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European Journal of Experimental Biology, 2013, 3(1):487-492



Morpho-physiological responses of common bean leaf to water deficit stress

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ABSTRACT

To investigate the traits and characteristics of leaves of common bean (*Phaseolus vulgaris* L.) genotypes under contrasting moisture regimes, a biennial experiment was conducted as split-plot based on randomized complete block design with four replications. Two irrigation levels (normal and water deficit) and eight genotypes (AND1007, Akhtar, D81083, COS16, KS21486, MCD4011, WA4531-17 and WA4502-1) were arranged in main plots and in sub-plots, respectively. The results indicated that WA4502-1 had the highest leaf area and LAI, and leaf biomass, and had the lowest chlorophyll content in the well watered treatments. Under drought stress, AND1007 showed the highest leaf relative water content (RWC), leaf number per plant, leaf angle, LAI and leaf biomass. In this case, the highest level of chlorophyll index was related to Akhtar genotype. Genotype WA4531-17 had the greatest specific leaf weight (SLW) in both conditions. Water deficit reduced the RWC, leaf wet weight, leaf dry weight, LAI, and plant leaf numbers until 8, 34, 31, 22, and 19 percentages, respectively. By contrast, leaf temperature and leaf angle of all genotypes increased up to 2°C and 24 degrees. In general, AND1007 and COS16 were superior genotypes than the others.

Keywords: Bean, drought, fluorescence, leaf angle, RWC

INTRODUCTION

Availability of water is one of the most important factors, which determine geographical distribution and productivity of plants [1]. At the whole plant level, the effect of stress is usually perceived as a decrease in photosynthesis and growth, and is associated with alteration in carbon and nitrogen metabolism [2]. Plants exposed to drought or water stress have evolved a series of morphological and physiological adaptations, which confer tolerance to these stresses [3]. Changes in leaf anatomical characteristics are known to alter the CO₂ conductance diffusion components to maintain photosynthetic rates despite low water status. Water deficiency limits photosynthesis because of the restriction of CO₂ diffusion from the external environment to the carboxylation site in chloroplast [4, 5]. Severe drought stress can also induce biochemical damage by reducing Calvin Cycle activity [4]. Much breeding for drought resistance in common bean for the warm tropics has until now focused on developing improved sources of drought resistance without regard for other varietal traits [6, 7]. New common bean cultivars have been developed through selection and incorporation of physiological, phenological, morphological and yield traits for drought tolerance [8, 9]. Leaf characteristics play critical roles in determining rates of photosynthesis and

transpiration [10, 11]. Leaves intercept the available amounts of solar energy and assimilate CO₂, hence due to their significance in crop growth and yield, attention to them and their characteristics is very important [12]. Drought accelerates leaf senescence, decrease canopy size, reduce photosynthesis and decrease yields [13]. Water deficit reduces leaf area and number of leaves per plant led to reduction in bean crop growth. Also, chlorophyll content which directly associated with biomass accumulation in bean crop decrease by water deficit [14]. Loss of leaf area, which could result from reduced size of younger leaves and inhibition of the expansion of developing foliage, is also considered an adaptation mechanism to drought [15]. Of course, reduced leaf area causes decrease of canopy temperature and cooling of plant growth environment [16]. Wentworth et al. [17] determined the photosynthetic characteristics of two contrasting varieties of common bean (*Phaseolus vulgaris*) under drought stress. Both varieties showed reduced chlorophyll content on a leaf area basis and a decrease in leaf area and an increase in leaf thickness. The capacity to avoid or minimize photo-inhibitory and photo-oxidative damages is due not only to biochemical processes but also to morpho-structural adaptations [18]. Among the different mechanisms of photo-protection evolved in plants, leaf movement (paraheliotropism) represents an efficient strategy that reducing light interception is able to minimize excitation pressure to reaction centers [19, 20]. Leaf angle, the angle a leaf deviates from horizontal to ground, directly affects the flux of solar energy per unit leaf area, and is thus an important factor in determination of maximum photosynthetic ability of a plant [11, 21]. According to Pastenes et al. [19], in beans (*Phaseolus vulgaris* L.), paraheliotropism seems to be an important character to avoid photo-inhibition. The extent of the leaf movement is increased as the water potential drops, reducing light interception and maintaining a high proportion of open PSII reaction centers. Chlorophyll fluorescence measurement has become a widely used method to study the functioning of the photosynthetic apparatus and is a powerful tool to study the plant's response to environmental stress [22]. The ratio of Fv/Fm provides an estimate of the maximum quantum efficiency of PSII photochemistry. Cornic and Briantais [23] demonstrated that photosynthetic gas exchange rate and Fv/Fm ratio in *Phaseolus vulgaris* were concomitantly decreased by water stress. De Souza et al. [24] studied the effect of water deficit on soybean and concluded that severe drought stress accelerate the leaf senescence by reduction of leaf nitrogen and chlorophyll contents.

