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## **ANALYSIS OF EARLY GROWTH CRITERION TO SCREEN FOUR FABACEAE PLANTS FOR THEIR TOLERANCE TO DROUGHT STRESS**

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**ABSTRACT:** Early growth criterion of four Fabaceae leguminous plants, were analyzed, in a glasshouse, under water stress condition, in pots on a substrate made of  $\frac{3}{4}$  of ground and  $\frac{1}{4}$  of sand, with two threeleaflets leaves seedlings. Experimental design is a randomized factorial with: four species (*Cajanus cajan*, *Phaseolus lunatus*, *Tephrosia vogelii* and *Vigna subterranea*), four levels of water stress: 90 (blank), 60, 30 and 15 % of field capacity, five replications per treatment. Various parameters analyzed since stage threeleaflets leaves or at the end of water stress for each species are: leaves number, leaves area and specific weight, dry biomass of roots, stems and leaves. Results obtained show that, water stress has affected negatively almost all seedlings parameters of growth studied with notable inter specific variations. Broadly *Vigna subterranea*, less touched by water stress effects seems to be most tolerant specie, whereas *Tephrosia vogelii*, most touched, seems to be most sensitive of four studied species. The studied parameters can be used as marker to identify Fabaceae tolerant to water stress.

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**KEYWORDS:** Water stress, early criterion, Growth, Fabaceae

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## 1. INTRODUCTION

In world in general and Africa in particular, leguminous plants are integral part of industrial and rural systems of farming [1, 2]. They are able to fix molecular nitrogen [3, 4] and are thus of great interest: for restoration of soils having low mineral nitrogen content [3, 5-7] for human and animal consumption as source of energy and proteins [8-10] and as huge income source in economic [11]. The world production of leguminous plants cultivated for their seeds and sometimes for their leaves (*Vigna unguiculata*) exceeds 300 million tons yearly [12, 13, 10]. Insufficiency and irregularity of rains in rainagriculture, differences in temperature, edaphic conditions, global climatic warming, constitute essential factors limiting agricultural production [14-16]. These factors limit yield in arid and semi-arid regions, likewise in areas victims of current climatic changes [17, 18]. Water stress settles when plant water requirements are higher than the quantities available for roots and when water content of plant lessens sufficiently to interfere with normal processes of plant [19, 20]. He affects growth, physiology and metabolism of plant [21, 19]. Use of water stress tolerant plants on low moisture soils, would constitute a means to maximize production per area unit [22, 23]. It is thus significant to determine, incidence of water stress on growth parameters of plants at stage where they are most prone to water deficit in order to evaluate their level of tolerance to this constraint. It is in this context that we studied the effect of water stress on some growth parameters of four Fabaceae leguminous plants at an early stage.

## 2. MATERIALS AND METHODS

Healthy seeds of each species, are disinfected, germinate, then are sown in plastic bacs filled with river sand and watering regularly with tap water until stage two threeleaflets leaves per seedling [24]. Substrate used consists of: crush dried ground taken at 20 cm depth, mixed with river sand in proportions  $\frac{3}{4}$  ground and  $\frac{1}{4}$  sand. This mixture having good drainage, good permeability and better water retention capacity, is used to grow seedlings. Its field capacity and water stress levels are determined by [24]. Seedlings with two threeleaflets leaves are transplanted in plastic pots of 3 l containing substrate, and various water stress levels are applied. Experiment is laid out in randomized factorial design with: 4 leguminous plants (*C. cajan*, *P. lunatus*, *T. vogelii* and *V. subterranea*); 4 watering levels: 90(blank), 60, 30 and 15 % of field capacity; that means 900, 600, 300 and 150 ml of water for 2.4 kg of dry substrate respectively. Each of 16 treatments is replicated 5 times; the experimental unit comprises 25 seedlings per treatment and 5 seedlings per pot. Eight pots without seedlings are used in the design to determine substrate water lost by evaporation at the level each pot. Various parameters are evaluated since stage threeleaflets leaves per plantlet, regularly till the end of experimentation [25, 26]. At the end of water stress period growth parameters: leaves number, leaf

area, leaf specific weight, dry weight of plant, stems, leaves and ratios DBR/DBS and DBL/DBS are assessed [27].

