Selecting key traits to indicrectly assess common bean growth under water stress

Alisson Marcos Fogaça[1*], Eduardo Augusto Agnellos Barbosa[1]

(1.Department of Soil Science and Agricultural Engineering, Ponta Grossa State University, Ponta Grossa 84030-900, Brazil)
*(*Corresponding author: Alisson M. Fogaça, M.Sc.,*
Department of Soil Science and Agricultural Engineering, Ponta Grossa State University, Ponta Grossa 84030-900, Brazil, Email: alifogaca@hotmail.com. Tel: +55 42 99974 5416.)

Abstract: Recognize water stress constraints on common bean growth may help to select tolerant genotypes, manage irrigation based on plant necessity and predict losses. This manuscript aims to select key traits that discriminate magnitudes of water stress on common bean and indicates plant growth constrains. To do so, an experiment was set in a greenhouse with two factors: crop evapotranspiration replenishment and cultivars. First factor was composed of 25%, 50%, 75%, 100%, 125% and 150% of crop evapotranspiration replenishment starting at flowering (32 days after germination), and second factor of the cultivars BRS Estilo and IPR Campos Gerais. A randomized block scheme with five replications was used, totalizing 60 experimental units. On the 55th day after germination, we analysed morphological (steam diameter, leaf area, and number of trifoliolates) and physiological (net assimilation of CO_2, stomatal conductance, leaf transpiration, and total content of chlorophyll) traits. A multivariate analysis of variance indicated single effect of crop evapotranspiration replenishment. Then, a canonical discriminant analysis was applied and the discrimination was confirmed by the Scott-Knott hierarchical clustering. We harvested the plants to correlate the selected traits with the aerial dry weight to determine its ability to assess plant growth indirectly. The traits related to leaf gas exchanges were the strongest discriminators of levels of water stress and had moderated correlation with common bean growth. For instance, when assessing the net assimilation of CO_2 during most stressful periods of the day, it was the most promisor trait that can be used singly that integrate both objectives.

keywords: *Phaseolus vulgaris* L., drought stress, net assimilation of CO_2, canonical discriminant analysis, crop evapotranspiration.

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1 Introduction

Adequate availability of water plays a major role in food security, however the climate change will be affecting the water regimes and water availability for agriculture, with disastrous effects over agricultural and food production (Wang et al., 2016). The increases in air temperature substantially change the frequencies and intensities of rainfall as well as its seasonal variabilities and spatial distributions (Haque et al., 2016; Singh and Kumar, 2019). Consequently, crops will be more often subjected to floods and droughts. Thus, quantifying the water stress on plants can guide strategies to manage water in agricultural system (Misra, 2014). One of the crops that is a model for this purpose is the common bean (*Phaseolus vulgaris* L.), because it has a role in the food security of the main source of proteins and nutrients (De la Vega et al., 2017), especially in developing countries.

Select physiological and morphological traits that

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[*] Corresponding author: **Alisson M. Fogaça**, M.Sc. Department of Soil Science and Agricultural Engineering, Ponta Grossa State University – UEPG, Ponta Grossa, 84030-900, Brazil. Email: alifogaca@hotmail.com.

strongly discriminate water regimes and are robust indicator of plant growth may be helpful to quickly recognize common bean genotypes that tolerate water stress (Polania et al., 2016), manage irrigation based upon plant necessity to save water (Anderson et al., 2016), and predict losses by water deficit (Lanna et al., 2016).

To select traits that are sensible to conditions of water stress it can be used the canonical discriminant analysis (CDA), which is a statistic tool that takes into consideration the intercorrelation between variables that are most discriminant (Cruz-Castillo et al., 1994). Authors used this method with success to evaluate different wheat lines under water stress (Safari et al., 2018), to distinguish water regimes for durum wheat (Lopes and Araus, 2006), and to select traits that respond to salinity stress (Yepes et al., 2018).

