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Differences in Salt Tolerance Between *Phaseolus vulgaris* and *Phaseolus coccineus* Cultivars

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Abstract: Diverse cultivars of *Phaseolus vulgaris* L. and *Phaseolus coccineus* L. were tested under saline conditions to evaluate yield performance and to estimate physiological differences (chlorophyll, stomatal conductance and transpiration rate). The study was carried out in Central Mexico in a low saline soil (pH 6.8-7.5 and EC of 2-5 dS m^{-1}) and high saline soil (pH 8-8.7 and EC of 5-8 dS m^{-1}). Three *P. vulgaris* cultivars and one *P. coccineus* cultivar were planted in a high saline soil during 2003, while thirteen *P. vulgaris* cultivars and three *P. coccineus* cultivars were planted in a low saline soil during 2004. The experimental design for both saline fields was a randomized complete block with four replicates. The *P. vulgaris* cv. Bayomex showed the highest seed yield, biomass and pod number under high saline conditions, while the *P. coccineus* cv. Ayocote Negro showed a lower seed yield and biomass, but higher seed weight. Canario-107 and Criollo were the *P. vulgaris* cultivars with the lowest yield during 2003. For the season 2004 under low saline conditions, four *P. vulgaris* cultivars (Zacatecas, Ojo de Cabra, Morito and Bayo-18) showed higher seed yield, biomass and seed weight, but the three *P. coccineus* cultivars showed major yield in all parameters. Generally, the cultivars of both crop species with high yield presented high chlorophyll levels than the sensitive cultivars under low and high saline conditions. In low salinity, the *P. coccineus* and *P. vulgaris* cultivars showed diversity in leaf stomatal conductance, transpiration rate and leaf temperature. Stomatal conductance explained yield differences among cultivars of both crop species showing two well defined groups (one for each crop species).

Key words: Stomatal conductance, seed yield, dry bean, runner bean, chlorophyll

INTRODUCTION

Soil salinity affects about 7% of diverse agricultural areas around the world and it is still increasing in dry irrigated areas affecting yield of diverse crops with losses that ranged from 10 to 50% depending of the crop species (Ghassemi *et al*., 1995; El-Saidi, 1997; Munns and Tester, 2008). Saline soils usually content high pH and electroconductivity (EC) major than 4 dS m^{-1} that reduces nutrient and water availability for roots (Szabolcs, 1994).

There are diverse agricultural areas located around the former Texcoco's Lake located East Mexico City that are well characterized as saline-alkaline soils (sodic soil) with high pH (6.8-8.7) and EC (2-14 dS m^{-1}) (Beltrán-Hernández *et al*., 1999). Common bean (*Phaseolus vulgaris* L.), an important human protein source in Mexico, is frequently cultivated in

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surrounding areas to the former Texcoco's Lake with low consideration of salt tolerance (Duranti and Gius, 1997; Gutierrez-Rodriguez *et al*., 2005). *Phaseolus vulgaris* is considered as a sensitive crop to saline conditions like many other leguminous species because seed yield is reduced at low salinity levels less than 2 dS m^{-1} (Subbarao and Johansen, 1994). In comparison, runner bean (*Phaseolus coccineus* L.) is considered as resistant species to saline conditions because seed yield is maintained in low-medium saline soil (EC >4 dS m^{-1}) (Subbarao and Johansen, 1994).

There are not many studies in relation to the salt tolerance and yield performance between *P. vulgaris* and *P. coccineus*. Ndakidemi and Makoi (2009) compared four cultivars of bean (Lyamungo 90, Jesca, Flor de Mayo and CAB-19) under diverse salt levels (NaCl) to assess plant growth and found that higher saline levels caused chlorosis and reduced plant height, dry matter and seed yield. Carbonell-Barrachina *et al.* (1997) reported the effects of different saline levels in a cultivar of common bean (cv. Buenos Aires) and found that increasing salinity levels cause a lower accumulation of some nutrients as nitrogen and phosphorous. Stephen *et al.* (2001) compared the salt tolerance of canola (*Brassica napus* cv. Cyclone), field pea (*Pisum sativum* cv. green-seed Radley and cv. yellow-seed Carneval), pinto bean (*Phaseolus vulgaris* cv. Othello) and durum wheat (*Triticum turgidum* cv. Kyle) under saline conditions reporting that *P. vulgaris* was one of the most sensitive species to salinity (low plant growth and seed yield). The symbiotic N_2 fixation is another process that is negatively affected by high salt content on soils as was reported by Rai (1992), where two *P. vulgaris* genotypes (HUR 137 and VL 63) showed less nodulation by two *Rhizobium* strains, low nitrogenase activity, small plant nitrogen content and reduced seed yield. Dos-Santos and Fageria (2007) reported that the nitrogen use efficiency and grain yield was higher in cultivars with larger growth seasons than in cultivars with early maturity of *P. vulgaris* genotypes under medium saline levels.

