agronomy

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Chlorophyll α Fluorescence Parameters as an Indicator to Identify Drought Susceptibility in Common Bush Bean

Alefsi David Sánchez-Reinoso, Gustavo Adolfo Ligarreto-Moreno and Hermann Restrepo-Díaz *

Departamento de Agronomía, Facultad de Ciencias Agrarias, Universidad Nacional de Colombia, Carrera 30 No. 45-03, Bogotá 11321, Colombia

* Correspondence: hrestrepod@unal.edu.co; Tel.: +57-316-5000

Received: 25 July 2019; Accepted: 6 September 2019; Published: 9 September 2019

Abstract: The common bean is susceptible to drought conditions and the evaluation of plant responses to low water availability can be difficult. The quantification of chlorophyll fluorescence as a sensitive trait to environmental stresses is an important alternative in the characterization of drought-susceptible genotypes. The objective of this study was to evaluate mainly the use of chlorophyll α fluorescence (maximum efficiency of PSII (F_v/F_m), photochemical quenching (qP), non-photochemical quenching (NPQ)) and rapid light-response curves (RLCs) (initial slope of the curve (α), minimum saturation irradiance (I_k) and maximum relative electron transport rate (ETR_{max})) parameters as tools for the identification of susceptible or tolerant bush bean cultivars to water deficit stress conditions in two different phenological stages. Using a randomized block design in a factorial arrangement, five bush bean cultivars (Cerinza, Bachue, NUA35, Bacata and Bianca) were evaluated under water deficit conditions by the suspension of irrigation for 15 days from 40 to 55 Days after Emergence (DAE) (vegetative stage) or 50 to 65 DAE (reproductive stage). The results showed that F_v/F_m and NPQ recorded the highest variation due to water deficit conditions, especially in the vegetative stage. The greatest reductions in F_v/F_m (0.67) and NPQ (0.71) were evidenced in cultivar NUA35 compared to its control plants (0.78 and 1.07, respectively). The parameters obtained from RLCs showed that cultivar Bacata registered the lowest α (0.17) and I_k (838.19 $\mu mol \cdot m^{-2} \cdot s^{-1}$) values compared to its control plants (α 0.23; I_k 769.99 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Differences were only obtained in ETR_{max} in the reproductive stage (50–65 DAE) in which cultivar NUA35 reached values of 158.5 in stressed plants compared to control plants (251.22). In conclusion, the parameters derived from RLCs such as α and I_k can be used as tools to identify drought susceptibility in the vegetative stage, whereas ETR_{max} can be used in the reproductive stage. In addition, PSII photochemistry (F_v/F_m and NPQ) can also help to understand the agronomic responses of common bush bean cultivars to drought conditions.

Keywords: light saturation point; *Phaseolus vulgaris*; quenching; rapid light-response curves

1. Introduction

The common bean (*Phaseolus vulgaris* L.) is the most cultivated and consumed grain legume in the world and plays an important role in the human diet due to its high protein and mineral content [1,2]. A worldwide production of 55,627 Mt in 38,038,865 ha was recorded for green and dry beans in 2017 [3]. Studies have projected a reduction in climate conditions due to heat and drought stress in a large part of bean crops in South America [4].

Plants are naturally exposed to different abiotic and biotic stress conditions [5]. Drought is the most decisive abiotic stress for crop yield and productivity, demanding a continuous knowledge of plant responses to this condition [6,7]. Plants under water deficit stress show a wide range of responses that include morphological, physiological, biochemical and molecular changes [8]. In addition, the decrease

in chlorophyll content under drought stress has been considered a typical symptom of oxidative stress and may be the result of pigment photooxidation and photosynthetic pigment degradation [5].

The monitoring, identification and quantification of plant responses to drought are highly demanded in breeding programs for the selection of tolerant and high-yielding genotypes [9]. In general, research on drought tolerance assessment is focused on plant survival [6]. Chlorophyll fluorescence is a highly informative technique of plant traits to cope with adverse environmental conditions [10]. In this sense, chlorophyll fluorescence uses information about the photochemical activity of plants, allowing the early detection of environmental stress [11]. This can be done because the chlorophyll molecule is fluorescent, which makes it possible to detect changes in electron transfer at the level of chloroplast membranes through photon dissipation [12]. One of the great advantages of this technique is that it is sensitive to disorders of the photosynthetic cell membrane without destroying the plant tissues of the species under study [13]. Rosenqvist and van Kooten [14] state that any variation in the values between 0.79 to 0.84 indicates that the PSII reaction centers are being affected. These authors also mention that photochemical and non-photochemical quenching (qP and NPQ) are parameters that help to understand acclimation mechanisms in plants under different environmental conditions. Finally, Flowers et al. [15] evaluated chlorophyll α fluorescence parameters (F_v/F_m, qP) in different snap bean genotypes under ozone stress, concluding that these parameters can help to determine snap bean sensitivity to high O_3.

Specialized equipment such as modulated fluorometers allows constructing Rapid Light-Response Curves (RLCs) which can be used to measure quantum yield in terms of irradiance [16]. From RLCs, parameters such as the initial slope of the curve (α), minimum saturation irradiance (I_k) and maximum relative electron transport rate (ETR_{max}) can be determined, indicating the photosynthetic efficiency of plants under stress conditions [17]. RLCs is a technique that has been used for characterization of plants under drought stress in in vitro or field conditions [18,19].

Knowledge of the mechanisms of genotype acclimation to adverse environmental conditions is an effective strategy to reduce vulnerability to climate change [4]. Techniques to facilitate the identification of drought-tolerant genotypes have become important in modern agriculture [7]. Therefore, the objective of this research was to evaluate the use of chlorophyll fluorescence parameters as a tool for the identification of susceptible or tolerant bush bean (*Phaseolus vulgaris* L.) cultivars to water deficit stress conditions in two different phenological stages (vegetative and reproductive) as a trait of interest in legume improvement programs.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

Seeds of five bush bean cultivars were used in the present study: (i) 'ICA-Cerinza' and'ICA-Bachue' (cultivars with at least 20 years of sowing history in traditional Colombian agriculture); (ii) 'NUA35' (cultivar with eight years of commercialization since its release); and (iii) 'Bianca' and 'Bacata' (recently released cultivars). Seeds were sown in the greenhouse of the Faculty of Agricultural Sciences at Universidad Nacional de Colombia located in the city of Bogotá at a height of 2556 m.a.s.l., (4°35'56" N and 74°04'51" W) from September 2015 to January 2016 in 2.1 m^2 plots (3 rows of 1 linear meter long). The plant spacing was 16 cm × 70 cm between plants and between rows, respectively (20 seeds per plot = 85,000 plants ha^{-1}). The physical and chemical characteristics of the soil in the greenhouse were: (i) sandy loam soil (0.26 sand, 0.42 silt, and 0.32 clay); (ii) chemical characteristics: Total N 0.36%, Ca: 10.6, K: 0.98, Mg: 1.75, and Na: 0.24 meq 100 g^{-1}, Cu: 1.67, Fe, 310, Mn: 3.21, Zn: 15.5, B: 0.48, and P: > 116 mg kg^{-1}; (iii) pH 5.4; and (iv) effective cation exchange capacity (ECEC) 13.8 meq 100 g^{-1}. Growth conditions in the greenhouse during the experiment were: a natural photoperiod of 12 h with a Photosynthetic Active Radiation (PAR) of 1000 µmol m^{-2} s^{-1}, average temperature of 27 °C and 60 to 80% relative humidity. Two edaphic fertilizers were used per plant: (i) 4 g/plant (350 kg ha^{-1}) of a 15–15–15 compound fertilizer (TRIAN 15® Yara, Colombia) as a nitrogen, phosphorus and potassium

source; (ii) and 2 g plant^{-1} (170 kg ha^{-1}) of microelements (granulated Agrimins, Colinagro®, Colombia) respectively, divided into two applications (half of the aforementioned dose at 15 and 40 days after emergence (DAE)). Finally, the composition of the Agrimins fertilizer was: Total N 8% (Urea N 7% and Ammonium N 1%), Assimilable P (P_2O_5) 5%, Ca (CaO) 18%, Mg (MgO) 6%, Total S 1.6%, Cu 0.75%, B 1%, Mo 0.005% and Zn 0.25%.