Relative water content (RWC) as an integrative indicator of internal plant water status under drought conditions has successfully been used to identify drought-resistant cultivars of common bean [25]. According to Kumar et al. [3], the high yielding bean cultivars displayed a smaller reduction in leaf water content but a larger reduction in leaf water potential than the poor yielder. Stoyanov [26] reported that water stress reduced RWC between 19% and 37% in the first trifoliate leaf. This reduction is higher in sensitive than resistant varieties [27]. Specific leaf weight (SLW) and canopy temperature have been proposed as potential surrogate tools for selecting genotypes with higher WUE in several legumes [15]. O'Neill et al. [28] considered leaf temperature as a potential indicator of plant water stress, since increasing plant water deficit leads to stomatal closure, decreases transpirational cooling and consequently increases leaf temperature.

Since the leaves are more sensitive and plastic to environmental changes than the other organs, and leaf traits also associate with the important leaf functions, evaluation of leaf characteristics can provide a lot of information on the crop growth and some stress tolerance mechanisms. Therefore, the objective of this research was to identify leaf responses of eight common bean genotypes to drought stress.

MATERIALS AND METHODS

Eight common bean genotypes including white group (WA4502-1 and WA4531-17), red group (Akhtar, AND1007 and D81083) and Chitti group (COS16, KS21486 and MCD4011) were investigated under normal irrigation and water deficit in the research farm of Seed and Plant Improvement Institute (SPII), Karaj, Iran. Normal irrigation was performed based on 55-60 mm evaporation from class A pan. Water stress was performed based on 100-110 mm evaporation from class A pan after seedling establishments from emergence of 3rd trifoliate leaf to maturity. Split-plot experiments were performed in randomized complete block design with four replications. The seeds were sown on June 2009 and 2010. Irrigation conditions and genotypes were in the main- and sub-plots, respectively. Seeds of each genotype were sown at 6 rows of 5 m length with plants space of 5 cm, separated by 50 cm. Leaf morphological traits such as leaf number per plant, leaf angle with the horizon, the leaf fresh and dry weight and leaf area were investigated. Number of leaves per plant in each genotype was determined in two stages (pre-flowering and flowering) by counting the leaflets. In flowering stage (R6), several traits were determined including leaf angle from horizon, fresh and dry biomass, LAI, relative water content of leaves (RWC), leaf chlorophyll index, chlorophyll fluorescence and SLW. Leaf chlorophyll index was measured using the chlorophyll meter (MINOLTA,

SPAD-502). To determine chlorophyll fluorescence, the central leaflets of each plant were taken in darkness and then were taken in the light and chlorophyll fluorescence was determined using a Fluorometer (model OS-30, USA). At the two stages of growth (flowering, R6; pod filling period, R8), leaf temperature of plants was determined based on Fischer *et al.* [29].

Data were analyzed based on experimental design model. Means comparison was performed based on Duncan's multiple range test ($P \leq 0.05$). All calculations were performed using SAS (version 9.1) and SPSS (version 16) software. Cluster analysis was performed based on UPGMA method.

