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Comparison of Water Relations, Leaf Gas Exchange and Assimilation of Three Grain Legumes under Reproductive Period Water Deficit

[1]Kindie Tesfaye, [2]S. Walker and [3]M. Tsubo
[1]Haramaya University, P.O. Box 134, Dire Dawa, Ethiopia
[2]Department of Soil, Crop and Climate Sciences, University of the Free State, P.O. Box 339, Bloemfontein 9301, South Africa
[3]Arid Land Research Center, Tottori University, 1390, Hamasaka, Tottori-City, Tottori, 680-0001, Japan

Abstract: A field study was conducted during 2002-2003 in a semi-arid environment in order to compare water relations, leaf gas exchange, assimilation and stomatal regulation of common bean (*Phaseolus vulgaris* L., cv. Roba-1), cowpea (*Vigna anguiculata* L., cv. Blackeye bean) and chickpea (*Cicer arietinum* L., cv. ICC-4958) under three water regimes. The water regimes were well-watered control (C) and water deficit imposed at flowering (MS) and pod-filling (LS) periods. Midday leaf water potential (Ψ_L) of chickpea was significantly more responsive to changes in Available Soil Water (ASW) than that of beans and cowpea. Both the MS and LS stresses were critical in reducing the rate of photosynthesis (A) and transpiration (E) in all species. Most of the variation in A was explained by ASW in chickpea and cowpea while it was largely explained by Ψ_L in beans. Stomatal closure was initiated when the ASW dropped to 51.5, 41.6 and 56.7% which corresponded to a Ψ_L of -1.33, -2.26 and -1.23 MPa and a stomatal conductance (g_s) of 0.066, 0.055 and 0.083 mol m^{-2} sec^{-1} in beans, chickpea and cowpea, respectively. The first sharp decline in A was observed when the g_s declined below 0.29, 0.38 and 0.33 mol m^{-2} sec^{-1} in beans, chickpea and cowpea, respectively. These values could be used to optimize water supply during irrigation of the crops and to develop grain legume simulation models and/or calibrate the existing ones to suit for dry environments.

Key words: Grain legumes, gas exchange, leaf water potential, soil water deficit, stomatal conductance

INTRODUCTION

Water shortage is a major constraint to crop production as crops are usually exposed to drought periods of varying duration and intensity during their growth (Sadras and Milroy, 1996), particularly in semi-arid regions. Semi-arid climates are characterized by fluctuating rainfall both in amount and distribution and plants grown under these climates are prone to frequent atmospheric drought, even when the soil water reserves are adequate (Maroco *et al*., 1997). In the semi-arid tropical regions, grain legumes are grown under rainfed conditions, so their yield depends on the amount of water transpired and the seasonal pattern of soil water availability (Tesfaye and Walker, 2004). Because of the erratic nature of the rainfall during the season, these crops can experience intermittent or continuous water deficits during their growth.

Plant productivity generally depends on the rate of CO_2 assimilation (Srivasta and Strasser, 1996; Costa Franca *et al*., 2000) and transpiration which serves as a major cooling mechanism for plant leaves through the evaporation process (Jalali-Farahani *et al*., 1993). Stomatal regulation is one of the prominent examples of plant responses to drought and stomata can be considered to be integrators of all environmental factors that affect plant growth (Morison, 1998). Stomata regulate water use and the development of water stress and influence plant growth rates through effects on availability of CO_2 assimilation. Thus, stomatal responses to drought have a substantial influence on plant adaptation in dry climates.

Leaf water status and humidity of the air are reported to have a major influence on stomatal conductance in the field (Turner, 1991). Stomatal conductance usually decreases when plants are subjected to soil water deficits (Lopez *et al*., 1988) and differences in stomatal conductance in response to leaf water potential have been reported in grain legumes (Cruz de Carvalho *et al*., 1998; Pimentel *et al*., 1999; Costa Franca *et al*., 2000; Nautiyal *et al*., 2002). Solane *et al*. (1990) reported that stomatal conductance and photosynthesis maintenance during leaf water deficit was associated with favorable seed yield in soybean genotypes.

Water flow in the soil-plant-atmosphere continuum is governed by differences in the water potential of the three systems and the resistances in the water flow

Corresponding Author: Kindie Tesfaye, Haramaya University, P.O. Box 134, Dire Dawa, Ethiopia
Tel: +251 255 530051 Fax: +251 255 530331

pathway. The soil water potential usually determines the upper limit of leaf water potential while the lower limit is set by the combined action of atmospheric variables, soil water content and the resistances to flow (Kirkham, 2004). Therefore, understanding of plant processes and reactions to water deficit requires determination of the quantitative relationships between soil-plant water relations, growth, gas exchange and assimilation rate (Sadras and Milroy, 1996). Several studies have attempted to understand the different response of grain legumes to water deficits under both controlled (greenhouse and laboratory) and field conditions (Vasquez-Tello *et al*., 1990; Cruz de Cravalho *et al*., 1998; Leport *et al*., 1998, 1999; Anyia and Herzog, 2004).

Common bean (*Phaseolus vulgaris* L.) and cowpea (*Vigna unguiculata* L.) are the major legumes that are grown in the cereal based water-limited areas of Ethiopia while chickpea (*Cicer arietinum* L.) is mostly grown as a second crop on residual moisture during the dry period. The actual yield of these crops is very low because the crops usually experience water deficit at one or more of their growth stages in the field. Understanding the water relations and gas exchange response of the species to water deficit under the same environmental conditions could help design appropriate crop-specific management practices that increase productivity in water-limited environments.

Although it is known that cowpea is more drought tolerant than beans (Vasquez-Tello *et al*., 1990; Cruz de Cravalho *et al*., 1998) and chickpea is more drought tolerant than many cool-season legumes (Singh, 1993; Leport *et al*., 1999), there is little information that shows the relative physiological response of the three species to water deficit in the semi-arid tropics where they are grown in the same field. Therefore, the aim of the present study was to undertake a comparative analysis of the effects of mid- and late-season water deficit on soil-plant water relations, leaf gas exchange, assimilation and stomatal regulation of the three grain legume species in semi-arid environment.