### Data analysis

Collected data are subjected to analysis of variance (ANOVA) to detect significant differences between averages. Averages are compared by using Student Newman-Keuls and Duncan test at 5 % level of significance. Software SAS (Statistical Analysis System) or SPSS 18.0 is used. The results, presented in form of histogram, of graph or table are carried out using the software Microsoft Excel 2007.

## 3. RESULTS AND DISCUSSION

### Leaves number of (LN)

Number of emitted leaves varies according to specie and decreases when water stress level increases (Fig. 1). For *C. cajan*, *V. subterranea* and *T. vogelii*, reduction of LN at 15<sup>th</sup> water stress day is significant from one treatment to another. In *P. lunatus*, this parameter decreases significantly ( $p < 0.05$ ): 24.05, 48.42 and 49.05 % for T1, T2 and T3 respectively.

### Leaf area (LA)

Leaf area (Fig. 2 and 3) of 3<sup>rd</sup> and 4<sup>th</sup>threeleaflets leaves decreases when water stress level increases for four studied species compared to unstressed plants. For *P. lunatus* and *C. cajan* this reduction is not significant for 3<sup>rd</sup> leaf on the 15<sup>th</sup> day of stress. The LA of 4<sup>th</sup> threeleaflets leaf is significantly lower than that of 3<sup>rd</sup> for T1, T2, T3 for *P. lunatus* and *C. cajan*; at T2 and T3 for *V. subterranea* and at T3 for *T. vogelii* (Fig. 3). The effect of water stress on LA is more marked on 4<sup>th</sup> leaf of four studied leguminous plants than on 3<sup>rd</sup> one (Fig. 2 and 3). During experimentation, withering and/or death of plants was observed starting from T2 (30 % FC) for four studied leguminous plants (Fig. 2 and 3).

### Leave specific weight (LSW)

Leave specific weight (Fig. 4) decreases with increase in water stress level for two studied leguminous plant species compared to unstressed. For *V. subterranea* it decreases significantly ( $p < 0.05$ ): 3.75 mg/cm<sup>2</sup> (14.24 %), 3.85 mg/cm<sup>2</sup> (14.62 %) and 7.20 mg/cm<sup>2</sup> (27.34 %) for T1, T2 and T3 respectively. There are no significant difference between LSW at T1 and T2. The highest reduction is observed at T3. For *C. cajan*, water stress doesn't have significant effect on LSW at T1 and T2; however, with T3, one observes a significant reduction in LSW of 5.45 mg/cm<sup>2</sup> (34.34 %).

### Dry biomass

Dry biomass of leaves, stems and roots varies according to water stress level and according to leguminous plant species (Table 1). Significant differences ( $p < 0.05$ ) of dry biomasses of leaves

compared to blank are noted for four leguminous plants. For *P. lunatus* and *V. subterranea*, losses of leaves dry biomass due to moderate water deficit are higher than those due to marked restriction. Treatments T1, T2 and T3 reduce leaves dry biomass respectively: 0.37 g, 0.53 g, 0.01 g for *P. lunatus* and 0.32 g, 0.23g, 0.08 g for *V. subterranea*. For *C. cajan* and *T. vogelii*, it is the contrary: 0.02 g, 0.03 g, 0.06 g of loss, and 0.02 g, 0.26 g, 0.10 g respectively. Dry biomass of leaves at T3 decreases more in condition of water stress for *P. lunatus* (0.91 g) and *V. subterranea* (0.89 g), these two species would be thus more sensitive to water stress than *C. cajan* (0.11 g) and *T. vogelii* (0.38 g). Senescence and leaves abscission are faster for stressed plants and increase with level of water stress. Water stress significantly decreases dry biomass of stem of four leguminous plants compared to T0; *P. lunatus* produces more and *V. subterranea* less (Table 1). In *V. subterranea* it doesn't have significant difference between T2 and T3 in term of accumulated biomass. Dry biomass of roots decreases for four leguminous plants subjected to water stress compared to T0 (Table 1). For *P. lunatus*, water stress causes a significant reduction ( $p < 0.05$ ) in roots dry biomass 0.74, 1.10 and 1.26 g respectively for T1, T2 and T3. In *T. vogelii*, at T1, no significant variation observes for RDB; for T2 and T3, significant reduction ( $p < 0.05$ ) 0.17 g and 0.25 g respectively is recorded. *Vigna subterranea*, at T1, T2 and T3 exhibits significant reduction in DBR 0.13, 0.20 and 0.23 g respectively; between T2 and T3, DBR registers no significant variation. For the three treatments, there is no significant variation of DBR for *C. cajan*. For four studied species, for DBL and DBR, *P. lunatus* accumulates always more biomass and *V. subterranea* accumulates less. Broadly studied leguminous plants produce and accumulate more dry biomass at level of above ground parts compared to underground parts. Plant dry biomass decreases significantly ( $p < 0.05$ ) when water stress level increases for four leguminous plants compared to T0 (Table 1). For *C. cajan*, water stress significantly decreases ( $p < 0.05$ ) DBP 0.12 and 0.22 g only for T2 and T3 respectively. For the four leguminous plants *P. lunatus* produces more DBP and *C. cajan* less. At the end of experimentation, various levels of water stress didn't significantly modify distribution of dry biomass between roots and stems (Table 1). The studied species accumulate more dry biomass on the level of roots, because ratio DBR/DBS is always higher than one. Ratio DBL /DBS is higher than one for all studied species, which means that they accumulate more biomass in leaves compared to stems. According to values of ratios DBR /DBS and DBL /DBS for T3 following classification can be made:

