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Effects of Drought Stress on Growth, Water-Use Efficiency and Leaf Gas-Exchange of Common Bean (*Phaseolus vulgaris* L.) Genotypes Differing in Drought Resistance

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Abstract

Growth, water-use efficiency (WUE) and leaf gas exchange responses of common bean (Phaseolus vulgaris L.) genotypes differing in degree of drought resistance were assessed when plants were subjected to drought stress at vegetative and early pod filling stages. A drought-resistant inbred line (SEA 15) and a drought-susceptible cultivar (BrSp) selected from an earlier screening trial were used for the study. The two genotypes were grown in a vegetation hall under drought stress and non-stress conditions during the summer of 2004. Despite differential genotypic responses found to the stress imposed, drought initiated at both growth stages had an adverse effect on leaf area expansion, above-ground biomass yield and biomass partitioning of the two genotypes. Seed yield reduction due to drought imposed during early pod-fill stage was 72% and 33% for BrSp and SEA 15, respectively. Drought stress at the vegetative stage increased the WUE of both genotypes, although the increase was considerably higher for SEA 15 (3.12 mg g^{-1}*) compared with BrSp (2.45 mg* g^{-1}*). On the other hand, seed yield based water-use efficiency (*WUE_{SY}*) was significantly higher for SEA 15 (1.81 mg* g^{-1}*) compared with BrSp (0.33 mg* g^{-1}*) under drought stress imposed at early pod-fill stage. Drought stress initiated during the reproductive phase significantly reduced the net photosynthetic rate (A) and stomatal conductance (*g_s*) of the two genotypes. Despite the similar response in leaf gas-exchange parameters (A and gs) between the two genotypes, the stress resulted in a significant and consistent decrease in* C_i/C_a *ratio of SEA 15 implying that the stress thresholds at which stomatal and metabolic limitations to A occurred differed between the two genotypes. Some of these specific adaptation traits related to growth, partitioning and water-use can be used in tandem with seed yield as selection criteria in breeding beans for drought conditions.*

Key words: drought stress, leaf gas exchange, *Phaseolus vulgaris*, relative water content, water-use efficiency

Introduction

As much as 60% of the common bean (*Phaseolus vulgaris* L.) production in the developing world occurs under conditions of significant drought stress (Graham and Ranalli, 1997). Consequently, the average global yield of beans remains less than 900 kg ha^{-1} (Singh, 2001). Drought-induced reductions in seed yield vary considerably depending on the timing and intensity of the stress (Shenkut and Brick, 2003). Seed yield-based genotypic differences for drought resistance are also reported for the crop (Terán and Singh, 2002). Past studies have shown that common bean genotypes selected for specific adaptations to drought conditions produce significantly higher seed yield compared with landraces and standard cultivars grown under similar drought conditions.

Shoot biomass accumulation is considered an important trait to attain high seed yield in grain legumes (Saxena *et al*., 1990). Significant differences have been observed for shoot biomass accumulation among dry bean cultivars grown under moderate to severe drought stress conditions (Rosales-Serna *et al*., 2002). Because plant biomass has moderate to high heritability and exhibits low genotype × environment interactions, it has been suggested that the trait could be used as an indirect selection criterion to improve and stabilize seed yield for low moisture areas (Shenkut and Brick, 2003). According to Chaves *et al* (2002), in addition to dry matter accumulation, the ability of genotypes to partition stored vegetative biomass to reproductive organs to a large extent determines sink establishment and economic yield under drought stress.

Under moisture-limiting environments, productivity in crop plants may be increased by improving water-use efficiency (WUE) (Ehleringer *et al*., 1993). To achieve this goal, it is important to identify the factors underlying variations in the WUE since they can either positively or negatively be correlated with productivity depending on the main processes determining changes in WUE (Udayakumar *et al*., 1998). Genotype variation for WUE has been demonstrated in common beans using carbon isotope discrimination ($\Delta^{13}C$) technique (Ehleringer *et al*., 1990). Nevertheless, key physiological traits that offer a potential to improve WUE in common bean are not thoroughly studied. Among the parameters that relate cellular water status to metabolism, relative water content (RWC) is often preferred as it can easily be measured, and is robust indicator of water status (Lawlor and Cornic, 2002). RWC as an integrative indicator of internal plant water status under drought conditions has successfully been used to identify drought-resistant cultivars of several crops including common bean (Costa França *et al*., 2000).

Photosynthesis is the main process responsible for dry matter accumulation that consequently affects plant growth and development, which are strongly affected by the environment. The results of several investigators (Kubiske and Abrams, 1993; Schulze, 1986) indicate that under drought stress plants of a drought-resistant genotype maintained higher rates of photosynthesis and stomatal conductance than plants of a drought-susceptible genotype. The differences in gas-exchange rate between the genotypes may lead to different rates of assimilate synthesis and availability for export to sink organs. In common bean, drought stress at its initial phase limits photosynthesis due to stomatal closure (Miyashita *et al*., 2005). However, as the stress progresses over a longer period, non-stomatal inhibition of photosynthesis may become more important (Lawlor and Cornic, 2002; Medrano *et al*., 2002). Increasing evidence suggests that down-regulation of different photosynthetic processes under drought stress depends more on CO_2 availability in the mesophyll (i.e. stomatal closure) rather than leaf water potential (ψ) or RWC (Medrano *et al*., 2002). Stomatal control is one of the main mechanisms for adapting to water stress in common bean (Laffray and Louguet, 1990). Information on a common pattern of photosynthetic response to drought for common bean is currently meagre.