2.2. Water Deficit Treatment

Three different groups of treatments were established in each cultivar: (i) plants irrigated throughout the crop cycle (control); (ii) plants with water deficit in the phenological stage 13–14 (vegetative) according to the BBCH (Bayer, BASF, Ciba-Geigy and Hoechst) scale [20] (formation of three to four fully expanded trifoliate leaves), which was reached at approximately 40 DAE; (iii) plants with water deficit during the reproductive stage according to the BBCH scale 63–64 (30 to 40% open flowers), which was reached at approximately 50 DAE. Water deficit was established by the total suspension of irrigation for 15 days. This period of water deficit stress was based on previously developed studies with variables such as photosynthesis and electrolyte leakage showed greater affectations [16]. Before and after the water deficit period, plants were irrigated in the morning (700 h to 900 h) with an equal amount of water. In the initial growth stages (up to 13–14 BBCH), plants were irrigated with 6 mm per week. During stages 15 to 55 BBCH, 12 mm were supplied per week. Finally, 18 mm were supplied per week from stage 56 to 89 according to the BBCH scale. All cultivars were irrigated at the same time with a watering can. The soil moisture content was constantly monitored by a humidity probe (Kelway HB−2 Soil Acidity and Moisture Tester, Kel Instruments Co., Inc. NJ, USA) at a depth of 20 cm during the stress period.

2.3. Photosynthetic Pigments and Chlorophyll Fluorescence Determination

The measurements of all photosynthetic pigments and chlorophyll fluorescence variables were taken at the end of each stress period (at 55 DAE for the vegetative stage and 65 DAE for the reproductive stage). In general, variables were estimated in the second fully expanded trifoliate leaf from the upper part of the canopy. Regarding the quantification of photosynthetic pigments, 500 mg of plant material were collected and homogenized with liquid nitrogen. Subsequently, samples were stored at −80 °C until their respective analysis. Finally, the experiment lasted approximately 115 days after the emergence of all seedlings.

Approximately, 30 mg of the stored samples were homogenized in 6 mL of acetone (80%) and centrifuged (Model 420101, Becton Dickinson Primary Care Diagnostics, MD, USA) at 5000 rpm for 10 min to remove particles [21]. The chlorophyll content was estimated at 663 and 646 nm, whereas carotenoids were determined at 470 nm.

A spectrophotometer (Spectronic BioMate 3 UV-vis Thermo, Madi-son, WI, USA) was used for both determinations. The content of leaf photosynthetic pigments was determined by the equations for acetone according to Wellburn [22].

The maximum efficiency of PSII (F_v/F_m), photochemical quenching (qP), non-photochemical quenching (NPQ) and rapid light-response curves (RLCs) were determined at 55 and 65 DAE using a modulated fluorescence chlorophyll meter (MINI-PAM, Walz, Effeltrich, Forchheim, Germany). Leaves were dark-adapted with leaf clips for 15 min. After dark adaptation, the fluorescence variables F_0, F_m, F_v/F_m, qP, and NPQ were determined. The minimal fluorescence (F_0) was recorded with modulated low intensity light (<0.1 μmol·m^{-2}·s^{-1}) without affecting the variable fluorescence. The maximal fluorescence (F_m) was estimated by a 0.8 s long saturating light pulse (2600 μmol·m^{-2}·s^{-1}) with 20,000 Hz frequency. The variable fluorescence was calculated by the difference between F_0 and F_m. F_v/F_m ratio was obtained from the F_v and F_m and represent potential maximal PSII quantum yield. The photochemical and non-photochemical quenching were calculated as $qP = (F_m' - F)/(F_m' - F_0)$, and $NPQ = (F_m - F_m')/F_m'$ [23].

Rapid light-response curves were constructed by plotting the electron transport rate (ETR), qP and NPQ versus the increasing actinic irradiance (from 1 to 1795 μmol·m^{-2}·s^{-1}) with intervals of 10 s between irradiance levels [24]. The parameters α, ETR_{max} and I_k were estimated using the model described by Xu et al. [17].

2.4. Experimental Design and Data Analysis

A randomized block design with a factorial arrangement was performed, with the phenological stage in which drought stress was initiated as the first factor and the five evaluated genotypes as the second factor, for a total of 10 treatments with four repetitions and 60 plots of 2.1 m^2 (3 rows of 1 linear meter long). Subsequently, when there were significant differences in the ANOVA, the comparative Tukey's test was used at $P \leq 0.05$. Data were analyzed using the Statistix v 9.0 software (analytical software, Tallahassee, FL, USA). The results of the analysis of variance are summarized in Table 1.

Table 1. Summary of the analysis of variance of the effects of water stress on the physiological behavior of five bush bean genotypes.

Parameter	Abbreviation	Variation Source					
		Vegetative Stage (55 DAE)			Reproductive Stage (65 DAE)		
		Stage	Cultivar	Stage × Cultivar	Stage	Cultivar	Stage × Cultivar
Total Chlorophyll	*Chl total*	***	***	***	NS	***	*
Carotenoids	*Cx + c*	***	***	*	**	***	***
Maximum efficiency of PSII	F_v/F_m	***	**	**	*	NS	NS
Photochemical quenching	qP	NS	NS	NS	NS	*	NS
Non-photochemical quenching	NPQ	NS	*	*	***	***	NS
Initial slope	α	NS	**	**	NS	*	NS
Maximum electron transport rate	ETRmax	NS	NS	NS	***	***	***
Light saturation point	I_k	NS	NS	**	NS	*	NS

*, **, and *** significantly different at 0.05, 0.01 and 0.001 probability levels, respectively. NS, not significant with $a = 0.05$; DAE, Days after Emergence.

3. Results

3.1. Chlorophyll and Carotenoids Contents

Figure 1 shows the results obtained in the quantification of the total chlorophyll and carotenoid content. In general, it can be observed that significant differences were obtained in the interaction Stage × Cultivar on the concentration of these evaluated photosynthetic pigments for the two sampling points. In the first sampling point (55 DAE), it was observed that the majority of control plants showed a leaf chlorophyll content around 2166.26 μg mg^{-1} Fresh Weight (FW). It is important to point out that there was a greater reduction of the total chlorophyll content in cultivar Bacata plants that were subjected to water deficit stress conditions during the vegetative stage (1517.65 μg mg^{-1} FW). In the reproductive stage, cultivar Bacata continued to show the lowest chlorophyll values compared to cultivar Bachue, which presented the highest values (1538.90 μg mg^{-1} FW and ~1709.29 μg mg^{-1} FW, respectively) (Figure 1A). Regarding the carotenoid content, a reduction in this group of photosynthetic pigments was observed especially in cultivars Bianca, Bacata and NUA35 compared to their controls (~306.91 μg mg^{-1} FW vs. 362.33 μg mg^{-1} FW) due to water stress in the vegetative stage. At 65 DAE, cultivar Bachue plants under water deficit in the reproductive stage also showed a higher total carotenoid concentration compared to their control (320.50 μg mg^{-1} FW vs. 230.39 μg mg $^{-1}$ FW, respectively) (Figure 1B).

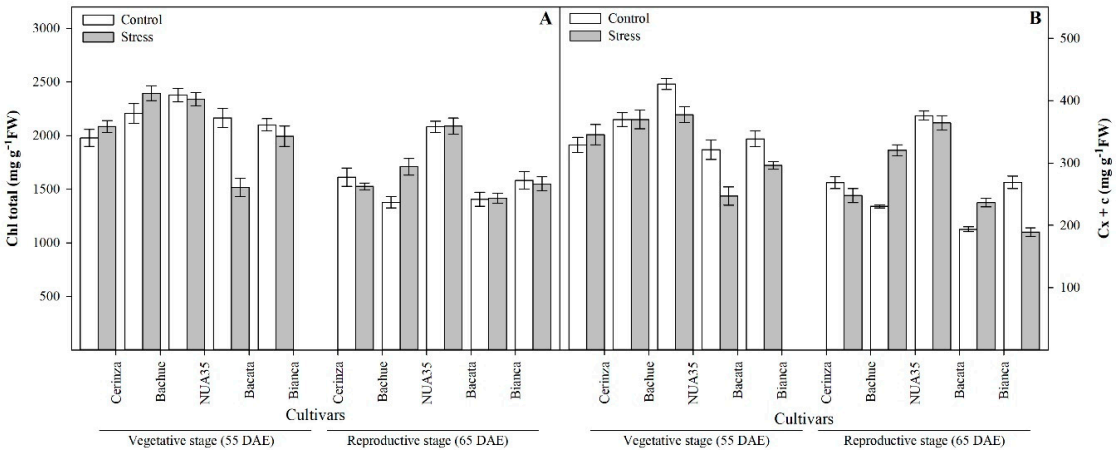

Figure 1. Effect of water deficit stress on two different phenological stages (vegetative (55 Days after Emergence (DAE)) and reproductive (65 DAE)) on total chlorophyll (**A**) and total carotenoids (**B**) in five bush bean cultivars. Bars represent the mean of four plants ± standard error; FW, Fresh weight.

