

# The effect of drought stress on yield, leaf gaseous exchange and chlorophyll fluorescence of dry beans (*Phaseolus vulgaris* L.)

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## Highlights

- Drought stress across all stress levels resulted in a reduction in dry matter production, leaf area index, number of seeds per plant, number of pods per plant, seed size and finally grain yield.
- The highest WUE was found in the treatment which was irrigated on fortnightly bases from 36 DAP. The results suggest that drought stress towards the end of the growing season may not cause serious harm in grain yield.
- The results suggest that drought stress can be practiced in dry bean production in areas where there is a challenge of irrigation water with consideration of the growth stage of the crop.
- The results of the study indicate that drought stress effects on photosynthetic rate were highly significant.
- Chlorophyll fluorescence was also affected by drought stress.

## ABSTRACT

Global food production relies on irrigation, especially in low rainfall areas such as South Africa. The study was conducted to determine the effect of drought stress on growth, yield, leaf gaseous exchange and chlorophyll fluorescence parameters of dry bean under field conditions and the after effects of drought stress upon lifting drought stress. A rain shelter field trial was conducted at the Hatfield Experimental Farm of the University of Pretoria, Pretoria, South Africa. Dry bean cultivar DBS 360 was subjected to five levels of moisture stress arranged in a randomized complete block design with six replications. The plants were exposed to the following drought stress levels: the control: Irrigated to field capacity (S1), Withholding irrigation from 36 days after planting (DAP) for 24 days (S2), Withholding irrigation from 49 DAP for 24 days (S3), Withholding irrigation from 73 DAP to the end of the growing season (S4) and irrigated to field capacity on a fortnightly bases for the rest of

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the season from 36 DAP to the end of the growing season (S5). The results revealed that drought stress reduced dry matter production, leaf area index, number of pods per plant, number of seeds per plant, hundred seed weight and grain yield. Treatments S1, S4 and S5 produced statistically similar grain yield. Drought stress towards the end of the growing season may not cause serious harm in grain yield. Drought stress resulted in a reduction in photosynthetic rate, intercellular carbon dioxide concentration, stomatal conductance and transpiration. Chlorophyll fluorescence was also affected by drought stress. The highest WUE was found in the treatment which was irrigated on fortnightly bases from 36 DAP. This indicates that with appropriate irrigation it is possible to save water without a great yield loss in dry bean.

Keywords: Moisture stress, photosynthesis, water stress, water use efficiency

## 1. INTRODUCTION

Dry bean is an important protein grain crop in South Africa grown mostly for human consumption. The average dry bean production in South Africa is about 65 thousand tons per annum while the average annual consumption is 129 thousand tons. This implies that the local market is only able to supply 51% of the local consumption requirements while the balance is met through imports (DAAF, 2012).

Dry bean require a minimum of 400-500mm of rainfall in the growing season (Liebenberg *et al.*, 2002). In high rainfall regions of South Africa dry bean is produced under dry land and in low rainfall regions of South Africa supplemental irrigation is necessary. Dry bean has been reported to be very sensitive to drought and on the other side water is scarce in South Africa (Liebenberg *et al.*, 2002). The impact of drought stress is determined by the severity of stress and the ability of plants to adapt to this stress (Rosales *et al.*, 2012). Drought stress has a considerable impact on dry bean growth and seed yield although the ranges of reductions are highly variable due to differences in the timing and intensity of the stress imposed and genotype used (Emam *et al.*, 2010).

Most dry bean production in the developing world occurs under conditions of recurring drought stress (Graham and Ranali, 1997). Drought is considered to be one of the main environmental factors that strongly limit growth and yield of plants worldwide (Chaves *et al.* 2003). Drought stress occurs when available water in the soil is reduced and transpiration continue to loose water without additional water by rain or irrigation. Drought stress is

characterized by the reduction of water content, diminished leaf water potential and turgor loss, closure of stomata and decrease in cell enlargement and growth (Jaleel et al., 2009). Drought stress is a problem because it limits plant production resulting in lower yields which leads to reduced food prices and high food prices. Water use efficiency (WUE) is one trait important for plant drought response (Edwards et al., 2012). Water use efficiency is defined as the ratio of dry matter production to water use (Hubick et al., 1986).

The introduction of drought stress reduced leaf area, chlorophyll content, dry matter and yield in two common bean cultivars (D81083 and Sayyard) in Iran (Emam et al. 2010) . Post flowering drought stress resulted in a reduction in seed yield, pods per plant and 100 seed weight in small red seeded common bean (Rezene et al. 2013). Drought stress reduced photosynthetic rate due to stomatal conductance, increased  $F_0$  accompanied by decrease in  $F_m$  and no change in  $F_v/F_m$  in the study conducted on beans (Zlatev and Yordanov 2004).

Therefore, the objective of the study was to determine whether the timing of the drought stress in plant development affects yield, leaf gaseous exchange and chlorophyll fluorescence parameters and also to check the possibility of saving water without losing biomass.

