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Canopy Reflectance, Stomatal Conductance, and Yield of *Phaseolus vulgaris* L. and *Phaseolus coccinues* L. Under Saline Field Conditions

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ABSTRACT

A field trial was carried out in Central México (rainfed conditions) to study the growth, photosynthesis and yield response of *Phaseolus vulgaris* L. cv. Bayomex and *Phaseolus coccineus* L. cv. Ayocote Negro under saline conditions. Both species were grown in a soil with high salinity (pH 8-8.7 and an electric conductivity (EC) of 7-14 dS m^{-1}) and low salinity (pH 6.8-7.5 and EC of 2-5 dS m^{-1}). In both species, the yield was reduced under high saline conditions due to a low gas exchange (low stomatal conductance). The normalized difference vegetation index (NDVI), green normalized difference vegetation index (GNDVI), and the photochemical radiation index (PRI) were associated to seed yield, biomass, and leaf area index under high and low salinity. Stomatal conductance was reduced under high salinity conditions (a reduction of 72% for *Phaseolus vulgaris,* and 42% for *Phaseolus coccineus*), and it also showed an association with the spectral reflectance indices.

Key Words: Infrared reflectance; Normalized vegetation difference index; Green normalized vegetation difference index.
Abbreviations: NDVI=Normalized vegetation difference index; GNDVI=Green normalized vegetation difference index; EC=Electric conductivity

INTRODUCTION

Soil salinity affects about 7% of the agricultural areas in the world, and it is still increasing as a result of irrigation or land clearing (Ghassemi *et al.,* 1995). Plant growth (biomass and yield) is reduced by high salt content on soils due to an increase of pH which reduces micronutrient availability (ion uptake by the roots) (Szabolcs, 1994). El-saidi (1997) reported that yield losses of 10%, 25% and 50% occurred by soil EC of 4 dS m^{-1} or more, but it depends of the crop species.

Soil of the former Lake Texcoco somewhat to the east of Mexico City is saline-alkaline (sodic soil) with pH >11 and EC >80 dS m^{-1} (Beltrán-Hernández *et al.,* 2001). Some of the agricultural regions surrounding the Lake Texcoco also had saline problems. The east region has saline soils with pH around 6.8-8.7 and EC of 2-14 dS m^{-1} where the common bean is sown (Escalante-Estrada *et al.,* 2003).

Common bean *(Phaseolus vulgaris* L.) is a significant source of dietary protein in many developing countries (Durante & Gius, 1997). However, it is sensitive to saline conditions like many other leguminous crops, and seed yield is reduced at soil salinity level less than 2 dS m^{-1} (Subbarao & Johansen, 1994). However, runner bean *(Phaseolus coccineus* L.) is considered as resistant to saline conditions (Subbarao & Johansen, 1994).

The assessment of crop growing under saline conditions has been evaluated with remote sensing techniques (spectral reflectance) in barley (Peñuelas *et al.,* 1997). Spectral reflectance indices provide a useful tool for the assessment of many physiological traits (i.e., leaf area, canopy biomass, absorbed radiation, chlorophyll content, photosynthetic capacity, and water status). These parameters can be determined with spectral reflectance measurements in the visible region (400-700 nm), and in the near-infrared region (700-1200 nm) (Araus *et al.,* 2001).

The most commonly known index for analyzing vegetation is the normalized difference vegetation index (NDVI; R_{900}-R_{680}/R_{900}+R_{680}) (i.e., Araus *et al.,* 2001). It is used as an indirect assessment of canopy biomass, leaf area index, light-absorption, and potential photosynthetic capacity (i.e., Gamon *et al.,* 1995; Peñuelas, 1998; Araus *et al.,* 2001). Alternatively, Gitelson *et al.* (1996) proposed the use of the green normalized difference vegetation index (GNDVI, R_{780}-R_{550}/R_{780}+R_{550}) which may prove to be more useful for estimating green crop biomass. In addition, several indices have been described for the estimation of chlorophyll content by canopy reflectance methods such as the index (R_{780}-R_{710}/R_{780}-R_{680}) reported by Datt (1999). This index gave the best results among several published indices (sixty indices) (Le Maire *et al.,* 2004). Spectral reflectance techniques may also allow the calculation of the photochemical reflectance index (PRI; R_{531}-R_{570}/R_{531}+R_{570}) used for quantifying the status of the xanthophyll pigments in the processes of excess radiation dissipation (Gamon *et al.,* 1992).

