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RESEARCH ARTICLE

ANALYSIS OF EARLY BIOCHEMICAL CRITERION TO SCREEN FOUR FABACEAE PLANTS FOR THEIR TOLERANCE TO DROUGHT STRESS

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ABSTRACT

Early biochemical criterion of four Fabaceae leguminous plants, were studied, in glasshouse, under water stress condition, in pots on a substrate made up of ¾ of ground and ¼ of sand, with seedlings having two three leaflets leaves. Experimental design was a factorial arranged in a completely randomized block with: four species (*Cajanus cajan, Phaseolus lunatus, Tephrosia vogelii and Vigna subterranea*), four watering levels: 90 (blank), 60, 30 and 15% of field capacity, replicated five times. Various parameters measured after 15 days of water stress, are content of: chlorophyll a+b, carotenoids, proline, total amino acids, total soluble proteins and total soluble sugars. Results obtained for studied parameters show that water stress differently modifies metabolism of plants according to genotype. Proline content and total soluble sugars content can be used like early indicators and relevant criteria of tolerance to drought, usable in varietal selection and like parameters of improvement of yield in arid areas.

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INTRODUCTION

Leguminous plants according to their quantitative and qualitative composition (Tsoata *et al*., 2015a), contribute in a significant way to animal and human nutrition everywhere in the world (Shao *et al*., 2009; Akédrin *et al*., 2010; Gao *et al*., 2012); especially in Latin America and Africa (Polania *et al*., 2016). Furthermore, they are multipurpose plants; their multiple functions were highlight in many works (Akédrin *et al*., 2010; Bertrand, 2009; Hindumathi and Reddy, 2012; Jensen *et al*., 2012; El Sohaimy, 2012; Bahadoran and Mirmiran, 2015). It's because of the huge importance of leguminous plants in society that UNO in its sixty-eighth general assembled proclaimed 2016 international year of leguminous plants, in order to sensitize on: many advantages which they offer, to stimulate their production and commercialization and to encourage new intelligent uses along the food chain (Anonyme, 2013). But production of leguminous plants is limited mainly by biotic and abiotic (Ceccarelli, 2010). Among abiotic stresses, water stress is most complex and devastator on a total scale (Pennisi, 2008; Zhang *et al*., 2011) and its frequency should increase as consequence of climatic change (Ceccarelli, 2010). Water stress can be defined as a situation in which water potential of plant and cell turgidity are reduced enough at the interface with normal functions (Shao *et al*., 2008). It can appear in form of rains stop at the middle of vegetative cycle, rains ceasing very early or occurring tardily, or weak precipitations (Katungi *et al*., 2010). Water stress can thus induce yield loss of 32 to 60 % (Katungi *et al*., 2010). Plants adapt by modifying various biochemical processes to adjust them self with stressing environment (Bohnert *et al*., 1995; Tsoata *et al*., 2016b). Many authors showed that, in plants, water constraint involves biochemical effects in which proteins, sugars, are implied, among other products (Shao *et al*., 2008; Tsoata *et al*., 2016b). To mitigate water stress, farmers use several methods among which, culture of tolerant species or varieties, or use of local varieties having a weak yield (Abate *et al*., 2011). However research finding that can be used to create elites genotypes adapted to local conditions or not very sensitive to water stress are scare. For these reasons it is necessary and urgent to intensify research tasks clarifying the features of adaptation, or tolerance to water stress for leguminous plants in particular and plants in general. It's in this context that we studied in glasshouse effects of water stress on biochemical parameters of four Fabaceae leguminous plants, in order to positively contribute to comprehension of behavior of those plants under water stress.