When subject to water stress, plants of common bean promptly close stomata to control water losses by transpiration (Osakabe et al., 2014). Stomatal closure not only reduces water losses but also limits leaf CO_2 diffusion into the leaf mesophyll, constraining photosynthesis (Lawson and Blatt, 2014). Alterations on the number and area of leaves, and stem morphology may be a later response from a reduction of carbon assimilation (Boutraa and Sanders, 2001). Water stress may also affect chlorophyll content due to oxidation by the reactive oxygen species, which are overproduced in this condition (Jaleel et al., 2009). However, fluctuation on the responses of chlorophyll are reported (Darkwa et al., 2016; Tairo et al., 2017).

Assessing traits under an integrative analysis such as CDA may indicate a set or even a single key variable that is robust evidence of water stress impact on common bean growth. This study aims to select key traits that discriminate magnitudes of water stress on common bean and indirectly access constrains on plant growth.

2 Material and methods

2.1 Experimental characterization

This study was carried in a greenhouse, located at the State University of Ponta Grossa, Brazil (25º5’23.88”S, 50º6’8”W, and 975 m a.s.l.), which was covered with EVA film of 150 microns equipped with four fans set to start automatically when the temperature was above 25°C. The local climate is Cfb according to Köppen-Geiger classification – warm temperature, fully humid and warm summer (Peel et al., 2007). We set a thermo hygrometer data logger HT 2000 (Perfect Prime, USA) in the center of the greenhouse to track it atmosphere variability. The daily average temperature and humidity recorded, followed by the standard error of the mean, were 24.28°C±0.37°C and 63.43%±1.36%.

The growth medium used was a Ferralsol sifted on an 8 mm mesh. Its chemical analysis (Pavan et al., 1992) showed a pH of 4.9; exchangeable Al^{3+}, Ca^{2+}, Mg^{2+}, and K^+ contents of 0.1, 3.2, 1.7, and 0.29 $cmol_c$ dm^{-3}, respectively; total acidity ($H^+ + Al^{3+}$) of 6.69 $cmol_c$ dm^{-3}; P (Mehlich1) of 6.5 mg dm^{-3}; and organic carbon of 33 g kg^{-1}. Its texture is composed of 540, 302 and 158 g kg^{-1} of clay, silt, and sand, respectively. It was incorporated 1.91 kg lime dm^{-3} to the growth medium to reach a base saturation of 70%.

The base fertilization was urea (46-00-00) with urease inhibitor and MAP+Zn (10-49-00) in the doses of 360 and 40 g pot^{-1}, respectively. We used the same urea to realize a topdressing fertilization at 20 days after germination (DAG) in the dose of 310 g pot^{-1}.

Five seeds were sowed on 12 liters pots, which were first filled with 1.5 kg of gravel and 2 kg of sand and then 10 dm^3 of soil. On 16/09/2017, the seeds germinated and we started to count the DAG. When plants had three trifoliolates (10 DAG), we thinned the plants and left the most vigorous.

2.2 Irrigation management

To impose the water stress on the plants, we determine the reference evapotranspiration (*ETo*) via class A pan (Doorenbos and Pruitt, 1977), which was placed next to the experiment in the greenhouse. We estimated the crop evapotranspiration (*ETc*) by multiplying the *ETo* by a common bean crop coefficient suggested by Bergamaschi et al. (1989). The volume of water applied was established considering the pot area and measured with a graduated cylinder. The water was distributed evenly over the entire pot area. All the plants received the same irrigation depth until the flowering (32 DAG).

2.3 Sources of variation and experimental design

The experimental design used was randomized blocks with five replicates, totalizing 60 experimental units. It was set in a factorial scheme with two factors: *ETc* replenishment (ER) and cultivars (CV). The levels of the first factor were 25%, 50%, 75%, 100%, 125% and 150% of *ETc* replenishment, starting at the flowering (32 DAG). The second factor was two cultivars most cultivated on Paraná – BR: BRS Estilo and IPR Campos Gerais. The first cultivar has an average of 90 days from germination to maturation and the second 88 days.