By periodic measurements of NDVI during the growing cycle of a crop, the agronomic yield has been predicted in wheat (Rudorff & Batista, 1990) and corn (Wiegand *et al.*, 1991). NDVI, and GNDVI also were associated with yield and biomass among bread wheat genotypes (Gutiérrez-Rodríguez *et al.*, 2004b). In a previous study with *Phaseolus vulgaris* grown under three nitrogen levels (0, 100 and 200 kg nitrogen ha^{-1}) we found a positive association between NDVI and seed yield (Gutiérrez-Rodríguez *et al.*, 2004a). However, there are not studies about spectral reflectance in *P. vulgaris* or *P. coccineus* under saline conditions.

In the present study, we determined four reflectance indices (NDVI, GNDVI, PRI, and one chlorophyll index) during pod filling stage in *Phaseolus vulgaris* and *Phaseolus coccineus* grown under high and low saline field conditions to: (i) examine the association between spectral reflectance indices and yield; (ii) determine whether stomatal conductance, transpiration rate, and leaf area index are related with the spectral reflectance indices.

MATERIALS AND METHODS

The study was carried out in Montecillo, Mexico (19^o19' N, 98^o54' W, 2250 m above sea level) under rainfed conditions (June-September, 2000) with a temperate climate. Seeds of *Phaseolus vulgaris* L. cv. Bayomex and *Phaseolus coccineus* L. cv. Ayocote Negro were sown in a plant density of 6.25 plants m^{-2}. The experimental design was a random block with four replications. Seeds of both crops were sown in a soil with high salinity (pH 8-8.7, and EC of 7-14 dS m^{-1}) and in a soil with low salinity (pH of 6.8-7.5 and EC of 2-5 dS m^{-1}). 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).

Canopy reflectance was measured from 350 to 1100 nm using a Field Spec spectroradiometer (Analytical Spectral Devices, Boulder, CO). All data collected were expressed as spectral reflectance after standardization by radiance of a leveled reference standard ($BaSO_4$) (Labsphere Inc., North Sutton, USA). Measurements were taken 0.5 m above the canopy with a 10º field of view foreoptic. Canopy reflectance measurements were taken at random places on each plot during the pod filling stage (80 days after the sowing) and were acquired on cloud-free days near solar noon.

SR indices were calculated following the equations with the wavelength (nm) described by several authors; $NDVI=R_{900}-R_{680}/R_{900}+R_{680}$ (Araus *et al.*, 2001); $GNDVI=R_{780}-R_{550}/R_{780}+R_{550}$ (Gitelson *et al.*, 1996); $PRI=R_{531}-R_{570}/R_{531}+R_{570}$ (Gamon *et al.*, 1992); and a chlorophyll index ($R_{780}-R_{710}/R_{780}-R_{680}$) (Datt, 1999).

Stomatal conductance, transpiration rate, and leaf temperature were measured on the central leaflet using a porometer Licor LI-1600 (LICOR Instruments, NB, USA). The measurements were made on portions of leaves exposed directly to the sunlight and the leaves were maintained at right angles to incident solar radiation. The measurements were acquired on cloud-free day near solar noon during pod-filling stage (80 days after the sowing).

The spectral reflectance indices and gas exchange parameters were taken after three raining days (precipitation accumulated of 13 mm) and one cloud-free day.

Leaf chlorophyll estimates were made *in situ* on the central leaflet using a portable chlorophyll meter (SPAD-502, Minolta, Tokyo, Japan), which uses light absorbance at specific wavelengths to estimate the amount of chlorophyll in the leaf. Means of four readings per leaf were recorded during pod filling stage.

Leaf area index (LAI) was also determined during pod filling stage in plants of both species. All the trifoliate leaves were removed from the shoot to measure area in a leaf area meter Licor LI-3100 (LICOR Instruments, NB, USA). Leaf area index was measured following the next equation: LAI=(leaf area per plant) (plant density) / land area sown.

At physiological maturity, aboveground biomass and seed yield were determined in every plot. The samples were oven-dried, weighed, and threshed, and the seed weight was recorded.

Meteorological data (maximum and minimum temperature, and precipitation) were recorded during the growth cycle in both species.