3.2. Fluorescence Parameters

Table 2 presents the results for F_v/F_m, qP, and NPQ. Significant differences were found in the Stage × Cultivar interaction for F_v/F_m and NPQ at 55 DAE. The greatest reductions in F_v/F_m (0.67) and NPQ (0.71) were evidenced in cultivar NUA35 plants with a period of 15 days of stress in the vegetative stage compared to the control plants of the same cultivar (F_v/F_m of 0.78 and NPQ of 1.07). Regarding qP and NPQ, significant differences were found mainly in the factor Cultivar at 55 DAE. In this regard, the lowest qP values were observed in cultivar Cerinza plants (0.56) and the highest ones in cultivar NUA35 plants (0.66). For NPQ, the lowest values were found in cultivar NUA35 plants (0.97) and the highest ones in cultivar Bacata plants (1.60). Significant differences were not obtained on qP and NPQ at 65 DAE.

Table 2. Effect of water deficit stress in two different phenological stages on fluorescence parameters of five bush bean cultivars (55 DAE and 65 DAE).

Treatment	Vegetative Stage (55 DAE)			Treatment	Reproductive Stage (65 DAE)		
	F_v/F_m	qP	NPQ		F_v/F_m	qP	NPQ
Stress stage				Stress stage			
Control	0.78 a[z]	0.61	1.10	Control	0.79 a	0.60	1.07
Vegetative	0.74 b	0.60	1.23	Reproductive	0.75 b	0.63	1.21
Significance	*	NS	NS[y]	NS	**	NS	NS
Cultivar				Cultivar			
Cerinza	0.77 a	0.63	1.12	Cerinza	0.77	0.56	1.27 ab
Bachue	0.76 ab	0.63	0.89	Bachue	0.75	0.65	1.10 ab
NUA35	0.74 b	0.62	1.16	NUA35	0.76	0.66	0.97 b
Bacata	0.77 ab	0.57	1.58	Bacata	0.75	0.58	1.60 a
Bianca	0.77 ab	0.58	1.29	Bianca	0.75	0.63	1.41 ab
Significance	**	NS	NS	Significance	NS	NS	***
Interaction				Interaction			
Cerinza × C	0.79 a	0.64	1.22 ab	Cerinza × C	0.79	0.55	1.47
Bachue × C	0.79 a	0.66	0.60 b	Bachue × C	0.80	0.65	0.78
NUA35 × C	0.78 a	0.62	1.07 ab	NUA35 × C	0.79	0.71	0.50
Bacata × C	0.79 a	0.62	0.92 ab	Bacata × C	0.78	0.54	1.25
Bianca × C	0.76 a	0.51	1.69 ab	Bianca × C	0.79	0.57	1.35
Cerinza × V	0.79 a	0.58	1.26 ab	Cerinza × R	0.77	0.57	0.98
Bachue × V	0.74 ab	0.64	1.23 ab	Bachue × R	0.73	0.65	1.14
NUA35 × V	0.67 b	0.65	0.71 ab	NUA35 × R	0.76	0.64	1.08
Bacata × V	0.75 a	0.54	1.86 a	Bacata × R	0.73	0.66	1.56
Bianca × V	0.77 a	0.61	1.11 ab	Bianca × R	0.77	0.62	1.27
Significance	*	NS	*	Significance	NS	NS	NS
[x]CV (%)	4.31	11.40	42.44	CV (%)	5.63	13.04	32.0

[z] a, b stand for the values are significantly different at $p \leq 0.05$ according to the Tukey test; [y] NS. = Not significant ($p \leq 0.05$); *, **, and *** significantly different at 0.05, 0.01 and 0.001 probability levels, respectively. [x] C.V, Coefficient of variation.

3.3. Rapid Light-Response Curves

RLCs showed significant differences between treatments at the end of the water deficit period in the vegetative (Figure 2) or reproductive (Figure 3) stages. A reduction of ETR values was registered with an increase of the actinic light. Cultivars Bachue and Bacata showed the greatest drops at 55 DAE under water deficit conditions in the vegetative stage, whereas cultivars NUA35 and Bacata were the most affected at 65 DAE.

Figures 4 and 5 show the variation of qP with respect to the intensity of the actinic irradiance at the end of the water deficit period in the vegetative or reproductive stage, respectively. In general, qP showed an inversely proportional behavior to the increase in actinic irradiance in both stress periods and genotypes evaluated. The water deficit stress period in the vegetative stage caused cultivars Cerinza and Bianca to show an increase in qP, while a reduction of this variable was observed in cultivar Bacata. At the end of the stress period in the reproductive stage, 'Bacata' was the only cultivar that showed a variation on qP, observing a slight increase of this variable compared to plants under control conditions.

Non-photochemical quenching showed a directly proportional tendency to the increase in actinic irradiance in the evaluated factors (genotypes and stress stage). The water deficit caused a reduction of the NPQ values in cultivars NUA35 and Bianca compared to plants under control conditions at the end of water stress in the vegetative stage (55 DAE). However, the water deficit produced an opposite tendency in cultivars Cerinza, Bachue and Bacata, in which an increase in the energy dissipation in the form of heat was observed in comparison to plants without stress (Figure 6). At 65 DAE (reproductive stage), water deficit caused two differential behaviors on NPQ in the evaluated genotypes. This variable showed a greater increase in cultivars Bachue and NUA35 under water deficit stress, whereas NPQ was lower in stressed 'Cerinza' plants (Figure 7).

Table 3 summarizes the parameters obtained from the rapid light-response curves (α, ETR_{max} and I_k) at 55 and 65 DAE, respectively. Significant differences were observed in the Stage × Cultivar interaction for the variables α and I_k in the stress period in the vegetative stage (40–55 DAE). At the end of the vegetative stage, the water deficit caused a reduction of α values in cultivar Bacata compared to plants under control conditions (0.23 and 0.17, respectively). For I_k, cultivars Bacata and Bianca showed the lowest values due to the stress condition (838.19 and 769.99 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively). Differences in the interaction of the evaluated factors were observed only on ETR_{max} in the reproductive stage (50–65 DAE). The water deficit caused a reduction only on ETR_{max} in cultivar NUA35, obtaining values close to 158.5 in stressed plants compared to control plants (251.22).