2.4 Physiological and morphological traits

At 55 DAG, we assessed the leaf gas exchanges with an infrared gas analyzer (IRGA) model LI-6400XT (LI-COR, USA), which provide the traits net assimilation of CO_2 (*A*, μmol CO_2 m^{-2} s^{-1}), stomatal conductance (*gs*, mol H_2O m^{-2} s^{-1}) and leaf transpiration (*E*, mmol H_2O m^{-2} s^{-1}). To control the atmospheric influence, we set the internal temperature of the chamber to 25°C, PPFD to 1200 μmol photons m^{-2} s^{-1}, CO_2 concentration to 400 μmol mol^{-1}, and flow to 400 μmol s^{-1}. These measurements were made on a young fully expanded leaf, which was also used to determine the total content of chlorophyll (*Chl*) with a portable chlorophyll meter model CFL 1030 (Falker, BR), which return a chlorophyll index.

The leaf area (*LA*, cm^2) was reached using the equation of Figueiredo et al. (2012) for brazilian common beans. We measure the steam diameter (*S*, mm), and then we counted the number of trifoliolate leaves (*NTL*) of each plant. Thereafter, we harvested the aerial part and dried at 65°C for 48 h to obtain the aerial dry weight (*ADW*, g).

2.5 Statistical analyses

We checked the normality of the residues of each trait with the Shapiro-Wilk normality test and then used Royston's multivariate normality test (Kormakz et al., 2014). No normal data was transformed by the square root function. A multivariate analysis of variance (MANOVA) with Pillai trace was used to check the significance of the factors (p <0.05). A CDA was realized for the levels of the significant factors and generated canonical discriminant functions (CDF) utilizing the R package *candisc* (in The Comprehensive R Archive Network, The R institute). To select the key discriminant traits, we interpreted the standardized coefficients and the correlations of the CDF's with the original traits. A *post-hoc* Scott-Knott clustering analysis was used to confirm the discrimination (Jelihovschi et al., 2014).

We correlated the canonical scores with the *ADW* using a Pearson's correlation (r) to quantify its ability to assess plant growth. Equally, we calculated r for the individual traits and compared with the r obtained for the canonical scores. All statistical analyses were performed on the R environment for statistical computing (R Core Team, 2017).

3 Results and discussion

3.1 Data normality and multivariate analysis of variance

The data collected is presented on Table 1 sorted by level of the studied factors. All traits showed normal distribution, except for *gs*, which was normalized by the square root function. Subsequently, the Royston's analysis showed normality with p-value equal to 0.56. The MANOVA revealed high significance of ER and CV with p-value equal $9.12X10^{-6}$ and $8.54X10^{-9}$, respectively. The multiplicative effect of the factors was not significant (p<0.05) (Table 2). Thus, it was applied the CDA procedure only for the levels of ER, because there is no significant difference of water stress between the studied cultivars.

Table 1 Summary of traits sorted by level of factor.

Levels	Traits			
	A (μmol CO2 m^{-2} s^{-1})	gs (mol H2O m^{-2} s^{-1})	E (mmol H_2O m^{-2} s^{-1})	Chl
25%	0.70±0.55†	0.005±0.004	0.08±0.08	42.37±1.96
50%	3.18±0.76	0.021±0.004	0.35±0.06	43.76±1.89
75%	10.21±1.12	0.134±0.042	1.68±0.37	41.79±2.

