Growth, phenology and chlorophyll fluorescence of habanero pepper (*Capsicum chinense* Jacq.) under water stress conditions

Crecimiento, fenología y fluorescencia de la clorofila del chile habanero (*Capsicum chinense* Jacq.) bajo condiciones de estrés hídrico

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Abstract

The irrigation methods offer a good alternative for increasing water use efficiency. The main purpose of this study was to identify the best irrigation level for Habanero pepper (*Capsicum chinense* Jacq.) plants based on available water capacity (AWC) of substrate. An experiment carried out with three irrigation levels (20, 40 and 60% of AWC) and five genotypes (H225, H241, H244, H226 and Jaguar). The irrigation level of 20% decreased the soil water potential to cause water stress in plants. At an irrigation level of 40%, average jaguar plant height (92 cm) and H241 (79 cm), genotypes were greater than under the other treatments. Average leaf area of H225, H241 and H244 plants was at least 48% greater than Jaguar plants. Average total dry matter of H241 and Jaguar plants, were 30 and 28% greater than H226 plants; and values of maximum photochemical quantum yield (F_v/F_m), relative electron transport rate and effective photochemical quantum yield of photosystem II were higher in H241, H244 and H226 plants; *i.e.* they are less probably due to photo inhibition damage. However, flowering time was earlier in plants with an irrigation level (IL) of 20% than plants with an IL of 40%, this was a consequence of stress due to water deficit. The 60% irrigation treatment caused flooding and all genotypes died before the inflorescence stage. These results suggest the enforcement of a suitable irrigation level based in available water capacity of substrate coupled with a vigorous genotype can encourage healthy plants and high water use efficiency.

Keywords: Drought stress, electron transport rate, flowering phenophase, irrigation level.

Resumen

Los métodos de riego ofrecen una buena alternativa para incrementar el uso eficiente del agua. El objetivo de este estudio fue identificar la mejor lámina de riego para las plantas de chile habanero (*Capsicum chinense* Jacq.) basado en la capacidad de agua disponible (AWC) del sustrato. Se realizó un experimento con tres láminas de riego (20, 40 y 60% de AWC) y cinco genotipos (H225, H241, H244, H226 y Jaguar). La lámina de 20% disminuyó el potencial hídrico del sustrato, causando estrés hídrico en las plantas. Con una lámina de 40%, la altura de planta de los genotipos Jaguar (92 cm) y H241 (79 cm) fue mayor que en los otros tratamientos; el área foliar de H225, H241 y H244 fue al menos 48% mayor que en las plantas de Jaguar; la biomasa total de las plantas de H241 y Jaguar fue 30 y 28% mayor que las de H226; el rendimiento cuántico máximo fotoquímico (F_v/F_m), la tasa relativa de transporte de electrones y el rendimiento cuántico efectivo del fotosistema II fueron superiores en las plantas de H241, H244 y H226; *i.e.* estos genotipos son menos susceptibles a sufrir daños por foto inhibición. Sin embargo, la floración fue más precoz en las plantas regadas con 20% que en las regadas con 40%, esto lo ocasionó el estrés por falta de agua. El tratamiento de riego a 60% causó inundación y las plantas de todos los genotipos murieron antes de la floración. Estos resultados sugieren que la aplicación de una adecuada lámina de riego acoplada con un genotipo vigoroso, pueden estimular plantas saludables con un alto uso eficiente del agua.

Palabras clave: Estrés por sequía, fenofase floración, lámina de riego, tasa de transporte de electrones

Introduction

Water for agriculture is critical for future global food security, because agriculture constitutes the largest user of fresh water, with irrigation withdrawals representing approximately 70% of total water use (Knox *et al.*, 2012). However, only half of this, is estimated to reach the intended crop, the rest being lost somewhere between the point of extraction and the crop (Turral *et al.*, 2010). The significant quantity of water used by agriculture for production raises the question as to whether agriculture could become more efficient in its water use (Hatfield, 2015), on the fact that most fresh water is stored as glaciers or deep groundwater (Oki & Kanae, 2006). Furthermore, global warming threatens to exacerbate the situation with a predicted reduction in water resources. In the Yucatan Peninsula, an increase in the drought severity index is expected (Orellana *et al.*, 2009), which could have a direct effect on crops. Habanero pepper (*Capsicum chinense* Jacq.) is the most important crop in the Yucatan Peninsula. As the hottest pepper, it represents one of the major spices traded on international markets due to its typical burning sensation, known as pungency, caused by the presence of capsaicinoids (Garruña-Hernández *et al.*, 2013). However, few studies of water use are available on this species. Quintal-Ortiz *et al.* (2012), applied five irrigation levels in pots with drainage and concluded that an irrigation level (IL) of 60% of available water capacity (AWC) of substrate increased both plant growth and fruit size. It is not clear in that study, was all the water used in evapotranspiration? What would happen when using pots without drainage? Movement of water throughout soil profile is known to occur when the amount of water exceeds the soil storage capacity and water moves downward under gravitational force. Respectively, in the search for a better water use efficiency, the aim of this research was to identify the best irrigation level for Habanero pepper plants based on AWC of substrate.