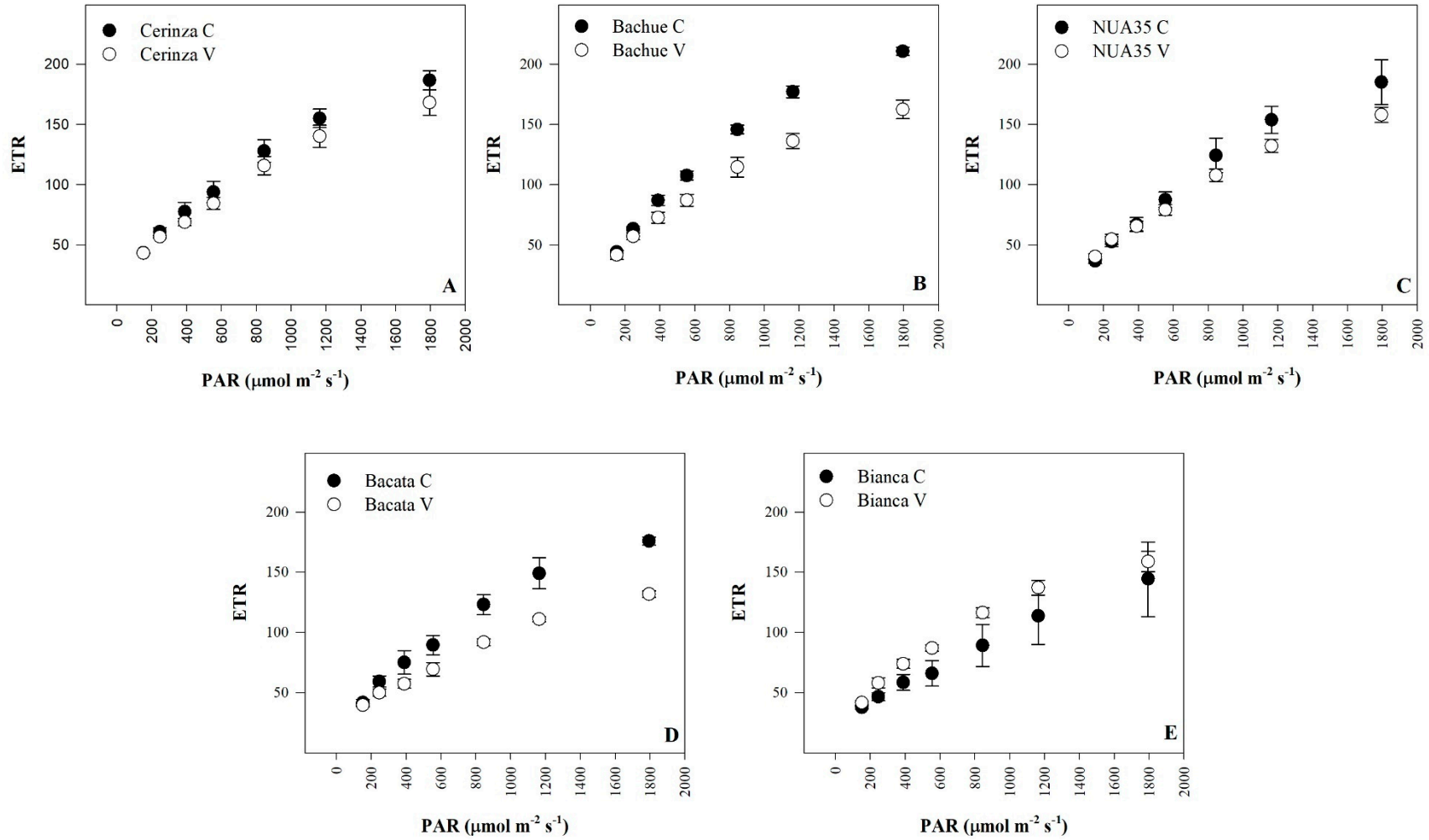

Figure 2. Electron transport rate (ETR) obtained from rapid light-response curves of 'Cerinza' (**A**), 'Bachue' (**B**), 'NUA35' (**C**), 'Bacata' (**D**) and 'Bianca' (**E**) under two water treatments (control (●) and water-stressed (○) plants) at the end of vegetative stage (55 DAE). Data represent the mean of four data points ± standard error; PAR, Photosynthetic Active Radiation.

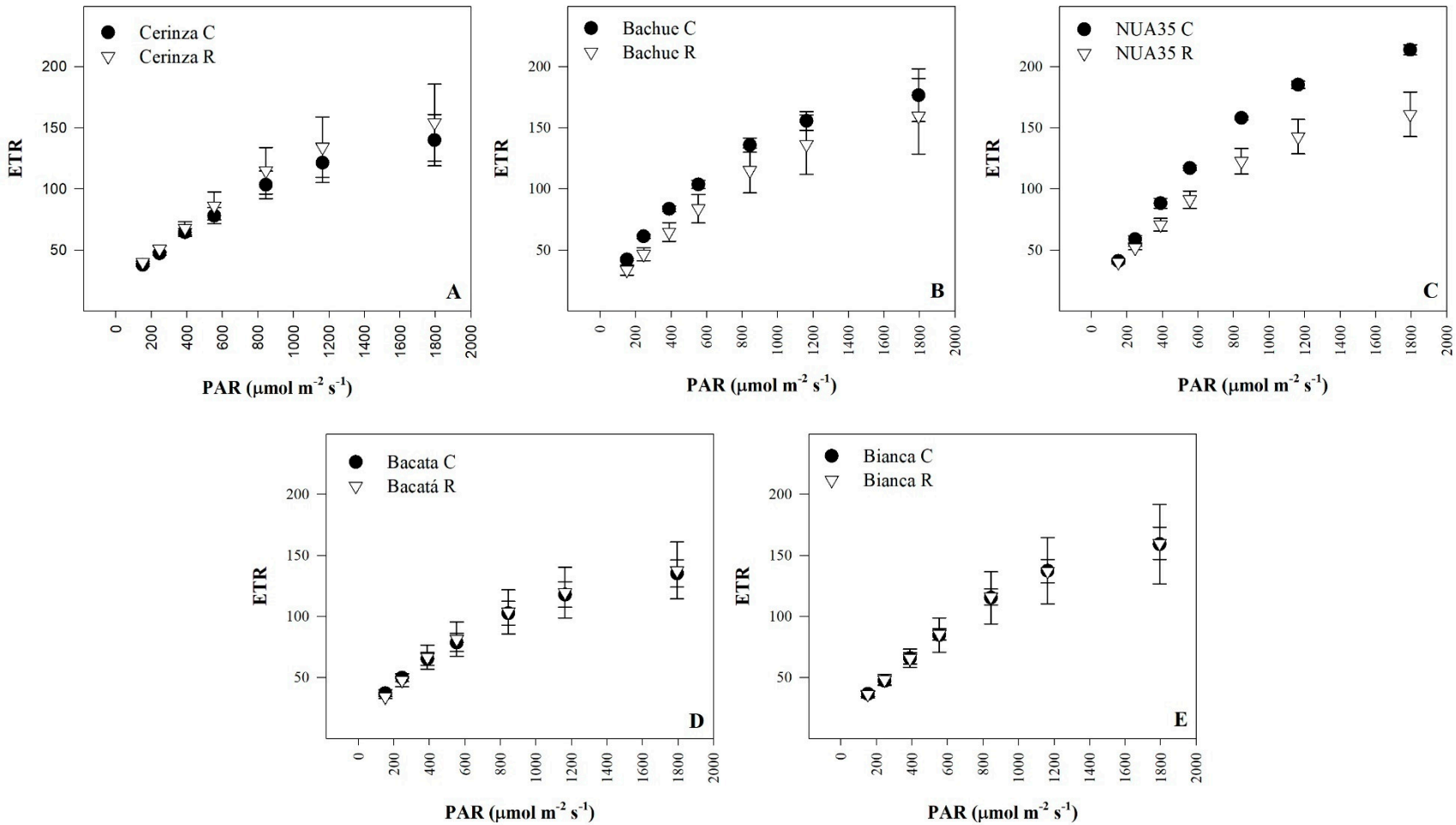

Figure 3. Electron transport rate (ETR) obtained from rapid light-response curves of 'Cerinza' (**A**), 'Bachue' (**B**), 'NUA35' (**C**), 'Bacata' (**D**) and 'Bianca' (**E**) under two water treatments (control (●) and water-stressed (▽) plants) at the end of reproductive stage (65 DAE). Data represent the mean of four data points ± standard error.

