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## ADAPTATION TRAITS IN DRY BEAN CULTIVARS GROWN UNDER DROUGHT STRESS\*

### CARACTERÍSTICAS DE ADAPTACIÓN EN VARIEDADES DE FRIJOL BAJO SEQUÍA

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#### ABSTRACT

Drought is the major constraint to common bean (*Phaseolus vulgaris* L.) production in Mexico. The objective of this study was to identify physiological and phenological traits related to drought adaptation in common bean. A field experiment was conducted under a rainout shelter at The Valley of Mexico Experimental Station near Texcoco, State of Mexico. Eight common bean cultivars from different genetic races and growth cycle and contrasted drought response were tested under drought stress and non-stress. Irrigation was withheld 55 days after sowing at the initiation of flowering, to induce the stressed treatment. Starting at this day, six consecutive nondestructive samplings were conducted at noon every other day. In each sampling, leaf water potential, stomata conductance and CO<sub>2</sub> assimilation rate were determined. Data on phenology were also recorded. At physiological maturity, seed yield and shoot biomass were measured. Harvest index was calculated. All cultivars exhibited a tendency to escape drought effects throughout accelerated reproductive development. This response was of small magnitude in Mesoamerican cultivars Negro Cotaxtla 91 and BAT 477 (type III) and significant in cultivars from the Durango race such as Pinto Zapata, Bayo Madero and Bayo Criollo del Llano. Significant difference among cultivars for stomata control was

observed with high sensitivity in BAT 477 and SEQ 12, cultivars from the Mesoamerican race and in ICA Palmar from Nueva Granada race. Bred and distinct cultivars ICA Palmar (type I) and Pinto Villa (type III) from Durango race, displayed high photosynthetic rate and harvest index, traits or mechanisms directly related to seed yield under stress and non-stress conditions.

**Key words:** *Phaseolus vulgaris* L., biomass, physiological traits, seed yield.

#### RESUMEN

La sequía es el factor que más limita la producción de frijol (*Phaseolus vulgaris* L.) en México. El objetivo del presente trabajo fue identificar características fisiológicas y fenológicas relacionadas con la adaptación a la sequía en este cultivo. Se estableció un experimento bajo una cubierta de plástico en el Campo Experimental Valle de México en Texcoco, Estado de México, México. Se evaluaron ocho variedades de frijol de diferente raza genética y hábito de crecimiento y contrastantes en su respuesta a la sequía, bajo dos condiciones: riego durante todo el ciclo y sequía. En sequía, el riego se suspendió a

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partir del inicio de la floración. A partir de esta etapa, se realizaron seis muestreos de hojas, en las que se determinaron potencial hídrico, conductividad estomática y tasa de asimilación de CO<sub>2</sub>. Se registraron datos fenológicos. A la madurez fisiológica se determinó el rendimiento de semilla y la biomasa del vástago. Se calculó el índice de cosecha. Las variedades mostraron tendencia de escape a la sequía mediante el desarrollo acelerado en la etapa reproductiva. Esta respuesta fue de menor magnitud en variedades de la raza Mesoamericana Negro Cotaxtla 91 y BAT 477 (tipo III) y fue más notoria en variedades de la raza Durango Pinto Zapata, Bayo Madero y Bayo Criollo del Llano (todas tipo III). Se observaron diferencias significativas entre variedades para el control estomático; se observó más alta sensibilidad en BAT 477 y SEQ 12, variedades de la raza Mesoamericana y en ICA Palmar, variedad de la raza Nueva Granada (tipo I). Las variedades ICA Palmar y Pinto Villa (tipo III) de la raza Durango, mostraron altos valores de tasa fotosintética e índice de cosecha, características directamente relacionadas con el rendimiento en condiciones de estrés hídrico y de no estrés.