## **2. Materials and methods**

### *2.1 Experimental site and treatments*

A field experiment was conducted inside a rain shelter at the Hatfield Experimental Farm of the University of Pretoria, Pretoria, South Africa (latitude 25°45'S, longitude 28°16'E and an altitude of 1327 m.a.s.l). The experiment was implemented in March 2013. Drought stress was applied through subjecting the dry bean (*Phaseolus vulgaris* L) cultivar DBS 360 to five levels of moisture stress arranged in a randomized complete block design with six replications. Drought stress treatments were as follows: the control: Irrigated to field capacity on a weekly bases throughout the growing season (S1), Irrigated to field capacity on a weekly bases and withholding irrigation from 36 days after planting (DAP) for 24 days, then irrigated to field capacity to the end of the growing season(S2), Irrigated to field capacity on a weekly bases and withholding irrigation from 49 DAP for 24 days, then irrigated to field capacity to the end of the growing season(S3), Irrigated to field capacity on a weekly bases and withholding irrigation from 73 DAP to the end of the growing season (S4) and Irrigated to field capacity on a weekly bases and Irrigated to field capacity on a fortnightly bases from 36

DAP (S5). The plots were 2 x 2.5m<sup>2</sup>, and an intra-row spacing of 30 cm and inter-row spacing of 7.5 cm were used. The soil was clay loamy. Top dressing was done 28 DAP using lime ammonium nitrate (LAN-28%N) at the rate of 30kg/ha. Weeding was done by hand.

## *2.2 Weather data*

Weather data was collected from an automated weather data station close to the experimental site. Daily solar radiation, maximum and minimum relative humidity, maximum and minimum temperatures and wind speed were collected. Soil moisture was monitored using a 503DR CPN hydro probe neutron water meter (Campbell Pacific Nuclear, California). Readings were taken twice a week, at 0.2m to a depth of 1.0m, from access tubes installed in the middle of each plot and positioned between the rows. A drip irrigation system was used for irrigating the trial.

## *2.3 Agronomic data*

The effect of drought stress on dry bean growth was monitored through harvesting three plants per plot at 48 DAP, 64 DAP and 92 DAP. The samples were divided into leaves, stem and pods. The leaf area was measured using a LI 3100 belt-driven leaf area meter (Li Cor, Lincoln, Nebraska, USA). Thereafter the samples were oven-dried for 72 hours at 65°C to determine dry matter yield (DM). The total above ground dry matter was determined by adding together the dry mass of the leaves, stems and pods. Grain yield and yield components were determined by harvesting 1 m<sup>2</sup> at 134 DAP, which were the time the trial was terminated. The plants were harvested by hand. The number of plants per plot, number of seed per plant and number of pods per plant were counted and hundred seed mass was measured. The moisture content of the seed was determined by using a multi grain moisture meter (Dickey John, Auburn, Illinois, USA). Shelled seed mass was measured to determine the shelling %. Yield was expressed at 10% seed moisture basis.

## *2.4 Leaf gas exchange parameters*

The following parameters were measured three times during the growing season: Net photosynthesis, transpiration, stomatal conductance and intercellular carbon dioxide concentration using a portable gas exchange measuring system (Li 6400, Li-Cor, USA). The leaf temperature was 20±28 °C, PPFD was 1000 μmolm<sup>-2</sup>s<sup>-1</sup>, relative humidity was 70% and the ambient CO<sub>2</sub> concentration was 400 μmolmol<sup>-1</sup>. The data was collected from the fully

expanded matured 3<sup>rd</sup> leaf on a sunny day between ten and twelve. The measurements were done at 63, 100 and 105 DAP. Chlorophyll content was measured using a portable chlorophyll content meter (CCM-200, Opti- Sciences, USA). The chlorophyll content measurements were done at 48, 53, 61, 77, 80, 89 and 104 DAP.

### *2.5 Chlorophyll fluorescence measurements*

Chlorophyll fluorescence was measured using a 6400-40 leaf chamber fluorometer. The measurements were taken from the top most expanded leaf. Minimal fluorescence ( $F_0$ ) was measured for 60 minute dark-adapted leaves and maximal fluorescence ( $F_m$ ) was measured after a 0.8s saturation light pulse for the same leaves. Maximal variable fluorescence ( $F_v = F_m - F_0$ ) and the photochemical efficiency of PSII ( $F_v / F_m$ ) for dark adapted leaves were calculated. Photochemical (qP) and non-photochemical (qN) quenching parameters were calculated according to Schreiber *et al.* (1986), using the nomenclature of Van Kooten and Snel (1990).

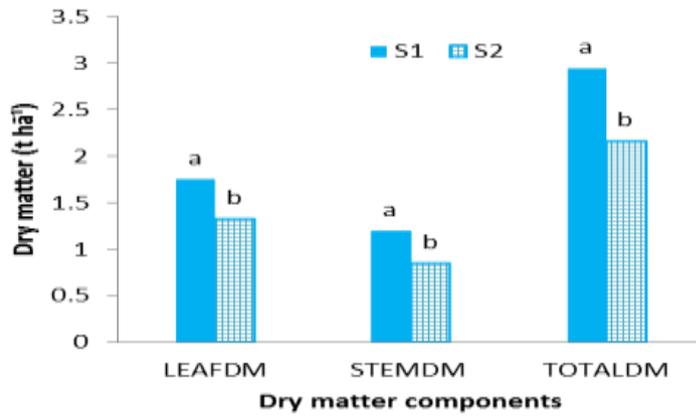
### *2.6 Statistical analysis*

The analysis of variance was performed using General linear models of the Statistical Analysis System software (SAS, 2010). Means were compared using the Tukey's least of significance differences (LSD) test at 5% probability level.