In a previous study, yield of one cultivar of *P. vulgaris* and one for *P. coccineus* were compared under low and high saline conditions in relation to the gas exchange (stomatal conductance), leaf area index and canopy reflectance patterns (normalized difference vegetation index ([NDVI) and other indices) (Gutiérrez-Rodríguez *et al*., 2005). However, there are no studies to compare the salt tolerance and yield performance among cultivars (genetic diversity) of both crop species in surrounding areas of the former Texcoco's Lake. The objective of the present work was; 1) to determine yield differences among *P. vulgaris* and *P. coccineus* cultivars commonly planted in a surrounded area of the former Texcoco's Lake that presented low and high saline soils and 2) to estimate some physiological differences (chlorophyll, stomatal conductance and transpiration rate) of both crops under low salinity.

MATERIALS AND METHODS

The study was conducted in Montecillo, Central Mexico (19°19' N, 98°54' W, 2250 m above sea level and temperate climate) in two saline soils under rainfed conditions. The research center called Postgraduate College (Colegio de Postgraduados) in Agriculture Sciences is located to the East region of the former Texcoco's Lake and presents soils with diverse salinity levels. In the North side of this research center, soils are highly saline (pH 8-8.7 and EC of 5-8 dS m^{-1}), while in the East side, soils are low saline (pH 6.8-7.5 and EC of 2-5 dS m^{-1}). Three cultivars of *Phaseolus vulgaris* L. (Bayomex, Canario-107 and Criollo) and one cultivar of *Phaseolus coccineus* L. (cv. Ayocote Negro) were planted in May 28, 2003 in a high saline soil and plants were harvested in September, 2003. During 2004 (May 22, 2004), thirteen *P. vulgaris* cultivars (Zacatecas, Ojo de Cabra, Morito, Bayo-18, Bayomex, Negro 98, Promesa, Pinto Cabaña, Pinto, Flor de Mayo, Flor de Durazno, Canario-107 and Peruano) and three *P. coccineus* cultivars (Ayocote Negro, Blanco and Morado) were planted in a low saline soil. The experimental design for both saline fields was a randomized complete block with four replicates. Seeds of both crop species were sown in a plant density of 6.25 plants m^{-2} in both growing seasons (2003 and 2004). Plots were well managed with respect to fertility (100 kg of nitrogen ha^{-1} as urea, 100 kg of P_2O_5 ha^{-1} given as triple superphosphate).

Leaf chlorophyll estimates were made *in situ* on the central leaflet using a portable chlorophyll meter (SPAD-502, Minolta; Tokyo, Japan). Means of four readings per leaf were recorded during pod filling stage in each plot during 2003 and 2004. Stomatal conductance, transpiration rate and leaf temperature were measured on the central leaflet using a porometer Licor LI-1 600 (Licor Instruments; Nebraska, USA). The measurements were made on portions of leaves exposed directly to the sunlight on cloud-free days (near solar noon) during pod-filling stage (80 days after the planting) just for the season 2004.

At physiological maturity, above ground biomass and seed yield were determined in each plot. The samples were oven-dried, weighed and threshed and seed weight was recorded. The weight for 100 seeds was recorded as well as height and pod number.

Weather data (maximum and minimum temperature and rainfall) were obtained from a local weather station for both growing seasons (Table 1).

RESULTS

Rainfall during May and August was higher for the year 2003 than for the same months of 2004 and consequently the total rainfall was higher for the season 2003 (602 nm) than for 2004 (472 mm) (Table 1).

Yield Performance under High Salinity

The *P. vulgaris* cv. Bayomex showed the highest seed yield, biomass and pod number under high saline conditions (Table 2). The *P. coccineus* cv. Ayocote Negro showed lower seed yield and biomass, but higher seed weight than cv. Bayomex. Canario-107 and Criollo (*P. vulgaris* cultivars) presented the lowest seed yield, biomass and seed weight. There were also differences in maturity where *P. vulgaris* cv. Bayomex and Criollo showed earlier maturity than the *P. coccineus* cv. Ayocote Negro and where Canario-107 showed the earliest maturity.