**Palabras clave:** *Phaseolus vulgaris* L., biomasa, características fisiológicas, rendimiento de semilla.

## INTRODUCTION

Most dry bean production in the world takes place under rainfed conditions and drought due to insufficient or unpredictable rainfall limits yield. Nearly 60% of bean production occurs in agricultural land prone to water deficit, where the costs of irrigation or the lack of precipitation are major difficulties for producers (Graham and Ranalli, 1997). Consequently, there is an increasing need to improve drought tolerance in common bean cultivars, where adaptive mechanisms to cope with drought stress include traits such as root architecture, growth habit, maturity acceleration, early flowering, shoot biomass accumulation and efficient assimilate redistribution towards seeds, contributing to an increased harvest index (Terán and Singh, 2002; Rosales-Serna *et al.*, 2004). Since seed yield is the main economic trait in common bean, the most practical way to screen for drought tolerant genotypes is the quantification of seed production, expressed as mean seed yield (Terán and Singh, 2002).

Differences in seed yield among common bean cultivars under drought stress can be associated with physiological

and biochemical responses, such as tissue water retention, osmotic adjustment, integrity of membrane system, protease activity and stomata adjustment (Costa Franca *et al.*, 2000; Hieng *et al.*, 2004; Lizana *et al.*, 2006).

In the highlands of Mexico, where water deficit is the main constrain for bean production, there is a bean-breeding program. New common bean cultivars have been developed through selection and incorporation of physiological, phenological, morphological and yield traits for drought tolerance (Beaver *et al.*, 2003). High drought tolerance levels have been found in bean cultivars from semiarid regions of Mexico; for example, in cultivars belonging to the Durango race (Teran and Singh, 2002). One of these is Pinto Villa, cultivar that has one of the highest mean seed yield under stress among a large selection of common bean genotypes (Teran and Singh, 2002).

In order to launch a successful common bean-breeding program for drought adaptation, the knowledge of physiological mechanisms involved in drought tolerance is important. Therefore, the objective of this research was to identify physiological and phenological traits such as leaf water potential, stomatal conductance, leaf assimilation rate, shoot biomass and seed yield of eight dry bean cultivars under drought stress and non-stress conditions.

## MATERIALS AND METHODS

### Location and experiment layout

The study was conducted at the Valle de Mexico Experimental Station, near Texcoco, State of Mexico (19° 20' N and 2 240 masl) during the summer season in 2000. The experiment was established under a rain-out shelter on June 11 in a soil classified as Eutric cambisol (FAO, 1989). The experimental unit was a single row 5.0 m in length with 52 cm between rows. Fertilizer was applied at sown at a rate of 40-40-00 units/ha of N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O<sub>5</sub>, respectively at 10 cm depth beside the row. The experiment was kept weed and pest free during the growing cycle. At fifty five days after sowing (DAS), two treatments of soil moisture were applied, one was well-watered throughout the growing cycle while in the other irrigation was withheld until the soil reached permanent wilting point (PWP) at a depth of 0-60 cm, monitored through the gravimetric method. Five days after PWP was reached (73 DAS), irrigation was reestablished and it continued until the physiological maturity of the cultivars.

Soil moisture content was determined ten times every other day during the water stress period. In order to measure soil moisture, soil samples were taken, weighted and immediately dried in at 120 °C for 48 h. The soil moisture retention curve was previously determined. A randomized complete block design arranged as split plot with three replicates was utilized, irrigation treatments were randomized in the main plots and cultivars in the subplots.

## Germplasm

Most of the cultivars utilized in this research were previously classified as drought resistant according to their yield response under rainfed and drought stressed conditions at multiple locations. With the exception of Bayo Criollo del Llano, the rest of the cultivars are bred cultivars (Table 1).

**Table 1. Racial classification and agronomic traits of eight dry bean cultivars.**

Cultivar	Race	Growth habit	Drought response
Negro Cotaxtla 91	Mesoamerican	II	Susceptible
BAT 477	Mesoamerican	III	Resistant
SEQ 12	Mesoamerican	III	Resistant
Pinto Zapata	Durango	III	Resistant
Pinto Villa	Durango	III	Resistant
Bayo Criollo del Llano	Durango	III	Resistant
Bayo Madero	Durango	III	Susceptible
ICA Palmar	Nueva Granada	I	Resistant

## Physiological traits

Measurements of leaf water potential, leaf stomatal conductance and leaf assimilation rate were performed at 55, 57, 59, 61, 63 and 65 DAS. Determinations were made at noon. Leaf stomatal conductance and leaf assimilation rate were determined on the terminal leaflet of the youngest fully expanded trifoliolate leaf in five irrigated and water stressed different plants with a portable open gas analyzer system (CIRAS-1, PPSYSTEMS). Leaf water potential was determined in five different leaves after the petiole was cut at 5 cm from the base and immediately inserted into a pressure chamber, Scholander type (Soil Moisture Equipment, Corp. Santa Barbara, CA.). The pressure in the chamber was raised slowly until a pressure balance was reached (xylem water potential) and recorded.