MATERIALS AND METHODS

Very young seedlings resulting from germination of healthy and disinfected seeds of each species are developed in plastic

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containers on river sand regularly humidified until the stage two three leaflets leaves. Then transferred in plastic pots of 3 L containing a substrate made up of ground ¾ and sand ¼ and various levels of water stress are applied. The experimental design is a randomized factorial with: 4 leguminous plants (*C. cajan*, *P. lunatus*, *T. vogelii* and *V. subterranea*); 4 levels of watering: 90(blank), 60, 30 and 15 % of field capacity; that is to say 900, 600, 300 and 150 ml of water for 2,4 kg of dry substrate; five replications. After water stress period of 15 days, following biochemical parameters were recorded: content chlorophyll a+b, carotenoids, proline, total amino acids, total soluble proteins and total soluble sugars (Tsoata *et al*., 2016b).

Data analysis

Data collected for various parameters are subjected to analysis of variance (ANOVA), to Student Newman-Keuls and Duncan test at 5 % level of significance for comparison of means, thanks to software SAS or SPSS 18.0. The software Microsoft Excel 2007 is used for graph (Tsoata *et al*., 2016b).

Leaves pigments content

Leaves pigments are extracted and proportioned according to (Lichtenthaler and et Buschmann, 2001; Tsoata *et al*., 2015c).

Total amino acids and proline

Total amino acids and proline are determined by ninhydrine reaction according to (Yemm and et Cocking, 1955; Tsoata *et al*., 2015a).

Total soluble proteins

Extraction of soluble proteins was made by crushing of 0.5 g of fresh leaves in a mortar in presence of 5 ml of tris-HCl 0.4 M (pH = 6.8) buffer, containing 1.5 M NaCl followed by centrifugation at 5000 rpm at 4 °C during 10 mn (Tsoata *et al*., 2015a). The supernatant was recovered and the titration of proteins done by the colorimetric method of (Bradford, 1976; Tsoata *et al*., 2015a).

Total soluble sugars

The titration of total sugars was carried out according to anthrone method (Yemm and Willis, 1954; Tsoata *et al*., 2015a).

RESULTS

Content of chlorophyll a+b and carotenoids

For chlorophyll a+b content, no significant difference is observed for all treatments (T1, T2 and T3) for *P. lunatus*, *V. subterranea* and *T. vogelii*. But for *C. cajan*, a significant reduction ($p < 0.05$) in Chlorophyll a+b content is recorded, this reduction is 1.15 mg.g^{-1} DW for T1 and 1.08 mg.g^{-1} DW for T2 compared to T0. There is no significant difference between Chlorophyll a+b content of T1 (0.91 mg.g^{-1} DW) and that of T2 (0.99 mg.g^{-1} DW). For the studied leguminous plants, carotenoids content and chlorophyll a / chlorophyll b (Chl. a/Chl. b) (Table 1) exhibit no significant difference for all treatments (T1, T2 and T3) compared to T0.

Proline and total amino acids

For *P. lunatus*, proline content is relatively constant whatever the level of stress (Fig. 1). For *V. subterranea*, proline increase is significant ($p < 0.05$) only for T3 (0.83 µg.g^{-1} DW). In *C. cajan*, significantly increment is observed for T2 (0.39 µg.g^{-1} DW) and T3 (1.39 µg.g^{-1} DW). *Tephrosia vogelii*, shows significant increase ($p < 0.05$) of proline content for all levels of water stress. For T3 (drastic stress), proline content increases considerably in leaves: 4 times (275 %) for *C. cajan*, 5 times (357 %) for *V. subterranea* and 6 times (524 %) for *T. vogelii*. The total amino acid content (TAA) of leaves varies under water stress for four studied leguminous plants compared to T0 (Table 2). For *C. cajan*, water stress didn't induce any significant variation of TAA content for T1; in T2, this content drops by 38.00 % before increasing by 30.42 % for T3. In *P. lunatus*, this content increases significantly ($p < 0.05$) compared to T0 for stressed plants. *Tephrosia vogelii* TAA content decreases in T1 and T2; with T3, it increases