Generally, understanding the morphological and physiological bases of drought resistance may help target the key traits that limit growth and yield of the crop. To this end, comparing genotypes differing in drought resistance and those released during different periods may serve as the most reliable and sound approach to identify potential traits. We suppose that physiological response to drought stress varies between common bean cultivars developed for wider agro-ecological adaptation and inbred lines selected for specific adaptation. This study was carried out to assess differential responses in growth, water-use efficiency (WUE) and leaf gas exchange of common bean (*Phaseolus vulgaris* L.) genotypes differing in degree of drought resistance when subjected to drought at different growth stages.

Materials and Methods

Plant materials

A drought-resistant inbred line (SEA 15) and a drought-susceptible cultivar (BrSp) selected from an earlier screening trial comprised of three adapted cultivars (Mex. 142, Roba 1 and BrSp) and three inbred lines (SEA 15, SEA 23 and BAT 881) were used in this study. The adapted (old) cultivar (Brown-speckled, hereafter abbreviated as BrSp) was chosen among varieties developed by the national bean research program of Ethiopia for wider adaptations to different agro-ecological conditions of the country whereas the inbred line (SEA 15) was obtained from the bean research program of CIAT.

Experimental treatments and design

Two parallel experiments were carried out at the experiment station of the University of Giessen, Germany during the summer of 2004. The purpose of setting the two experiments was to impose drought stress during different growth phases of the crop. In the first experiment (hereafter referred as the vegetative phase experiment), drought stress was initiated when bean plants attained a growth stage of V6 (plants had six trifoliate leaves). Plants were harvested 5 and 10 days after drought imposition. In the second experiment (hereafter referred as reproductive phase experiment), the stress treatment began at early pod-filling stage (plants had at least one pod that had grown to maximum length). In the later set of experiment, plants were harvested 5, 10 and 20 days after drought stress was initiated. In both sets of experiments, the two genotypes were grown under drought stress and non-stress conditions. Drought stress was imposed by withholding the amount of water applied in order to keep the soil moisture level at about 30% of the maximum water-holding capacity (WHC). For non-stressed (control) treatments, the soil moisture was maintained at 70% of the maximum WHC until the plants were harvested.

Seeds of the test genotypes were grown in *Ahr* pots filled with 13 kg of Kleinlindener soil. At the time of planting, the soil was fertilized with Blaukorn (12.0% N, 5.2% P, 14.1% K, 1.2% Mg and 6.0% S). Eight seeds per pot were initially sown and later thinned to four plants when the first trifoliate leaves were unfolded. Plants were raised in a vegetation hall. The pots were weighed daily and watered to restore the appropriate moisture by adding a calculated amount of water. Additions of water (equivalent to the amount of water lost) to each pot were recorded daily to calculate the total water consumed (kg plant^{-1}) by the genotypes under contrasting soil moisture regimes. For both experiments, the treatments were laid in a completely randomized designed with four replications.

Data collection

Biomass and seed yield: Plants were harvested 5 and 10 days (d) after the imposition of the stress (for the vegetative phase experiment) and 5, 10 and 20 days after the initiation of drought at pod-filling stage. Above-ground dry weight was obtained by adding up various plant parts (leaves, stems, pod walls and seeds) harvested separately and dried at 80 °C for 48 h. Biomass partitioning ability of the genotypes was evaluated by computing the ratio of reproductive structures (pods/pod walls + seeds) to vegetative biomass (leaf + stem dry weight). Seed yield (g plant^{-1}) was calculated as a product of the yield components (number of productive pods per plant, number of seeds per pod and seed weight). Harvest index (HI) was calculated as the proportion of seed weight to the above-ground dry weight (stem + leaves + pod + seed) at harvest.

Water-use efficiency and leaf-water relations: Water-use efficiency (WUE, mg g^{-1}) of the bean plants during the vegetative phase was calculated according to the following formula adapted from Anyia and Herzog (2004):

$$WUE = (w_2 - w_1) / T$$

where w_1 and w_2 are the total dry weights at the end of 5 and 10 days stress, respectively, and
T is the total amount of water used for transpiration between the first and the second harvest.

Instantaneous water-use efficiency (IWUE, µmol mol^{-1}) was calculated as the ratio of net photosynthetic rate (A) to stomatal conductance (g_s) determined during the reproductive phase.

Leaf growth and water relation parameters were determined on young expanding trifoliate leaves. During the vegetative phase, these leaves were located at the 7th and 8th (5 d stress) and at the 8th and 9th (10 d stress) main stem nodes of BrSp and SEA 15, respectively. The central leaflets of the selected trifoliate leaves were cut and fresh weight (FW) taken immediately. The weighed leaves were then placed in a petri-dish containing wet filter paper and kept in the dark. After 24 h, the turgid weight (TW) was obtained. For the dry weight (DW), the leaflets were oven-dried at 80 °C for 48 h. The second leaflet from the same trifoliate leaf used for fresh weight determination was cut and the leaf area (LA) was measured using AM200 leaf area meter (ADC BioScientific Ltd., UK). Leaf relative water content (RWC, %) was calculated as follows:

$$RWC = [(FW-DW) / (TW-DW)] \times 100;$$

where FW, DW, TW and LA are the fresh weight, dry weight, turgid weight and leaf area, respectively.