## Agronomic traits

Days to flowering (DF) was recorded when 50% of the plants in a specific plot had at least one open flower; days to physiological maturity (DPM) was recorded when 90% of the pods in 50% of the plants lose their green pigmentation. From those traits the number of days of seed filling was calculated (DSF= DPM-DF).

At harvest, seed yield and shoot biomass were determined on plot basis (g m). All plant parts, except seeds, were

oven-dried at 70 °C for 72 h. Harvest index was calculated (HI= seed yield/total biomass, excluding roots and fallen leaves). The drought intensity index [DII= 1-(Xd/Xp)] was also calculated, where Xd is the mean yield under drought and Xp is the mean yield under non-stress (Fischer and Maurer, 1978).

## Data analysis

Phenological traits, seed yield, shoot biomass and harvest index were analyzed as a factorial with split-plot arrangement with soil moisture treatments as main plots and cultivars as subplots with the MSTATC Program for microcomputers (Freed *et al.*, 1991). Individual analyses of variance for each irrigation treatment were conducted for the rest of measured and calculated traits.

## RESULTS AND DISCUSSION

### Drought intensity

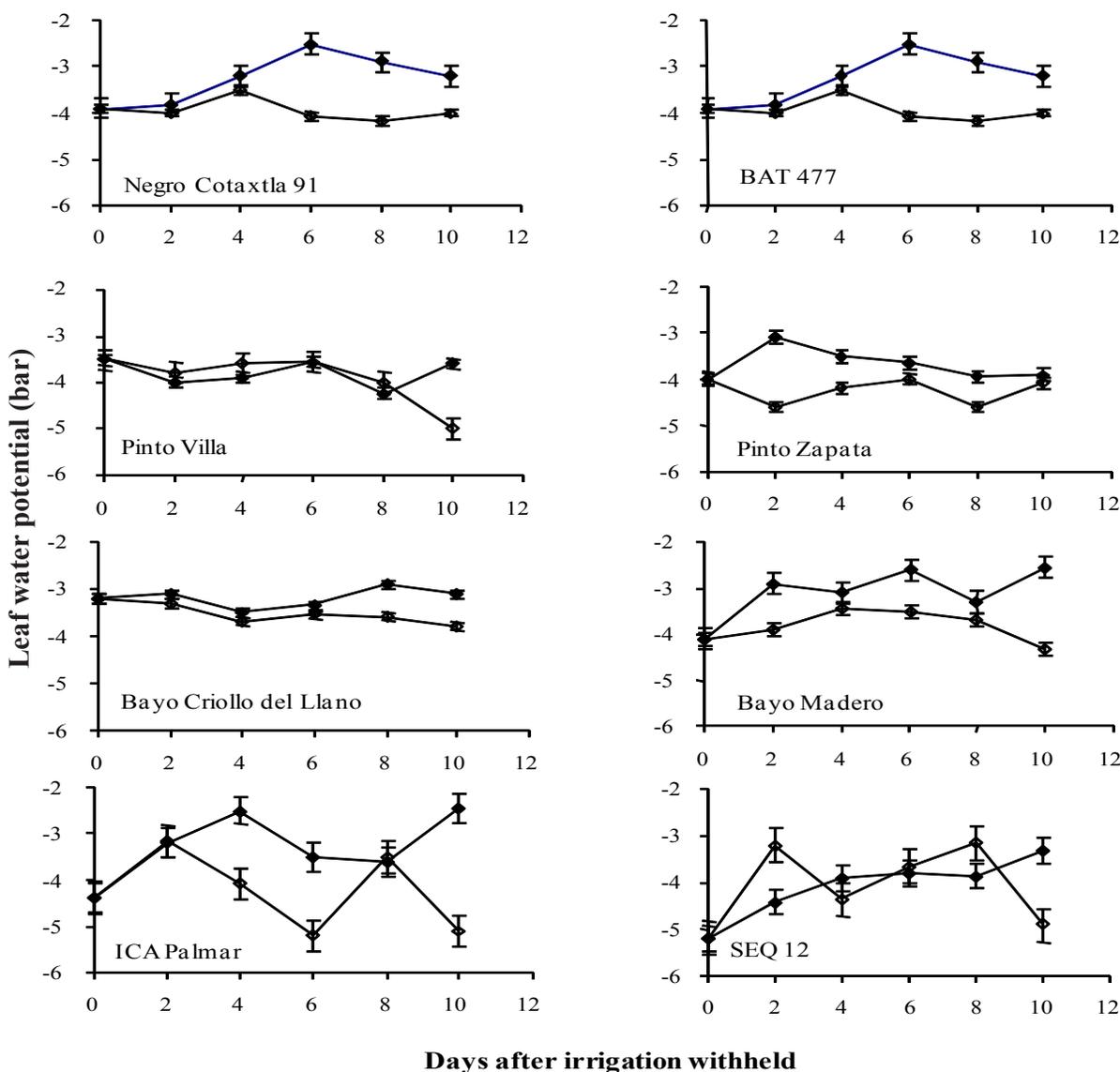
The drought treatment showed a reduction in yield in all cultivars compared with the full irrigated treatment; the reduction represented by DII= 0.50 value. This stress was comparable with previous experiments conducted with beans under rain-fed conditions at the highlands of Mexico (DII= 0.49, Schneider *et al.*, 1997; 0.48, Rosales-Serna *et*