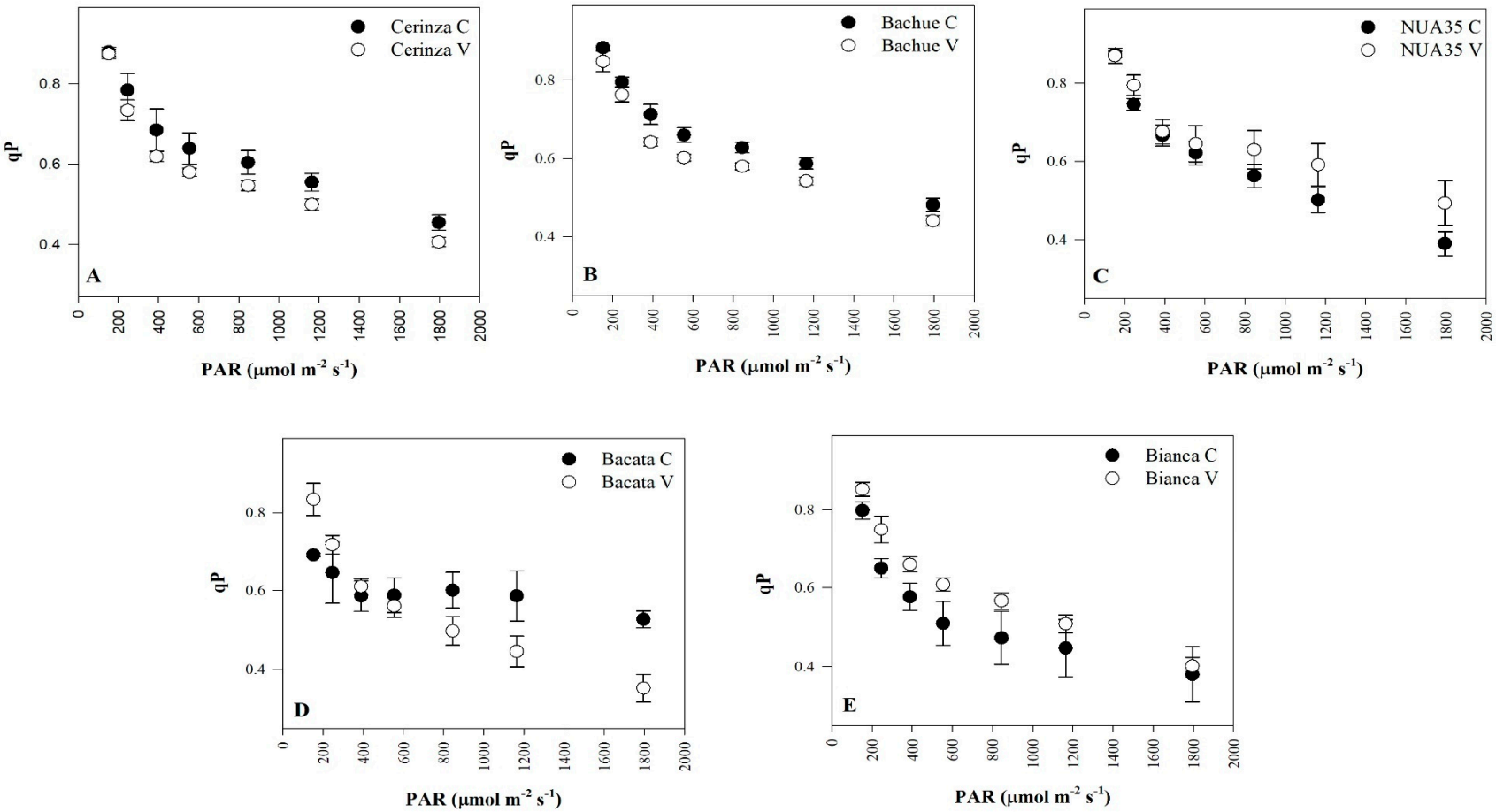

Figure 4. Photochemical quenching (qP) obtained from rapid light-response curves of 'Cerinza' (**A**), 'Bachue' (**B**), 'NUA35' (**C**), 'Bacata' (**D**) and 'Bianca' (**E**) under two water treatments (control (●) and water-stressed (○) plants) at the end of vegetative stage (55 DAE). Data represent the mean of four data points ± standard error.

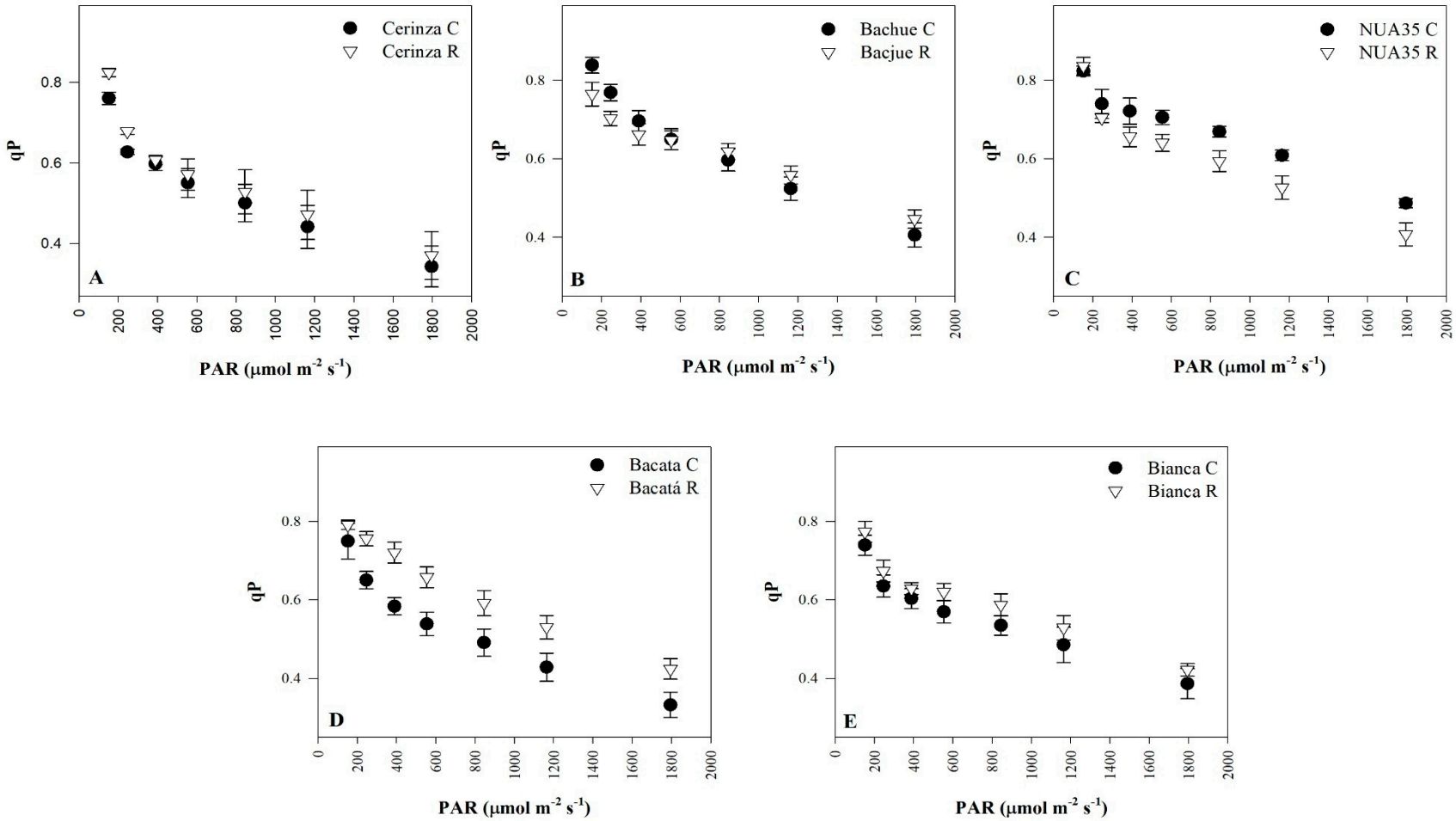

Figure 5. Photochemical quenching (qP) obtained from rapid light-response curves of 'Cerinza' (**A**), 'Bachue' (**B**), 'NUA35' (**C**), 'Bacata' (**D**) and 'Bianca' (**E**) under two water treatments (control (●) and water-stressed (▽) plants) at the end of reproductive stage (65 DAE). Data represent the mean of four data points ± standard error.

Table 3. Effect of the interaction between water stress and common bush bean (*Phaseolus vulgaris*) cultivars on the initial slope of the curve (α), maximum electron transport rate (ETR_{max}), and minimum saturation irradiance (I_K).

Treatment	Vegetative Stage (55 DAE)			Treatment	Reproductive Stage (65 DAE)		
	α ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	ETRmax	I_k ($\mu mol \cdot m^{-2} \cdot s^{-1}$)		α ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	ETRmax	I_k ($\mu mol \cdot m^{-2} \cdot s^{-1}$)
Stress stage				Stress stage			
Control	0.23 a [z]	207.42	936.47	Control	0.24	190.38 a	795.48
Vegetative	0.21 ab	191.87	876.60	Reproductive	0.22	165.71 b	811.98
Significance	NSy	NS	NS	Significance	NS	***	NS
Interaction				Interaction			
Cerinza	0.22 ab	241.35	1122.83	Cerinza	0.22 b	151.75 b	768.83 ab
Bachue	0.24 a	209.75	896.54	Bachue	0.23 ab	194.09 a	830.91 ab
NUA35	0.22 ab	204.39	916.10	NUA35	0.24 a	204.87 a	893.33 ab
Bacata	0.19 b	169.94	850.35	Bacata	0.19 b	150.75 b	685.04 b
Bianca	0.21 b	203.09	976.53	Bianca	0.21 b	188.75 a	850.14 a
Significance	**	NS	NS	Significance	*	***	*
Interaction				Interaction			
Cerinza × C	0.23 abc	232.35	1052.72 ab	Cerinza × C	0.21	167.64 bcd	773.82
Bachue × C	0.27 a	221.03	838.06 ab	Bachue × C	0.27	195.83 b	728.18
NUA35 × C	0.24 a	185.92	786.85 b	NUA35 × C	0.28	251.22 a	910.54
Bacata × C	0.23 abc	207.85	884.87 ab	Bacata × C	0.21	159.22 cd	707.97
Bianca × C	0.16 c	189.95	1119.85 a	Bianca × C	0.21	188.56 bc	856.91
Cerinza × V	0.21 abc	201.21	953.87 ab	Cerinza × R	0.23	145.86 d	758.85
Bachue × V	0.23 ab	196.73	844.59 ab	Bachue × R	0.20	196.91 bc	905.40
NUA35 × V	0.21 abc	235.22	976.33 ab	NUA35 × R	0.24	158.5 bcd	774.97
Bacata × V	0.17 bc	148.54	838.19 ab	Bacata × R	0.21	152.89 bcd	718.17
Bianca × V	0.23 abc	177.65	769.99 b	Bianca × R	0.21	188.93 bc	902.48
Significance	**	NS	**	Significance	NS	***	NS
CV[x] (%)	12.50	19.66	13.16	CV[x] (%)	14.22	10.21	13.76

[z] Values within a column followed by different letters are significantly different at $P \leq 0.05$ according to the Tukey test. [y] N.S. = Not significant ($P \leq 0.05$); *, **, and *** significantly different at 0.05, 0.01 and 0.001 probability levels, respectively. C.V[x], Coefficient of variation.