Photosynthetic parameters: Gas-exchange characteristics, net photosynthetic rate (A), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were measured on the central leaflets of fully-matured upper canopy leaves of both stressed and non-stressed treatments using a portable photosynthesis system (Li-COR LI-6200, Li-Cor, Inc., Lincoln, NE) assembled with an infra-red gas analyzer (Li-COR LI-6250) and data logger. Measurements were made on the 5th and 10th days of the stress imposition during the vegetative phase experiment. Five measurements were made during the reproductive phase beginning on day two of the stress imposition and continued on alternate days until 10th day. Leaf gas-exchange measurements were initiated (usually between 09.30 and 13.30 h) at ambient relative humidity and temperature, when CO_2 concentration in the 0.25 L leaf chamber approached ambient concentration. When the photosynthetically active radiation (PAR) was below 800 µmol m^2 s^{-1}, leaflets were illuminated by a light source to maintain a saturating irradiation of up to 1200 µmol m^{-2} s^{-1} PAR.

Data analysis

Data were analyzed using the MSTAT-C statistical package (MSTAT-C, 1989). Data were subjected to analysis of variance to assess the significance of differences among treatments for various parameters. Means of the treatments that exhibited significant differences were separated using the least significant difference (LSD) test. The differences between control and drought-stressed treatment means were tested for statistical significance using the t-test. Relationships between selected parameters were determined using the Pearson's simple correlation test. For all analyses, $P \leq 0.05$ was used for declaring statistical significance

Results and Discussion

Effects on yield, growth and biomass partitioning

Drought stress imposed during early pod-filling stage significantly reduced seed yield of BrSp and SEA 15 by 72% and 33%, respectively (Table 1). A comparable higher drought-induced reduction in seed yield was also found in a previous study for other adapted old cultivars compared with the inbred lines. A marked effect of the stress on harvest index of the susceptible cultivar was also found (Table 1). Under drought stress, SEA 15 had higher (1.81 mg g^{-1}) seed yield-based WUE (WUE_{SY}) than BrSp (0.33 mg g^{-1}).

The inbred line SEA 15 gave higher yield under both growth conditions. This was in accordance with its superior performance found under field conditions in selected drought-prone areas of South and Central America (CIAT, 2002). A cultivar from the race Durango, represented by SEA 15 in the present study, had previously been reported to possess significant levels of drought tolerance owing to its evolutionary origin in semi-arid and semi-humid regions of the Mexican highlands (Terán and Singh, 2002).

Drought stress initiated during the vegetative as well as reproductive phases significantly reduced above-ground dry weights of the two genotypes (Table 2). When the plants were subjected to the stress during the vegetative phase, drought-induced reductions in above-ground dry weights were higher for SEA 15 (36–40%) than for BrSp (24-33%) (Table 2). On the other hand, the reduction for the same parameter due to drought imposed at pod-filling stage was comparable between the genotypes (24 - 33% for BrSp *versus* 17-29% for SEA 15). Under drought as well as non-stress growth conditions, SEA 15 maintained higher reproductive (pods + seeds) to vegetative (leaves + stems) biomass ratio than BrSp (Fig. 1).

Table 1 The effect of drought stress imposed at early pod-filling stage on seed yield, harvest index and seed yield based water-use efficiency (WUESY) of the two genotypes

Genotype	Seed yield (g plant $^{-1}$)		Harvest index (%)		WUE_{SY} (mg DM g^{-1} H_2O)	
	Control	Stress	Control	Stress	Control	Stress
BrSp	8.4±0.9	2.4±0.5**	28.4±2.4	13.5±2.2*	0.74±0.07	0.33±0.06**
SEA 15	20.5±0.6	13.8±1.4**	64.0±1.2	63.4±4.2	2.01±0.06	1.81±0.19

*, ** Indicate significant differences between drought stressed and control treatments at 5 and 1% levels of probability, respectively, according to t-test. Data are means±S.E. of four replications.

Biomass reduction due to drought stress imposed during the vegetative phase was proportional to the drought-induced impairment of leaf area measured on individual leaves of the genotypes (Fig. 2 and Table 2). The higher rate of decrease in leaf area caused higher degrees of reduction in total leaf area and total leaf biomass weight per plant in SEA 15 compared with BrSp. The results of this study concur recent reports of Turtola *et al*. (2006) on *Salix* species in which higher biomass reduction was found for fast growing species than for slow growing counterparts when subjected to drought stress at early growth stage. Moreover, diversion of biomass to plant parts other than leaves is considered as an adaptational response to drought stress of resistant genotypes during early growth stage (Fernández *et al*., 2002). Furthermore, the restriction in leaf area expansion is often considered as an avoidance mechanism employed to limit further water losses when the stomata are closed (Ruiz-Sanchez *et al*., 2000).