MATERIALS AND METHODS

Field experiments: Two irrigation field experiments were conducted at the research center of Haramaya University in Dire Dawa, Ethiopia (latitude 9°6'N, longitude 41°8' E, altitude 1197 m above sea level) during the periods from late March to early July 2002 (first season) and from mid-October 2002 to early February 2003 (second season). The station lies in the semi-arid belt of the eastern Rift Valley escarpment with a long-term average rainfall of 612 mm. The rainfall distribution is bimodal in which 39 and 44% of the rainfall is received during the months of March to May and July to September, respectively (Tesfaye and Walker, 2004). The period between July to October is used for the production of cereal crops with some intercropping of grain legumes. Grain legumes are also grown between March to May under rainfed condition and between October to February under irrigation. The soil is classified as Eutric Regosol with a gentle slope (3-8%) (Amede, 1998). The texture and structure of the topsoil (0-30 cm) are clay loam and sub angular blocky, respectively. The soil has an average pH (H_2O 1:2.5) of 8.52 and organic matter content of 1.18%.

Common bean (cv. Roba-1), chickpea (cv. ICC-4958) and cowpea (cv. Blackeye bean) were planted on March 27, 2002 and October 17, 2002, for the first and second seasons, respectively. All cultivars had semi-indeterminate growth habit. Roba-1 is an improved and relatively drought tolerant bean variety (Tesfaye, 1997). Blackeye bean is a well-adapted cowpea variety used as a standard check by the Lowland Pulse Research Program at Haramaya and Melkasa research stations. ICC-4958 is a registered drought resistant chickpea cultivar (Saxena *et al*., 1993) currently grown in Ethiopia.

Nitrogen and phosphorus fertilizers were applied to the soil before planting in the form of Urea and Di-ammonium Phosphate at a rate of 30 kg ha^{-1} each. Hand weeding was done throughout the growing periods to keep the plots free of weeds. Sumathion (20 mL active ingredient (ai)/10 L of water) and Maneb (10 g ai/10 L of water) were applied twice during each season to control insect pests and fungal diseases, respectively.

Experimental design: The experiments had three water regime treatments, viz., well-watered control (C), mid-season/flowering period water stress (MS) and late season/pod-filling period water stress (LS). The experimental design was randomized complete block with a split plot arrangement of treatments by using the water stress treatments as main plot and the crop species, viz, common bean (*Phaseolus vulgaris* L.), cowpea (*Vigna anguiculata* L.) and chickpea (*Cicer arietinum* L.) as sub-plot. Each treatment was replicated three times. Each sub-plot (4×6 m) had 10 rows and the plant population was 25 plants m^{-2}. A 4 m wide strip of guard plants surrounded the whole experimental area.

Irrigation application: Plots were irrigated immediately after planting to ensure uniform seedling establishment. The field capacity and the permanent wilting point of the soil at 600 mm depth were 69 and 32 mm giving available soil water content of 37 mm. The available soil water at

600 mm depth was kept above 90% in the non-stressed treatment (C). Stress was imposed in the MS treatment by withholding irrigation and rainfall from the plots during the flowering and pod setting periods. The plots were re-watered when the available soil water depletion reached between 23-25%. The LS treatment plots were not irrigated and also protected from rainfall for the period from pod filling to maturity. Detail explanation on the degree and duration of stress in each treatment is given in Tesfaye *et al.* (2006). A simple rainout shelter was used to protect stress plots from rainfall. Lateral water movement between plots was protected by 1×40 m polythene plastics buried to a depth of 1 m between the plots.

Measurements: The soil water content to a depth of 600 mm was monitored every day using Time Domain Reflectometery (TDR) (Soil Moisture Equipment Corp., CA, USA) throughout the experimental periods. Three soil water measurements were taken per plot at a distance of 1 m in the central row.

The effects of available soil water depletion on plant water status, leaf gas exchange, assimilation and stomatal regulation of each species were monitored by measuring leaf water potential, internal CO_2, rates of photosynthesis and transpiration and stomatal conductance of well-watered and stressed plants simultaneously as described below.

Leaf water potential of the stressed and non-stressed plots was measured between 12:00 and 13:00 local time on upper fully exposed leaves of five plants per plot using a pressure chamber (PMS Instrument Company, Oregon, USA) every other day throughout each stress period. The leaves were covered with a white polythene bag before excision and then water potential measurement of each leaf was completed within the next 2 min to avoid evaporative water loss that affect the readings.

Rate of photosynthesis, transpiration and stomatal conductance were measured using an Infrared Gas Analyser, IRGA, Type LCA-4 (ADC Bio Scientific Ltd., UK) between 12:00-13:00 local time (GMT +3) every other day during each stress treatment. The measurements were made from two upper fully expanded central trifoliate leaves per plant and five plants per plot giving a total of 10 measurements per plot.

Weather conditions: Weather parameters were measured at the Dire Dawa International Airport weather station (latitude 9°36' N, longitude 41°51' E and altitude 1260 m above sea level; 500 m away from the experimental site) during the first season and from an automatic weather station which was set in the field during the second season. Both maximum and minimum temperatures were higher in the first than in the second season. Accordingly, the first season was less humid (high vapor pressure deficit, VPD) than the second season (low VPD) but received higher rainfall. The length of the growing period was shorter in the first (107 days) than in the second season (120 days). Because of long period of overcast conditions in the late season of the second experiment, water stress intensity in the LS treatment for the second season was lower than the first season.

Data analysis: Statistical analyses were made using Number Cranture Statistical System, NCSS 97 (Hintze, 1997). Differences between slopes of linear regressions were tested using the t-test. Cutoff values for a given variable were calculated as an intersection point of two linear regression lines obtained from data points clustered based on similarity of trend. Non-linear regressions were analyzed and tested for significance using GraphPad Prism Software, version 5.01 (GraphPda Software Inc.).

RESULTS

Leaf water potential (Ψ_L), stomatal conductance (G_s), internal CO_2 concentration (C_i), photosynthesis (A) and transpiration (E): During the flowering period, the midday Ψ_L of plants in the C treatments remained above -1.43 MPa in all species in both seasons while it reached as low as -1.58 MPa in beans and cowpea and -3.37 MPa in chickpea (in 2002) when plants were stressed until ASW reached to a level of 32% (Table 1). Among species, chickpea had the lowest while beans had the highest Ψ_L at 32% ASW in the LS treatment in 2002 (Table 2).