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100%	8.58±1.39	0.092±0.017	1.33±0.22	37.50±2.17
125%	15.48±1.67	0.408±0.087	3.53±0.49	40.68±2.11
150%	13.44±1.57	0.273±0.049	2.95±0.38	38.88±1.92
BRS Estilo	8.09±1.12	0.150±0.033	1.65±0.28	37.92±1.20
IPR Campos Gerais	9.10±1.26	0.160±0.039	1.66±0.30	43.74±0.89
	S (mm)	LA (cm^2)	NTL	ADW (g)
25%	3.52±0.14	17.42±1.55	9.20±0.70	14.27±0.41
50%	3.33±0.11	15.01±1.77	8.60±0.54	15.08±0.67
75%	3.39±0.07	16.74±1.39	8.40±0.75	16.53±0.57
100%	3.38±0.09	15.75±1.50	8.00±0.58	17.27±0.78
125%	3.34±0.09	14.79±1.24	9.70±0.86	17.53±0.90
150%	3.56±0.08	16.23±0.85	9.50±0.58	19.77±1.01
BRS Estilo	3.37±0.06	13.83±0.65	7.70±0.31	15.64±0.46
IPR Campos Gerais	3.47±0.05	18.16±0.73	10.10±0.34	17.84±0.52

Note: † n = 10 and 30 for means of ER and CV, respectively, followed by the standard error of the mean. A: net assimilation of CO_2; gs: stomatal conductance; E: leaf transpiration; Chl: total content of chlorophyll; S: steam diameter; LA: leaf area of the five leaflets; NTL: number of trifoliolate leaves; ADW: aerial dry weight.

Table 2 Results of multivariate analysis of variance of the traits collected

Source of Variation	Df	Pillai trace	~F	Num Df	Den Df	*p*-value
Block	4	1.44	3.29	28	164	$1.07\text{X}10^{-6}$
CV	1	0.58	7.67	7	38	$9.12\text{X}10^{-6}$
ER	5	1.85	3.52	35	210	$8.54\text{X}10^{-9}$
CV*ER	5	0.95	1.41	35	210	0.076
Residuals	44					

Note: CV: cultivar; ER: crop evapotranspiration replenishment; Df: degree of freedom; ~F: approximated F; Num Df: numerator degree of freedom; Den Df; denominator degree of freedom, *p*-value: probability.

3.2 Canonical discriminant analysis

The CDA for the levels of ER showed that the first, second and third CDF are significant, containing 68.65%, 21.06% and 8.59% of total variance, respectively. For the first CDF, it was observed the greater weights of the standardized coefficients for *gs, A* and *E* (Table 3). Furthermore, these three traits were well represented since all of them have high correlation with the CDF (Table 4).

Table 3 The statistical results of the canonical discriminant analysis for the levels of crop evapotranspiration replenishment (ER)

Statistics	1st CDF†	2nd CDF	3rd CDF	4th CDF	5th CDF
Eigenvalue	4.41	1.35	0.55	0.08	0.03
%	68.65	21.06	8.59	1.23	0.47
Num Df	35	24	15	8	3
Den Df	204.35	172.15	138.43	102	52
~F	6.33	3.55	2.01	0.69	0.52
p-value	$2.2\text{X}10^{-16}$	$6.6\text{X}10^{-7}$	0.018	0.695	0.666

Note: CDF: canonical discriminant functions; Num Df: numerator degree of freedom; Den Df; denominator degree of freedom; ~F: approximated F; *p*-value: probability.

Although the high standardized coefficients, it was reasonable to drop the second and third CDF of the interpretation due to its weak correlations with all original traits (Tables 3 and 4) and low variance content. Further investigation for these CDF's would not be helpful to select key traits to discriminate the levels of ER.

Table 4 The canonical discriminant coefficient parameters for the significant (*p*<0.05) canonical discriminant functions

Trait	Standardized coefficients			Correlations		
	1st CDF	2nd CDF	3rd CDF	1st CDF	2nd CDF	3rd CDF
A	-0.98	-1.22	1.44	-0.94	0.13	-0.04
gs	-1.00	-2.58	-2.62	-0.94	0.22	-0.19
E	0.89	4.08	1.30	-0.89	0.37	-0.12

Chl	0.45	-0.03	-0.90	0.19	0.14	-0.32
S	0.10	-0.27	0.25	0.04	0.18	0.33
LA	-0.31	0.15	0.55	0.07	0.15	0.17
NTL	-0.03	0.49	-0.17	-0.13	0.44	-0.09

Note: CDF: canonical discriminant functions; *A*: Liquid assimilation of CO2; *gs*: stomatal conductance; *E*: transpiration; *Chl*: chlorophyll content; *S*: steam diameter; *LA*: leaf area; *NTL*: number of trifoliolated leaves.