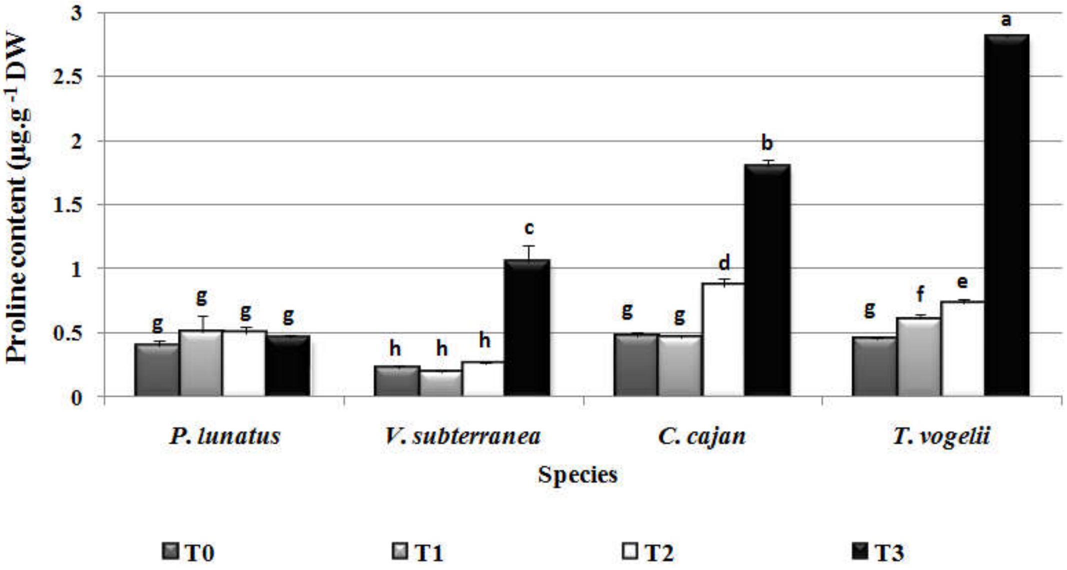

Fig.1. Effect of water stress on proline accumulation

Table 1. Chlorophyll and carotenoïd content

Treatments Parameters	Species	T0 (90%FC)	T1 (60 %FC)	T2 (30 %FC)	T3 (15%FC)
Chlorophyll a+b	*C. cajan*	2.06 ± 2.49 a	0.91 ± 0.03 ab	0.99 ± 0.15 ab	nd
	P. lunatus	0.60 ± 0.02 ab	0.81 ± 0.02 ab	0.84 ± 0.03 ab	0.78±0.04 ab
	T. vogelii	0.64 ± 0.02 ab	0.31 ± 0.02 ab	0.24 ± 0.12 ab	nd
	V. subterranea	0.40 ± 0.02 ab	0.50 ± 0.06 ab	0.56 ± 0.08 ab	0.97± 0.04 ab
Chl. a/Chl. b	*C. cajan*	0.22 ± 1.33 a	0.87 ± 0.09 a	0.92 ± 0.34 a	nd
	P. lunatus	0.89 ± 0.01 a	1.18 ± 0.24 a	1.36 ± 0.41 a	2.29 ± 1.91 a
	T. vogelii	1.68 ± 1.97 a	0.93 ± 0.13 a	0.68 ± 0.12 a	nd
	V. subterranea	0.73 ± 0.20 a	0.87 ± 0.38 a	0.57 ± 0.34 a	0.95 ± 0.08 a
Carotenoïds	*C. cajan*	0.63 ± 1.05 a	0.00 ± 0.01 a	0.01 ± 0.06 a	nd
	P. lunatus	0.02 ± 0.01 a	0.05 ± 0.01 a	0.06 ± 0.01 a	0.08 ± 0.05 a
	T. vogelii	0.01 ± 0.09 a	0.02 ± 0.01 a	0.00 ± 0.07 a	nd
	V. subterranea	0.02 ± 0.01 a	0.00 ± 0.02 a	0.02 ± 0.03 a	0.02 ± 0.02 a