The average g_s for the well-watered plants (ASW>90%) of the three species ranged from 0.38-0.52 mol m^{-2} sec^{-1} during the flowering period and from 0.44-0.91 mol m^{-2} sec^{-1} during the pod-filling period in the two seasons (Table 1, 2). The g_s recorded at 32% ASW during the MS treatment in both seasons was less than 0.03 mol m^{-2} sec^{-1} in all the species (Table 1) while it ranged between 0.03 (beans) and 0.09 (chickpea) mol m^{-2} sec^{-1} in the LS treatment in 2002 (Table 2). The g_s measured at 50% ASW in the LS treatment in 2002 was similar for the three species while it had a wide range among species in 2002/2003 (Table 2). Cowpea exhibited the lowest stomatal conductance during the flowering period at 32% ASW in the second season while beans had the lowest g_s during the pod-filling period stress in both seasons showing some variation of stomatal conductance with plant age in the two species.

Differences in midday Ψ_L and g_s were significant ($p<0.05$) among the species in the MS and LS treatments in both seasons. However, there was no significant

Table 1: Mean leaf water potential (Ψ_L), stomatal conductance (g_s), internal CO_2 concentration (C_i) and rates of midday photosynthesis (A) and transpiration (E) of bean, chickpea and cowpea at different levels of available soil water (ASW) during the flowering period

Season	Species	ASW[ə] (%)	Ψ_L (MPa)	g_s (mol m^{-2} sec^{-1})	C_i (vpm)	A (μmol m^{-2} sec^{-1})	E (mmol m^{-2} sec^{-1})
2002	Bean	>90[§]	-1.23±0.06	0.48±0.055	247±20.7	15.10±0.90	8.11±0.38
		60	-1.44±0.04	0.05±0.020	395±9.20	7.48±0.79	3.40±0.46
		50	-1.48±0.02	0.04±0.003	145±6.60	4.22±0.35	2.73±0.18
		32	-1.58±0.03	0.02±0.001	181±22.4	2.50±0.59	2.54±0.37
	Chickpea	>90	-1.43±0.10	0.47±0.039	257±26.1	20.63±0.94	10.71±0.78
		60	-2.34±0.04	0.06±0.010	375±8.90	5.12±0.46	3.58±0.67
		50	-2.74±0.07	0.04±0.020	202±25.4	2.66±0.91	2.84±0.71
		32	-3.37±0.10	0.02±0.010	198±27.9	2.27±0.81	1.65±0.28
	Cowpea	>90	-0.99±0.14	0.52±0.129	227±17.8	17.61±1.10	7.95±0.93
		60	-1.48±0.03	0.06±0.020	230±11.3	8.80±1.15	4.94±0.69
		50	-1.57±0.04	0.05±0.020	165±10.2	5.87±1.10	3.28±0.52
		32	-1.57±0.04	0.01±0.010	158±20.0	3.70±0.85	1.82±0.49
2002/03	Beans	>90	-1.07±0.03	0.41±0.087	174±21.6	18.40±0.28	8.45±0.50
		60	-1.30±0.02	0.14±0.037	131±23.6	11.40±0.96	4.30±0.71
		50	-1.38±0.01	0.04±0.009	130±19.6	7.40±0.46	2.90±0.33
		32	-1.49±0.02	0.03±0.004	127±28.3	3.90±0.50	1.74±0.14
		25	-1.66±0.02	0.01±0.003	150±14.5	1.75±0.30	0.69±0.10
	Chickpea	>90	-1.21±0.06	0.41±0.070	180±18.8	16.42±0.89	6.47±0.59
		60	-1.76±0.01	0.16±0.069	178±13.1	8.40±1.23	3.20±0.39
		50	-2.11±0.02	0.08±0.007	154±40.6	5.82±0.42	2.38±0.49
		32	-2.57±0.03	0.02±0.004	152±43.6	2.73±0.36	1.20±0.21
		25	-3.02±0.02	0.01±0.002	206±31.2	2.66±0.61	0.57±0.14
	Cowpea	>90	-0.93±0.04	0.38±0.090	190±16.6	16.85±0.21	6.23±0.22
		60	-1.20±0.03	0.05±0.003	174±9.30	10.00±0.37	4.80±0.21
		50	-1.35±0.02	0.01±0.003	131±26.0	7.83±0.29	2.64±0.21
		32	-1.50±0.02	0.01±0.003	114±23.7	4.64±0.28	2.35±0.12
		25*	-1.46±0.02	0.02±0.004	188±19.6	3.22±0.43	1.91±0.18

[ə]The ASW values indicated range between±2%, [§]The Ψ_L, r_s, A and E values indicated at the high ASW (>90%) are means for measurements taken between 90-100% ASW. Values next to means are standard errors, *Measurements taken on a day with very low air vapor pressure deficit

Table 2: Mean leaf water potential (Ψ_L), stomatal conductance (g_s), internal CO_2 concentration (C_i) and rates of midday photosynthesis (A) and transpiration (E) of bean, chickpea and cowpea at different levels of available soil water (ASW) during the pod-filling period[δ]

Season	Species	ASW (%)	Ψ_L (MPa)	g_s (mol m^{-2} sec^{-1})	C_i (vpm)	A (μmol m^{-2} sec^{-1})	E (mmol m^{-2} sec^{-1})
2002	Bean	>90	-1.13±0.14	0.46±0.140	174±12.3	16.70±1.02	9.46±0.38
		60	-1.40±0.03	0.08±0.008	156±7.20	8.75±0.52	4.00±0.29
		50	-1.57±0.04	0.04±0.003	140±10.6	4.62±0.46	3.62±0.24
		32	-1.70±0.03	0.03±0.010	179±27.5	2.97±0.86	3.32±0.65
	Chickpea	>90	-0.63±0.36	0.91±0.222	196±14.6	21.85±1.26	10.94±0.45
		60	-2.35±0.09	0.18±0.030	172±12.3	10.54±1.04	7.59±0.48
		50	-2.50±0.15	0.10±0.010	163±15.7	8.22±1.12	6.25±0.55
		32	-2.90±0.03	0.09±0.009	164±23.6	3.53±1.06	4.44±0.22
	Cowpea	>90	-0.84±0.15	0.52±0.122	176±11.2	18.92±1.15	8.67±0.77
		60	-1.43±0.04	0.17±0.030	172±7.70	10.68±1.29	6.09±0.63
		50	-1.53±0.02	0.07±0.020	133±16.1	7.36±1.36	4.93±0.65
		32	-1.60±0.03	0.05±0.020	120±7.20	5.70±1.42	3.19±0.51
2002/03	Bean	>90	-0.76±0.02	0.57±0.070	176±16.8	19.70±0.60	8.70±0.30
		60	-1.18±0.04	0.15±0.022	143±19.6	13.30±0.57	4.90±0.35
		50	-1.21±0.02	0.03±0.005	82±20.70	5.78±0.54	1.73±0.22
	Chickpea	>90	-1.00±0.17	0.44±0.048	195±17.8	19.41±0.55	6.97±0.98
		60	-1.23±0.02	0.22±0.029	102±19.8	11.70±2.08	5.28±0.40
		50	-1.55±0.07	0.15±0.039	144±21.1	5.25±0.50	1.77±0.07
	Cowpea	>90	-0.67±0.05	0.56±0.014	179±17.7	18.69±1.43	7.21±0.84
		60	-0.85±0.03	0.19±0.026	173±25.3	12.22±1.20	4.70±0.21
		50	-1.05±0.02	0.07±0.008	111±17.2	9.66±0.86	3.40±0.13