*al.*, 2004) but was smaller than the reported under a rain-shelter controlled drought treatment at Michigan (DII=0.63, Ramirez-Vallejo and Kelly, 1998).

**Physiological traits**

Physiological traits were measured during the first ten days after irrigation was withheld. A differential effect of water stress was observed among bean cultivars. Leaf water potential fluctuated between -3 to -5 bars for both non-stressed and stressed treatments. In some

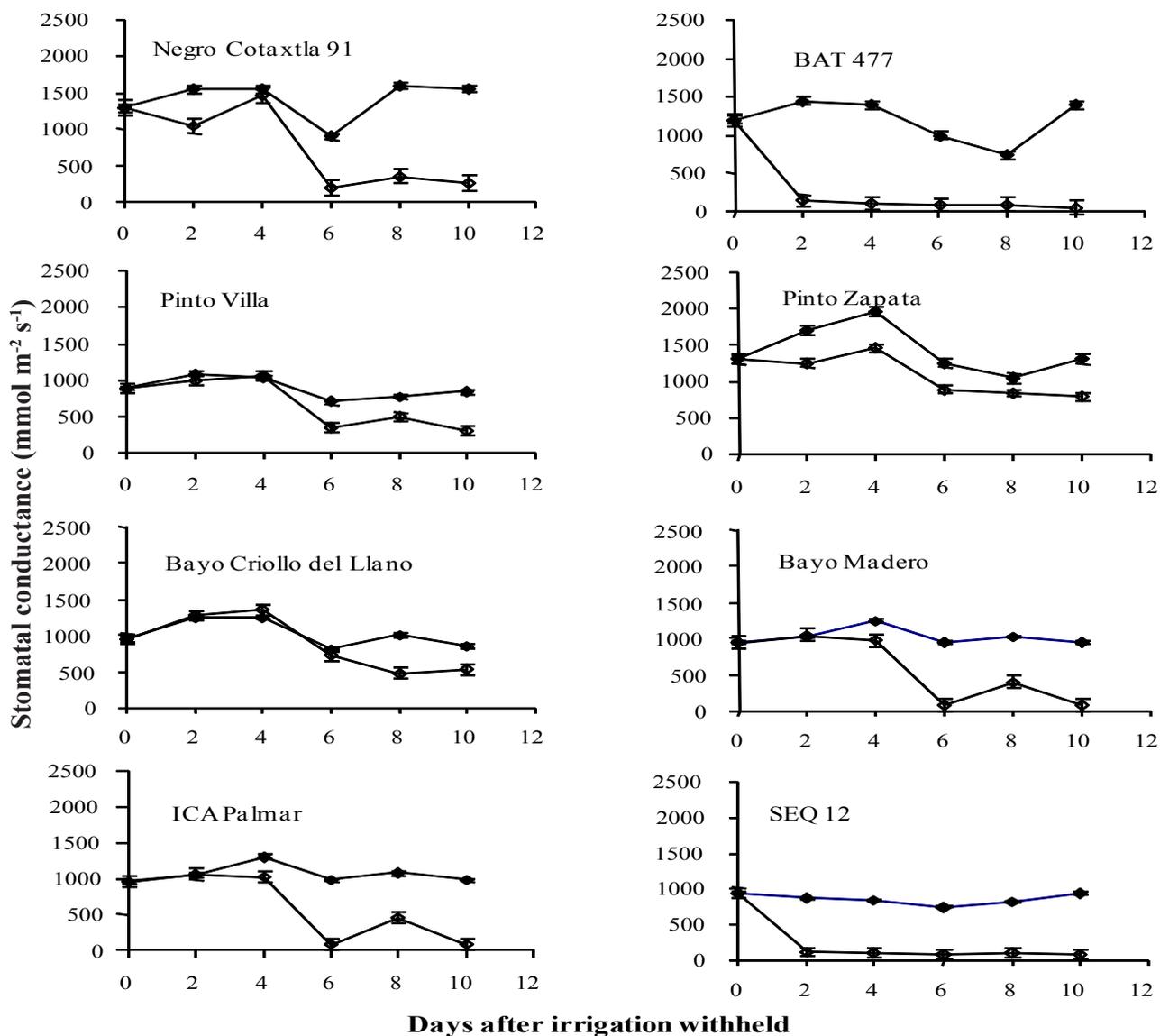
cultivars, the soil drying did not show any effect on the leaf water potential at least for the first eight days after irrigation was withheld. That was the case of the bred line SEQ 12, from the Mesoamerican race and the cultivars Pinto Villa, Bayo Criollo del Llano and Bayo Madero, all type III from the Durango race (Figure 1). In contrast, bred lines BAT 477, from the Mesoamerican race, Pinto Zapata, Durango race and ICA Palmar, Nueva Granada race, displayed an early reduction in leaf water potential at day four, while the cultivar Negro Cotaxtla 91, from the Mesoamerican race, showed reduction in leaf water potential at day six.



**Figure 1.** Effect of irrigation withheld (day 0) on leaf water potential of eight bean cultivars. Closed and open symbols represent irrigated and drought stressed plants, respectively. Each point represents the average of five replicates, ± s. e.

Bred lines BAT 477, ICA Palmar and SEQ 12 exhibited high sensitivity for stomatal conductance and showed stomata closure at the second day of soil drying even though leaf water potential remained unaffected (Figure 2). This reduced stomata conductance

was maintained during the stress treatment. The cultivars Pinto Zapata, Negro Cotaxtla 91, Pinto Villa, Bayo Criollo del Llano and Bayo Madero showed a gradual reduction in stomata conductance after the day four of the stress treatment (Figure 2).