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

Table 2. Total amino acid content of leaves

Treatments Species	T0 (90 % FC)	T1 (60 % FC)	T2 (30 % FC)	T3 (15 % FC)
C. cajan	554.00 ±35.63bcd	516.33 ±39.73 bcd	343.50 ± 32.45 de	722.50 ± 6.61 b
P. lunatus	429.67 ± 43.77 cd	561.50 ±150.70 bcd	522.67 ± 31.48 bcd	539.17± 118.68bcd
T. vogelii	581.33 ± 80.51 bc	535.00 ±119.48 bcd	476.33± 182.85 cd	1592.00 ± 107.39 a
V. subterranea	213.83 ± 42.56 ef	127.67 ± 21.73 f	224.50 ± 44.25 ef	179.33 ± 44.89 ef

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

Table 3. Total soluble protein content

Treatments Species	T0 (90 % FC)	T1 (60 % FC)	T2 (30 % FC)	T3 (15 % FC)
C. cajan	3.12 ± 0.13 f	4.73 ± 0.35 e	1.62 ± 0.71 g	1.47 ± 0.61 g
P. lunatus	6.88 ± 0.31 cd	7.28 ± 0.32 c	6.93 ± 0.57 cd	6.20 ± 0.28 d
T. vogelii	8.54 ± 0.13 b	5.23 ± 0.21 e	5.18 ± 0.57 e	7.03 ± 0.43 cd
V. subterranea	8.50 ± 0.39 b	8.46 ± 0.42 b	10.14 ± 0.45 a	9.33 ± 0.47 b

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

Table 4. Total soluble sugars content

Treatments Species	T0 (90 % FC)	T1 (60 % FC)	T2 (30 % FC)	T3 (15 % FC)
C. cajan	1.46 ± 0.08 de	1.48 ± 0.12 de	1.69 ± 0.32 de	1.44 ± 0.34 de
P. lunatus	0.75 ± 0.08 e	1.71 ± 0.11 de	2.21 ± 0.25 de	5.69 ± 0.22 c
T. vogelii	13.67 ± 1.35 a	13.18 ± 3.64 a	3.76 ± 0.17 cd	2.86 ± 0.14 de
V. subterranea	2.96 ± 0.17 de	5.53 ± 0.36 c	8.61 ± 0.11 b	3.54 ± 0.63 cd

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

significantly ($p < 0.05$) by 173.85 %. For *V. subterranea*, this content decreases significantly by 40.29 % for T1; for T2 and T3, there is no significant difference. Compared to T0, TAA content is higher in T3 for *C. cajan, P. lunatus and T. vogelii*. *Tephrosia vogelii* and *C. cajan* accumulate more TAA than 2 other leguminous plants.

Total soluble proteins

A great variability is observed on accumulation of total soluble proteins (TSP) for four studied leguminous plants compared to T0 (Table 3). For *C. cajan*, TSP content increases significantly ($p < 0.05$) by 1.67 $\mu g.g^{-1}$ DW for T1; with T2 and T3, this content decreases significantly by 1.50 $\mu g.g^{-1}$ DW and 1.64 $\mu g.g^{-1}$ DW respectively. In *P. lunatus*, TSP content increases by 0.39 $\mu g.g^{-1}$ DW with T1, before dropping of 0.68 $\mu g.g^{-1}$ DW at T3,compared to T0. For *T. vogelii*, a significant reduction ($p < 0.05$) in this content is recorded, 3.31 $\mu g.g^{-1}$ DW at T1, of 3.36 $\mu g.g^{-1}$ DWF in T2 and 1.51 $\mu g.g^{-1}$ DW in T3, compared to T0. In *V. subterranea*, there is no significant difference between TSP accumulated for T1 and T3; for T2 a significant increase ($p < 0.05$) of 1.64 $\mu g.g^{-1}$ DW is observed, compared to T0.