[δ]Explanations as in Table 1

difference ($p>0.05$) in the minimum g_s attained at the end of the MS treatment in 2002 despite a significant difference in Ψ_L among the species.

The C_i declined with soil water depletion until the remaining available soil water reached 32% in both the MS and LS treatments in both seasons except at 60% ASW in the MS treatments in 2002 (Table 1, 2). However, an increase in C_i was observed in all species when 75% of the available water was depleted in the MS treatment in 2002/03 (Table 1).

The maximum midday A values recorded in the control plots were 20.5, 23.6 and 23.9 in 2002 and 19.7, 20.2 and 21.1 μmol m^{-2} sec^{-1} in 2002/2003 in beans, chickpea and cowpea, respectively. The average value of A measured in the control treatment during the flowering period was the highest in chickpea in 2002 and in beans in 2002/2003 (Table 1). At 32% ASW, the values of A in the MS treatment were reduced by 79-83% in beans, 83-89% in chickpea and 72-79% in cowpea relative to the average measurements taken in the control treatments in the two seasons. Beans had the lowest while cowpea had the highest A value at the severe stage of the water stress (25% ASW) during the flowering period in 2002/2003. Relative to the average records in the well-watered treatments, the values of A recorded at 50% ASW during the LS treatment in the two seasons (Table 2) indicated a reduction of A by 71-72, 62-73 and 48-61% in beans, chickpea and cowpea, respectively. In 2002, a decline in ASW from 50 to 32% in the LS treatment resulted in a further decline of A by 10, 21 and 9% in beans, chickpea and cowpea, respectively.

Under well-watered conditions, chickpea and beans had the highest E value in 2002 and 2002/2003 seasons, respectively during the whole reproductive period (Table 1, 2). When the ASW dropped to 32% in the MS treatment, the E values were reduced by 69, 85 and 79% in 2002 and by 79, 82 and 62% in 2002/2003 in beans, chickpea and cowpea, respectively. Similarly, the E values recorded in 2002 at 32% ASW in the LS stress treatment were reduced by 65% in beans, 59% in chickpea and 63% in cowpea relative to the mean values in the control treatments. The E values recorded at 50% ASW during the LS treatment (Table 2) were lower than the mean values recorded under well-watered conditions by 62, 43 and 43% in 2002 and by 80, 75 and 53% in 2002/2003 for beans, chickpea and cowpea, respectively.

Relationships between ASW, Ψ_L, g_s, A and E: Data were combined for the well-watered (C) and stressed (MS and LS) treatments to investigate the relationships among the variables during the whole reproductive period of the three species. There was a linear relationship between Ψ_L and ASW (%) in all species as expected (Fig. 1). ASW explained 48, 65 and 53% of the variability in Ψ_L in beans, chickpea and cowpea, respectively. Chickpea had significantly lower Ψ_L than beans and cowpea when the ASW dropped below 80%. As a result, the average decline in Ψ_L with available soil water was significantly ($p<0.01$) higher in chickpea than in beans and cowpea. Differences in Ψ_L between beans and cowpea were not significant ($p>0.05$) at any level of available soil water depletion.

The relationship between ASW and g_s was explained better by an exponential function than a linear one in all species (Fig. 2a). Cowpea had a higher exponential decline of g_s with ASW followed by beans and chickpea in descending order. This shows prompt stomatal response to a slight decline in soil water in cowpea followed by beans. Further analysis of the data shown in Fig. 2a indicated that stomatal closure was initiated when ASW reached 51.5, 41.6 and 56.7% at which the Ψ_L dropped to -1.33, -2.26 and -1.23 MPa (Fig. 1) in beans, chickpea and cowpea, respectively.

There was strong linear relationship between A and ASW in all species (Fig. 2b). The ASW explained 77, 83 and 82% of the variability in A in beans, chickpea and cowpea, respectively. Chickpea generally had higher A values than beans and cowpea at high ASW (>80%) but

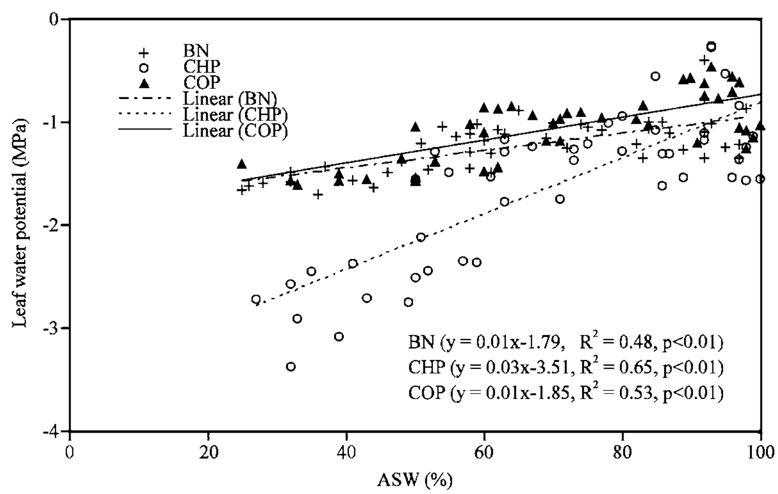

Fig. 1: Relation between available soil water (ASW) and leaf water potential in beans (BN), chickpea (CHP) and cowpea (COP) under water deficit during the reproductive period