## **3. Results and discussion**

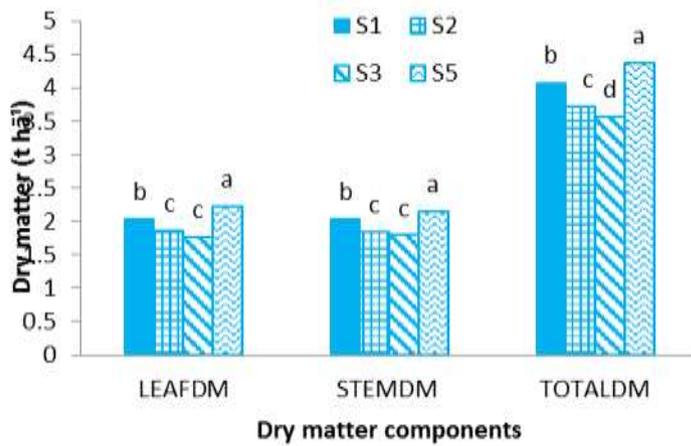
### *3.1 Dry matter*

The dry matter partitioning was highly affected by drought stress introduced at 36 DAP at  $P \leq 0.05$  (Figure 1). The treatment S2 resulted in a reduction of dry matter of leaves, stem and the total of 24, 29 and 26 % respectively compared to S1. The results also revealed that drought stress highly affected dry matter partitioning at 64 DAP (Figure 2). The treatment S5 resulted in the highest dry matter in terms of leaves, stem and the total. The results revealed that at 64 DAP S1, S2 and S3 resulted in a 6, 15 and 18 % reduction in total dry matter partitioning respectively compared to S5. Both S2 and S3 resulted in a significant lower dry matter yield for all the components as compared to S1 and S5. The results further revealed



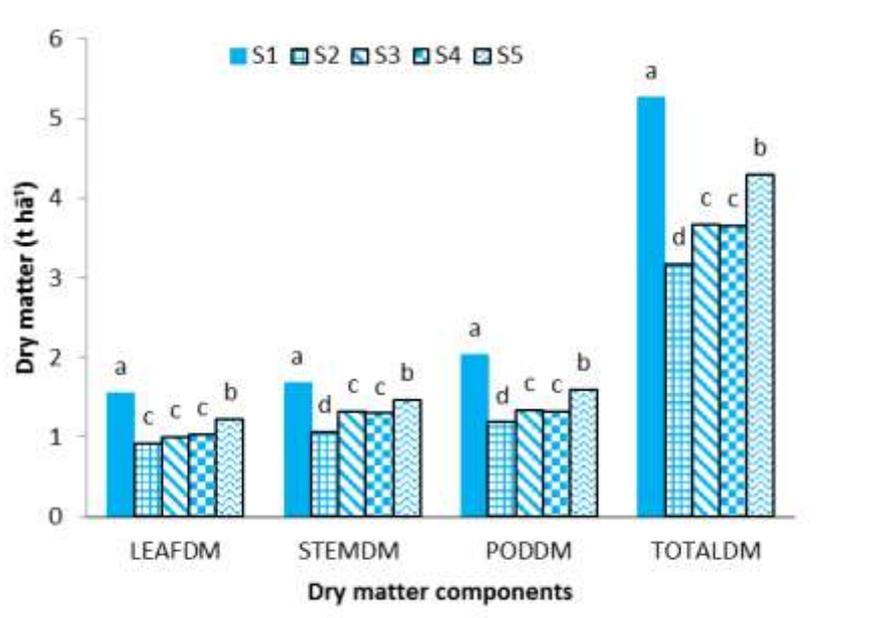
**Figure 1** Effect of drought stress on dry matter production of dry bean at 48 DAP

Note: Means of bars of the same plant part with the same letter are not significantly different, DAP=Days after planting



**Figure 2** Effect of drought stress on dry matter production of dry bean at 64 DAP

Note: Means of bars of the same plant part with the same letter are not significantly different, DAP=Days after planting



**Figure 3** Effect of drought stress on dry matter production of dry bean at 92 DAP

*Note: Means of bars of the same plant part with the same letter are not significantly different, DAP=Days after planting*

that at 92 DAP dry matter partitioning was still affected by drought stress (Figure 3). The highest dry matter in terms of leaves, stem, pods and total was found in S1 (the well watered or control). The lowest dry matter for each of the components was found in S2. Applying water stress later in the season (S3 and S4) also resulted in a significant reduction in the different components as compared to S1 and S5. However, apart from the leaf dry matter yield, it did better than when water stress was applied early in the season (S2). The highest dry matter of leaves and stems was found at 64 DAP and the lowest was found at 95 DAP.

### 3.2 Leaf area index

The results revealed that water stress resulted in a reduction in leaf area index (Table 1). At 48 DAP the leaf area index of S1 treated plants were significantly higher than that of S2 ( $P \leq 0.05$ ). At 64 DAP treatment S1 resulted in the highest LAI compared to the other treatments  $P \leq 0.05$ . The treatments S2, S3 and S5 resulted in a 21, 49 and 20 % reduction of LAI respectively, as compared to S1. At 92 DAP S1 resulted in the highest LAI with S2, S3, S4, and S5 resulting in 66, 32, 14 and 38% reduction respectively ( $P \leq 0.05$ ).