RESULTS AND DISCUSSION

The growing period for *P. vulgaris* was 118 days after the sowing, and 132 days for *P. coccineus.* The precipitation accumulated during the growing season of *P. vulgaris* was 369 mm and 378 mm for *P. coccineus*, and mean temperature oscillated from 9.0 to 24^oC in both growing cycles.

Canopy reflectance determined during the pod filling stage of *P. vulgaris* and *P. coccineus* was reduced under high saline conditions (Fig. 1). Plants of both species grown under low saline conditions developed a higher canopy (leaf area index) than the plants in high salinity (Table I). The major changes of canopy reflectance occurred in the infrared region (>750 nm) in both species under high saline conditions. Spectral reflectance is relatively low in the visible wavelengths (400-700 nm) due to the absorption by photosynthetic pigments, and all leaves reflect less blue and red light than they did green (Hume *et al.*, 2002). The variation of the near-infrared light balance is related to leaf thickness because transmittance decline with leaf thickness (Hume *et al.*, 2002).

Phaseolus coccineus produced a higher biomass than *Phaseolus vulgaris* under low salinity, but they did not show differences in seed yield (Table I). Under high saline conditions, *P. coccineus* had the lowest biomass and seed yield. The biomass production and seed yield were reduced in both species under high salinity.

In both species, the two vegetative indices (NDVI and GNDVI) were higher in plants grown under low salinity than under high salinity. A high NDVI or GNDVI value was associated with leaf area index (high canopy), biomass, and seed yield (Table III), and they could be used to assess yield of *P. vulgaris* and *P. coccineus* under saline conditions. In a previous study with *Phaseolus vulgaris* grown under three nitrogen levels (0, 100 and 200 kg nitrogen ha^{-1}) we found a positive association between NDVI (determined during the pod filling stage) and seed yield, biomass, leaf area index, and intercepted radiation (Gutiérrez-Rodríguez *et al.*, 2004a). The vegetative indices (NDVI and GNDVI) have also been associated with yield, and biomass in bread wheat genotypes under drought and well-irrigated conditions (Gutiérrez-Rodríguez *et al.*, 2004b).

The PRI index was lower under low salinity than under high salinity (Table II). The PRI index has been used to indicate radiation dissipation on the canopy in plants grown under saline conditions (Gamon *et al.*, 1992). In our study, the PRI index was increased under high salinity indicating an apparent high radiation dispassion on the canopy in both species by the stomatal closure and an increase of leaf temperature (Table IV). The PRI index showed an inverse relationship with leaf area index, yield, stomatal conductance, and transpiration.

Table I. Leaf area index, and yield of *Phaseolus vulgaris* and *Phaseolus coccineus* grown under saline field conditions

Species	Salinity	Leaf area index at pod filling	Biomass ($g\ m^{-2}$)	Seed yield ($g\ m^{-2}$)
Phaseolus	Low	1.9 b	251.0 b	145.8 a
vulgaris	High	1.6 b	210.7 c	114.5 b
Phaseolus	Low	2.3 a	305.6 a	152.5 a
coccineus	High	1.3 c	116.4 d	50.0 c

Means with different letters are significantly different from one another ($p \leq 0.05$); (n=4).

Table II. Vegetative indices (NDVI and GNDVI) and photochemical radiation index (PRI) measured during pod filling in *Phaseolus vulgaris* and *Phaseolus coccineus* grown under saline field conditions

Species	Salinity	NDVI	GNDVI	PRI
Phaseolus	Low	0.916 a	0.738 a	0.012 a
vulgaris	High	0.874 b	0.666 b	0.039 b
Phaseolus	Low	0.931 a	0.774 a	0.015 a
coccineus	High	0.833 b	0.702 b	0.036 b

Means with different letters are significantly different from one another ($p \leq 0.05$); (n=4)

Table III. Relationship between spectral reflectance indices and yield, and gas exchange parameters in *Phaseolus vulgaris* and *Phaseolus coccineus* grown under saline field conditions

	Leaf area index	Biomass	Yield	Stomatal conductance	Transpiration
NDVI	0.97**	0.98**	0.98**	0.73	0.73
GNDVI	0.82	0.68	0.58	0.91*	0.75
PRI	-0.81	-0.76	-0.76	-0.97**	-0.96**

*Correlation coefficient significant at the 0.05 level of probability; **Significance level 0.01.