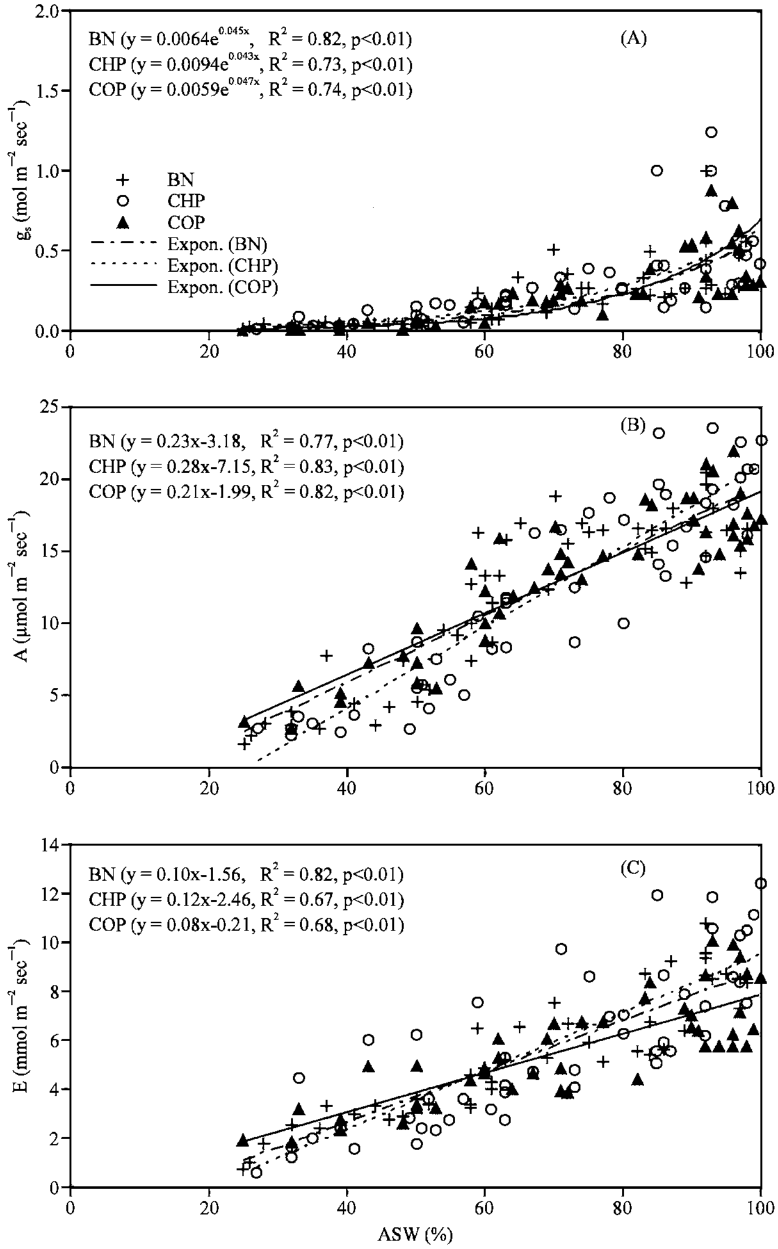

Fig. 2: The relation of available soil water (ASW) with stomatal conductance, g_s (A), midday rate of photosynthesis, A (B) and transpiration, E (C) in beans (BN), chickpea (CHP) and cowpea (COP) under water deficit during the reproductive period. Slopes of linear regression equations are significantly different at 5% p level for all species with n ranging between 40 and 50 for the different species

had lower values when the ASW dropped below 70%. As a result, the average decline in the rate of photosynthesis with the depletion of available soil water was significantly ($p<0.05$) higher in chickpea than in beans and cowpea which showed a similar response (Fig. 2b). Available soil water also explained 82, 67 and 68% of the variability in E in beans, chickpea and cowpea, respectively (Fig. 2c). Significant differences ($p<0.05$) were observed between chickpea and cowpea when the ASW was above 80% and below 50% while beans showed intermediate response in the decline of its rate of transpiration with soil water availability. Compared to the other two species, cowpea exhibited conservative water use (Fig. 2c).