$$V. subterranea > P. lunatus > C. cajan > T. vogeli$$

## DISCUSSION

Leaves intervene in processes controlling growth and development of plants, such as photosynthesis, respiration, transport of nutritive substances among others [28, 29]. They constitute thus main propeller of plants growth because of photosynthesis which is crucial process that supports crop growth and development [29]. Growth parameters: leaves number, leaf area, leaves specific weight; dry biomass of: leaves stems, roots, whole plant and ratio DBR/DBS, DBL/DBS; of four studied leguminous plants decrease as water stress rises. This decrement was previously observed: on lentil [30], on leguminous plants [31-33], on *Stevia rebaudiana* [34], on *Ocimum basilicum* [35]. These reductions could be an avoidance strategy to water stress. The effect of water stress can be expressed, according to adaptive strategy of each species by morphological modifications. These modifications affect underground or above ground parts: reduction of LA and rolling up of leaves [36]. Vegetative development is very disturbed under conditions of water starvation [37]. Many authors [38-40] report a significant depressive effect of water stress on number of leaves per plant and leaf area. Similar effect was observed on voandzou [41, 42]. Reduction of leaves number would be consequence of stop and/or reduction of production of new leaves [26, 43] or due to reduction of cellular enlargement or more leaves senescence caused by lessen of turgid pressure [44]. Plant would prioritize maintenance of its existing structures to its increase. Reduction of LA of third and fourth three leaflets leaves can be allotted to combination of growth reductions of young leaves and formation of new ones [45]. The reduction of leaves area could be explained by reduction of leaf size due to an early senescence of these leaves in water limiting condition. It can also be due to slowdown of mitotic activity of epidermis cells which leads to reduction of total number of leaves cells. This reduction is one plants answer to dehydration; it contributes to conservation of water resources, which allows survival of plant [46]. Plants leaves subjected to water stress usually reach apparent final sizes smaller compared to controls [47]. This result is similar to those of [45] on *Sophora davidii* and [48] on *Eucalyptus globulus*. Leaves specific weight is one of significant plants markers in response to water starvation and can be regarded as a simple criterion of selection of species presenting great water use efficiency in water stress conditions. Under water stress, LSW decreases; this result is similar to that observed for durum wheat Vit genotype [26] and suggests a significant reduction in leaves weight. Dry biomass of plant, constitutes one of realistic criteria to determine the response of plant to various types of stress including water stress [49, 33]. Water stress induced in plants specific responses like: reduction of vegetative growth, closing of stomata, reduction in photosynthesis [50-52], cells divisions and expansion [53, 54]. These various answers permit plant to reduce water loss or to optimize its absorption [50-52]. In this work reduction of dry biomass is observed for leaves, stems,

roots and entire plant under water stress compared to blank. This result corroborates those of: [30] on lentil; [55] on bambara groundnut; [56, 57] on bambara groundnut and [54] on *Cicer arietinum*. Reduction in growth would be explained by reduction of photosynthesis caused by water stress; but also by deficit of mineral nutrition (N, P especially) due to reduction of elements flow towards roots [58, 59]. By reducing cells division and their expansion, water stress slows down plant growth [54, 60]. This reduction of growth would constitute an adaptive mechanism allowing plant to survive in water stress condition. It would allow plant photosynthetic products and energy towards production of molecules enabling him to fight against stress [61] and/or to maintain roots growth to improve absorption of water [62]. A prolonged water stress can limit growth and production of biomass and even cause plant death [63]. Reduction of ratio DBR/DBS recorded for *P. lunatus* would be consequence of reduction of biomass allocated to roots. This result is not consistent with those of [64] on *Chamaecytisus palmensis*, [65] on coffee-tree. Differences observed for leguminous plants studied in water stress condition, were already reported for chickpea [54] and would be due to genotypic differences [54].