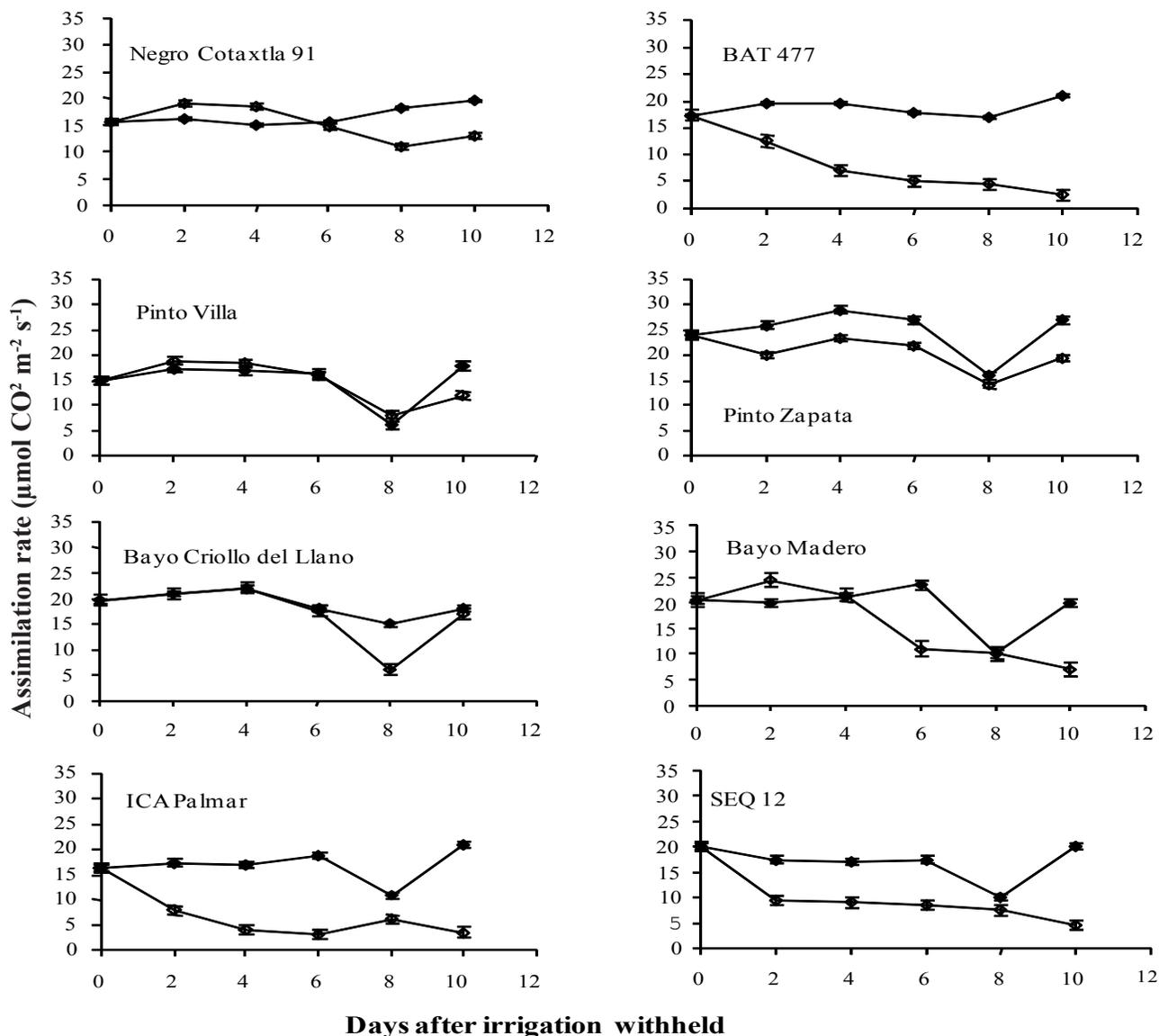
**Figure 2.** Effect of irrigation withheld (day 0) on stomatal conductance of eight dry bean cultivars. Closed symbols represent irrigated plants and open symbols drought stressed plants. Each point is the average of five replicates,  $\pm$  s. e.

Drought stress caused a decrease in leaf assimilation rate. This trait showed a similar response to that found in stomatal conductance. Assimilation rate of the bred lines BAT 477, ICA Palmar and SEQ 12 decreased from

the second day of soil drying (Figure 3). In the cultivars Pinto Zapata, Negro Cotaxtla 91, Pinto Villa, Bayo Criollo del Llano and Bayo Madero this physiological trait was only slightly affected by the stress treatment. These

results indicate high stomata sensitivity; mainly, in bred lines BAT 477, ICA Palmar and SEQ 12. It was clear that the leaf water potential could not had

elicited the stomata response since it had not been affected at the time stomata closure was observed (Acosta-Diaz *et al.*, 2004).



**Figure 3.** Effect of irrigation withheld (day 0) on CO<sub>2</sub> assimilation rate of eight dry bean cultivars. Closed symbols represent irrigated plants and open symbols drought stressed plants. Each point is the average of five replicates, ± s. e.