Table IV. Chlorophyll estimation measured during pod filling in *Phaseolus vulgaris* and *Phaseolus coccineus* grown under saline field conditions

	Salinity	SPAD reading	R_{780}-R_{710}/R_{780}-R_{680}
Phaseolus	Low	37.5 a	0.72 a
vulgaris	High	40.9 a	0.65 a
Phaseolus	Low	42.4 a	0.72 a
coccineus	High	36.5 a	0.66 a

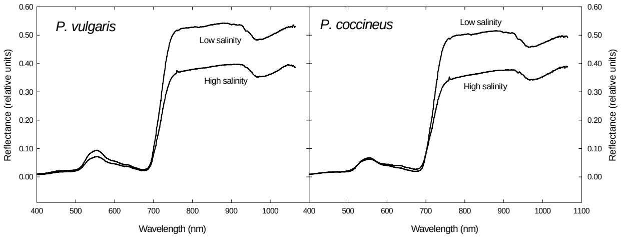
Means with different letters are significantly different from one another ($p \leq 0.05$); (n=4)

Table V. Stomatal conductance, transpiration rate, and leaf temperature in *Phaseolus vulgaris* L. and *Phaseolus coccineus* L. grown under saline field conditions

Species	Salinity	Stomatal conductance ($mmol\ m^{-2}\ s^{-1}$)	Transpiration rate ($mmol\ H_2O\ m^{-2}\ s^{-1}$)	Leaf temperature (°C)
Phaseolus	Low	326.8 a	11.3 a	22.3 c
vulgaris	High	93.2 d	3.4 c	27.4 a
Phaseolus	Low	299.4 b	8.1 b	23.0 c
coccineus	High	175.8 c	4.7 c	24.7 b

Means with different letters are significantly different from one another ($p \leq 0.05$); (n=4); Average radiation=1880 ($\mu mol\ m^{-2}\ s^{-1}$).

Fig. 1. Canopy reflectance determined during pod filling in *Phaseolus vulgaris* and *Phaseolus coccineus* grown under saline field conditions



Chlorophyll estimated with the spectral chlorophyll index and with the SPAD meter did not show differences in the two saline conditions in both species (Table IV). The chlorophyll content on leaves of both species was not affected by the salinity level.

Stomatal conductance, and transpiration rate of *P. vulgaris* and *P. coccineus* were lower under high salinity than low salinity, but leaf temperature was higher due to the stomata closure by the salinity level (Table V). In fact, the transpiration process on leaves is an important regulator of leaf temperature that expends energy by evaporating moisture (Paulsen, 1995). Bayuelo-Jiménez *et al.* (2003) found that an increase of salinity level caused a gradual decreased of leaf water vapor conductance, and CO_2 assimilation. Two-thirds of this reduction in CO_2 assimilation rate at high salt level (80 mM NaCl) was attributable to stomatal conductance in several *Phaseolus* species (Bayuelo-Jiménez *et al.*, 2003).

In our study, both *Phaseolus* species reduced biomass and seed yield in high salinity due to a low gas exchange (low stomatal conductance) under low salinity than they did under high salinity (Tables I, V). It was a reduction of 72% in stomatal conductance in *P. vulgaris* comparing the values of high and low salinity, while the reduction was of 42% in *P. coccineus*. Although *P. vulgaris* had a lower stomatal conductance than *P. coccineus* in high salinity, it had a higher seed yield. Also, there was a clear association between spectral reflectance indices and stomatal conductance, and transpiration rate in both species (Table III).

Both species of *Phaseolus* were able to grow in soils with high salinity level (EC >5 dS m^{-1}). The high salinity in soils increases osmotic potential and water potentials, and roots extract water less easily from soil aggravating water stress conditions (Letey, 1985). The water provided by rainfall is the only way to minimize salt loading of the underlying groundwater to crop water needs. In the current study, all the physiological measurements (i.e., reflectance indices, stomatal conductance, leaf area index, etc.) were collected after three raining days, and the osmotic and water potential are surely reduced in the soil with a precipitation of 13 mm accumulated.

CONCLUSIONS

In conclusion, biomass and seed yield were reduced under high saline field conditions, and the two vegetative indices (NDVI, and GNDVI) could be used to assess the plant growth reduction in *P. vulgaris* and *P. coccineus*. Low gas exchange (stomatal conductance) was involved in the low yield of both species under saline conditions, and it also was related with NDVI, GNDVI, and PRI.

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