4. Discussion

PSII photochemistry is affected when plants are under abiotic stress conditions and the fluorescence parameters of chlorophyll α are potentially useful for detecting genotypes with tolerance or susceptibility traits [19,25]. Chlorophyll α fluorescence parameters (NPQ and F_v/F_m) are also considered physiological markers that provide information about the acclimation of the photosynthetic apparatus to stresses [26,27]. The present study showed that F_v/F_m and NPQ were the parameters that showed differences between the studied factors under water deficit conditions, especially in the vegetative stage. In general, F_v/F_m and NPQ showed a decrease in the genotypes used (Table 3). Sharma et al. [28] found that wheat genotypes susceptible to drought had lower F_v/F_m values. Hazrati et al. [29] also reported that *Aloe vera* plants with a prolonged water deficit period (plants irrigated at 20% field capacity for two months) recorded reductions in the NPQ. The continuous decrease in these two parameters (F_v/F_m and NPQ) indicates that the photo-protection mechanisms of energy dissipation are being affected by the stress condition, especially in cultivar Bacata, whose values presented the greatest reductions in the vegetative stage [26,30].

RLCs provide detailed information on the plant photosynthetic behavior through the study of the electron transport saturation characteristics [28]. In the present study, RLCs (ETR, qP and NPQ) better described the effects of water deficit between genotypes in the two different development stages studied compared to chlorophyll α fluorescence parameters. In this sense, cultivar Bacata mainly showed the greatest variations in its RLCs, with lower ETR and qP under water deficit, whereas NPQ was generally higher in the two stress periods. Exposure to high irradiance points in RLCs activates the NPQ mechanisms to minimize photo-damage [31,32]. Serôdio et al. [33] conclude that this increase in NPQ after exposure to different actinic light intensities indicates a recovery process after a stress period.

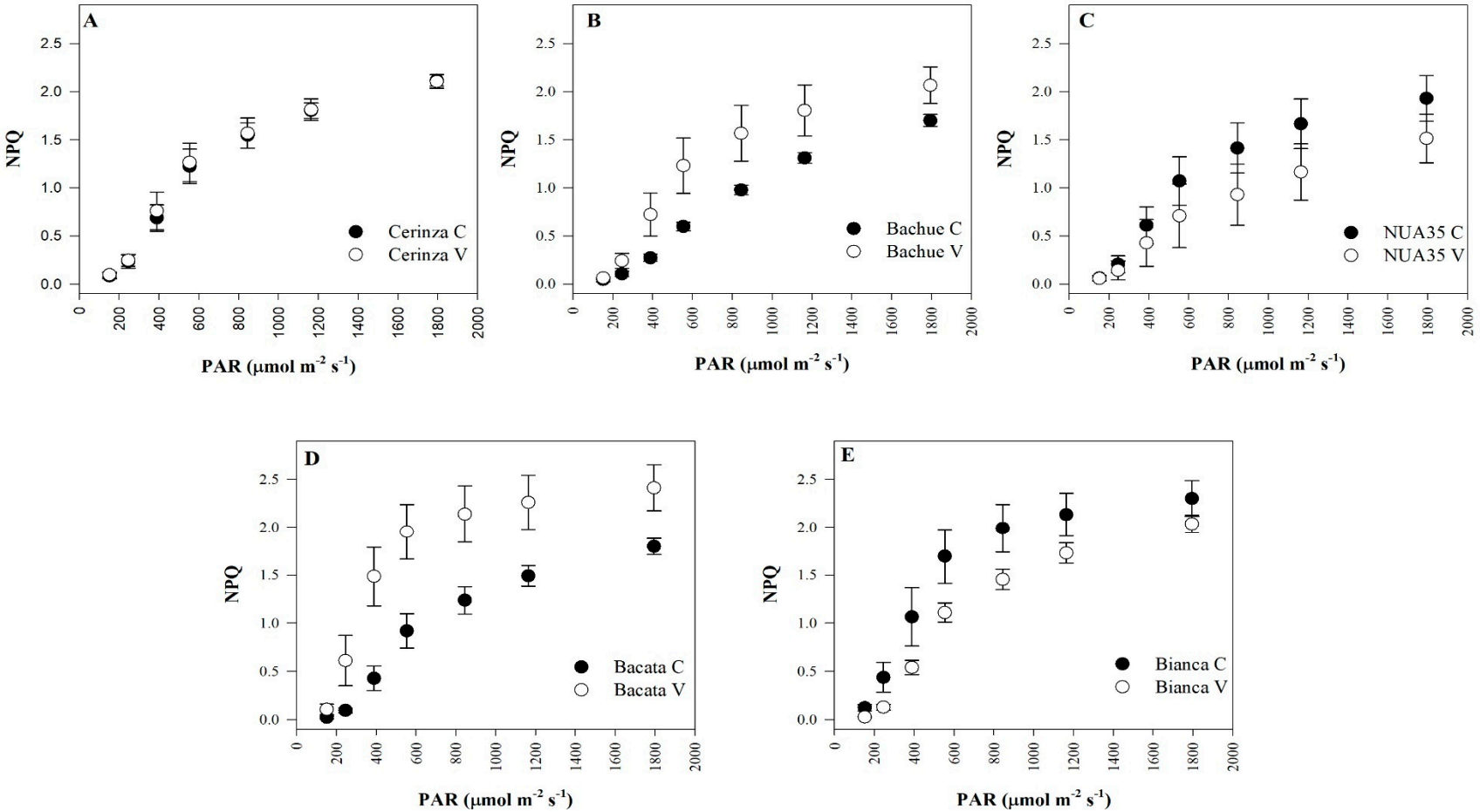

Figure 6. Non-photochemical quenching (NPQ) obtained from rapid light-response curves of 'Cerinza' (**A**), 'Bachue' (**B**), 'NUA35' (**C**), 'Bacata' (**D**) and 'Bianca' (**E**) under two water treatments (control (●) and water-stressed (○) plants) at the end of vegetative stage (55 DAE). Data represent the mean of four data points ± standard error.

