenuation of the negative effects of the water stress on growth would be allotted to: increase drought tolerance of plant (Al-Karaki et Clark, 1998; Ruiz-Lozano *et al.*, 1995); by increasing motionless nutrients absorption (Ghazi Al-Karaki *et al.*, 2004); other factors associated with mycorrhizal colonization could influence resistance to dryness (Ghazi Al-Karaki *et al.*, 2004; Perner *et al.*, 2007).

Conclusion

On the basis of result of this study, leave and growth parameters are strongly correlated positively with the increase in mycorrhiza activity observed by the means of root colonization. Thus mycotrophes leguminous plants exposed to drought or temporary period of dryness would largely benefit from mycorrhization of their roots in term: of tolerance to dryness, improvement of growth and mineral-water absorption on dry soil with low availability in nutriment. The mycorrhizal inoculum used is thus an effective microbial material making it possible for these two leguminous species to tolerate water stress. Research work is still necessary to specify the most efficient strains.

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