**Table 1** Effect of drought stress on dry bean leaf area index

Stress level	48 DAP	64 DAP	92 DAP
S1	2.23a	3.13a	1.052a
S2	1.96b	2.46c	0.361d
S3		1.60d	0.712c
S4			0.907b
S5		2.68b	0.654c
Cv %	3.85	5.05	16.29
LSD	0.119*	0.153**	0.144**

*Note: Means for values in a column with the same letter are not significantly different, DAP=Days after planting, \*: significant at  $p \leq 0.05$ , \*\*: significant at  $p \leq 0.001$*

### 3.3 Yield and Yield components

In comparison to the control (S1), the number of pods per plant was significantly reduced by drought stress ( $P \leq 0.01$ ) (Table 2). Although S5 resulted in significant less pods than the control, it did lead to a significantly higher number of pods than when plants were stressed in the late reproductive stages (S3 and S4). The number of pods for the S5 plants did, however, not differ significantly from those plants experiencing stress in the early vegetative stage (S2), nor were the differences significant amongst S2, S3 and S4 treated plants. The number of seeds per plant was significantly ( $P \leq 0.05$ ) influenced by drought stress (Table 2). The results revealed that S1 and S5 treated plants had similar number of seeds per plant and that the number at S5 was similar to S3 and S4 (Table 2). The lowest number of seeds was found at S2. The introduction of drought stress at 36, 49, 73 DAPS and irrigating once in two weeks resulted in a reduction of 22, 16, 18 and 10 % respectively compared to S1. The results revealed that the largest seeds were produced by S1 (Table 2) and the smallest was produced by S2, which is similar to S3 and S3 is similar to S4, which is also similar to S5. The reduction in the hundred seed weight from S1 to S2 was 19.74 %. This suggests that introducing drought stress at 36 DAP can results in a serious reduction of seed size. The non-significant effect of drought stress for S5 might have resulted from the fact that stress was not so heavy to can disrupt the translocation process of the stresses plants. There was no effect of drought stress on the shelling percentage.

The effect of moisture stress on grain yield was highly significant at ( $P \leq 0.05$ ) (Table 2). All the yield parameters were reduced by drought stress which results to the reduction in grain

yield. There was no significant loss in irrigating the crop once every two weeks (S5) or by introducing water stress late in the reproductive cycle (S4) as compared to the control (S1). The results therefore suggest that stress level S5 and S4 can be adopted without compromising grain yield. The treatment S2 and S3 were significantly affected by moisture stress resulting in the 23-42% reduction in grain yield as compared to S1.

**Table 2** Effect of drought stress on dry bean yield and yield components of dry beans collected at final harvest (134 DAP)

Stress level	Yield (t ha <sup>-1</sup> )	Pods plant <sup>-1</sup>	Seeds plant <sup>-1</sup>	Hundred seeds weight	Shelling %	Water use (mm)	WUE (kg ha <sup>-1</sup> mm <sup>-1</sup> )
S1	3.20a	10.45a	36.43a	39.03a	78	420.65a	7.61b
S2	2.48b	8.63bc	28.31c	31.57d	77	275.95d	9.00b
S3	1.84c	8.35c	30.58bc	32.81cd	64	382.48b	4.83c
S4	2.89ab	8.41c	29.91bc	35.21bc	79	330.36c	8.73b
S5	3.04a	9.43b	32.75ab	37.43ab	77	251.38e	12.11a
Cv%	14.88	8.37	9.82	7.34	16.94	3.69	14.87
LSD	0.48**	0.913**	3.73*	3.11*	ns	14.77***	1.516***

*Note: Means for values in a column with the same letter are not significantly different, DAP: Days after planting, \*: significant at  $p \leq 0.05$ , \*\*: significant at  $p \leq 0.01$ , \*\*\*: significant at  $p \leq 0.001$ .*

### 3.4 Water use efficiency

The effect of drought stress on water use was highly significant at  $P \leq 0.05$  (Table 2). The results revealed that the highest amount of water (420.65 mm) was used by treatment S1 and the lowest amount of 251.38 mm by S5. The results further indicated that the effect of drought stress on WUE was highly significant at  $P \leq 0.05$ . The highest WUE was obtained by S5 (12.11 kg ha<sup>-1</sup>mm<sup>-1</sup>) followed by S2 (8.98 kg ha<sup>-1</sup>mm<sup>-1</sup>), S4 (8.74 kg ha<sup>-1</sup>mm<sup>-1</sup>), S1 (7.61 kg ha<sup>-1</sup>mm<sup>-1</sup>) and finally S3 (4.81 kg ha<sup>-1</sup>mm<sup>-1</sup>) (Table 2). The treatment S3 resulted in significantly the lowest WUE.

### 3.5 Chlorophyll content

The effect of drought stress on chlorophyll content was significantly high across all the stressed treatments. S2 was the most affected treatment in all days except at 77 and 104 DAP, resulting in between 11 and 39% reduction in chlorophyll content. The results indicated that the maximum chlorophyll content was found at 80 DAP from there it started declining. The results also suggest that treatment S2 fail to recover after re-watering (Table 3) on day 61 DAP.

**Table 3** Effect of drought stress on chlorophyll content of dry beans

Treatment	48 DAP	53 DAP	61 DAP	77 DAP	80 DAP	89 DAP	104 DAP
S1	12.02a	11.00a	17.22a	20.66a	24.29a	23.21a	20.34a
S2	10.51b	9.81b	14.19c	15.17c	19.46c	14.13c	13.58cd
S3	-	-	15.90b	14.42d	20.11bc	15.02c	13.25d
S4	-	-	-	-	-	17.41b	13.94c
S5	-	-	15.68b	16.96b	20.67b	18.33b	15.17b
Cv %	2.11	3.77	3.91	3.52	4.29	5.73	3.41
LSD	0.35**	0.69*	0.98**	0.72**	1.11**	1.21**	0.62**

*Note: Means in a column with the same letter are not significantly different, DAP: Days after planting, Cv: coefficient of variation, \*: significant at  $p \leq 0.01$ , \*\*: significant at  $p \leq 0.001$*

### 3.6 Photosynthesis ( $P_n$ )

The introduction of drought stress had a significant effect on photosynthesis at all three measurement days ( $P \leq 0.01$ ) (Table 4). The highest photosynthetic rates were found in S1 (63 DAP) and the lowest in S3 and S4 (both at 100 and 105 DAP). These results suggest that drought stress during any growth stage of dry bean can results in serious reduction of photosynthetic rates. The reduction can be as high as 45% with treatment S3 being the most affected.