Material and methods

Plant material

The study was conducted at the Instituto Tecnológico de Conkal, Yucatan, Mexico. Habanero pepper (*Capsicum chinense* Jacq.) seeds from five genotypes (4 landrace: H225, H241, H244, H226; and 1 commercial: Jaguar) were germinated in 200-well polystyrene trays in a peat moss substrate. After 45 days, the seedlings were transplanted to 6 Kg pots (without drainage) containing a soil-poultry manure-henequen bagasse mix (2:1:1 v/v) and placed in a greenhouse for the experimental phase. The environmental conditions of the greenhouse during all crop cycles were registered with a portable micro meteorological station (HOBO; Onset Computer Corporation Massachusetts, USA) (Figure 1).
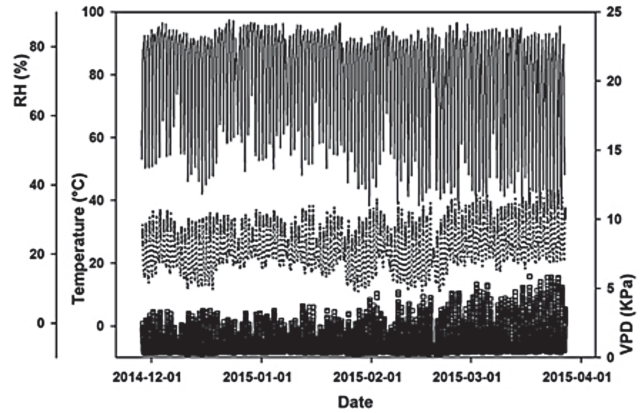

Figure 1. Relative humidity (RH), temperature and vapor pressure deficit (VPD) during the crop cycle of habanero pepper in a greenhouse.

Irrigation treatments

After transplanting, all irrigated plants at field capacity, were subject to homogenize the substrate, two days later the start of irrigation treatments. The field capacity (θ_{fc}) and permanent wilting point (θ_{wp}), were estimated with gravimetric method based on conventional oven-dry weight of substrate. To calculate the irrigation level, the equation of Wallender & Grimes (1991), was modified using θ_{fc}-θ_{wp} instead of AWC and pot depth instead of soil depth (Equation 1).

$$IL = \frac{(\theta fc - \theta wp)\, xPD \times BD}{WD \ X \ 100}$$

Equation 1

Where: IL = irrigation level (cm), θ_{fc} = field capacity (%), θ_{wp} = permanent wilting point (%), PD = pot depth (cm), BD = bulk density (g cm^{-3}), WD = water density (g cm^{-3}). To convert IL to water volume (WV) in cm^3, IL, was multiplied per pot area (cm^2). The water volume calculation to be applied per treatment (Equation 2).

$$FIL = \frac{WV \ X \ IA}{100}$$

Equation 2

Where: FIL = final irrigation level in volume (cm^3) and IA = irrigation amount (in this research: 20, 40 or 60% of AWC).

Growth, phenology and water potential

When plants were in fruiting stage (150 days after sowing), the stem diameter and plant height, were measured with a digital vernier and measuring tape respectively. In order to obtain the leaf area and dry weight (matter), were evaluated accordingly to Garruña-Hernández *et al.* (2014).

The inflorescence stage was considered when more than half (50% + 1) of the plants in each treatment exhibited flowers (Garruña-Hernández *et al.*, 2013). Soil water potential (Ψs) was estimated at 8:00 and 14:00 hours. 100 g of substrate in plastic bags, stored in a cooler with ice, carried to the laboratory, and then water potential measured with a WP4 dewpoint potentiometer (Decagon Devices, Washington, USA).