Total soluble sugar: The increase in water stress levels doesn't influences accumulation of total soluble sugars (TSS) for *C. cajan* (Table 4). This content increases with increase of water stress level in *P. lunatus*; the highest accumulation of TSS is observed with T3 (5.69 $mg.g^{-1}$ DW). In *T. vogelii*, reduction in TSS content is noticed when level of water stress increased; for T1, reduction is not significant; but significant 9.91 $mg.g^{-1}$ DW for T2 and 10.81 $mg.g^{-1}$ DW for T3, compared to T0. In *Vigna subterranea*, a significant ($p < 0.05$) increase of TSS content is noticed, 2.56 $mg.g^{-1}$ DW for T1, 5.65 $mg.g^{-1}$ DW for T2 and 0.58 $mg.g^{-1}$ DW for T3.

DISCUSSION

Photosynthetic pigments are essential for production of plants organic nutriments and life of plants cells depends on photosynthetic activity (Bezerra *et al*., 2008). Their amount in plants is often correlated with the impact of several environmental stresses, because their quantity is related to stress visual symptoms and to plant photosynthetic productivity (Jain *et al*., 2013). Water stress modifies photosynthetic pigments and their composition, Anjun *et al*. (2003), Farooq *et al*. (2009) report a chlorophyll reduction in water stressed plants. Chlorophylls, carotenoids and ratio chl. a /chl. b of leaves, are good indicators for stress detection and plants tolerance to stress (Chakhchar, 2015). Results of this experiment show that compared to control, water stress didn't induce significant variation of foliar pigment and ratio chl. a /chl. b, except *C. cajan* for chl. a+b. These results do not corroborate those of: Yadav *et al*. (2013) on *V. mungo*; Allahverdiyev (Allahverdiyev, 2015)) on durum wheat; Tsoata *et al*. (2015c) on voandzou, and suggest that for *P. lunatus*, *V. subterranea* and *T. vogelii,* under conditions of this experimentation, water stress, at this development stage, doesn't affect leaves pigments. This result is similar to those of Mabbaudhi *et al*. (2013) on *V. subterranea*, reporting that, chlorophyll content was not sensitive to water stress. Development stage of plants subjected to water stress would have an influence on chlorophylls and carotenoids amount in leaves tissue. Content of chlorophyll, carotenoids and ratio Chl. a /chl. b, would thus not be early indicators of tolerance to water stress for these three species. Quantity of leaves chlorophyll can be influenced by factors such as leaf age, position and environmental factors: light, temperature and availability of water (Hikosaka *et al*., 2006). The decrease of chl. a+b observed for *C. cajan* corroborates results of Anjun *et al*. (2003) on barley; Atti *et al*. (2004) on *Glycine max*, Mekliche (2003) on durum wheat; Kiani *et al*. (2008) on sunflower. The decrement of chlorophyll content is the consequence of reduction of stomata opening aiming at limiting water losses by transpiration and increase in resistance to entry of atmospheric CO_2 necessary to photosynthesis (Bousba *et al*., 2009). Closing of stomata, with time lowers CO_2 absorption as well as photosynthesis and consequently reduced chlorophyll content of stressed plants (Makakheri *et al*., 2010). This decrease of pigments content could also be due to very weak biosynthesis following drop of activity of glutamate synthetase, enzyme implied in biosynthesis of glutamate, which is precursor of photosynthetic pigments (Tahri El Houssine *et al*., 1998). Moreover, the decrement could be due to fast decomposition of chlorophyll, as well as to modifications of structure of thylakoïdal membrane (Bacelar *et al*., 2006). Results on photosynthetic pigments emphasize variability existing between studied leguminous plant species with regard to their response to water stress.