In contrast to the vegetative phase, the decrease in above-ground biomass due to drought initiated at pod-filling stage was comparable between the genotypes except at 20 days stress (Table 2). The significant decrease in total above-ground biomass yield of SEA 15 at the final harvest was accompanied by a remarkable increase in reproductive to vegetative biomass ratio (Fig. 1). On the other hand, continued vegetative growth after the start of the reproductive phase resulted in relatively lower reproductive to vegetative biomass ratio for BrSp. These results demonstrate that the drought-susceptible genotype (BrSp) has lower sink strength than SEA 15. According to Zhang *et al*. (2005), mobilization of reserves is dependent on sink strength, which varies with the genotype, and is affected by the environment (e.g. water availability). In line with this, that the mechanisms underlying differences in drought resistance of the bean genotypes could be partly related to the selections made for efficient biomass partitioning to reproductive structures rather than biomass accumulation ability *per se.*

Table 2. The effect of drought stress imposed at vegetative and early pod filling stages on above-ground dry weights (g plant $^{-1}$) of two common bean genotypes

Duration of stress (days)	Treatment		Growth phase	
			Vegetative	Reproductive
5	Br Sp	Control	6.1±0.2 ^{c}	27.3±0.5 ^{a}
		Stress	5.1±0.2 ^{c}	20.9±0.9 ^{b}
	SEA 15	Control	11.6±0.8 ^{a}	24.9±1.0 ^{a}
		Stress	7.4±0.2 ^{b}	20.7±1.4 ^{b}
10	Br Sp	Control	9.9±0.4 ^{b}	28.5±0.6 ^{a}
		Stress	7.0±0.5 ^{c}	20.3±0.8 ^{b}
	SEA 15	Control	17.8±0.8 ^{a}	30.7±1.8 ^{a}
		Stress	10.7±0.1 ^{b}	21.7±0.6 ^{b}
20	Br Sp	Control	-	35.4±0.7 ^{a}
		Stress	-	23.7±1.3 ^{b}
	SEA 15	Control	-	24.3±0.6 ^{b}
		Stress	-	17.8±0.5 ^{c}

Means within the same column and for the same duration of stress followed by similar letters are not significantly different according to LSD test at P≤0.05. Data are the means±S.E. of four replications

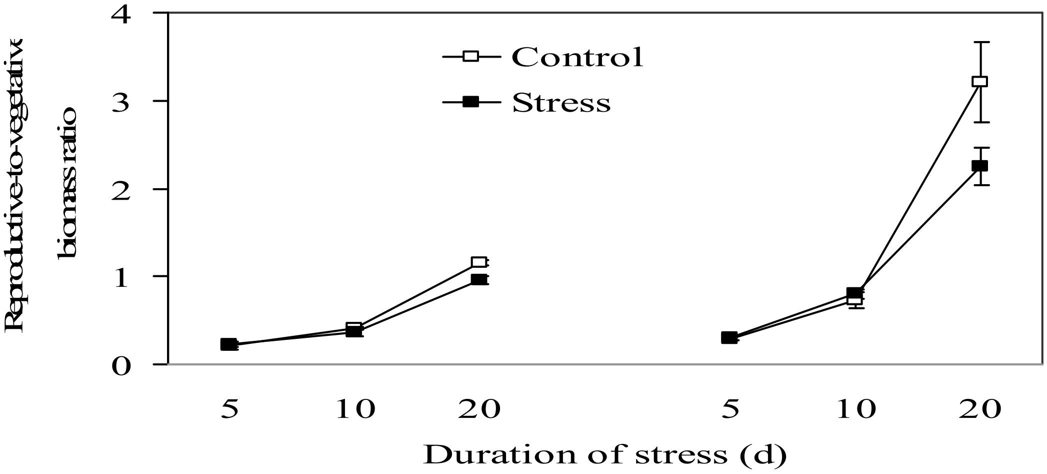

Fig. 1. The effect of drought stress initiated at early pod-filling stage on reproductive to vegetative biomass ratio of two genotypes. Vertical bars show ±S.E. of four replications.

The main effects due to genotypes and soil moisture regimes were significant for leaf area determined during the vegetative growth phase of the crop. SEA 15 responded to the stress imposed with an enormous (about 65%) leaf area reduction relative to the control treatment at both sampling times compared with BrSp, with only about 40% reduction (Fig. 2).

Effects on leaf water relations and water-use efficiency

Relative water content (RWC), as a key reference parameter of leaf water status, exhibited a positive and significant correlation with net photosynthetic rate (r = 0.54, $p < 0.05$) and stomatal conductance (r = 0.57, $p < 0.01$) during the reproductive phase. However, the degree of association between RWC and net photosynthetic rate (*A*) was smaller (R^2=0.33, p<0.01) as compared with the relationship between *A* and stomatal conductance (g_s) ($R^2 = 0.89$, $p < 0.01$).

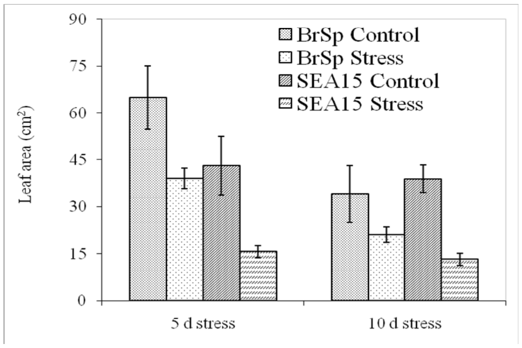

Fig. 2. The effect of drought stress imposed at vegetative stage on leaf area of the two genotypes. Means followed by the same letter during the same duration of stress are not significantly different according to LSD test at P≤0.05. Vertical bars are ± S.E. of four replications.