Chlorophyll fluorescence parameters

The maximum photochemical quantum yield of photosystem II (F_v/F_m) was measured with a portable pulse amplitude modulation fluorometer (PAM Walz, Effeltrich, Germany) at pre-dawn (5:00 h), where F_v is variable fluorescence (F_m – F_0), F_0 is initial fluorescence, and F_m maximum fluorescence. Fluorescence measurements performed on the third or fourth leaf from the shoot apex. Measurements performed at 145 days after sowing (100 days after starting the water deficit treatment). The saturation pulse intensity was 6000 µmol photons m^{-2} s^{-1} and the pulse length was 0.8 s. Light intensity during the measurement was 204 µmol photons m^{-2} s^{-1}. Light response curves of the relative electron transport rate (ETR_{PSII}) and the effective photochemical quantum yield of PSII (Φ_{PSII}) measurement were obtained through the application of a series of nine saturation pulses under increasing actinic irradiance (PPFD) from 0 to 1500 µmol photons m^{-2} s^{-1}, obtaining ETR_{PSII} by equation 3.

$$ETR\,PSII = \frac{PAR \; X \; ETR-Factor \; x \; Ppsii}{Pps \; x \; \emptyset PSii}$$

Equation 3

Where PAR = Photosynthetic active radiation. ETR-Factor = Ratio of photons absorbed by photosynthetic pigments to incident photons (a value of 0.84 which matches the average absorptance in the visible range of many green leaves). P_{PS2}/P_{PPS} = Photons absorbed by PSII relative to photons absorbed by photosynthetic pigments (A value of 0.5 is reasonable if one assumes the presence of only linear electron transport and, hence, equal electron transport rates in PSI and PSII. The Φ_{PSII} obtained by equation 4.

$$\emptyset \; PSII = \frac{Fm'-F'}{Fm'}$$

Equation 4

Where: F_m´ = Maximum fluorescence levels during a treatment is induced by saturating light pulse application, which closes all PSII reactions centers, and F´ = Is the level of the fluorescence curve during treatments and shortly before a saturating pulse is applied.

Experimental design

A randomized complete block design with bifactorial arrangement was performed employing

Table 1. Dry weight and leaf area of five genotypes of habanero pepper plants at two irrigation levels (20% and 40%). Data are means ± SE. Different letters in the same column represent statistically significant differences (Tukey, α=0.05). *n*=5.

| Treatments	Dry weight (g)				Leaf area (cm²)
	Root	Stem	Leaf	Total	
H225 20%	2.82 ± 0.7 c	9.04 ± 2.5 bc	3.77 ± 0.5 cd	16.8 ± 3.2 c	991.8 ± 362 bc
H241 20%	1.24 ± 0.1 c	8.33 ± 1.3 bc	4.10 ± 0.5 c	15.1 ± 0.8 c	1036.3 ± 102 bc
H244 20%	1.90 ± 0.4 c	7.06 ± 0.3 c	3.85 ± 1.4 cd	13.8 ± 1.9 c	1020.7 ± 606 bc
H246 20%	2.05 ± 0.2 c	8.84 ± 0.9 bc	3.62 ± 0.7 cd	15.4 ± 1.0 c	1335.4 ± 693 bc
Jaguar20%	1.93 ± 0.2 c	9.23 ± 1.0 bc	1.55 ± 0.6 d	16.3 ± 1.0 c	385.2 ± 153 c
H225 40%	4.47 ± 1.1 ab	13.09 ± 1.9 bc	12.34 ± 0.9 a	33.4 ± 3.1 ab	2842.5 ± 171 a
H241 40%	5.42 ± 0.2 a	15.77 ± 2.4 b	11.07 ± 0.6 a	37.4 ± 0.5 a	2665.8 ± 106 a
H244 40%	6.22 ± 0.9 a	11.69 ± 1.8 bcd	11.41 ± 0.7 a	32.1 ± 2.8 ab	2846.7 ± 180 a
H246 40%	5.49 ± 0.5 a	11.09 ± 1.6 bcd	7.35 ± 0.4 b	26.6 ± 4.1 b	2164.5 ± 483 ab
Jaguar40%	6.04 ± 0.2 a	22.90 ± 1.2 a	5.96 ± 0.4 bc	36.6 ± 0.9 a	1401.7 ± 560 bc

three irrigation levels (20, 40 and 60%) and five genotypes (H225, H241, H244, H226, Jaguar). Four replicates were established (15 plants each). Data were examined by two-way ANOVA and means were compared using Tukey's test at P ≤ 0.05 (Statistic Six Sigma, Release 7, StatSoft).