Chlorophyll levels were lower in the two sensitive *P. vulgaris* cultivars (Canario-107 and Criollo) presenting chlorosis on leaves caused by the high salinity levels (Table 2). The *P. coccineus* cv. Ayocote Negro showed the highest chlorophyll content of all the cultivars planted during the season 2003.

Table 1: Mean, maximum and minimum temperature (°C) and monthly total rainfall (mm) for two growing seasons (2003 and 2004) in Montecillo, Mexico

Cycle	May	June	July	August	September	Mean/sum
Season 2003						
Temperature (°C)						
Min	39.0	36.0	33.0	31.0	33.0	34.40
Max	-3.0	5.0	4.0	2.0	-1.0	1.40
Average	33.8	30.1	30.3	27.7	28.3	30.04
Rainfall (mm)	100.0	176.9	84.3	152.1	88.6	601.90
Season 2004						
Temperature (°C)						
Min	37.0	33.5	33.5	34.5	32.5	34.20
Max	2.5	5.0	3.0	4.0	2.5	3.40
Average	32.6	31.0	30.2	30.8	30.6	31.04
Rainfall (mm)	44.0	164.4	77.0	90.4	96.0	471.80

Table 2: Seed yield, biomass and other components measured in cultivars of *Phaseolus coccineus* L. and *Phaseolus vulgaris* L. grown under low and high saline conditions during 2003 and 2004

Crop species	Seed yield ($g\ m^{-2}$)	Biomass ($g\ m^{-2}$)	100 seeds (g)	Pods (m^2)	Height (cm)	Flowering (days)	Mat (days)	Chlorophyll (SPAD)
Season 2003 (pH 8-8.7 and EC of 5-8 dS m^{-1})								
Phaseolus coccineus L.								
Ayocote Negro	132.2	371.1	65.6	107.5	-	42	132	41.2
Phaseolus vulgaris L.								
Bayomex	179.3	353.7	27.3	211.4	-	51	118	39.3
Canario-107	41	206.1	23.0	104.6	-	42	90	31.4
Criollo	86	260.2	23.7	139.7	-	73	132	35.3
LSD	40.3	86.2	3.6	78.2		5.8	9.8	4.8
Significance	**	**	**	**		**	**	*
Season 2004 (pH of 6.8-7.5 and EC of 2-5 dS m^{-1})								
Phaseolus coccineus L.								
Ayocote Blanco	653.1	1721.8	200.4	62.5	129.0	46	122	32.5
Ayocote Morado	563.6	1604.8	175.6	61.3	155.0	50	120	34.6
Ayocote Negro	522.9	1379.9	170.9	62.5	128.0	52	119	35.8
Phaseolus vulgaris L.								
Zacatecas	313.0	640.7	45.8	120.0	83.0	82	105	32.9
Ojo de Cabra	293.0	455.9	49.1	78.8	65.6	78	98	32.3
Morito	290.1	620.4	53.7	62.5	77.0	75	100	30.2
Bayo-18	287.8	534.5	28.9	126.3	70.8	75	96	29.2
Bayomex	243.3	408.9	42.9	67.5	65.4	69	99	31.8
Negro 98	241.9	466.6	37.1	105.0	42.2	76	95	29.3
Promesa	217.9	396.2	45.5	66.3	48.8	80	96	21.1
Pinto Cabaña	197.3	412.5	28.5	101.3	59.6	74	94	29.1
Pinto	150.8	345.3	30.9	73.8	50.6	76	95	37.1
Flor de Mayo	143.8	234.6	25.5	77.5	32.4	72	105	27.0
Flor de Durazno	138.7	255.1	34.5	56.3	36.4	46	84	33.5
Canario-107	95.2	162.1	38.0	53.8	29.0	46	84	25.2
Peruano	68.1	182.2	41.6	35.0	25.4	45	88	25.5
LSD	61.2	111.7	13.1	2.8	10.9	2.6	4.5	7.6
Significance	*	*	**	**	**	**	**	*

*p = 0.05, **p = 0.01

Yield Performance under Low Salinity

Zacatecas, Ojo de Cabra, Morito and Bayo-18 were the *P. vulgaris* cultivars with higher seed yield, biomass and seed weight under low saline conditions during the season 2004, while Canario-107 and Peruano were the cultivars with the lowest yield parameters (Table 2). The rest of *P. vulgaris* cultivars were intermediate in yield. Among the three *P. coccineus* cultivars evaluated under low saline soils, cv. Ayocote Blanco showed higher yield than the other two cultivars (Ayocote Morado and Negro) (Table 2).