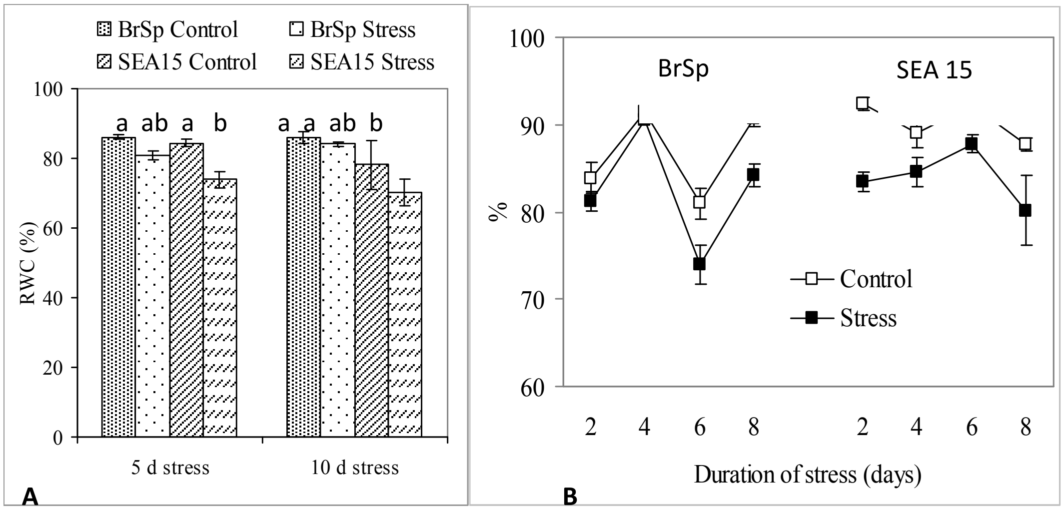

Fig. 3. Leaf relative water content (RWC) of the two genotypes under drought stress and non-stress growth conditions during vegetative (A) and reproductive (B) growth phases. Means within the same duration of stress followed by the same letter are not significantly different according to LSD at P≤0.05. Vertical bars are ± S.E. of four replications.

When plants of both genotypes were subjected to drought stress, the leaf dehydration (expressed by leaf relative water content, RWC) was smaller for BrSp than for SEA 15 (Figs, 3A and B). This is in contrast to the findings of Costa Franća *et al*. (2000) which disclosed higher leaf tissue water retention capacity by a drought-resistant cultivar compared with a susceptible genotype. The bean genotypes exhibited marked differences in terms of the response of stomatal conductance (g_s) and photosynthetic rate (A) to leaf RWC. i.e. the role of leaf water status in driving stomatal closure under drought stress appeared to differ between the two bean genotypes. The decrease in leaf RWC of the drought-resistant genotype, SEA 15, was accompanied by a smaller decrease relative to BrSp in g_s and A (Figs, 6A and B) implying that leaf water status had a leading (feedback control) role over stomatal closure. Leaf gas-exchange parameters (g_s and A) of BrSp decreased earlier (at 2 days stress) than the decrease in RWC, which was detected only 6 days after drought stress was initiated (Fig. 3B). According to Schulze (1986), closure of stomata under dehydrating conditions could result either from a feedback response to the generation of water deficits in the leaf itself that is transmitted to the guard cells, or from a feed-forward control before any alteration in leaf tissue water status takes place (perhaps the case with BrSp here). The result of this study supports the proposal of Flexas *et al.* (2004) that g_s rather than RWC is a more reliable indicator of the level of stress in plant leaves; hence, g_s determined the rate of photosynthetic rate under drought stress more than RWC did.

The effects of genotypes and soil moisture supply regimes were highly significant for water-use efficiency (WUE, mg dry matter produced per g water used) determined at vegetative growth stage. Drought stress imposed during the same period increased WUE by about 35% and 37% for BrSp and SEA 15, respectively (Fig. 4B). Nevertheless, the increase in WUE owing to drought stress during the vegetative phase was significantly higher for SEA 15 (3.12 mg g^{-1}) compared with BrSp (2.45 mg g^{-1}).

The main effects due to genotype and watering regime were highly significant for photosynthetic (instantaneous) water-use efficiency (IWUE, ratio of net photosynthetic rate to stomatal conductance). Relative to control treatments, the IWUE of SEA 15 increased under drought stress by about 29% (average of five sampling dates) (Fig. 5). On the other hand, drought-induced increase in IWUE of BrSp (by about 12%) was not significantly different from the control treatment. IWUE exhibited closer association with stomatal conductance (g_s) ($R^2 = 0.42$, $p < 0.01$) rather than with net photosynthetic rate (A) ($R^2 = 0.20$, $P < 0.05$).