Each cultivar showed different degree of expression of the physiological mechanisms, which contributed to avoid dehydration by reducing water loss. The most important of these was stomatal adjustment to water loss in response to declining stomata conductance and assimilation rate,

for which there appeared to be substantial differences among cultivars in reply to drought stress (Acosta-Diaz *et al.*, 2004). The high sensitivity of cultivars BAT 477, SEQ 12 and ICA Palmar suggests that the Mesoamerican and Nueva Granada races have evolved under the pressure of different biotic

stresses but drought, in contrast to cultivars from the Durango race, developed at the semiarid highlands of Mexico, which showed stomata control under slight drought stress. The mechanism maintains high values of stomatal conductance, suggesting high water use efficiency in these cultivars (Lizana *et al.*, 2006).

Another possibility is that stomata of *Phaseolus vulgaris* L. are sensitive to very small increase in ABA concentration, although this sensitivity must operate against a high background concentration of ABA (Trejo and Davies, 1991; Lizana *et al.*, 2006). An interesting aspect is that stomata adjustment showed a great dynamic range among the studied cultivars. In addition, bred lines BAT 477 and SEQ 12, which displayed sensitive stomata to water stress, showed the highest stomata frequency and the smaller stomata size (data non shown). It is not clear whether frequency and/or size are related to sensitivity; however, it seems that those traits can be readily transmitted through generations since SEQ 12 is a progeny from BAT 477 (CIAT, 1995).

### Agronomic traits

No significant differences were observed among irrigation treatments and cultivars for days to flowering but significant differences ( $p \leq 0.01$ ) were observed due to irrigation treatments and cultivars for days to maturity and the duration of reproductive period (data not shown). The drought treatment caused a reduction in flowering and maturity period in all cultivars compared with the non-stress treatment. Negro Cotaxtla 91 of indeterminate growth habit type II, Mesoamerican race, that had been classified as drought susceptible, was developed for rain-fed conditions in the tropical lowlands of Mexico, was late in flowering and maturity in contrast to early cultivars from the Durango race of indeterminate growth habit type III and drought resistant, Pinto Zapata, Pinto Villa and Bayo Madero (Table 2). These late cultivars were developed for rain-fed conditions in the semiarid highlands of Mexico and had been classified as drought resistant (Acosta-Díaz *et al.*, 1994; Acosta-Gallegos *et al.*, 1995; Rosales-Serna *et al.*, 2000; Rosales-Serna *et al.*, 2004).

**Table 2. Days to flowering, maturity and reproductive period of eight dry bean cultivars grown under two irrigation treatments during the reproductive stage, Texcoco, Estado de Mexico. 2000.**

Cultivar	Days to flowering		Days to maturity		Length of eproductive period d <sup>-1</sup>	
	Non-stress	Stress	Non-stress	Stress	Non-tress	Stress
Negro Cotaxtla 91	55	56	109	103	54	47
BAT 477	53	53	105	90	52	37
Pinto Zapata	44	44	100	90	56	46
Pinto Villa	44	45	98	87	54	42
Bayo C. del Llano	50	53	105	97	55	44
Bayo Madero	46	46	99	90	53	44
ICA Palmar	49	49	105	100	56	51
SEQ 12	47	48	103	100	56	52
Average	48	49	103	95	55	45
LSD (0.05)	3.1	2.0	1.73	1.26	3.55	2.1

The drought stress treatment accelerated physiological maturity, particularly of cultivars BAT 477, Pinto Villa, Pinto Zapata, Bayo Criollo del Llano and Bayo Madero (Table 2). In contrast, the effect on SEQ 12 and ICA Palmar were only three and five day reduction, respectively. The number of days to physiological maturity was associated with the variation of the duration of the reproductive period.

The tested cultivars responded to drought stress in several ways and the degree of expression varied among cultivars. In general, cultivars exhibited a tendency to escape from the effects of drought through a faster development in response to the stress. Similar effects of drought stress on plant phenology have previously been observed (Acosta-Gallegos and Kohashi-Shibata, 1989; Ramírez-Vallejo and Kelly, 1998). Therefore, the matching of crop phenology to

environmental conditions, mainly rainfall pattern, has been recognized as an important criterion for improving drought adaptation in common bean (Acosta-Gallegos and Adams, 1991; Acosta-Gallegos and White, 1995; Ramirez-Vallejo and Kelly, 1998; Rosales-Serna *et al.*, 2000; Acosta-Díaz *et al.*, 2004; Rosales-Serna *et al.*, 2004).