Results and discussion

The irrigation level (IL) affected early plant growth, the IL of 60% could not be evaluated because pots became flooded and plants did not survive. A previous study on IL in *C. chinense* (Quintal-Ortiz *et al.*, 2012) concluded that 60% of AWC increased both plant growth and fruit size, but pots used for this study had drainage.

Irrigation treatments affected significantly plant growth (plant height, stem diameter, leaf area and dry matter). However, plant genotype did not have a significantly effect on plant growth parameters. Accordingly to González-Dugo *et al.* (2007), the pepper growth is closely linked to the water amount applied. Our results showed plant height of the jaguar genotype irrigated at 40%, was statistically similar to H241 (92±3.7 and 79±4.8 cm respectively) genotypes, but different to other treatments.

Decreasing the IL from 40 to 20% produced a negative effect on plant height in both Jaguar and H241 genotypers (Figure 2A). The same occurred for stem diameter in all genotypes (Figure 2B). Similar responses were observed by Achten *et al.* (2010), who applied dry treatments to *Jatropha curcas* and showed there was a decrease in both stem length and diameter. In addition, dry matter was higher in the roots. However, in our study, there was a higher root dry weight in plants with high irrigation levels (Table 1). All genotypes had similar leaf area at an IL of 20%, but at an IL of 40%, leaf area of genotypes H225, H241 and H244 had at least 48% greater leaf area than Jaguar plants (Table 1). Similarly, there were no significantly differences in total dry matter among genotypes from an IL of 20%, but with an IL of 40% the total dry weight of H241 and Jaguar plants was 30 and 28% greater than plants of H226. Clearly, an increase in dry matter was produced by raising the IL (Table 1), such as occurs in tomatoes, where total dry matter accumulation is significantly depressed by soil water deficit (Patanè *et al.*, 2011).