#### **4. CONCLUSION**

At early growth stage, water stress has affected negatively almost all studied growth parameters, with notable inter specific variations. Statistical analysis reveals a positive and significant correlation between growth parameters of four leguminous plants. Broadly *Vigna subterranea*, less touched by water stress seems to be most tolerant specie, whereas *Tephrosia vogelii*, specie most touched, seems to be most sensitive of four studied species. These parameters can be used as marker to identify Fabaceae tolerant to water stress.

#### **CONFLICT OF INTEREST**

The authors have no conflict of interest.

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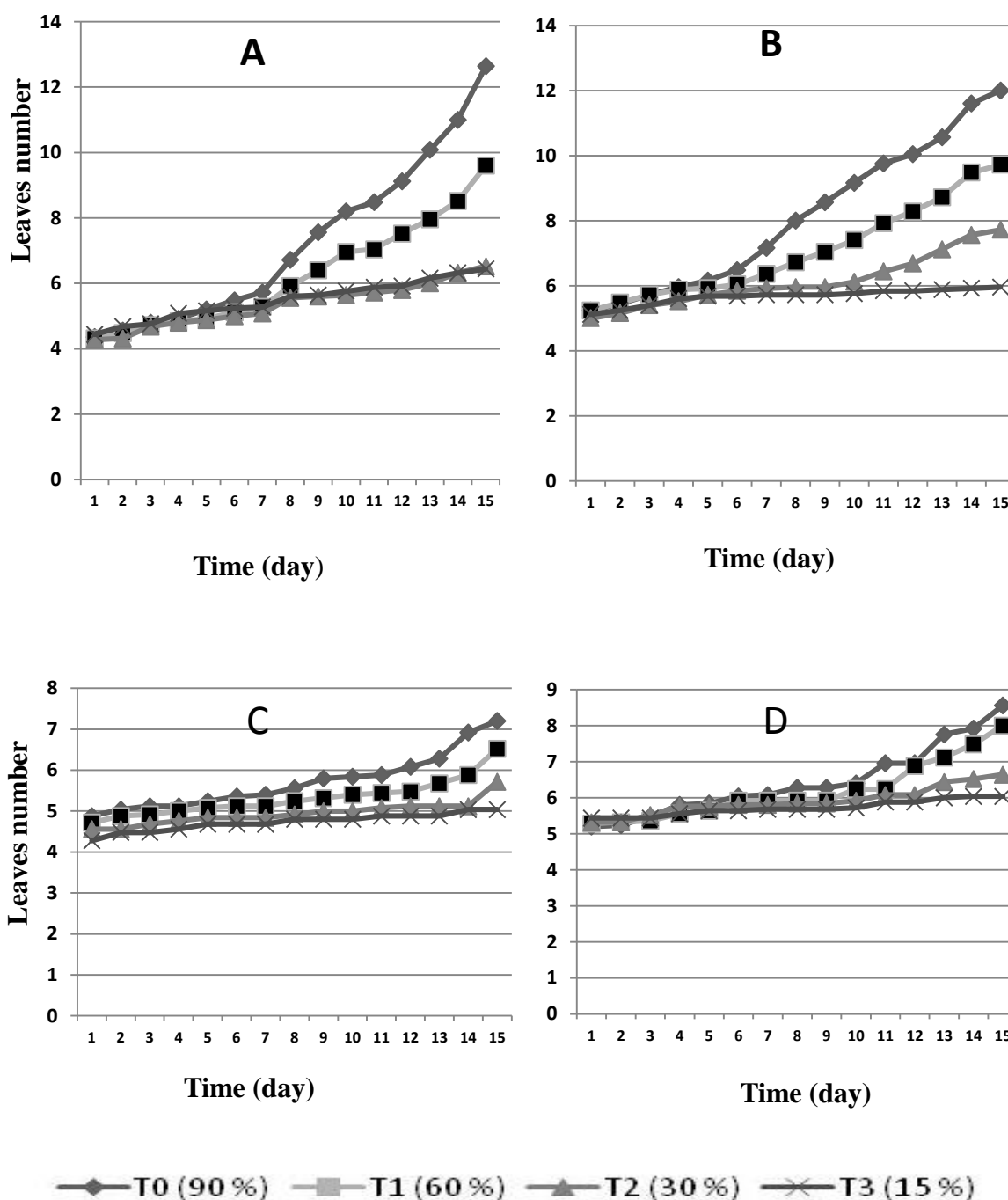
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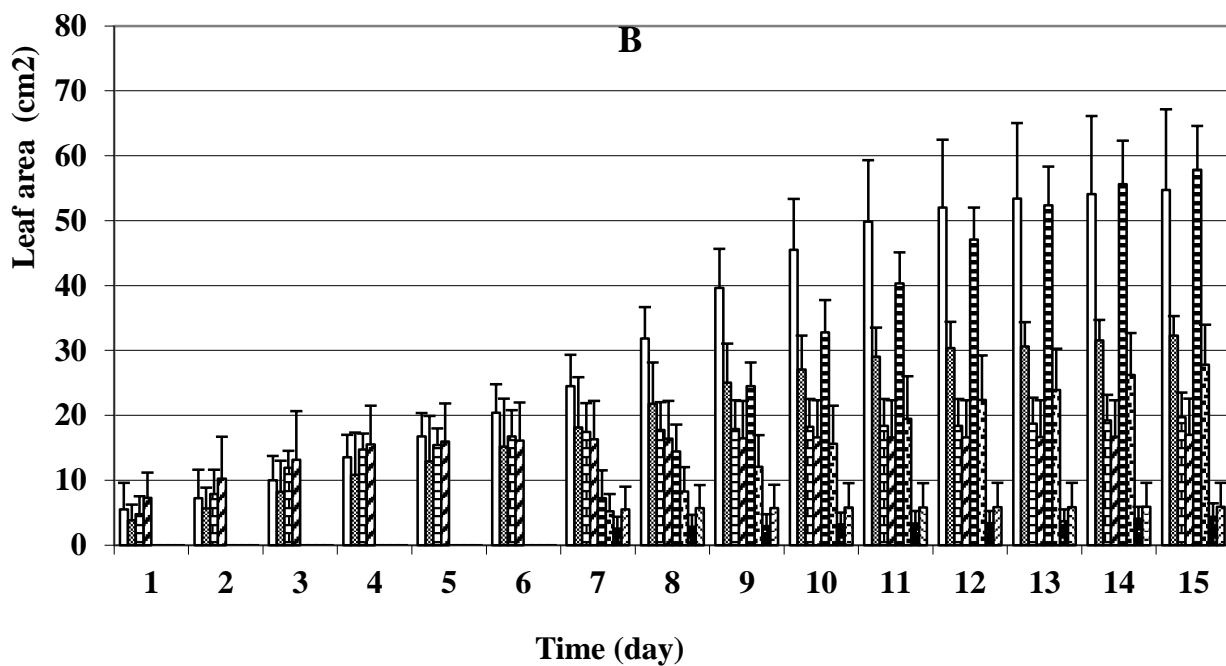
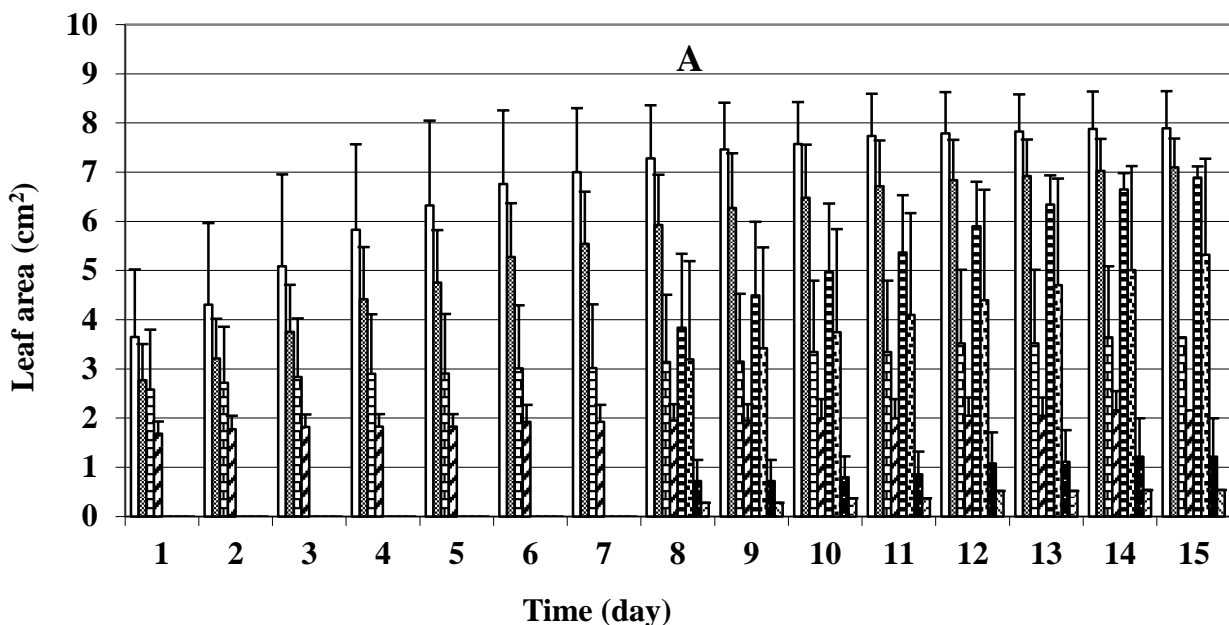
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**SUPPLEMENTARY FILES**