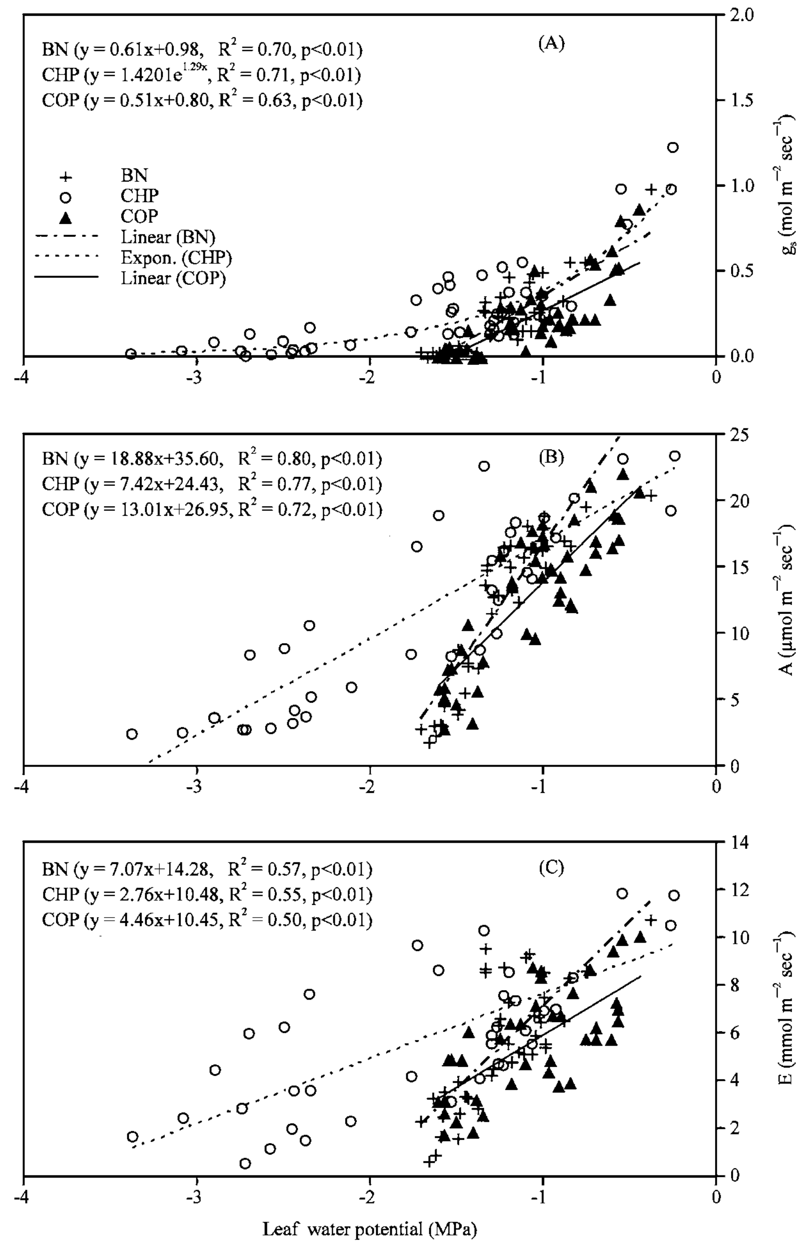

Fig. 3: The relation of leaf water potential with stomatal conductance, g_s (A), midday rate of photosynthesis, A (B) and transpiration, E (C) in beans (BN), chickpea (CHP) and cowpea (COP) under water deficit during the reproductive period. Slopes of linear regression equations are significantly different at 1% p level for all species with n ranging between 40 and 50 for the different species

There was a significant linear relationship between g_s and Ψ_L in beans ($R^2 = 0.70$) and cowpea ($R^2 = 0.63$) in which the g_s declined with Ψ_L at a similar rate in both species (Fig. 3a). On the other hand, the g_s in chickpea declined exponentially with Ψ_L (Fig. 3a) indicating a faster decline rate of g_s at higher Ψ_L values than at lower values. A complete closure of stomata occurred at Ψ_L of ca. -1.61 MPa in beans and ca. -1.55 MPa in cowpea while closure commenced at -2.26 MPa in chickpea.

There was also a linear relationship between A and Ψ_L in all species (Fig. 3b). The Ψ_L explained most of the variability in A in beans (82%) compared to the one in cowpea (72%) and chickpea (77%). The reduction of A with a decline in Ψ_L was significantly ($p<0.01$) different among the three species that beans showed the highest rate of decline while chickpea showed the lowest (Fig. 3b). Chickpea was able to photosynthesize at lower Ψ_L (<-3.0 MPa) than beans and cowpea in which

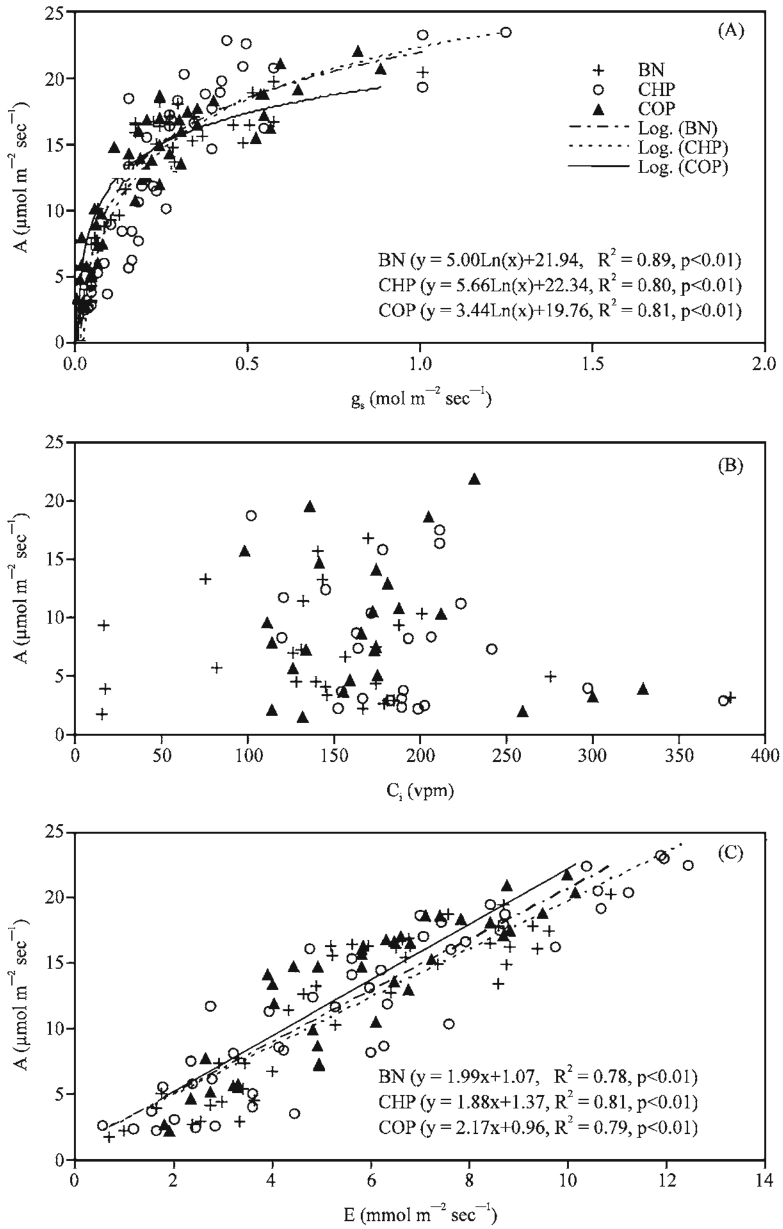

Fig. 4: The relation between midday rate of photosynthesis (A) vs. stomatal conductance, g_s (A), midday rate of photosynthesis vs. internal CO_2, C_i (B) and midday rate of photosynthesis vs. midday rate of transpiration, E (C) in beans (BN), chickpea (CHP) and cowpea (COP) under water deficit during the reproductive period

photosynthesis was dropped to zero at Ψ_L of ca.-1.9 and -2.1 MPa, respectively. E was weakly correlated to Ψ_L in all species (Fig. 3c) when it is compared to A (Fig. 3b). The rate of decline of E with Ψ_L was significantly ($p<0.01$) lower in chickpea than in beans and cowpea which responded similarly.