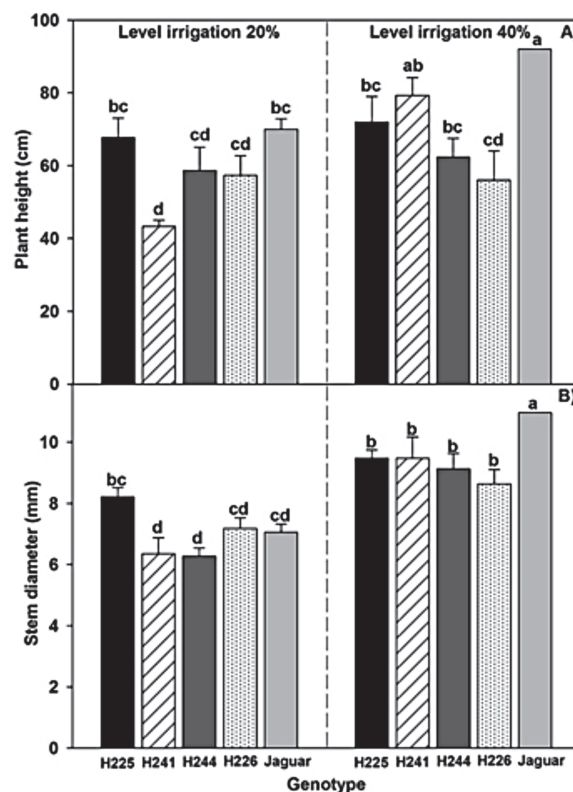
Figure 2. A) Plant height. B) Stem diameter of five habanero pepper genotypes at two irrigation levels (20% and 40%). Data are means ± SE. Different letters represent statistically significant differences (Tukey, ⬚=0.05).
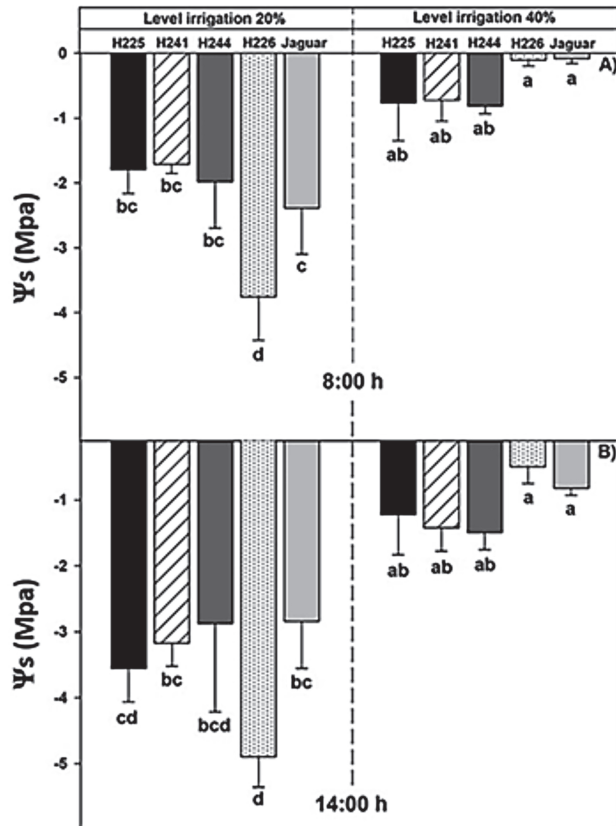
In all genotypes, the inflorescence stage was earlier in plants with an IL of 20%, compared to an IL of 40% (Figure 3). This was a stress consequence due to water deficit. The degree of crop sensitivity to water deficit during the different developmental stages of a plant species is a significant characteristic to consider (Jaimez *et al.*, 2000). Morales *et al.* (2013), found that raspberry under drought stress showed earlier inflorescence, while Ávila *et al.* (2012), observed that flowering was increased by water stress in *Citrus*. However, Jaimez *et al.* (2000), observed that low water availability delays the occurrence of maximum flowering in *C. chinense*. Our results showed the earlier inflorescence time was in response to water stress, H241, H244 and H226 genotypes, showed only two days of delay in flowering development under both irrigation treatments (Figure 3). Inflorescence time could be influenced by genotype.
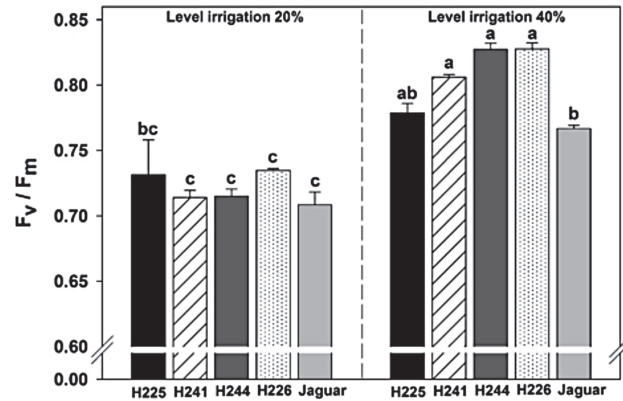
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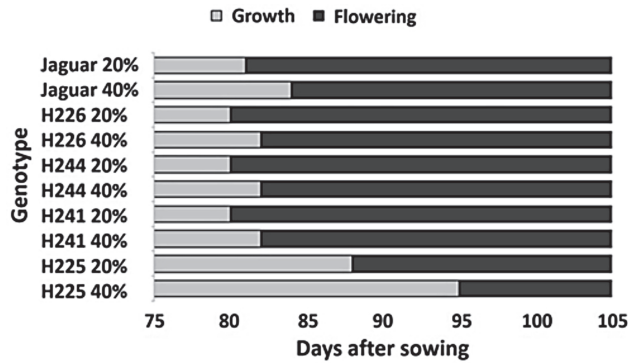
Figure 3. Flowering phenophase of five genotypes of Habanero pepper at two irrigation levels (20% and 40%). Data are means ± SE. Different letters represent statistically significant differences (Tukey, ▯=0.05)

The soil water potential (Ψs) decreased from morning to afternoon, but it was lower in IL of 20% both at 8:00 (Figure 4A) and 14:00 h (Figure 4B). Therefore, water stress conditions were evident in the most restrictive treatment (IL of 20%).