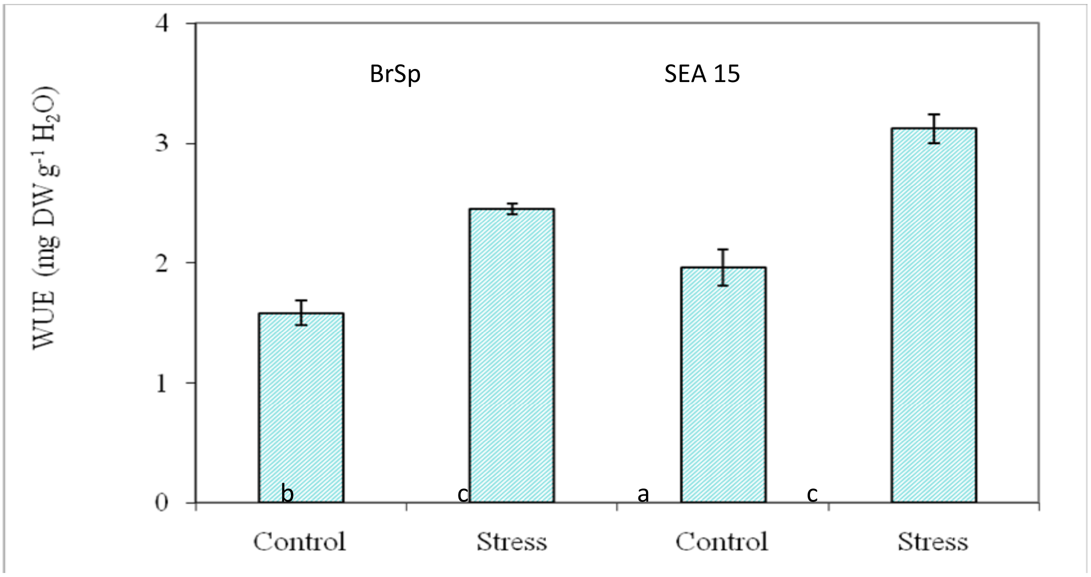

Fig. 4. Water-use efficiency of the two genotypes 10 days after drought stress was imposed at the vegetative growth stage. Means followed by the same letter are not significantly different according to LSD test at P≤0.05. Vertical bars are ± S.E. of four replications.

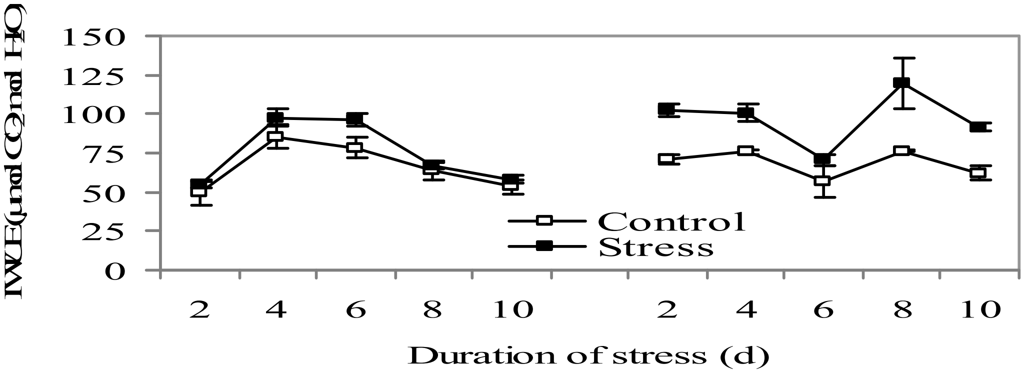

Fig. 5. Instantaneous water-use efficiency (IWUE) of two common bean genotypes grown under drought stress imposed at reproductive stage and non-stress growth conditions. Bar graphs on the right side are average measurements of five sampling dates. Vertical bars are ± S.E. of four replications. *, ** The differences between drought-stressed and non-stressed treatments are significant at 5 and 1% levels of probability, respectively, according to t-test.

The results of this study showed that substantial improvement of WUE_{SY} occurred in inbred line (SEA 15) selected for specific adaptation to drought stress compared with the old adapted cultivar (BrSp). The higher WUE_{SY} under both soil moisture supply regimes for the inbred line was associated with higher harvest index and relatively smaller quantity of water consumed during the entire growth period. Consistent with higher WUE_{SY} found for the inbred line over the adapted bean cultivar in the present study, Siddique *et al*. (1990) reported similar differences between modern and old wheat cultivars. In both improved (modern) bean and wheat genotypes, higher WUE_{SY} was augmented by two key features such as early flowering and the subsequent use of a larger proportion of available water for the maintenance of reproductive growth.

Relative to the non-stressed treatments, water consumption per unit leaf area for drought-stressed (imposed at vegetative stage) BrSp and SEA 15 was about 58% and 44%, respectively. This implies that SEA 15 has much greater transpirational water control than BrSp under drought situations. Faster and higher vegetative biomass accumulation of SEA 15 was achieved through luxurious consumption of water when grown under non-limiting soil moisture supply regime (Fig. 4A). When the genotype was subjected to drought during the same growth phase, biomass yield (weight) and water consumption were much more depressed in SEA 15 than in BrSp. This demonstrates that the drought-resistant genotype was an ‘opportunist’ in relation to available water, having higher rates of transpiration and growth when soil moisture was adequate but having marked reductions of both water loss and growth when soil moisture was limiting. Higher correlation of IWUE with g_s than with A implied that drought-induced increase in water-use efficiency of the bean genotypes was attained mainly due to efficient stomatal closure as a water conservation strategy. The predominance of stomatal control of IWUE over that by carbon assimilation capacity has been reported for several crops (e.g. Anyia and Herzog, 2004).