**Table 4** Effect of drought stress on dry bean photosynthesis rate, intercellular carbon dioxide concentration, stomatal conductance and transpiration ( $\mu\text{molm}^{-2}\text{s}^{-1}$ )

Treatment	Photosynthesis			Intercellular carbon dioxide concentration			Stomatal conductance			Transpiration		
	63 DAP	100 DAP	105 DAP	63 DAP	100 DAP	105 DAP	63 DAP	100 DAP	105 DAP	63 DAP	100 DAP	105 DAP
S1	22.92a	9.89a	12.62a	286a	312c	249b	0.55a	0.32ab	0.18a	5.87a	3.33a	2.97ab
S2	15.40d	7.14bc	-	259b	339ab	-	0.29c	0.36a	-	3.86c	2.87a	-
S3	17.44c	5.36d	-	288a	355a	-	0.39b	0.36a	-	4.70b	3.01a	-
S4	-	6.93cd	8.88c	-	331bc	259ab	-	0.32ab	0.13b	-	2.32b	2.65b
S5	21.26b	8.68ab	10.50b	282a	318bc	265ab	0.47ab	0.29b	0.16a	5.21b	3.05a	3.21a
Cv%	6.50	18.6	4.01	4.09	5.61	3.22	18.84	11.00	15.40	13.00	13.93	10.04
LSD	1.54**	1.70*	0.55**	14.05**	22.38**	10.70*	0.09**	0.04*	0.03*	0.78**	0.49**	0.38*

Note: Means in a column with the same letter are not significantly different, DAP: Days after planting, Cv: coefficient of variation, \*: significant at  $p \leq 0.01$ , \*\*: significant at 0.001

### 3.7 Intercellular carbon dioxide concentration ( $C_i$ )

The introduction of drought stress had a significant effect on intercellular carbon dioxide concentration ( $C_i$ ) ( $P \leq 0.01$ ) (Table 4). The results indicated that at 63 DAP drought stress reduced  $C_i$  with S2 resulting in the lowest of  $259.31 \mu\text{mol mol}^{-1}$ . At 100 DAP S3 resulted in the highest  $C_i$  of  $355.51 \mu\text{mol mol}^{-1}$  and the lowest at S1. The results further indicates that at 100 DAP S3 and S2 had statistically similar  $C_i$ . At 105 DAP  $C_i$  was increased by drought stress where S1 ( $249.67 \mu\text{mol mol}^{-1}$ ) resulted in the lowest concentration. The results revealed that that severe drought stress increases  $C_i$  and mild drought stress reduces it.

### 3.8 Stomatal conductance ( $g_s$ )

The stomatal conductance at 63, 100 and 105 DAP ( $P \leq 0.05$ ), was significantly affected by drought stress (Table 4). The results indicated that  $g_s$  was reduced by drought stress with S2 resulting in  $0.287 \text{ mmol m}^{-2}\text{s}^{-1}$ . This was a 48 % reduction as compared to S1. At 100 DAP S3 and S2 had the highest value of  $g_s$  ( $0.362 \text{ mmol m}^{-2}\text{s}^{-1}$ ) which was not significantly different from S1 and S4. The treatment S5 had the lowest  $g_s$  of  $0.293 \text{ mmol m}^{-2}\text{s}^{-1}$  at 100 DAP indicating that the plants in S5 were the most drought stressed at this time. The highest  $g_s$  was observed when the plant was still small and reduced as the plant grows. This is in agreement with previous results observed in mung bean (Uprety and Bhatia, 1989). At 63 and 105 DAP there was a very strong relationship ( $r^2 = 0.956$ ,  $r^2 = 0.940$ ) between photosynthetic rates and stomatal conductance, while at 100 DAP it was weak ( $r^2 = 0.480$ ).

### 3.9 Transpiration

The results revealed that at 63 DAP drought stress reduced transpiration rate ( $P \leq 0.001$ ) by 34% for S2 (Table 4). The treatment S1 resulted in the highest transpiration rate. The treatment S3 and S5 were statistically the same. At 100 DAP drought stress reduced transpiration rates ( $P \leq 0.01$ ) by 30% at S4. The treatments S1, S2, S3 and S5 were statistically the same. At 105 DAP the transpiration of S5 was not significantly different to S1 but significantly different from S4. At 63 DAP the stomatal closure was the most prominent determinant for the increased transpiration efficiency ( $r^2 = 0.999$ ). The positive correlation between transpiration and stomatal conductance suggests that the reduction of transpiration at S2 was due to stomatal closure. At 100 and 105 DAP there were weak relationship between

transpiration and stomatal conductance with  $r^2=0.007$  and  $r^2=0.481$  respectively. The results also revealed that at 63 DAP there was a strong correlation between transpiration and photosynthesis ( $r^2=0.951$ ). At 100 and 105 DAP there were weak relationship between transpiration and photosynthesis with  $r^2=0.256$  and  $r^2=0.247$  respectively.

### 3.10 Minimal chlorophyll fluorescence ( $F_0$ )

The effect of drought stress on  $F_0$  was significant during 52, 93 and 100 DAP ( $P \leq 0.01$ ) (Table 5). The results revealed that drought stress increased  $F_0$  at all data collection dates. At 52 DAP S3 resulted in a 13% increase in  $F_0$  and S2 with 4% increase compared to S1. The treatment S3 resulted in an increased  $F_0$  of 5.7% at 93 DAP. At 100 DAP S4 resulted in a 13% increase in  $F_0$ .