RESULTS AND DISCUSSION

Number of leaves and leaf angle

These traits were significantly different in the genotypes and irrigation factor. Water deficit reduced number of leaves per plant until 18% and increased leaf angle from horizon until 24 degree. In normal condition, AND1007 and Akhtar had the highest and lowest leaves per plant at the pre-flowering (vegetative stage) and R6 (reproductive stage), respectively. In stressed plants, the highest and the lowest leaves per plant at the vegetative stage were related to above mentioned genotypes, while at the R6 stage WA4502-1 had the lowest leaves per plant (Table 1). According to many reports [30, 31], low irrigation level reduces total number of leaves per plant. Under drought condition, leaf angle was increased such that AND1007 and D81083 had greater leaf angles (Table 1). Leaf angle directly affects the flux of solar energy per unit leaf area, and is thus an important factor in determining the maximum photosynthetic ability of a plant [19, 32]. Greater leaf angles decrease transpiration and heat damage by decreasing direct exposure to high-intensity light [21]. Our results are in agreement with findings of and Lizana *et al.* [27] that Leaf angle increase with increasing water deficit.

Leaf fresh and dry weight

Water stress significantly decreased leaf fresh and dry weight until 34% and 31%, respectively. The highest and lowest percentage reductions in LFW were related to WA4502-1 and KS21486, respectively. D81083 showed the lowest reduction in LDW. At the flowering stage, the highest LFW and LDW were related to WA4502-1 in well watered treatments, while at stress condition AND1007 had the highest values (Table 1). Barrios *et al.* (2005) also concluded that under water stress, dry weight of leaves located on branches was reduced by 56.3% as compared to 15.0% for the leaves located on the main stem.

Leaf area and LAI

Both leaf area (LA) and leaf area index (LAI) were significantly affected by irrigation levels and genotype. Water deficit reduced these traits until 27%. LAI_{max} reduced 22% in all genotypes. WA4502-1 had the highest levels of LA, LAI at R6 and LAI_{max} in well watered treatments. At the stress condition, AND1007 showed the highest LA and LAI. The greatest reduction in LA was related to WA4502-1 consequently resulted in its LAI reduction (Table 2). Barrios *et al.* [33] reported that leaf area of dry beans reduces when the plants expose to drought stress. Furthermore, Nielsen and Nelson [30] observed significant LAI reductions in black bean (*Phaseolus vulgaris* L) under drought stress condition. Hopkins *et al.* [11] stated that leaf size can increase with increasing air temperature. Also, large leaves have thicker boundary layers of air around their surfaces which insulate and decrease water loss through transpiration.

Table 1- The means of leaf No./plant, leaf angle from Horizon, leaf fresh (LFW) and dry (LDW) weights under normal (N) and water stress (S) conditions

Genotype	Leaf No./Plant (pre-flowering)		Leaf No./Plant (flowering stage)		Leaf angle (Degrees)		LFW (g)		LDW (g)	
	N	S	N	S	N	S	N	S	N	S
	WA4502-1	29.75 ab	21.13 abc	52.50 ab	41.25 b	41.0 b	69.3 abc	16.79 a	8.67 d	5.53 a
WA4531-17	22.88 dc	20.88 bc	34.38 c	27.88 c	31.5 c	57.0 de	15.83 bcd	11.15 b	3.41 d	2.67 b
Akhtar	21.88 d	18.38 c	33.88 c	29.88 c	38.5 b	55.8 e	16.20 abc	11.26 ab	4.13 c	3.37 a
D81083	29.63 ab	23.88 abc	54.75 ab	46.13 ab	40.0 b	73.0 ab	15.69 cd	10.11 c	3.29 d	2.74 b
AND1007	32.00 a	27.00 a	62.63 a	51.13 a	50.5 a	75.8 a	16.02 bc	11.76 a	4.72 b	3.43 a
KS21486	31.75 a	25.25 ab	35.62 c	31.00 c	44.3 ab	65.8 bcd	11.83 e	8.67 d	2.21 e	1.45 d
COS16	28.00 abc	20.63 bc	43.13 bc	28.38 c	42.0 b	61.5 cde	16.39 ab	10.54 c	3.60 d	2.17 c
MCD4011	24.75 bcd	23.00 abc	43.88 bc	39.88 b	40.5 b	66.0 bcd	15.32 d	10.12 c	3.78 cd	2.64 b

Different letters at each column for genotypes indicating significant difference at $p \leq 0.05$.