Effects on leaf gas-exchange

Drought stress initiated during the reproductive phase significantly reduced the net photosynthetic rate (A) and stomatal conductance (g_s) of the two genotypes (Fig. 6A and B). The decreases in both parameters (A and g_s) owing to drought stress were observed at all sampling times during the course of the stress. Although significant differences were not found between the genotypes, the average reduction of A across the stress period was higher for the drought susceptible genotype (BrSp, about 62%) as compared with the drought tolerant genotype (SEA 15, only about 50% reduction relative to control plants) (Fig. 6A). Similarly, g_s for drought-stressed plants of both genotypes decreased by about 40% relative to the corresponding control plants (Fig. 6B). The correlation of the two gas-exchange parameters, A and g_s, was high and significant ($r = 0.95$, $p<0.01$).

The strong correlation detected between A and g_s under drought conditions suggests that drought-induced decline in A was largely a consequence of stomatal limitation. Such robust association between the two variables, A and g_s, is commonly reported implying that the decrease in g_s is the dominant factor responsible for the decline in A until drought conditions become very severe (Monneveux *et al*., 2006; Lawlor and Cornic, 2002). Moreover, such a close correspondence can also be the consequence of the co-regulation of both parameters in response to drought (Osório *et al*., 2006). Drought-sensitivity of gas-exchange was comparable between the two genotypes (Fig. 6). This is in contrast to previous observations, where different rates of *A* and g_s were reported among drought-resistant and susceptible bean genotypes (Wentworth *et al*., 2006). Only for BrSp, drought-induced reduction in *A* corresponded with a decrease in leaf sugar concentrations (data not presented). Therefore, in line with direct associations often reported between the two parameters (Amede and Schubert, 2003), the shortage of assimilates resulting from reduced carbon assimilation could be responsible for the decrease in growth and yield of this genotype under drought stress.

Despite comparable effects of drought stress on g_s of the two bean genotypes, significant decrease in leaf intercellular CO_2 concentrations (C_i) due to the stress was found only for SEA 15 (data not shown). Because the ambient CO_2 concentration (C_a) during measurements changes, C_i/C_a ratios instead of C_i are presented (Fig. 7). Corresponding with the C_i levels, drought-induced reduction of the ratio C_i/C_a was higher and more consistent for SEA 15 than it was for BrSp (Fig. 7).

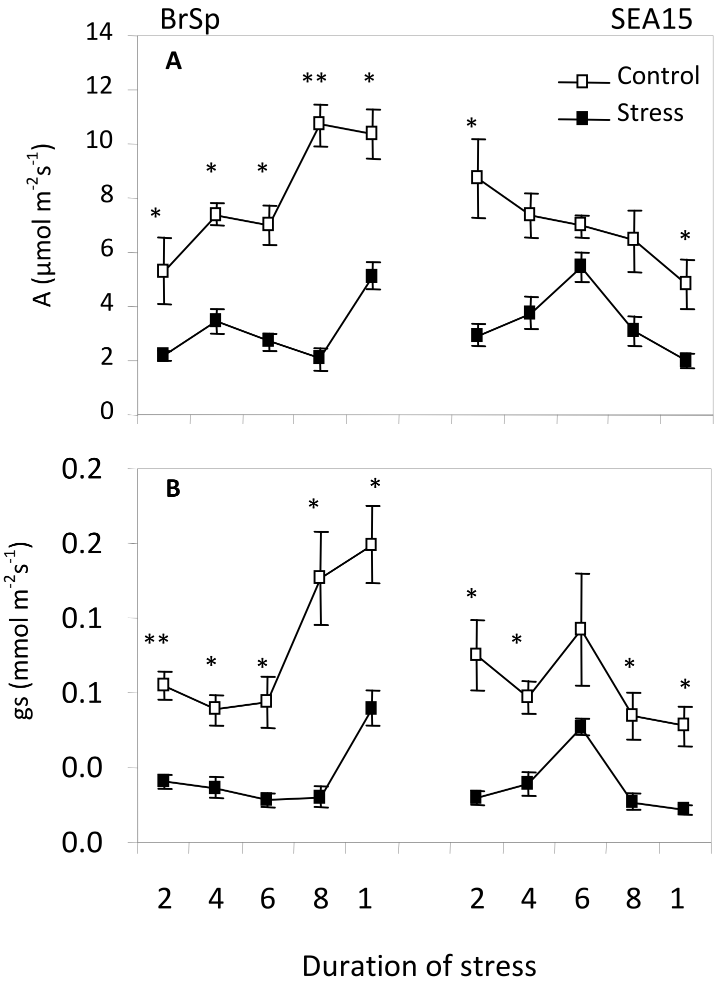

Fig. 6. The effect of drought stress imposed at early pod-filling stage on net photosynthetic rate (A) and stomatal conductance (B) of two genotypes. *, ** indicate significant (t-test) differences between drought stressed and control treatments at P≤0.05 and P≤0.01, respectively, according to t-test. Vertical bars are ± S.E. of four replications.