High yielding *P. vulgaris* cultivars generally showed high chlorophyll levels than low yielding cultivars under low saline conditions (Table 2). There were not greatest differences in chlorophyll content between two *P. coccineus* cultivars (Ayocote Morado and Negro), but Ayocote Blanco showed the highest chlorophyll levels of all cultivars evaluated during the season 2004.

The *P. vulgaris* cultivars showed diversity to reach maturity, but cultivars with higher yield reached maturity around 100 days and low yield cultivars showed a shorter growth season (around 85 days) (Table 2). The three *P. coccineus* cultivars showed longer maturity (around 120 days).

Comparing Both Crop Species for Low and High Saline Conditions

When both crop species grown under low and high saline conditions were compared, seed yield, biomass and other parameters were higher under low saline than under high saline conditions (Table 3). The average of the three *P. vulgaris* cultivars compared to the only

Table 3: Average of yield components of *Phaseolus vulgaris* L. and *Phaseolus coccineus* L. grown under low and high saline field conditions during 2003 and 2004

Species	Genotype No.	Yield (g m^{-2})	Biomass (g m^{-2})	100 seeds (g)	Pods (m^{-2})	Flowering (days)	Mat (days)	Chlorophyll (SPAD)
High salinity for season 2003								
Phaseolus coccineus L.	1	132.2	371.1	65.6	107.5	42	132	41.4
Phaseolus vulgaris L.	3	102.1	273.3	24.7	151.9	55	113	35.2
Low salinity for season 2004								
Phaseolus coccineus L.	3	579.9	1568.8	182.3	62.1	49	120	34.3
Phaseolus vulgaris L.	13	206.2	393.5	38.6	78.8	69	95	29.6

Table 4: Average of stomatal conductance, transpiration rate and leaf temperature determined during pod filling of *Phaseolus vulgaris* L. and *Phaseolus coccineus* L. cultivars and their association with seed yield and biomass grown under low saline conditions during 2004

Crop species	Stomatal conductance	Transpiration rate	Leaf temperature (°C)
	------------------(mmoles/m^2/sec)------------------		
Phaseolus vulgaris L.	239.10a	6.90a	25.50b
R with seed yield	0.59*	0.63*	-0.16
R with biomass	0.40	0.45	-0.11
Phaseolus coccineus L.	149.10b	4.6b	26.4a
R with seed yield	0.88	0.82	-0.85
R with biomass	0.99**	0.98*	-0.98*

*, **Significant at the 0.05 and 0.01 probability level, respectively

P. coccineus cultivar was lower in seed yield and biomass under high saline conditions during 2003 (Table 3). Even though the *P. vulgaris* cv. Bayomex showed the biggest yield, the two cultivars with lower yield caused a reduced average yield. For the season 2004 under low saline conditions, the differences between *P. vulgaris* and *P. coccineus* cultivars were stronger because *P. coccineus* had the highest yield including chlorophyll content (Table 3).

Stomatal Conductance and Transpiration Rate under Low Saline Conditions

The *P. coccineus* cultivars showed a higher leaf stomatal conductance, transpiration rate, but low leaf temperature compared to the *P. vulgaris* cultivars (Table 4, Fig. 1a, b). When the stomatal conductance and transpiration rate were associated to seed yield and biomass, they were correlated in both crop species. The three *P. coccineus* cultivars showed a well defined group with higher stomatal conductance values compared to the *P. vulgaris* cultivars that were clustered in another group. The same tendency was presented when transpiration rate and yield parameters were associated (data not shown).

DISCUSSION

Yield Performance under High Salinity

Even though there were a higher rainfall for the experiment conducted under high salinity during 2003 (602 mm) than under low salinity during 2004 (472 mm), the high soil salt content reduced yield parameters of *P. vulgaris* and *P. coccineus* cultivars (Table 1, 2).