### 3.11 Maximal chlorophyll fluorescence ( $F_m$ )

The effect of drought stress was significant at 52 DAP, 93 DAP and 100 DAP ( $P \leq 0.05$ ) (Table 5). At 52 DAP S5 and S2 resulted in a 4.3 and 27% increase in  $F_m$  respectively as compared to the control, but S3 resulted in a 25% reduction. At 93 DAP S3 resulted in a 29 % reduction in  $F_m$  while S1, S2, S4 and S5 were statistically similar. At 100 DAP drought stress resulted in an 11, 28, 31 and 33 % reduction at S4, S2, S3 and S5 respectively. At both dates S3 resulted in a serious reduction of  $F_m$ . Throughout all the data collection dates S3 fail to recover from water stress.

### 3.12 Maximum quantum efficiency of PSII photochemistry ( $F_v/F_m$ )

This parameter is widely considered to be a sensitive indication of plant photosynthetic performance (Kalaji & Guo, 2008). Drought stress might result in a decrease in  $F_v/F_m$ , but other factors might be involved also. For example the lowest  $F_v/F_m$  value after 52 DAP is found in S3, which only just started the drought stress period three days earlier and therefore most likely was not water stressed at 52 DAP. Plants in S2 and S5 were not affected at all (no decrease in  $F_v/F_m$ ) even though they had been drought stressed for 16 days at 52 DAP. .

**Table 5** Effect of drought stress on minimal chlorophyll fluorescence ( $F_0$ ), maximal chlorophyll fluorescence ( $F_m$ ) and maximum quantum efficiency of PSII photochemistry ( $F_v/F_m$ ) of dry bean

Treatment	$F_0$			$F_m$			$F_v/F_m$		
	52 DAP	93 DAP	100 DAP	52 DAP	93 DAP	100 DAP	52 DAP	93 DAP	100 DAP
S1	157.48c	164.15b	139.74d	516.87c	545.38a	537.64a	0.69a	0.69a	0.73a
S2	164.17b	164.17b	145.20c	539.27b	539.27a	386.80c	0.69a	0.69a	0.62bc
S3	177.77a	173.55a	152.68b	386.18d	496.30a	367.46d	0.53b	0.51c	0.58c
S4	-	160.53b	158.69a	-	388.19b	474.88b	-	0.63b	0.66b
S5	159.29c	163.42b	141.64cd	636.52a	521.11a	355.39e	0.72a	0.64b	0.60c
Cv %	1.44	2.68	1.76	1.77	10.70	0.51	7.08	2.47	5.93
LSD	3.81**	6.82*	4.01**	14.93**	82.12**	3.33**	0.074**	0.02**	0.06**

Note: Means in a column with the same letter are not significantly different, DAP = Days after planting, Cv= coefficient of variation, \*\*: significant at  $p=0.01$ , \*: significant at  $p\leq 0.05$

The results revealed that drought stress resulted in a reduction in Fv/Fm ratio during 93 and 100 DAPs (Figure 10). Liu et al. (2012) also observed a decline in Fv/Fm ratio in drought stressed plants of two maize cultivars.

### 3.13 Coefficient of photochemical quenching (qP)

Coefficient of photochemical quenching is an indication of the proportion of open PSII reaction centers, and translates light quantum energy into chemical energy process, which reflects the photosynthetic efficiency and the light use situation of plant (Liu *et al.*, 2012). At 93 DAP there was no significant difference among treatments. At 100 DAP the effects of drought stress are significant with S3 resulting in the lowest which was not significantly different to S2 and S4. The results revealed that at 100 DAP there was no significant difference between S1 and S4 and S5 (Table 6), S4 was still going through drought stress.

**Table 6** Effect of drought stress on coefficient of photochemical quenching (qP) and coefficient of non-photochemical quenching (qN)

Treatment	qP		qN	
	93 DAP	100DAP	93 DAP	100DAP
S1	0.995a	1.014ab	0.235c	3.795a
S2	0.994a	0.990bc	0.303b	2.402cd
S3	0.956a	0.985c	0.379a	2.209d
S4	0.993a	1.007abc	0.349a	3.438ab
S5		1.020a		2.870bc
Cv	2.46	1.33	6.42	14.51
LSD	ns	0.02*	0.032**	0.658*

Note: Means for values in a column with the same letter are not significantly different, DAP=Days after planting, \*: significant at  $p \leq 0.01$ , \*\*: significant at  $p \leq 0.001$

### 3.14 Coefficient of non-photochemical quenching (qN)

The effect of drought stress on qN was significant ( $P \leq 0.05$ ) (Table 6). At 93 DAP S3 resulted in the highest qN followed by S4 which was not statistically different from each

other. S3 resulted in a 48% increase in qN compared to S1. At 100 DAP S1 and S4 resulted in the highest and also statistically similar qN values. The increase in qN might have been caused by the large proportion of absorbed light energy not being used by plants in the photosynthesis process.

#### **4. Discussion**

The introduction of drought stress resulted in the significant reduction in dry matter production on all treatment except S5. The reduction in dry matter production suggests that a decline in photosynthesis resulted in the reduction in leaf development and expansion leading to reduced light interception and smaller plants. The reduction of dry matter production at podding stage has been found to be associated with the translocation of photo-assimilates to pods (Fageria and Santos, 2008). Previous reports indicated the reduction of dry matter production due to drought stress in dry bean (Emam et al. 2012) and soybean (Ghassemmi-Golezani and Lofti 2012).