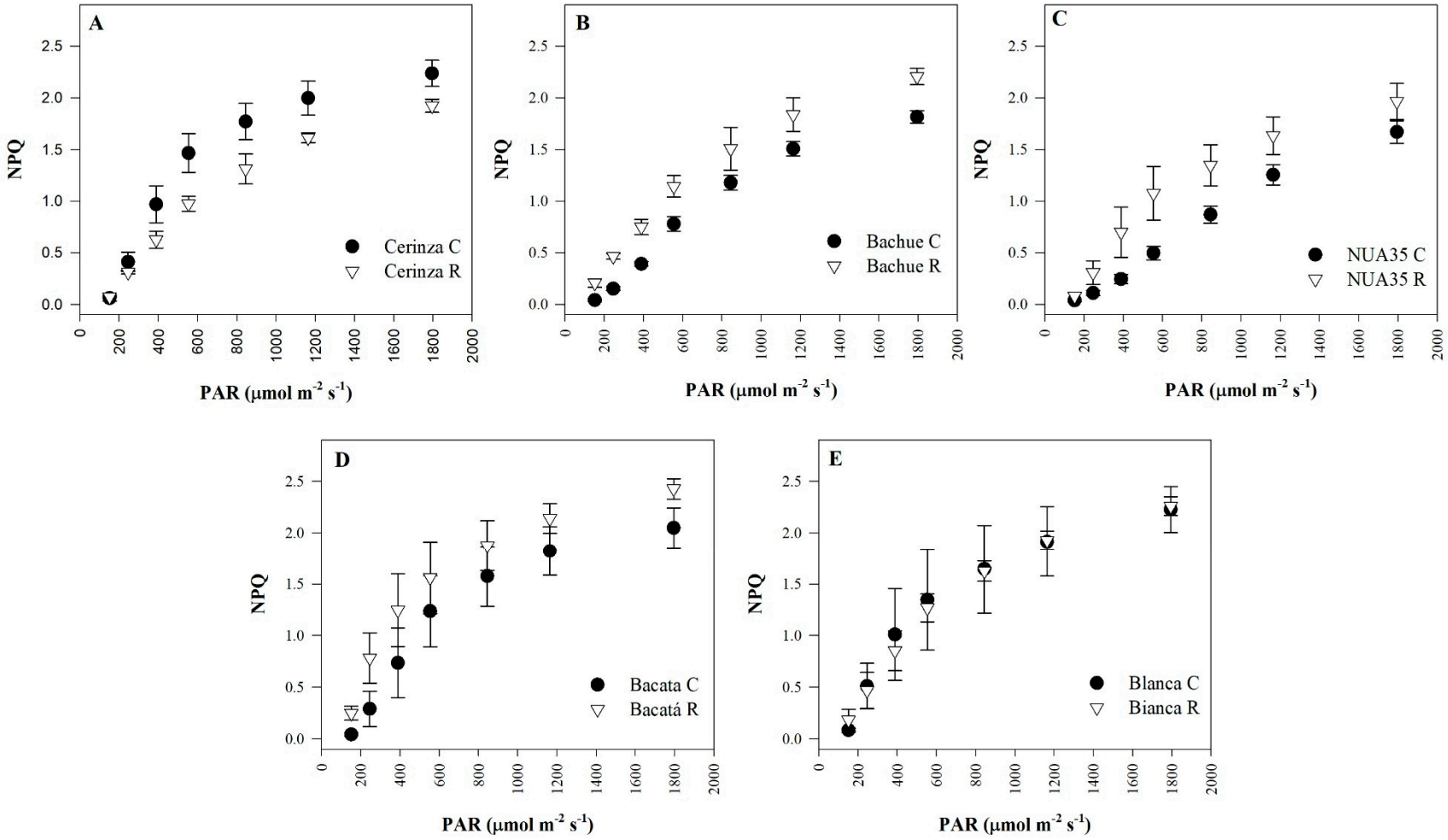

Figure 7. Non-photochemical quenching (NPQ) obtained from rapid light-response curves of 'Cerinza' (**A**), 'Bachue' (**B**), 'NUA35' (**C**), 'Bacata' (**D**) and 'Bianca' (**E**) under two water treatments (control (●) and water-stressed (▽) plants) at the end of reproductive stage (65 DAE). Data represent the mean of four data points ± standard error.

Photosynthetic pigments (chlorophylls and carotenoids) play a photoprotective role since they eliminate reactive oxygen species, disperse excess energy in the form of heat or suppress lipid peroxidation [34]. The obtained results showed that water deficit caused a reduction of the chlorophyll content in susceptible genotypes such as Bacata, whereas cultivar Bachue showed an increase in this variable. Reductions in the chlorophyll content have been reported in susceptible *Phaseolus vulgaris* L. genotypes to drought stress [35]. A reduction of chlorophyll under drought stress conditions is due to an overproduction of reactive oxygen species in the thylakoids [36]. In this sense, decreases in the chlorophyll content are considered a typical symptom of oxidative stress due to drought [37].

Variations in the content of carotenoids were recorded in the present work, with cultivars Bachue and Bacata showing an increase of this variable, whereas 'NUA35' and 'Bianca' showed reductions. A high carotenoid production has been reported in drought-tolerant wheat genotypes [38]. On the other hand, reductions in the carotenoid content have also been documented in *Solanum aethiopicum* and *Solanum macrocarpon* genotypes that are susceptible to water deficit [36]. Murtaza et al. [37] state that increases in pigment content help plant tolerance to drought. However, these authors also state that carotenoids present a high oxidative degradation in plants susceptible to water deficit, which is reflected in a lower foliar concentration of this pigment.

5. Conclusions

The parameters derived from chlorophyll α fluorescence (F_v/F_m, NPQ and ETR_{max}) and the development of RLCs are useful for the characterization of bush bean cultivars that are tolerant or susceptible to water deficit. In this sense, the results also support previous reports [39] in which cultivar Bacata proved to be a susceptible genotype since it showed considerable reductions in the different components of grain yield. The above suggests that PSII photochemistry is a tool that helps to understand the agronomic responses of common bush bean cultivars to drought conditions. Additionally, the photosynthetic pigment variables explain mechanisms of acclimation to water stress conditions and can be considered as a trait for the selection of genotypes in bean breeding programs.

Author Contributions: Conceptualization, A.D.S.-R. and H.R.-D.; Methodology, A.D.S.-R.; Software, A.D.S.-R.; Validation, A.D.S.-R., G.A.L.-M. and H.R.-D.; Formal Analysis, A.D.S.-R.; Investigation, A.D.S.-R.; Resources, G.A.L.-M.; Data Curation, A.D.S.-R.; Writing-Original Draft Preparation, A.D.S.-R.; Writing-Review & Editing, H.R.-D.; Visualization, G.A.L.-M.; Supervision, H.R.-D.; Project Administration, H.R.-D.; Funding Acquisition, H.R.-D.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Khoury, C.K.; Bjorkman, A.D.; Dempewolf, H.; Ramirez-Villegas, J.; Guarino, L.; Jarvis, A.; Rieseberg, L.H.; Struik, P.C. Increasing homogeneity in global food supplies and the implications for food security. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 4001–4006. [CrossRef] [PubMed]
2. Torres, T.; Farah, A. Coffee, maté, açaí and beans are the main contributors to the antioxidant capacity of Brazilian's diet. *Eur. J. Nutr.* **2017**, *56*, 1523–1533. [CrossRef] [PubMed]
3. FAOSTAT. Area Harvested, Yield and Production Quantity 2017: Beans, Dry; Beans, Green. Available online: http://www.fao.org/faostat/en/#data/QC (accessed on 15 May 2019).
4. Heinemann, A.B.; Ramirez-Villegas, J.; Stone, L.F.; Didonet, A.D. Climate change determined drought stress profiles in rainfed common bean production systems in Brazil. *Agric. For. Meteorol.* **2017**, *246*, 64–77. [CrossRef]
5. Anjum, S.; Xie, A.; Wang, Y.; Saleem, L.C.; Man, M.F.; Lei, W. Morphological, physiological and biochemical responses of plants to drought stress. *Afr. J. Agric. Res.* **2011**, *6*, 2026–2032.
6. Mishra, K.B.; Mishra, A.; Novotná, K.; Rapantová, B.; Hodaňová, P.; Urban, O.; Klem, K. Chlorophyll a fluorescence, under half of the adaptive growth-irradiance, for high-throughput sensing of leaf-water deficit in Arabidopsis thaliana accessions. *Plant Methods* **2016**, *12*, 46. [CrossRef]