Phaseolus vulgaris has been reported as a sensitive crop to low saline conditions (EC <2 dS m^{-1}) (Subbarao and Johansen, 1994). In our study, we found that *P. vulgaris* cv. Bayomex showed a better yield under high salinity than *P. coccineus* cv. Ayocote Negro during 2003 (Table 2), which has been reported as tolerant crop to high salinity (EC 2-4 dS m^{-1}) (Subbarao and Johansen, 1994). It means that *P. coccineus* was less tolerant to high salinity than the *P. vulgaris* cv. Bayomex (Table 2). There are many reports on the

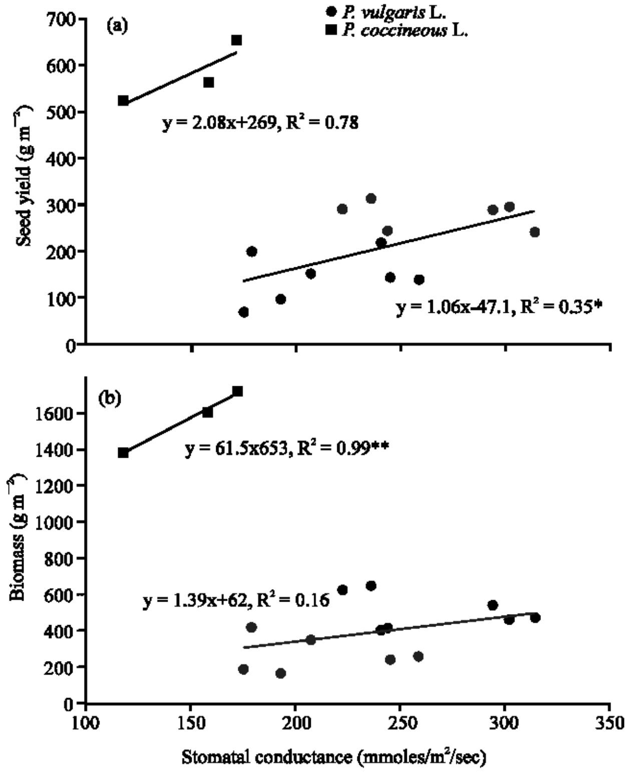

Fig. 1: Relationship of stomatal conductance with seed yield (a) and biomass (b) in *Phaseolus vulgaris* L. and *Phaseolus coccineous* L. grown under low saline conditions during 2004. *p = 0.05, **p = 0.01

literature about the low salt tolerance of *P. vulgaris*, but few studies about salt resistance. Ndakidemi and Makoi (2009) established that there is a high genetic potential in salt tolerance among *P. vulgaris* cultivars. In fact, several national breeding programs in Africa have been established to find dry bean genotypes with better salt tolerance, plant growth, chlorophyll levels and high seed yield (Ndakidemi and Makoi, 2009). Dry bean is a pretty common crop among small scale farmers in Africa and other developing countries and some economical benefits could be exploited in soil affected by salinity (Herridge *et al.*, 1995).

Phaseolus vulgaris cv. Canario-107 and Criollo resulted highly sensitive to the saline conditions with lower seed yield, biomass, seed weight and pod number than cv. Bayomex and cv. Ayocote Negro (Table 2). Clear symptoms of susceptibility to salinity were the chlorosis presented in cv. Canario-107 and Criollo (lower chlorophyll content) (Table 2). Ndakidemi and Makoi (2009) reported that tolerant *P. vulgaris* cultivars to high salinity exhibit higher plant growth and less signs of leaf injury as chlorosis compared to susceptible cultivars. In current study, cv. Canario-107 presented an earlier maturity than other cultivars under high saline conditions, but cv. Criollo with a longer maturity also presented clear chlorosis symptoms caused by salinity.

A high salt concentration in the soil decreases water availability (low soil osmotic potential) that reduced root water uptake, especially after several days without rainfall (Pascale and Barbieri, 1997). Another consequence of high salt content in soils is that root growth is stopped by the toxic effects of salts accumulated in cells reducing biological N_2 fixation (Munns and Tester, 2008). The ion accumulation on cells causes an osmotic stress effecting growth and water uptake and reduces plant growth at low and moderate salinity levels (Munns and Tester, 2008). Some crop species are higher tolerant to salinity than others and diverse comparisons among crops have been conducted for comparing yield (Munns and Tester, 2008). Stephen *et al.* (2001) compared the salt tolerance of canola, pea, pinto bean and durum wheat finding that durum wheat and canola were better under moderate and severe saline conditions than the other crops with higher biomass and grain yield.