The reduction in leaf area index might have resulted from drought stress inhibiting the development of new leaves and leaf senescence. The reduction in leaf area index could have been the result of reduced leaf size through the decrease in expansion of individual leaves (Akyeampong, 1986) and the number of leaves through the cessation of development of new leaves (Acosta- Gallegos, 1988) and premature senescence. The acceleration of leaf senescence has been previously associated with drought stress in soybean (Brevedan and Egli, 2003) and in dry beans (Emam et al., 2010).

The reduction of number of pods in this experiment might have resulted from flower senescence and flower abortion due to drought stress. Previous reports indicated that drought stress resulted in the decline of photosynthesis leading to senescence of flowers, preventing flower development and pod filling leading to pod abortion finally reducing the number of pods per plant in chickpea (Fang et al. 2010) and common bean (Emam et al. 2012; Rezene et al. 2013).

The number of seed per plant for treatment S2, S3 and S4 was significantly affected by drought stress. The reduction of number of seeds might have been caused by flower senescence and flower abortion. Previous reports indicated that drought stress resulted in the reduction of seeds per plant in dry beans (Singh 1995) and white bean (Habibi, 2011).When

moisture stress was introduced during effective flowering stage the reduction is much greater as compared to the well irrigated treatment in dry beans (Miller and Burke, 1983; Rezene et al., 2013) in chickpea (Fang et al., 2010). The number of seeds for S5 was not significantly affected by drought stress and it might be due to the fact that the available water in the soil maintained the crop until the next irrigation. Previous reports confirmed that partial root drying improved fruit quality in grape tree (Dry and Loveys, 1999).

The reduction in hundred seed weight suggests that drought stress accelerated maturity and results in the development of small seeds. Previous reports has reported the reduction of 100 seed weight in field beans (McEwen et al., 1981) and dry bean (Miller and Burke, 1983; Singh, 1995; Gohari, 2013). On the contrary, drought stress resulted in non-significant effect on 100 seed weight in dry bean (Acosta-Gallegos and Shibatha, 1989; Boutraa and Sanders, 2001).

The reduction in grain yield (S2 and S3) is resulting from the reduction of number of pods per plant, number of seeds per plant and hundred seed weight. Previous results indicated the production of lowest grain yield resulting from treatments stressed during flowering and grain filling stages in soybean (Maleki et al., 2013). Several previous studies also reported a reduction in grain yield due to drought stress in soybean, (Brevedan and Egli 2003; Ghassemmi-Golezani and Lofti, 2012), pinto bean (Ghassemmi-Golezani et al., 2010; and in legumes (Faroog et al., 2016). This reduction might be resulting from the fact that beans responds to drought stress by shedding off leaves, flowers and young pods (Adams et al., 1985). The reduction of grain yield due to moisture stress is variable due to differences in the timing and intensity of stress imposed and the genotype used (Frahm et al., 2004). The non-significant effect of drought stress on grain yield of S5 is resulting from the fact that there was no significant effect of drought on number of seeds per plant and hundred seeds weight for S5. For S4 the results suggests that drought stress introduced late in the growing season may not cause a significant loss in grain yield.

The highest water use efficiency by S5 might be due to the production of substantial yield with minimum water. Previous results reported the highest water use efficiency when wheat was irrigated after 21 days (Sarkar et al., 1987). The lowest WUE in S3 was due to the lowest grain yield produced by this treatment. Previous results reported higher WUE in drought stressed plant compared to well watered plants in dry beans (De costa and Liyanage, 1997; Gohari, 2013; Khonok 2013). Contrasting results were reported indicating

that the highest WUE was found in irrigated treatments than non- irrigated tomato plants (Begum et al., 2001).

The reduction in chlorophyll content might have resulted from leaves being damaged and turning yellowish due to drought stress. A decrease in chlorophyll content due to drought stress has been reported in wheat (Talebi, 2011), pea (Inaki-Iturbe *et al.*, 1998), maize (Mohammadkhani and Heidari, 2007), chickpea (Mafakheri *et al.*, 2010), soybean (Makbul et al., 2011) and rice (Chutia & Borah, 2012). The damage to leaf pigments as a result of water deficit has been reported in acacia (Montagu and Woo, 1999). The decrease in chlorophyll content is resulting from the damage to the chloroplasts caused by active oxygen species (Smirnoff, 1995). Drought stress leads to the production of reactive oxygen species (ROS) such as O<sub>2</sub><sup>-</sup> and H<sub>2</sub>O<sub>2</sub>, which lead to chlorophyll destruction (Mirnoff, 1993; Foyer *et al.*, 1994).

The reduction in photosynthetic rates resulted from stomatal and non-stomatal factors. The reduction of photosynthesis due to drought stress has been reported in faba bean (Girma and Haile, 2014), in grain legumes (Faroog et al. 2016) and dry bean (Lanna et al., 2016). During drought stress water deficit inside the plant tissue develops, leading to a significant inhibition of photosynthesis. A reduction in bean photosynthetic rates due to stomatal closure has been reported (Sharkey and Seemann, 1989). Tang *et al.* (2002) argued that a combination of stomatal and non-stomatal effects on photosynthesis exists, depending on the extent of drought stress (Yu *et al.* 2009). Tezera *et al.* (1999) concluded that water stress inhibits photosynthesis through diminished ribulose-1, 5-bisphosphate (RuBP) supply caused by low ATP synthesis. Considering the biochemical reactions, water deficit can also increase the oxygenase activity of the RuBP carboxylase/oxygenase (Rubisco), reducing carboxylation efficiency. Therefore, decreases in the rate of photosynthesis in drought-stressed plants can be caused by stomatal closure (*i.e.* reduction of CO<sub>2</sub> availability) and/or impairments in photochemical (*i.e.* decrease in NADPH and ATP supply) and/or biochemical (*i.e.* reduced RuBP regeneration and carboxylation efficiency) reactions (Tezera et al. 1999).