7. Todaka, D.; Shinozaki, K.; Yamaguchi-Shinozaki, K. Recent advances in the dissection of drought-stress regulatory networks and strategies for development of drought-tolerant transgenic rice plants. *Front. Plant Sci.* **2015**, *6*, 84. [CrossRef] [PubMed]
8. Zandalinas, S.I.; Mittler, R.; Balfagón, D.; Arbona, V.; Gómez-Cadenas, A. Plant adaptations to the combination of drought and high temperatures. *Physiol. Plant.* **2018**, *162*, 2–12. [CrossRef] [PubMed]
9. Goltsev, V.; Zaharieva, I.; Chernev, P.; Kouzmanova, M.; Kalaji, H.M.; Yordanov, I.; Krasteva, V.; Alexandrov, V.; Stefanov, D.; Allakhverdiev, S.I.; et al. Drought-induced modifications of photosynthetic electron transport in intact leaves: Analysis and use of neural networks as a tool for a rapid non-invasive estimation. *Biochim. Biophys. Acta (BBA)-Bioenerg.* **2012**, *1817*, 1490–1498. [CrossRef] [PubMed]
10. Govindjee, E. 63 Years since Kautsky-chlorophyll-a fluorescence. *Aust. J. Plant Physiol.* **1995**, *22*, 131–160.
11. Marques, M.C.; Nascimento, C.W.A.; da Silva, A.J.; da Silva Gouveia-Neto, A. Tolerance of an energy crop (*Jatropha curcas* L.) to zinc and lead assessed by chlorophyll fluorescence and enzyme activity. *S. Afr. J. Bot.* **2017**, *112*, 275–282. [CrossRef]
12. Do Nascimento, C.W.A.; Marques, M.C. Metabolic alterations and X-ray chlorophyll fluorescence for the early detection of lead stress in castor bean (*Ricinus communis*) plants. *Acta Sci. Agron.* **2018**, *40*. [CrossRef]
13. Silva, A.J.D.; Nascimento, C.W.; Neto, G.; da Silva, A.; Silva Junior, E.A. Effects of silicon on alleviating arsenic toxicity in maize plants. *Rev. Bras. Ciênc. Solo* **2015**, *39*, 289–296. [CrossRef]
14. Rosenqvist, E.; van Kooten, O. Chlorophyll fluorescence: A general description and nomenclature. In *Practical Applications of Chlorophyll Fluorescence in Plant Biology*; Springer: Boston, MA, USA, 2003; pp. 31–77.
15. Flowers, M.D.; Fiscus, E.L.; Burkey, K.O.; Booker, F.L.; Dubois, J.J.B. Photosynthesis, chlorophyll fluorescence, and yield of snap bean (*Phaseolus vulgaris* L.) genotypes differing in sensitivity to ozone. *Environ. Exp. Bot.* **2007**, *61*, 190–198. [CrossRef]
16. Sanchez-Reinoso, A.D.; Ligarreto-Moreno, G.A.; Restrepo-Diaz, H. Physiological and Biochemical Responses of Common Bush Bean to Drought. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2018**, *46*, 393–401. [CrossRef]
17. Xu, W.Z.; Deng, X.P.; Xu, B.C.; Gao, Z.J.; Ding, W.L. Photosynthetic activity and efficiency of *Bothriochloa ischaemum* and *Lespedeza davurica* in mixtures across growth periods under water stress. *Acta Physiol. Plant.* **2014**, *36*, 1033–1044. [CrossRef]
18. Rascher, U.; Liebig, M.; Lüttge, U. Evaluation of instant light-response curves of chlorophyll fluorescence parameters obtained with a portable chlorophyll fluorometer on site in the field. *Plant Cell Environ.* **2000**, *23*, 1397–1405. [CrossRef]
19. Kalaji, H.M.; Jajoo, A.; Oukarroum, A.; Brestic, M.; Zivcak, M.; Samborska, I.A.; Cetner, M.D.; Lukasik, I.; Goltsev, V.; Ladle, R.J. Chlorophyll a fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. *Acta Physiol. Plant.* **2016**, *38*, 102. [CrossRef]
20. Feller, C.; Bleiholder, H.; Buhr, L.; Hack, H.; Hess, M.; Klose, R.; Meier, U.; Stauss, R.; Van den Boom, T.; Weber, E. Phänologische Entwicklungsstadien von Gemüsepflanzen: II. Fruchtgemüse und Hülsenfrüchte. Nachrichtenbl. Deut. *Pflanzenschutzd* **1995**, *47*, 217–232.
21. Sims, D.A.; Gamon, J.A. Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. *Remote Sens. Environ.* **2002**, *81*, 337–354. [CrossRef]
22. Wellburn, A.R. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *J. Plant Physiol.* **1994**, *144*, 307–313. [CrossRef]
23. Schreiber, U.; Bilger, W.; Neubauer, C. Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis. In *Ecophysiology of Photosynthesis*; Schulze, E.D., Caldwell, M.M., Eds.; Springer: Heidelberg, Germany, 1994; pp. 49–70.
24. Wu, Q.; Su, N.; Shen, W.; Cui, J. Analyzing photosynthetic activity and growth of *Solanum lycopersicum* seedlings exposed to different light qualities. *Acta Physiol. Plant.* **2014**, *36*, 1411–1420. [CrossRef]
25. Kalaji, H.M.; Rastogi, A.; Živčák, M.; Brestic, M.; Daszkowska-Golec, A.; Sitko, K.; Alsharafa, K.Y.; Lotfi, R.; Stypinski, P.; Samborska, I.A.; et al. Prompt chlorophyll fluorescence as a tool for crop phenotyping: An example of barley landraces exposed to various abiotic stress factors. *Photosynthetica* **2018**, *56*, 953–961. [CrossRef]
26. Chavez-Arias, C.C.; Gómez-Caro, S.; Restrepo-Díaz, H. Physiological, Biochemical and Chlorophyll Fluorescence Parameters of Physalis Peruviana L. Seedlings Exposed to Different Short-Term Waterlogging Periods and Fusarium Wilt Infection. *Agronomy* **2018**, *9*, 213. [CrossRef]

27. Martinez, V.; Nieves-Cordones, M.; Lopez-Delacalle, M.; Rodenas, R.; Mestre, T.; Garcia-Sanchez, F.; Rubio, F.; Nortes, P.A.; Mittler, R.; Rivero, R. Tolerance to stress combination in tomato plants: New insights in the protective role of melatonin. *Molecules* **2018**, *23*, 535. [CrossRef] [PubMed]
28. Sharma, D.K.; Andersen, S.B.; Ottosen, C.O.; Rosenqvist, E. Wheat cultivars selected for high Fv/Fm under heat stress maintain high photosynthesis, total chlorophyll, stomatal conductance, transpiration and dry matter. *Physiol. Plant.* **2015**, *153*, 284–298. [CrossRef] [PubMed]
29. Hazrati, S.; Tahmasebi-Sarvestani, Z.; Modarres-Sanavy, S.A.M.; Mokhtassi-Bidgoli, A.; Nicola, S. Effects of water stress and light intensity on chlorophyll fluorescence parameters and pigments of *Aloe vera* L. *Plant Physiol. Biochem.* **2016**, *106*, 141–148. [CrossRef] [PubMed]
30. Belshe, E.F.; Durako, M.J.; Blum, J.E. Photosynthetic rapid light curves (RLC) of *Thalassia testudinum* exhibit diurnal variation. *J. Exp. Mar. Biol. Ecol.* **2007**, *342*, 253–268. [CrossRef]
31. Ralph, P.J.; Gademann, R. Rapid light curves: A powerful tool to assess photosynthetic activity. *Aquat. Bot.* **2005**, *82*, 222–237. [CrossRef]
32. Ihnken, S.; Eggert, A.; Beardall, J. Exposure times in rapid light curves affect photosynthetic parameters in algae. *Aquat. Bot.* **2010**, *93*, 185–194. [CrossRef]
33. Serôdio, J.; Vieira, S.; Cruz, S.; Coelho, H. Rapid light-response curves of chlorophyll fluorescence in microalgae: Relationship to steady-state light curves and non-photochemical quenching in benthic diatom-dominated assemblages. *Photosynth. Res.* **2006**, *90*, 29–43. [CrossRef]
34. Hussain, S.; Rao, M.J.; Anjum, M.A.; Ejaz, S.; Zakir, I.; Ali, M.A.; Ahmad, N.; Ahmad, S. Oxidative stress and antioxidant defense in plants under drought conditions. In *Plant Abiotic Stress Tolerance*; Springer: Cham, Switzerland, 2019; pp. 207–219.
35. Kusvuran, S.; Dasgan, H.Y. Effects of drought stress on physiological and biochemical changes in *Phaseolus vulgaris* L. *Legum. Res.* **2017**, *40*, 55–62.
36. Mibei, E.K.; Ambuko, J.; Giovannoni, J.J.; Onyango, A.N.; Owino, W.O. Carotenoid profiling of the leaves of selected African eggplant accessions subjected to drought stress. *Food Sci. Nutr.* **2017**, *5*, 113–122. [CrossRef] [PubMed]
37. Murtaza, G.; Rasool, F.; Habib, R.; Javed, T.; Sardar, K.; Ayub, M.M.; Ayub, M.A.; Rasool, A. A review of morphological, physiological and biochemical responses of plants under drought stress conditions. *Imp. J. Interdiscip. Res.* **2016**, *2*, 1600–1606.
38. David, O.A.; Osonubi, O.; Ajiboye, A.A.; Ajewole, T.O. Agronomic Components of Drought Stressed Wheat Plants under Different Soil Properties. *Vegetos-An Int. J. Plant Res.* **2018**, *31*, 82–91. [CrossRef]
39. Sánchez-Reinoso, A.D.; Ligarreto-Moreno, G.A.; Restrepo-Díaz, H. Evaluation of drought indices to identify tolerant genotypes in common bean bush (*Phaseolus vulgaris* L.). *J. Integr. Agric.* **2019**, *18*, 2–10. [CrossRef]