**Fig. 1** Average leaves number per plant (A: *P. lunatus*, B: *V. subterranea*, C: *C. cajan*, D: *T. vogelii*)



□ T0 3rd L   ■ T1 3rd L   ▨ T2 3rd L   ▩ T3 3rd L   □ T0 4th L   ■ T1 4th L   ▨ T2 4th L   ▩ T3 4th L

Fig. 2 Average leaf area for *Cajanus cajan* (A) and *Phaseolus lunatus*(B)

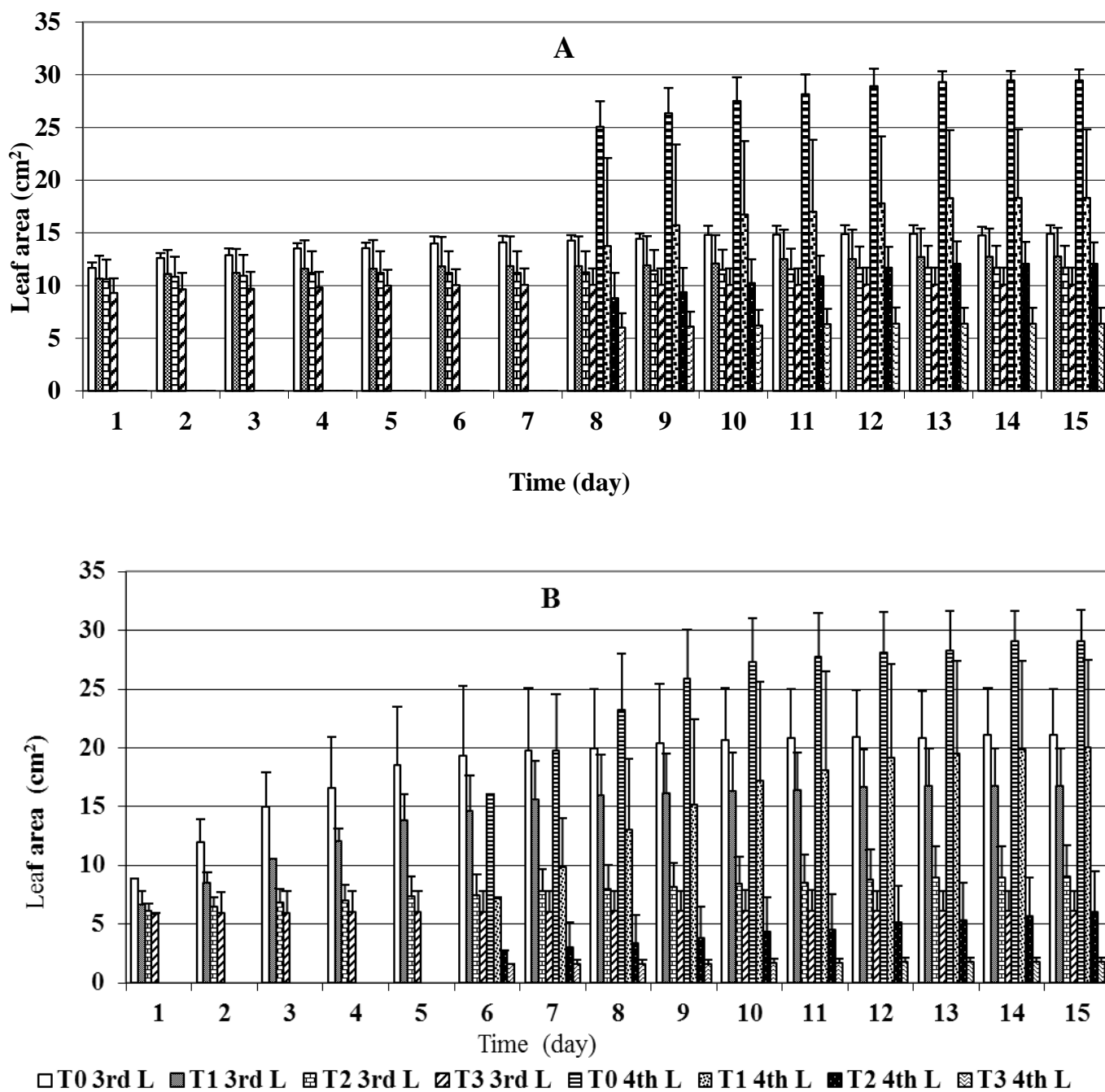
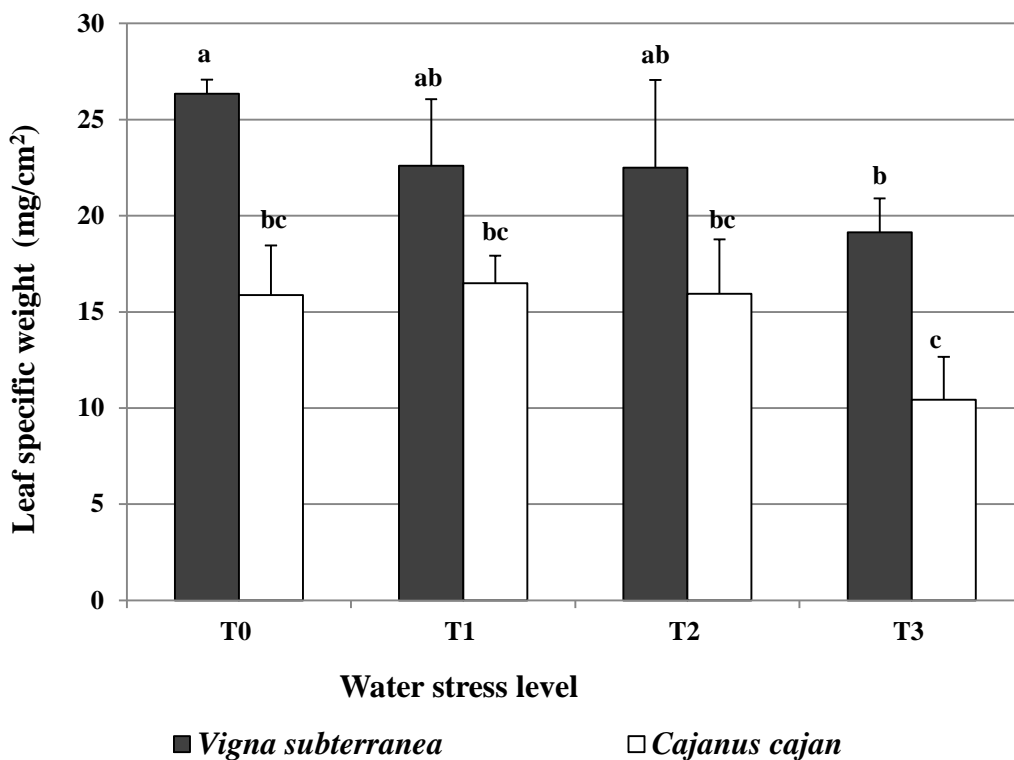


Fig . 3 Average leaf area of *Tephrosia vogelii*(A) and *Vigna subterranea* (B) under water stress