Regarding the chlorophyll (Chl) fluorescence parameters, the values of F_v/F_m reflect the potential quantum efficiency of PSII and are performed as a sensitive indicator of plant photosynthetic performance (Maxwell and Johnson, 2000). With the exception of genotype H225, F_v/F_m in leaves (Figures 5, 6) was higher in plants in an IL of 40% than in an IL of 20%. Plant leaves of H241 (0.81), H244 (0.83) and H226 (0.83) had higher F_v/F_m values. An F_v/F_m value of around 0.83 is an optimal measurement for most plant species (Maxwell & Johnson, 2000). Likewise, H244, H226 and H241 plants at an IL of 40% exhibited better performance both in terms of electron transport rate (ETR) and effective photochemical quantum yield of photosystem II (Φ_{PSII}). Genotypes H225 and Jaguar therefore showed greater physiological susceptibility to the lack of water. Penella *et al.* (2014), did not find any differences among control and water stress treatments in the F_v/F_m of 18 pepper genotypes. However, Ogaya & Peñuelas (2003), showed drought treatment produced a decrease in F_v/F_m values of *Quercus ilex* and *Parentucellia latifolia* plants.

Figure 5. Maximum photochemical quantum yield of photosystem II (F_v/F_m) in five genotypes of Habanero pepper at two irrigation levels (20% and 40%). Data are means ± SE. Different letters represent statistically significant differences (Tukey, *p*=0.05)

Figure 4. Soil water potential (Ψs) of five genotypes of habanero pepper at two irrigation levels (20% and 40%) at A) 8:00 and B) 14:00 hours. Data are means ± SE. Different letters represent statistically significant differences (Tukey, ▯=0.05)
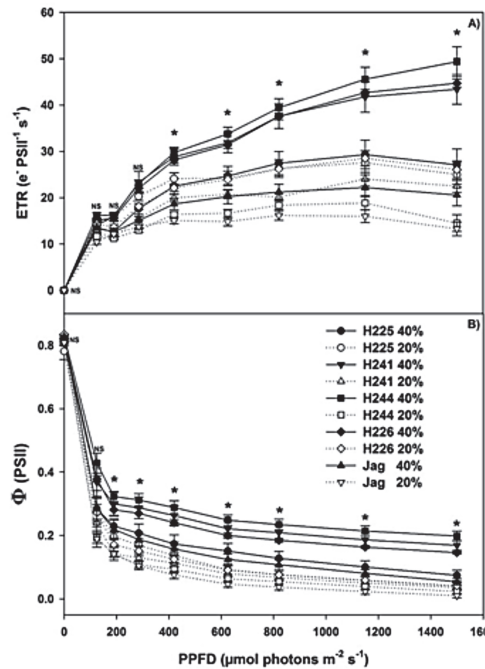
Figure 6. Response curves of A) Electron transport rate (ETR) and B) Effective photochemical quantum yield of photosystem II (Φ_{PSII}) to photosynthetic photon flux density in five genotypes of Habanero pepper at two irrigation levels (20% and 40%). Data are means. NS: not significant. *: significant (ANOVA, P ≤ 0.05).

Moreover, drought treatment also produced a slight decrease in ETR values for both species, and the highest PSII Yield was in the season when no drought stress occurred (Ogaya & Peñuela, 2003). The effects of abiotic and biotic stresses on plant photosynthetic performance lead to fitness and yield decreases. The maximum quantum efficiency of photosystem II (F_v/F_m) is a parameter of Chl fluorescence classically used to track changes in photosynthetic performance (Bresson *et al.*, 2015). If Chl fluorescence parameters can provide useful tests of photosynthetic performance *in vivo* and indicate the extent to which performance is limited by photochemical and non-photochemical processes (Baker, 2008), H241, H244 and H226 plants at an IL of 40% can be considered to show excellent photosynthetic performance, avoiding damage by photo inhibition.

Conclusion

The IL of 20% decreased the soil water potential (Ψs) sufficiently to cause water stress in plants and H241 and Jaguar plants showed good performance on growth parameters at an IL of 40%. However, inflorescence time was earlier in H241, H244 and H226 plants. In addition, Chl fluorescence measurements confirmed that genotypes H241, H244 and H226 had higher physiological performance than Jaguar; *i.e.*

they are less probably due to photo inhibition damage. Meanwhile, the IL of 60% caused flooding and plant death without drainage. These results suggest the enforcement of a suitable irrigation level based in available water capacity of substrate (40% in this research) coupled with a vigorous genotype can encourage healthy plants and high water use efficiency.

Acknowledgments

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