The results revealed that mild stress reduced C<sub>i</sub> and severe stress increased it. When C<sub>i</sub> increases it suggests the predominance of non-stomatal limitation to photosynthesis. Previous report indicated that severe water stress increases C<sub>i</sub> and mild water stress decreases C<sub>i</sub> (Lawlor, 1995). The decrease in C<sub>i</sub> indicates the stomatal limitations dominated, with moderate drought stress (Flexas & Medrano, 2002).

The reduction in  $g_s$  might have resulted from the stomatal closure which prevent  $CO_2$  from entering the leaf and photosynthetic carbon assimilation is decreased in favour of photorespiration. That drought can cause a decrease in  $g_s$  is in agreement with previous results where a 70% reduction of  $g_s$  after 22 days of drought stress was observed in dry bean (Rosales et al. 2012). The strong relationship between  $P_n$  and  $g_s$  indicates that the reduction in  $P_n$  was regulated mostly by stomatal closure and weak relationship indicates that the reduction in  $P_n$  was regulated by non-stomatal factors (Siddique *et al.*, 1999). The decrease in transpiration due to drought stress is in agreement with previous reports observed in *Eucalyptus globulus* clones (Osorio *et al.* 1998), wheat (Yordanov *et al.* 2001) and dry bean (Aroca *et al.* 2006).

An increase in  $F_0$  due to drought stress is in agreement with previous report observed in bean (Zlatev and Yordanov, 2004) and in cattail (Li et al., 2004). An increased  $F_0$  is a characteristic of PSII inactivation (Baker and Horton, 1987). The increased  $F_0$  might have resulted from the reduced plastoquinone acceptor ( $Q_A^-$ ), unable to be oxidized completely because of the electron flow retardation through PSII (Velikova et al., 1999). Even after termination of drought stress the  $F_0$  values were higher than for the control (e.g S3 at 93 DAP) which suggests that recovery was taking place slowly. The decrease in  $F_m$  due to drought stress is in agreement with previous results observed in bean (Zlatev and Yordanov, 2004). The decrease in  $F_m$  may be related to a decrease in the activity of the water splitting enzyme complex (Aro *et al.*, 1993). Throughout all the data collection dates S3 fail to recover from water stress. It should be considered that it has been found that measurement of  $F_v/F_m$  will only work for severe drought stress measurement in C3 plants due to photorespiration (Flexas et al. 2000). Photorespiration protects desiccating leaves against photo-inhibition not only acting as a sink for equivalents but also preventing over-reduction of the electron carriers between PSII and PSI (Katona et al., 1992).

The results that  $F_v/F_m$  for S2 and S5 was not affected by drought is in agreement with previous report observed in cattail and dry beans (Li et al., 2004; Terzi et al., 2010). A decrease in  $F_v/F_m$  indicates down regulation of photosynthesis (Zlatev and Lidon, 2012). Liu et al. (2012) also observed a decline in  $F_v/F_m$  ratio in drought stressed plants of two maize cultivars. The decreases in  $F_v/F_m$  ratio during 93 DAP for S5 and 100 DAP for S2 suggests that the recovery from water stress is accompanied by structural damage (Schapendonk *et al.*, 1989). This occurrence of chronic photo-inhibition is due to photo-inactivation of PSII centers (Zlatev & Yordanov, 2004). In bean leaves which has gone through drought, photo-

inhibitory impact on PSII could occur due to increased light intensity under stress conditions, which usually limits photosynthetic activity (Verhoeven *et al.*, 1997).

The decrease in qP might have been caused by an increase in the proportion of closed PS II centers (Zlatev and Lidon, 2012). The results for the reduction in qP due to drought was also observed by previous studies in dry beans (Zlatev and Yordanov, 2004; Terzi *et al.*, 2010).

The increase in qN might have been caused by the large proportion of absorbed light energy not being used by plants in the photosynthesis process. The increase in qN due to drought stress is in agreement with previous studies observed in barley, *Kalanchoë daigremontiana* and dry beans (Vassilev and Manolov 1999; Lu *et al.*, 2003; Zlatev and Yordanov, 2004).

## **5. Conclusions**

The introduction of drought stress during effective flowering (S2) and pod filling (S3) stages can result in serious reduction in yield. Drought stress across all stress levels resulted in a reduction in dry matter production, leaf area index, number of seeds per plant, number of pods per plant, seed size and finally grain yield. The highest WUE was found in the treatment which was irrigated on fortnightly bases from 36 DAP. This indicates that with appropriate irrigation it is possible to save water without a great yield loss in this crop. The results suggest that drought stress towards the end of the growing season may not cause serious harm in grain yield. The results suggest that drought stress can be practiced in dry bean production in areas where there is a challenge of irrigation water with consideration of the growth stage of the crop. The results of the study indicate that drought stress effects on photosynthetic rate were highly significant. The reduction was up to 45%. The reduction of photosynthesis at 63 and 105 DAP was greatly due to stomatal conductance. Drought stress resulted in a reduction in intercellular carbon dioxide concentration, stomatal conductance and transpiration. Chlorophyll fluorescence was also affected by drought stress. Drought stress can have serious effects on leaf gaseous exchange rate and chlorophyll fluorescence depending on the growth stage of the plant and the duration of drought stress.

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