Significant differences ( $p \leq 0.05$ ) were observed among irrigation treatments and cultivars for shoot biomass and

seed yield (Table 3). The drought treatment caused a reduction in shoot biomass in all cultivars compared with the non-stress treatment. Biomass reduction was larger in the resistant cultivars, ICA Palmar, SEQ 12, Pinto Zapata and Pinto Villa. High shoot biomass under drought stress coupled to high harvest index may be useful as a selection criterion for drought resistance in common bean (Rosales-Serna *et al.*, 2000; Rosales-Serna *et al.*, 2004).

**Table 3. Shoot biomass, seed yield and harvest index of eight dry bean cultivars grown under non-stress and water stress treatments. Texcoco, Estado de Mexico. 2000.**

Cultivar	Shoot biomass (g m <sup>2</sup> )		Seed yield (g m <sup>2</sup> )		Harvest Index (%)	
	Non-stress	Stress	Non-stress	Stress	Non-stress	Stress
Negro Cotaxtla 91	581	436	250	169	45	39
BAT 477	608	370	265	141	44	38
Pinto Zapata	673	313	336	136	50	42
Pinto Villa	496	326	249	138	50	44
Bayo C. del Llano	691	551	303	149	44	32
Bayo Madero	575	451	311	128	54	40
ICA Palmar	543	242	197	111	36	46
SEQ 12	538	276	256	101	45	37
Average	584	342	271	134	46	40
LSD (0.05)	151.2	84.3	83.4	34.7		

Overall, yield reductions due to drought were larger in the resistant cultivars, SEQ 12 and ICA Palmar, and in the susceptible cultivar, Bayo Madero; whereas susceptible cultivar Negro Cotaxtla 91 and resistant cultivars Bayo Criollo del Llano, BAT 477, Pinto Villa and Pinto Zapata were the highest yielding cultivars (Table 3).

Results suggest that the reduction of seed yield due to the effect of drought stress was independent of the growth cycle, probably due to the fact that the differences in the duration of the cycle among cultivars were not significant. Seed weight of SEQ 12 was less affected by the stress treatment in comparison with cultivars of larger seed size, such as Bayo Madero and Pinto Villa. The average reduction in seed weight was by far smaller than the decrease in seed yield, suggesting that other yield components, such as the number of pods per plant and seeds per pod were also affected by the stress.

Most of the cultivars showed a reduction in harvest index under the drought stress treatment (Table 3). Foster *et al.* (1995) observed that the harvest index in common bean

was not reduced under mild stress and significantly reduced under severe stress. In this study, cultivars ICA Palmar and Pinto Villa showed a high harvest index under drought stress conditions in comparison with the others cultivars. These cultivars had previously been classified as drought resistant (Acosta-Díaz *et al.*, 1994; Acosta-Gallegos *et al.*, 1995; Rosales-Serna *et al.*, 2000; Rosales-Serna *et al.*, 2004). The observed response was in part due to shoot biomass adjustment in coordination with the duration of the reproductive stage since these cultivars showed early maturity (Table 2). Acosta-Gallegos and Kohashi-Shibata (1989) reported similar findings in a study with cultivars from the Durango race.

The results indicate that under drought stress conditions, cultivars from the Durango race displayed a high seed mass to biomass ratio as compared to cultivars from the Mesoamerican race. Drought resistant cultivars that display high yield under stress are more efficient in photoassimilate remobilization (Samper and Adams, 1985; Rosales-Serna *et al.*, 2000; Acosta-Díaz *et al.*, 2004; Rosales-Serna *et al.*, 2004).

## CONCLUSIONS

The studied bean cultivars showed a tendency to escape from the effects of drought throughout a faster development; particularly, reducing the number of days to maturity and the duration of the reproductive period.

Each cultivar exhibited a different degree of expression of the physiological mechanisms, to avoid dehydration, the most effective were stomata control of water loss in response to declining stomata conductance and assimilation rate.

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