Accumulation of proline is a significant indicator of plants tolerance to drought. Several authors show that increase in content of proline is directly related to application of water stress (Mekliche *et al*., 2003; Cechin *et al*., 2006). Increase in proline would be a protective response of plants to all factors which involve a water reduction in cytoplasm. An increase in proline content in parallel with severity of water stress is noted for three studied leguminous plants. This result corroborates those of many works reporting an increase in proline content to a significant degree for many species and in various situations of stress (osmotic, water, thermal): olive-tree (Sofo *et al*., 2004; Boughallleb and Mhamdi, 2011); poplar (Yin *et al*., 2005); rice (Choudhary *et al*., 2005; Mostajeran and Rahimi-Eichi, 2009); voandzou (Tsoata *et al*., 2015a). Proline thus accumulated, as compatible inert osmolyte would play several roles in stressed plant: protection of subcellular structures, membranes and proteins (Kavi-Kishor *et al*., 2005; Ashraf and Foolad, 2007); elimination of free radicals, reactive oxygen species and neutralization of singulet oxygen (Okuma *et al*., 2004; Chen *et al*., 2006; Matysik *et al*., 2002); improvement of activity of several enzymes and would act as antioxidant (Matysik *et al*., 2002; Szabados and Savoure, 2009). Proline can confer stress tolerance to plants by development of an antioxidant system which can play a role of indicator of osmotic adjustment (Eliane *et al*., 2007). This type of adaptation allows plants to perform its normal physiological functions in spite of degradation of its intern water state caused by drought. Plants which accumulate most proline are regarded as most stress tolerant. Thus *T. vogelii* and *C. cajan* would be more tolerant to water stress than *V. subterranea*. Proline quantity didn't vary significantly for *P. lunatus* under water stress; this result doesn't corroborate those of Tsoata *et al*. (2015a) on voandzou; Sofo *et al*. (2004) on olive tree, Mostajeran (2009), Boughallds and Mhamdi (2011) on rice. It suggests that *P. lunatus* under osmotic stress would use mechanisms where little proline is needed, for protection against factors lessening cytoplasm water. The accumulation of proline however varies according to plant and even variety as we observed for studied species; this is in agreement with Sithole and Modi (2015) results. For some plants, like: *V. sbterranea*, *C. cajan* and *T. vogelii* proline plays major role in osmotic adjustment, whereas for others it represents only weak part of total active osmolytes, that is the case for tomato Claussen (2005) and *P. lunatus* in this work.

Several authors report changes in expression, accumulation and synthesis of proteins for several water stressed plants species during growth phase (Chen and Tabaeizadeh, 1992; Cheng *et al*., 1993; Sithole and Modi, 2015). Quantitative and qualitative proteins modifications were observed during drought (Riccardi *et al*., 1998). Researchers, by measuring quantity of TSP according to water deficit showed that it can decrease (Hsiao, 1973; Zerrad *et al*., 2008) or increase (Zerrad *et al*., 2008). Proteins synthesized in response to drought are implied in the response of plant to water scarcity (Riccardi *et al*., 1998) and in physiological adaptation to water starvation (Riccardi *et al*., 1998; Bray, 1993; Han and Kermode, 1996); they are called dehydrines (dehydration induced) (Close and Chandler, 1990). Results of this experiment emphasize TSP decrease for *C. cajan, P. lunatus* and *T. vogelii* under water stress. This result doesn't corroborate those of Riccardi *et al*. (1998) on corn, Sithole and Modi (2015) on *Lagenaria siceraria* and can be due to a severe reduction in photosynthesis under water stress. Indeed decrement of photosynthesis reduces among of compounds necessary to proteogenesis and consequently proteins synthesis decreases and can even stop (Havaux *et al*., 1987). In general, water stressed plants always show deficiency in nitrogenize nutriments, leading to an inhibition of proteins synthesis (Javed *et al*., 2013). Reduction of quantity of soluble proteins can also be due to the process of proteolysis under water stress (Munns, 2002), which leads to increase in soluble amino acids (Javed *et al*., 2013). For *V. subterranea*, significant increase in TSP content at T2, was already observed on corn (Riccardi *et al*., 1998) and on wheat (Hamid *et al*., 2010). This increment could be due to expression of stress proteins allowing plant to adapt to unfavorable environmental conditions (Javed *et al*., 2013).