**Fig. 4 Leaf specific weight**

**Table 1. DRY BIOMASS: STEM; LEAVE; ROOT; PLANT; DBR/DBS and DBL/DBS**

Parameters \ Treatments		T0 (90 % FC)	T1 (60 % FC)	T2 (30 % FC)	T3 (15 % FC)
DBL (g)	<i>C. cajan</i>	0.16 ± 0.02 c	0.14 ± 0.06 c	0.11 ± 0.02 c	0.05 ± 0.02 c
	<i>P. lunatus</i>	1.27 ± 0.21 a	0.90 ± 0.15 ab	0.37 ± 0.14 bc	0.36 ± 0.09 bc
	<i>T. vogelii</i>	0.45 ± 0.07 bc	0.43 ± 0.05 bc	0.17 ± 0.05 c	0.07 ± 0.02 c
	<i>V. subterranea</i>	1.05 ± 0.91 a	0.47 ± 0.21 bc	0.24 ± .04 c	0.16 ± 0.02 c
DBS (g)	<i>C. cajan</i>	0.12 ± 0.01 e	0.11 ± 0.02 ef	0.07 ± 0.01 ef	0.07 ± 0.01 ef
	<i>P. lunatus</i>	0.58 ± 0.07 a	0.41 ± 0.07 b	0.23 ± 0.03 cd	0.25 ± 0.03 c
	<i>T. vogelii</i>	0.26 ± 0.02 c	0.19 ± 0.02 d	0.12 ± 0.03 e	0.10 ± 0.01 ef
	<i>V. subterranea</i>	0.09 ± 0.01 ef	0.06 ± 0.01 ef	0.03 ± 0.01 f	0.03 ± 0.01 f
DBR (g)	<i>C. cajan</i>	0.13 ± 0.02 e	0.15 ± 0.04 e	0.11 ± 0.01 e	0.09 ± 0.02 e
	<i>P. lunatus</i>	1.66 ± 0.06 a	0.92 ± 0.07 b	0.55 ± 0.08 c	0.40 ± 0.08 d
	<i>T. vogelii</i>	0.37 ± 0.11 d	0.32 ± 0.05 d	0.20 ± 0.04 e	0.11 ± 0.02 e
	<i>V. subterranea</i>	0.33 ± 0.10 d	0.20 ± 0.02 e	0.13 ± 0.03 e	0.10 ± 0.03 e
DBP (g)	<i>C. cajan</i>	0.42 ± 0.04 def	0.39 ± 0.08def	0.30 ± 0.03 ef	0.20 ± 0.03 f
	<i>P. lunatus</i>	3.50 ± 0.29 a	2.22 ± 0.14 b	1.15 ± 0.21 cd	1.00 ± 0.13 cdef
	<i>T. vogelii</i>	1.07 ± 0.20 cde	0.94 ± 0.02 cdef	0.49 ± 0.01 def	0.28 ± 0.03 ef
	<i>V. subterranea</i>	1.47 ± 1.02 c	0.73 ± 0.18 def	0.39 ± 0.04 def	0.29 ± 0.04 ef
DBR/DBS	<i>C. cajan</i>	1.11 ± 0.12 c	1.39 ± 0.30 c	1.48 ± 0.11 c	1.30 ± 0.21 c
	<i>P. lunatus</i>	2.98 ± 0.14 ab	2.39 ± 0.31 bc	2.39 ± 0.36 bc	1.67 ± 0.14 c
	<i>T. vogelii</i>	1.41 ± 0.42 c	1.87 ± 0.28 c	1.71 ± 0.35 c	1.17 ± 0.20 c
	<i>V. subterranea</i>	3.85 ± 1.19 a	3.69 ± 0.42 a	4.11 ± 0.85 a	3.69 ± 0.89 a
DBL/DBS	<i>C. cajan</i>	1.64 ± 0.12 d	1.33 ± 0.17 d	1.48 ± 0.10 d	0.68 ± 0.05 d
	<i>P. lunatus</i>	2.23 ± 0.26 d	2.24 ± 0.40 d	1.66 ± 0.18 d	1.49 ± 0.18 d
	<i>T. vogelii</i>	1.75 ± 0.14 d	2.35 ± 0.26 d	1.44 ± 0.40 d	0.68 ± 0.07 d
	<i>V. subterranea</i>	11.80 ± 1.32 a	8.45 ± 1.10 b	7.76 ± 1.78 b	5.79 ± 1.06 c

Values follow by same letter on one line are not significant at  $p < 0.05$  %.