First CDF for the levels of ER were interpreted as the leaf gas exchanges due to have high correlation with the three traits obtained from the gas exchanges measurements with IRGA. The visual discriminations indicate that plants that received 125% and 150% of ER tend to have high values of *A*, *gs* and *E*, followed by 75% and 100%, 50% and 25% (Figure 1).

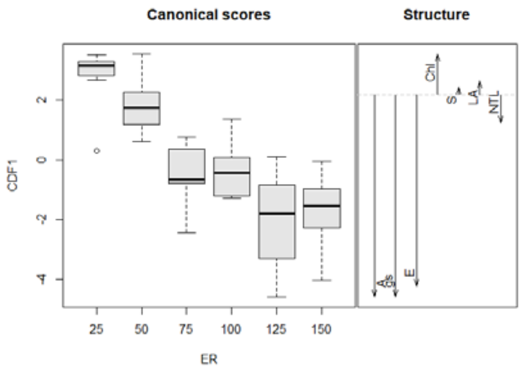

Figure 1 Scores of the first CDF grouped by levels of ER (left sided box) and structure of correlations with original traits (right sided box)

To confirm the discrimination, the scores obtained by the first CDF were subjected to ANOVA with ER and block as factors and, if significant, to Scott-Knott clustering procedure. The ANOVA showed high significant effect for ER with *p*-value equal $2.3X10^{-15}$ and clusters confirmed the interpretation of the visual method (Figure 2).

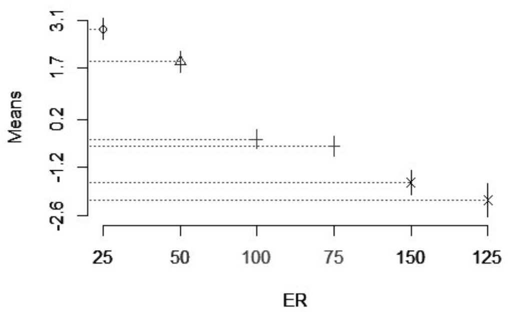

Figure 2 Means of scores from the first CDF by level of ER. Equal symbols represent the same group (p<0.05) and bars represent the standard error of the mean

Even though *Chl* slightly increased with water stress and had a positive correlation with *S* and *LA*, the standardized coefficients and correlations (Table 4) indicate that those traits are not helpful to distinguish levels of ER. On the other hand, Darkwa et al. (2016) observed moderate correlation of chlorophyll with plant height, number of pods per plant, seeds per pod and yield per hectare of 64 common bean genotypes under water stress. Thus, the chlorophyll content measurements show inconsistence between studies, reason to be disregarded as an indicator of water stress in common bean. This consideration corroborates with Tairo et al. (2017), who also observed variability between studies or between seasons of leaf chlorophyll content of common ben extracted with dimethylsulphoxide.

There was a weak correlation between *S*, *LA* and *NTL* (Table 4). Data suggests a propensity of plants under water stress tend to have a slightly decrease on the number of trifoliolates leaves, but with a slightly larger area and steam diameter. Data from Lanna et al. (2016) contrast with our finds, who observed a significant reduction on the leaf area when plants of common bean are under water stress from vegetative to maturation. Such variability may be linked with genotypes responses, regardless of the same species is used. Although morphological traits are an integrative response of a series of physiological processes, our finds did not point *S*, *LA* or *NTL* as robust discriminants, what corroborates with Poorter and Nagel (2000) that compiled thirty experiments with seventy observations and concluded that under water stress allocation of mass to leaves are modest. Thus, these morphological variables are not expressive to discriminate water stress.