The relation between A and g_s was explained by a logarithmic function ($R^2 > 0.80$) in all species (Fig. 4a). The logarithmic decline of midday photosynthesis with g_s was lower in cowpea than in beans and chickpea indicating the ability of cowpea to continue its photosynthesis under low g_s. Clustering of the data shown in Fig. 4a into two linear relations indicated that A was detrimentally affected when the g_s declined below 0.29, 0.38 and 0.33 mol m^{-2} sec^{-1} in beans, chickpea and cowpea, respectively. Calculation of the intrinsic water use efficiency (A/g_s) in the stress treatments indicated that cowpea (94 μmol mol^{-1}) was more efficient than beans

Table 3: Relative stomatal conductance (g_s), rate of midday transpiration (E) and photosynthesis (A) and difference in leaf temperature (T_L) between the control and stress treatment before and after re-watering of mid-season water stressed beans, chickpea and cowpea in 2002/2003+

Parameters	Measurement	Bean	Chickpea	Cowpea
g_s	Before re-watering	0.05	0.04	0.03
	After re-watering	0.50	0.47	0.33
	Recovery (%)	45.00	43.00	30.00
E	Before re-watering	0.13	0.09	0.17
	After re-watering	0.57	0.76	0.59
	Recovery (%)	44.00	67.00	42.00
A	Before re-watering	0.10	0.26	0.14
	After re-watering	0.56	0.60	0.59
	Recovery (%)	46.00	34.00	45.00
T_L (difference from control, °C)	Before	-1.80	-7.20	-4.30
	After	2.60	-4.90	0.70

+The values indicated are relative (or difference for temperature) to the control just before re-watering and three days after re-watering. Percent of recovery is the difference between the relative values before and after re-watering

(58 μmol mol^{-1}) and chickpea (33 μmol mol^{-1}) in the MS stress treatment whereas beans (85 μmol mol^{-1}) was more efficient than cowpea (50 μmol mol^{-1}) and chickpea (43 μmol mol^{-1}) in the LS stress treatment.

There was no correlation between C_i and A under water deficit (Fig. 4b) because of the differential response of C_i to mild and severe soil water deficits as opposed to the consistent decline of A with available soil water depletion.

The Instantaneous Water Use Efficiency (IWUE), calculated as the slope of the linear regression between A and E, is shown in Fig. 4b. The IWUE of chickpea (1.88 μmol mmol^{-1}) was significantly lower ($p<0.05$) than that of cowpea (2.17 μmol mmol^{-1}) but similar to that of beans (1.99 μmol mmol^{-1}).

Post stress recovery: While the recovery of transpiration was higher in chickpea (67%) than in beans and cowpea (42-44%), the recovery of midday rate of photosynthesis was higher in beans and cowpea (45-46%) than in chickpea (34%). On the other hand, the recovery of g_s was higher in beans and chickpea (43-45%) compared to cowpea (30%). The leaves of the stressed plants were hotter than the controls by 1.8, 7.2 and 4.3°C in beans, chickpea and cowpea, respectively during the stress but became cooler than the controls by 2.6°C in beans and by 0.7°C in cowpea after three days of re-watering (Table 3). However, the previously stressed leaves of chickpea remained hotter than their control counter parts by 4.9°C after re-watering. Comparison of the temperature differences between stressed and control plants before and after re-watering showed that the cooling of leaves up on re-watering was higher in cowpea (5°C) than in beans (by 4.4°C) and chickpea (2.3°C).

DISCUSSION

The Ψ_L values observed under well-watered conditions in the present study were lower than the value (-0.6 MPa) reported for six unstressed cool-season grain legumes including chickpea in a Mediterranean-type environment (Leport *et al*., 1998) which could be due to varietal differences. In agreement with previous reports (Nwalozie and Annerose, 1996; Diallo *et al*., 2001; Leport *et al*., 1998, 1999), the range of Ψ_L variation observed between the well-watered and stressed plants was smaller in beans and cowpea than in chickpea.

Significantly lower g_s values in the stressed than in the well-watered plants in all the species indicate the role of stomatal regulation to the drought adaptation mechanism of grain legumes as reported in other studies (Trejo and Davies, 1991; Barradas *et al*., 1994; Cruz de Carvalho *et al*., 1998; Costa Franca *et al*., 2000). However, the degree of stomatal regulation varied considerably among the species that cowpea and beans closed their stomatal aperture at higher soil and plant water status than chickpea which is in agreement with previous studies in beans (Cornic and Briantias, 1991; Trejo and Davies, 1991; Barradas *et al*., 1994) and cowpea (Diallo *et al*., 2001). However, the Ψ_L value at which beans closed its stomata in the current study was lower than the values reported (-0.6 to -0.9 MPa) for complete stomatal closure in the same plant (Costa Franca *et al*., 2000). Beans also closed its stomata at a similar Ψ_L value to that of cowpea in the present study which contradicts with previous reports that beans closed its stomata at higher leaf water potential than two cowpea cultivars in a controlled experiment (Cruz de Carvalho *et al*., 1998). These contradictions could be a result of cultivar and/or environmental differences between the studies.

Rapid stomatal regulation in response to a slight decline in Ψ_L in beans and cowpea (Fig. 1b) indicates a water conservation (dehydration postponement) mechanism employed by the species which help them maintain nearly constant plant water status during drought (Squire, 1990; Cruz de Carvalho *et al*., 1998; Taiz and Zeiger, 1998). On the other hand, slow stomatal response under severe water deficits in chickpea suggests the low contribution of stomatal closure to the drought avoidance of the crop compared to the other two species studied. The trigger for stomatal closure under periods of water stress is believed to be associated with root-to-shoot communication via Abscisic acid (ABA) translocation in many crops (Davies *et al*., 1990; Davies and Zhang, 1991; Ribaut and Pilet, 1991; Blum and Johnson, 1993) and decreases in hydraulic conductance of the soil-leaf continuum (Sperry, 2000; Salleo *et al*., 2000; Hubbard *et al*., 2001). Therefore, differences in the speed of stomatal closure among the species could also reflect differences in one or more of the above-mentioned mechanisms. On the other hand, osmotic adjustment is reported to be the major mechanism by which chickpea prevents cell desiccation and maintains photosynthesis under very low Ψ_L (Morgan *et al*., 1991; Leport *et al*., 1998) as opposed to cowpea in which osmotic adjustment is absent (Diallo *et al*., 2001).