Increase and reduction of TSP under water stress, corroborate results of: Riccardi *et al*. (1998), Ti-da *et al*. (2006) on corn;

Bensen *et al*. (1988) on *Glycine max*. These authors report that drought increases synthesis of some proteins and decreases those of others. During water stress, leaves increase their content of low molecular weight soluble proteins more than that of high molecular weight proteins (Farshadfar *et al*., 2008). Actually it's well known that proteins accumulated under water stress in plants fill up additional energy requirements in response to environmental stress and are useful like antioxidant enzymes. Furthermore they would intervene in stabilization of membrane proteins and osmotic adjustment (Close, 1996; Carpenter and Crowe, 1988), in the protection of cells against dehydration stress (Close and Chandler, 1990) and ensure sequestration of ions under water stress as well as the control of concentration of dissolved substances in cytoplasm. Amino acids (AA), first products of assimilation of nitrogen gas, are essential for proteins and nucleic acids synthesis (Shao *et al*., 2009). Reduction in content of TAA observed in this work has been already reported by Zerrad *et al*. (2008) on durum wheat and would be due to denaturation of proteins sensitive to dehydration following reduction of moisture in the pots of stressed plants. The increase of TAA content corroborates results of Hsu and Kao (2003) on *Oryza sativa*. Their accumulation reduces osmotic potential, thus facilitating entry of water in plant (Iqbal *et al*., 2011). They are used to manufacture proteins and other essential molecules to growth (Ashraf *et al*., 1996). Because of irregularity in progression of accumulation of AA and TSP with increasing water stress level, these two parameters would not be early indicators of tolerance to water stress. The significant increase in TSS content for water stressed plants constitutes an adaptive mechanism to stress (Javed *et al*., 2013). Soluble sugars are osmolytes providing a double function: equilibration of osmotic adjustment and osmoprotectants of proteins under water stress (Ashraf and Harris, 2003; Iqbal *et al*., 2011), thus reducing aggregation of denatured proteins (Ashraf and Harris, 2003). Stressed plants of *P. lunatus* and *V. subterranea* reacted by increase in TSS quantities of their cells in parallel with rise in water stress level. This result is similar to those of several researchers on durum wheat (Mekliche *et al*., 2003); on safflower (Mouellef, 2010; Javed *et al*., 2013). Accumulation of soluble sugars is a means adopted by plants in case of stress, to tolerate environmental constraints (Loretti *et al*., 2001); this accumulation allows maintenance of high cellular integrity (Loretti, 1993).

Soluble sugars (glucose, galactose, saccharose and fructose) are indicators of level of stress, because of their significant increase for a severe water stress, these sugars allow tolerance to various stresses (Zerrad *et al*., 2008). Soluble sugars protect membranes against dehydration under water stress condition and contribute in lowering of the osmotic potential. Accumulated sugars would originate from hydrolysis of polysaccharides under condition where water is scare (Clifford *et al*., 1998), allowing stressed plants to make osmotic adjustment. For *C. cajan* soluble sugars among doesn't vary significantly, but decreases for *T. vogelii* when the level of stress rises; suggesting thus that for these two species, soluble sugars would not have priority in osmotic adjustment under water stress. Total soluble sugars content lessen for *T. vogelii* and *V. subterranea* for T3. Generally under water stress, TSS content can remain constant for severe stress or increase for a moderate stress, because decrease in carbon assimilation, slows down also growth and export of photosynthesis compounds (Chaves and Oliveira, 2004). Under severe water stress soluble sugar content could lessen (Pinheiro *et al*., 2001).

Conclusion

Objective of this work was to analyze early biochemical indicators for tolerance to water stress of four Fabaceae leguminous plants. Results obtained for content of leaves pigments, proline, TAA, TSP and TSS allow concluding that water stress differently modifies metabolism of plants according to genotype. Contents of proline and TSS can be used like early indicators and relevant criteria of tolerance to drought usable in varietal selection and like parameters of improvement of yield in arid areas.

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Conflict of Interest

The authors declare no conflicts of interest.

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