Although stomatal closure appears to be one of the predominant factors limiting *A* as observed in other C_3 plants subjected to comparable intensity of drought stress we used (Flexas *et al*., 2004, Lawlor and Cornic, 2002), non-stomatal inhibition of *A* was also evident from the ratio C_i/C_a (Fig. 7). A lower ratio of C_i/C_a under drought stress relative to the control treatment for SEA 15 suggests that the decline in *A* was due to limited availability of CO_2 caused by stomatal closure. Contrary to this, the decrease in g_s due to drought was not accompanied by reduced C_i available for photosynthesis (i.e. the ratio C_i/C_a was unaffected) for BrSp. According to Lawlor and Cornic (2002), C_i levels similar or higher in values under drought stress relative to control treatments imply metabolic limitations to *A* caused by non-stomatal effects under drought conditions. However, there are questions about whether assessments of metabolic limitations based on C_i analysis are reliable under drought. Two main problems have been described related to C_i calculations in stressed leaves: patchy stomatal closure (Buckley *et al*., 1997); and the increase of the relative importance of cuticular transpiration when stomata are closing in drying leaves (Boyer *et al*., 1997). Assuming that uniform stomatal closure did occur in response to drought in the bean plants due to slow imposition of the stress as has been observed by Gimenez *et al*. (1992), the difference in the ratio C_i/C_a found between the bean genotypes appears to imply that the stress thresholds at which stomatal and metabolic limitations to *A* occur varied between the two common bean genotypes.

The difference in photosynthetic change under drought stress observed between the bean genotypes, therefore, may be useful in identifying drought-resistant cultivars. While photosynthetic inhibition due to stomatal closure is largely reversible upon re-watering, metabolic inhibition involves an impairment of biochemical processes, which may retard CO_2 fixation even after recovery (Loreto *et al*., 1995) or even cause irreversible effects leading to death of the leaf tissues (Lawlor and Cornic, 2002). This could, therefore, be an important consideration in determining the ability of plants to withstand drought. Although genotypic differences were not significant for photosynthesis inhibition under similar levels of drought stress, the causes that have led to inhibition of photosynthesis may differ and as such may have significant consequences with respect to the productivity of the bean genotypes under water limiting conditions.

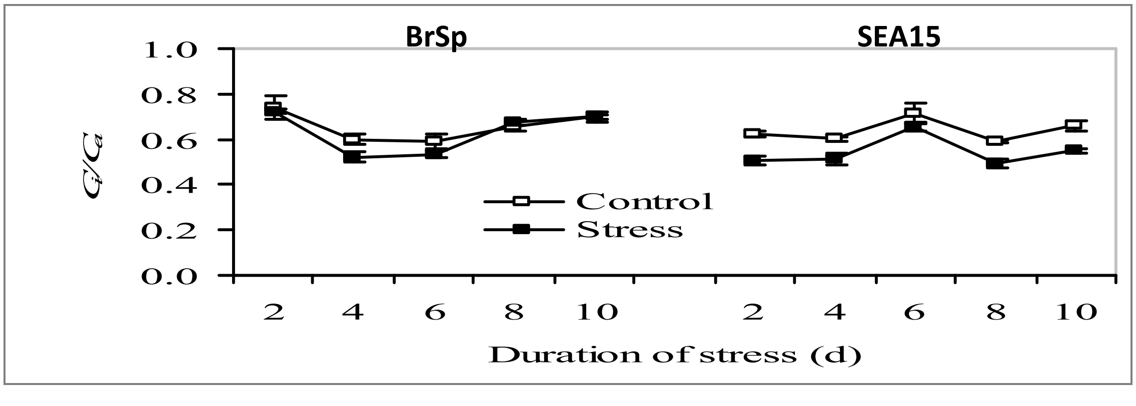

Fig. 7. The effect of drought stress imposed at early pod-filling stage on the ratio of leaf intercellular to ambient CO_2 concentration (C_i/C_a) of the two genotypes. *, **indicate significant differences (t-test) between drought stressed and control treatments at $P \leq 0.05$ and $P \leq 0.01$, respectively, according to t-test. Vertical bars are ± S.E. of four replications.

Conclusions

Drought stress imposed at different growth stages of the crop generally had an adverse effect on growth, water-use efficiency and leaf gas exchange parameters of the two genotypes. Nevertheless, significant differences were found between the adapted cultivar released for wider adaptation (BrSp) and the inbred line developed for specific adaptation to drought conditions (SEA 15) in their response to the stress imposed. Higher yield produced by the resistant genotypes under drought stress was related to the maintenance of the ability to partition vegetative biomass to reproductive structures as demonstrated by significantly higher reproductive to vegetative biomass ratio and harvest index relative to the susceptible cultivar.

Higher water-use efficiency of SEA 15 under drought conditions was attributed to higher biomass and seed yield produced with less water consumed compared with BrSp. Differences observed for leaf gas exchange characteristics demonstrated that the causes and stress threshold levels for drought-induced inhibition of photosynthesis were different between the two genotypes. Along with seed yield, these specific adaptation traits related to growth, partitioning and water-use can be used as selection criteria in breeding beans for drought conditions.

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