A decrease in C_i due to a change in stomatal conductance causes a decrease in the rate of CO_2 assimilation which is proportionally smaller than that in stomatal conductance (Farquhar *et al*., 1989). Hence the curvilinear relationship between A and g_s (Fig. 4a) indicates a stomatal limitation of A below a g_s of about 0.29, 0.38 and 0.33 mol m^{-2} sec^{-1} in beans, chickpea and cowpea, respectively. In the present study, however, the C_i increased in all species when 75% of the available soil water was depleted indicating the role of non-stomatal factors in reducing A under severe water deficit conditions. Similar to the present result, the major reason for the reduction of A in beans and cowpea has been related to stomatal closure under mild water deficit (Cruz de Carvalho *et al*., 1998; Costa Franca *et al*., 2000) whereas it has been related to non-stomatal factors (reduced rubisco activity and increases in internal CO_2 concentration) under severe water deficit (Bordribb, 1996; Vu *et al*., 1998; Anyia and Herzog, 2004).

Species were characterized by a similar rate of photosynthesis under well-watered conditions. The average values of A found under well-watered conditions were lower than the values reported for chickpea (21-27 μmol m^{-2} sec^{-1}) in a Mediterranean-type environment (Leport *et al*., 1998) but higher than the values reported for beans (10.7 μmol m^{-2} sec^{-1}) and cowpea (7.8-11.2 μmol m^{-2} sec^{-1}) under fully hydrated state conditions in a controlled experiment (Cruz de Carvalho *et al*., 1998; Anyia and Herzog, 2004) which could be due to varietal and/or environmental differences between the experiments. The rate of photosynthesis was almost negligible when the ASW dropped to 25, 14 and 10% in chickpea, beans and cowpea, respectively (Fig. 2b). Reductions in A due to water stress have also been reported for a number of grain legumes including chickpea, beans and cowpea (Leport *et al*., 1998; Cruz de Cravalho *et al*., 1998).

The decline in A with Ψ_L in cowpea was lower than beans mainly due to the ability of cowpea to close its stomata slowly at higher Ψ_L (Fig. 3a) and continue photosynthesis at negligible stomatal opening under lower Ψ_L than beans did (Cruz de Carvalho *et al*., 1998). This could be due to the fact that assimilation rate in cowpea is strongly associated with the maintenance of high relative water content under severe water deficit (Anyia and Herzog, 2004). The ability of cowpea to continue photosynthesis and transpiration after stomatal closure could also suggest a possible occurrence of partial stomatal opening (due to stomata patchiness) and/or cuticular transpiration as reported in other species (Boyer *et al*., 1997). The rate of photosynthesis was found to be more sensitive to the decline of soil water than did the rate of transpiration in all species. Since metabolic reactions are affected differently at different temperature ranges (Pastenes and Horton, 1996), this could be explained by the sensitivity of photosynthesis to increased leaf temperatures even before the stomata close completely.

High IWUE can be achieved either through lower stomatal conductance or high photosynthetic capacity or a combination of both (Condon *et al*., 2002). As shown by high A/g_s values in the stressed treatments, high IWUE in beans and cowpea is mainly a result of reduced stomatal conductance during water deficit. In chickpea, the maintenance of low but positive photosynthesis activity in the leaves under very low Ψ_L did not result in higher IWUE of the crop as compared to the other two species in which A dropped to zero at high Ψ_L. However, the maintenance of low level of A at very low Ψ_L in chickpea during reproductive development may be critical in providing the energy required to maintain translocation of assimilates from the source (leaves, stems and roots) to the developing sink, the seed (Leport *et al*., 1998).

Despite low g_s, rapid recovery of A in cowpea indicates the existence of reversible non-stomatal factors that could reduce photosynthesis during periods of water deficit. One of such factors, which affect photosynthesis indirectly, is leaf temperature. Theretofore, fast recovery of photosynthesis from stress in cowpea may be due to the fast decline of leaf temperature while the recovery in beans could be due to both lowering of leaf temperature in the stressed leaves and rapid recovery of stomatal conductance. Although the recovery of stomatal conductance was followed by a high recovery of transpiration in chickpea, the recovery of A and T_L was small compared to the other two species which could be due to the horizontal leaf structure of chickpea that absorbs high incident radiation (Tesfaye *et al*., 2006). In others studies, slower and partial recovery of photosynthesis following rehydration was observed in beans (Cruz de Carvalho *et al*., 1998). In comparing beans and cowpea, Cruz de Carvalho *et al*. (1998) found higher recovery of photosynthesis in cowpea than in beans. The fast recovery of A in beans, which is similar to the recovery in cowpea, in the present study could be due to the relatively drought tolerant bean cultivar used in the study. In other crops such as pigeonpea, a complete recovery of photosynthesis has been observed after seven days of re-watering (Lopez *et al*., 1988).

CONCLUSION

The data presented showed some distinct differences in the response of the three species to water deficit imposed during their reproductive periods. Although the species have similar responses under well-watered conditions, they do differ in stomatal regulation and maintenance of plant water status, photosynthesis and transpiration rates under declining available soil water conditions during their flowering and pod-filling periods. Beans and cowpea showed a fast early decline in stomatal conductance than chickpea but the rate of photosynthesis was markedly affected at higher stomatal conductance in chickpea than in beans and cowpea. Beans and cowpea maintain photosynthesis under water deficit by maintaining high plant water status through prompt stomata regulation (drought avoidance resulting in high IWUE) while chickpea tolerates desiccation and continue photosynthesis under low plant water status (desiccation tolerance resulting in low IWUE). Although the degree of limitation varies among species, stomatal limitation of CO_2 assimilation is dominant in the range of 30-67% available soil water depletion while non-stomatal limitations become important as the available soil water depletion falls below 67% in all species. The responses of beans and cowpea are similar for most of the parameters measured indicating closer performance of the species under dry environments. The variations observed in plant water status, gas exchange and assimilation measurements among the species and the different parameters determined in this study could be used to optimize water supply during irrigation of the crops and to develop grain legume simulation models and/or calibrate the existing ones to suit for dry environments.

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