Leaf chlorophyll content and fluorescence

The effect of irrigation was significant on leaf chlorophyll index. Water deficit reduced leaf chlorophyll index until 7%. This percentage varied among genotypes and WA4502-1 with 4.21% reduction and Akhtar and MCD4011 genotypes with 1.58% and 1.74% reductions had the highest and lowest reductions in leaf chlorophyll (Table 2). According to Mafakheri *et al.* [34] report, the decrease in chlorophyll under drought stress is mainly due to damage to chloroplasts caused by active oxygen species. Leaf fluorescence (Fv/Fm) was significantly influenced by both factors. In normal conditions, there were no significant differences among genotypes. Water deficit intensified leaf fluorescence. Akhtar leaves showed the lowest fluorescence (Table 2). On the other hand, this variety has the highest quantum yield and damage to its photosynthetic system (PSII) is less than other genotypes. Findings of Santos *et al.* [35] revealed that moderate water deficit did not promote photo-inhibition, as both drought-stressed and non-stressed plants presented Fv/Fm higher than 0.725 at the maximum water deficit.

Relative water content

Both factors had a significant effect on leaf relative water content (RWC). Reduction in RWC by water deficit was 8%. In both conditions, Akhtar and AND1007 had the highest RWC. WA4531-17 genotype showed the lowest RWC in stressed treatments. The lowest RWC reduction in stress condition was related to the MCD4011 (Table 3), i.e. the ability to retain water in the leaves of this genotype is higher than others. Khan *et al.* [36] concluded that water deficit stress resulted in a considerable decline in RWC (18%; from 83% in normal condition to 68% in stressed plants).

Table 2- The means of LAI, LAI_{max}, leaf chlorophyll and fluorescence (Fv/Fm) under normal (N) and water stress (S) conditions

Genotype	LAI		LAI _{max}		Chlorophyll (SPAD)		Fv/Fm	
	N	S	N	S	N	S	N	S
WA4502-1	5.11 a	2.76 bc	9.37 a	7.21 a	39.96 b	37.11 b	0.893 a	0.658 bc
WA4531-17	2.81 d	2.20 de	8.12 bc	6.78 bc	41.49 a	37.21 b	0.885 a	0.653 c
Akhtar	3.74 bc	3.07 ab	8.49 bc	6.86 bc	40.56 ab	38.98 a	0.878 ab	0.690 a
D81083	3.07 d	2.46 cd	5.30 d	3.82 e	40.15 ab	37.05 b	0.875 ab	0.655 c
AND1007	4.18 b	3.35 a	8.69 ab	6.93 ab	40.19 ab	37.40 ab	0.845 d	0.653 c
KS21486	1.75 e	1.38 f	4.54 e	3.43 f	40.75 ab	38.55 a	0.865 bc	0.673 abc
COS16	3.01 d	2.07 e	8.99 ab	6.41 c	41.64 a	37.85 ab	0.848 cd	0.653 c
MCD4011	3.28 cd	2.47 cd	6.65 c	5.39 d	40.63 ab	38.89 a	0.865 bc	0.683 ab

Different letters at each column for genotypes indicating significant difference at p≤0.05.

Leaf temperature and specific leaf weight

Factors had not significant effect on leaf temperature. Of course, at R6 stage under water stress there were significant differences among genotypes. In this stage, D81083 and Akhtar had the warmest and coolest leaves, respectively. Leaf temperature at R6 and R8 (pod filling period) stages increased until 2°C by water deficit. According to Khan *et al.* [36], water stress resulted in considerable increases in leaf temperature. Water deficit raised the leaf temperature in all genotypes. Also, the drought tolerant genotypes showed lower stomatal conductance associated with warmer leaves, while in sensitive lines were observed higher conductance and cooler leaves. Specific leaf weight (SLW) significantly influenced by both factors. Decreasing water availability led to a reduction in SLW until 6%. WA4531-17 had higher SLW under water stress than the others (Table 3).