The traits *A*, *gs* and *E* were robust discriminant of water stress. Plants that were subjected to lower water replenishment showed lower values of these three traits (Figure 1). Water depletion affect instantly gas exchanges due to stomatal closure (Osakabe et al., 2014). However, stomata response is quickly and gas exchange traits may vary across the day (Lawson and Blatt, 2014). It is crucial to collect gas exchanges data during times that stomatal is mostly affected (warm and sunny days), otherwise it may not evidence the magnitude of water stress. Previous studies agreed that water stress influences on *A* (Darkwa et al., 2016; Dipp et al., 2017). Thus, this promisor trait, when avail under times that the water stress is maximum, can be used to assess common bean growth.

3.3 Plat growth indirect assessment

The Pearson's correlation showed high significance with moderate negative relationship of *ADW* and the scores of the first CDF (Table 5). This correlation suggest that plants that have the highest scores showed the lowest *ADW*. Consequently, plants with highest gas exchanges tend to have highest *ADW* (Figure 1). For the single traits, only *A* is as strong and significant correlated with *ADW* as the scores obtained from the CDF (Table 5).

Table 5 Pearson's correlations between aerial dry weight (*ADW*) and variables.

Statistic	Trait							CDF1
	A	gs	E	Chl	S	LA	NTL	Scores†
r	0.52	0.45	0.46	0.26	0.20	0.33	0.29	-0.48
p-value	$210X^{-5}$	$3X10^{-4}$	$2X10^{-4}$	0.04	0.12	0.01	0.02	$8X10^{-5}$

Note: A: net assimilation of CO2; gs: stomatal conductance; E: leaf transpiration; Chl: total content of chlorophyll; S: steam diameter; LA: leaf area of the five leaflets; NTL: number of trifoliolate leaves. The scores were obtained from the first canonical discriminant function of the levels of crop evapotranspiration replenishment (ER).

Our data contrasts with data obtained by Cuellar-Ortiz et al. (2008), who reported no significant effect of water stress on the canopy biomass of a drought tolerant and a drought susceptible cultivar of common bean under 30%, 60% and 100% of field capacity during 23 days. It is possible that setting the water stress during flowering made possible for us to find correlation of *ADW* with gas exchanges, because it is the growth stage that water use efficiency is the lowest and water scarcity mostly affect plant production (Calvache et al., 1997). This hypothesis is sustained by the data from Lanna et al. (2016), who reported constrains on the shoot dry mass, leaf area and grain yield of two drought tolerant cultivars of common bean subjected to water stress from the vegetative until the pod filling period.

Our data indicates that *A* as a single trait or the leaf gas exchanges as a set of traits (Figure 1 and Table 5) are the most robust traits to discriminate and assess plant growth during the flowering. However, different result can occurs if the stress occurs out of flowering period (Cuellar-Ortiz et al., 2008; Lanna et al., 2016) and the collection of the traits is not done during the most stressful time (Lawson and Blatt, 2014). This variability is linked with the influence of cicardian clock over the transcription of genomes that regulates growth under water stress, which are preferably expressed during the day. Furthermore, the time of the day influences the magnitude and specificity of gene expression (Dubois et al., 2017). Along these evidences, the time choice to measure *A* may lead to a robust discrimination of water stress.

The aboveground biomass during flowering are well correlated with grain yield, mainly during water stress, as demonstrated by Polania et al. (2016). We suggest that indirect assess of this trait may aid breeders, researchers and farmers to quickly drawn strategies on water manage, contributing to food security in a climate change scenario.

4 Conclusion

Measurements of leaf gas exchanges are useful to distinguish water stress magnitudes and indirectly assess plant growth. The instantaneous measurement of net assimilation of CO_2 is a promisor trait to be used singly that matches both objectives, however data should be collected during the most stressful periods of the day when the magnitude of response is greater.

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