Another considerable negative effect of salinity over *Phaseolus* and other legume species is the reduction on nodulation by *Rhizobium* (Rai, 1992). The symbiotic N_2 fixation by *Rhizobium* in saline sodic soils was reduced in *P. vulgaris* genotypes causing lower nodulation and a minor nitrogenase activity (Rai, 1992). In consequence, nitrogen levels on shoots and seeds are decreased in saline conditions. In the current study, two *P. vulgaris* cultivars (Canario-107 and Criollo) showed clear chlorosis symptoms on leaves under high salinity during 2003 (Table 2) indicating that plants were unable to uptake nutrients from soil such as nitrogen to have higher chlorophyll contents. Carbonell-Barrachina *et al.* (1997) found that the nutrient absorption and their accumulation in plants are also diminished under saline conditions, especially nitrogen and phosphorous on leaves but other nutrients such as K, Na, Ca and Mg increased their levels. Salinity effects depend on the crop growth stage and salt stress intensity and Adiku *et al.* (2001) modeled the salt effects in common bean to simulate the negative influence on water uptake at diverse plant growth stages.

Yield Performance under Low Salinity

Under low saline conditions, *P. vulgaris* cultivars showed diversity in seed yield, biomass, seed weight and pod number in our study (Table 2). Some cultivars showed high and low seed yield being Zacatecas, Ojo de Cabra and Morito the high yielding cultivars, while Canario-107 and Peruano were low yielding. The three *P. coccineus* cultivars showed better yield components compared to the *P. vulgaris* cultivars, but cv. Ayocote Blanco was the best in seed yield and biomass of all cultivars planted in low salinity (Table 2). Canario-107 was again the cultivar with the highest sensibility to the saline conditions.

Comparing Both Crop Species for the Saline Conditions

When all cultivars of both crop species in the two growing seasons (2003 and 2004) were compared, the average yield of *P. coccineus* cultivars were higher compared to the *P. vulgaris* cultivars (Table 3). During the season 2003, the average yield of the three *P. vulgaris* cultivars were lower compared with the yield of *P coccineous* cv. Ayocote Negro even though *P. vulgaris* cv. Bayomex presented the highest yield (Table 3). Under low saline conditions, the *P. coccineus* cultivars presented larger plants (height) than the *P. vulgaris* plants and in consequence major seed yield, biomass and seed weight (Table 2, 3).

In general, the *P. coccineus* cultivars presented major chlorophyll content than *P. vulgaris* cultivars in both saline conditions (Table 2, 3).

Stomata Conductance and Transpiration Rate under Low Saline Conditions

The yield diversity in low salinity presented in the *P. vulgaris* cultivars was also displayed in stomatal conductance, transpiration rate and leaf temperature (Fig. 1a, b, Table 4). The *P. coccineus* cultivars presented a shorter range in gaseous exchange compared to the *P. vulgaris* cultivars. The yield differences among cultivars in both crop species were also evident for stomatal conductance and transpiration rates during pod filling. The *P. coccineus* cultivars presented higher rates in stomatal and transpiration rates than *P. vulgaris* cultivars (Fig. 1). Seed yield and gaseous exchange were highly associated for both crop species grown under low salinity including leaf temperature (Table 4, Fig. 1a, b). The two groups in stomatal conductance permitted to differentiate *P. coccineus* cultivars from *P. vulgaris* cultivars (Fig. 1a, b). The higher yield of *P. coccineus* was explained by a higher gaseous exchange as has been previously reported for other crops such as wheat (Fischer *et al.*, 1998). In another study, the photosynthetic traits (photosynthetic rate and stomatal conductance) were evaluated in segregating population of *P. vulgaris* founding high heritability for these traits (Bressan-Smith and Pereira, 2003). If there is a genetic influence on photosynthetic traits in dry bean, some genotypes with high yield could present high gaseous exchange, especially under low salinity as was demonstrated in our study. Pascale and Barbieri (1997) found that common bean plants were less affected in low salinity than under high salinity and established a major carbohydrate partitioning (photosynthetic assimilates) to pods deriving major seed yield.

CONCLUSION

There were clear differences in salt tolerance between *P. coccineus* and *P. coccineus* cultivars under low and high saline conditions. The *P. vulgaris* cv. Bayomex was the best for yield under high saline conditions, while *P. coccineus* cultivars presented better yield under low salinity. Chlorophyll levels of high yielding cultivars were higher than low yielding cultivars for both crop species in two salinity levels. Under low saline conditions, the *P. coccineus* cultivars showed a higher leaf stomatal conductance and transpiration rate than the *P. vulgaris* cultivars. Stomatal conductance and transpiration rate were associated to seed yield and biomass showing clear differences between both crop species.

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