Table 3. The means of RWC, leaf temperature at R6 and R8 stages, and SLW under normal (N) and water stress (S) conditions

Genotype	RWC (%)		Leaf temperature at R6 (°C)		Leaf temperature at R8 (°C)		SLW (g cm ⁻²)	
	N	S	N	S	N	S	N	S
WA4502-1	77.22 abc	71.96 ab	33.45 a	36.33 ab	36.98 b	39.00 ab	4.28 c	4.02 c
WA4531-17	72.84 bc	66.57 c	33.79 a	35.03 bc	36.75 b	38.68 ab	4.95 a	5.02 a
Akhtar	81.36 a	74.03 a	33.23 a	33.44 d	37.50 a	38.63 b	4.42 bc	4.40 bc
D81083	77.07 abc	69.68 abc	33.61 a	36.78 a	36.85 b	39.63 a	4.28 c	4.53 b
AND1007	79.41 ab	74.04 a	34.13 a	35.53 abc	37.48 a	39.13 ab	4.51 bc	4.11 bc
KS21486	72.39 c	67.58 bc	33.80 a	36.54 a	36.50 b	38.38 b	4.70 ab	4.15 bc
COS16	77.41 abc	69.33 abc	33.54 a	34.80 c	37.23 a	38.75 ab	4.95 a	4.23 bc
MCD4011	73.66 bc	71.15 abc	33.15 a	34.48 cd	37.68 a	38.83 ab	4.63 abc	4.21 bc

Different letters at each column for genotypes indicating significant difference at p≤0.05.

R6: flowering; R8: pod filling duration.

Akhtar is one of the varieties that well adapted to the Iran climate. In this variety, we observed the highest values of leaf chlorophyll content and RWC. However, its leaf fluorescence resulted from stress was lower than the others, suggesting its higher photosynthetic efficiency under stress condition. This genotype had also the coolest leaves and the lowest leaves per plant. In contrast, D81083 had warmer leaves and lower pre-flowering number of leaves than others under drought stress. In this condition, KS21486 indicated the lowest amounts of leaf fresh and dry weight, leaf area and LAI that due to its small size, the results were predictable. According to the results, one of the most adaptable genotypes is AND1007 that has greater values of leaves per plant, leaf fresh and dry weight, leaf area, LAI, and RWC than the others. Due to water deficit, WA4502-1 showed the highest reductions in pre-flowering number of leaves per plant (29%), leaf fresh (48%) and dry weight (50%) and leaf area per plant (46%), and the lowest reductions in leaf chlorophyll index (2.7%). The lowest reduction in LAI_{max} (16%) and the greatest reduction in leaf chlorophyll index (10.3%) were related to WA4531-17. Akhtar leaves had the lowest angles from horizon. In contrast, it had the lowest reduction in leaf area (18%) and the coolest leaves at both R6 and R8 stages. The greatest reductions in LAI_{max} (28%) was related to D81083. Also, this genotype indicated the greatest increase in leaf angle from horizon (33 degrees) and the lowest ratio of Fv/Fm. AND1007 had the lowest reductions in leaf fresh weight (26%). The highest percentage reductions in the number of leaves at R8 stage (34%), LAI_{max} (28%) and RWC (10.4%) were related to COS16. MCD4011 having suitable characteristics such as lower reductions in leaf numbers in both vegetative (7%) and reproductive (9%) stages, RWC (3.4%) and less fluctuation in the fluorescence than other genotypes, and suitable plant type and its good grain marketing, is good option for introduction to areas similar to Iran climate conditions.

CONCLUSION

Comparisons among the genotypes revealed that white beans are more drought-susceptible than red and Chitti beans. Intra-grouping evaluations showed that WA45021-1 has a relative advantage compared to the other white bean. In general, AND1007 and COS16 were superior genotypes than the others.

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