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Phothochemical quenching is indicative of ionic stress on 'Tahiti' acid lime grafted on citrus genotypes[1]

Quenching fotoquímico é indicativo de estresse iônico em limeira ácida 'Tahiti' enxertada em genótipos de citros

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HIGHLIGHTS:
Salinity increases photochemical heat loss in citrus plants.
Genotypes that provide great tolerance lose less energy.
'Tahiti' acid lime can be grown under moderate natural salinity when tolerant rootstocks are used.

ABSTRACT: The objective was to study the chlorophyll 'a' fluorescence and the photochemical quenching from 'Tahiti' acid lime grafted in new citrus genotypes under irrigation with three types of water during prefloration phase of the first year of cultivation, purposing to verify if the chlorophyll 'a' fluorescence and the photochemical quenching are indicatives of the beginning of ionic stress. An experiment was set up with three irrigation water salinity levels (0.14, 2.40 and 4.80 dS m^{-1}), and 13 citrus rootstock, corresponding to 'Rangpur Santa Cruz' lime, three Citrandarins, the 'Sunki Tropical' mandarin and eight citrus hybrids, all grafted with the 'Tahiti' acid lime, using randomized block design with four replications. At 70 days after transplanting, the chlorophyll a fluorescence of the plants were evaluated. Photochemical quenching indicates begin of ionic saline stress in citrus plants; The 'Tahiti' acid lime grafted on TSKC x (LCR x TR) - 059, 'Rangpur Santa Cruz' lime, the Citrandarins 'Riverside' and 'San Diego' and TSKC x TRBK - 007 genotypes has greater activation of protective mechanisms through transient fluorescence.

Key words: *Citrus* spp., fluorescence, quantic efficiency, saline waters

RESUMO: Objetivou-se estudar fluorescência da clorofila 'a' e o quenching fotoquímico da limeira ácida 'Tahiti' enxertada em novos genótipos de citros sob irrigação com três tipos de água na fase de prefloração do primeiro ano de cultivo, no intuito de verificar se a fluorescência da clorofila 'a' e o quenching fotoquímico são indicativos do início do estresse iônico. No experimento, foram avaliados três condutividades elétricas da água de irrigação (0,14, 2,40 e 4,80 dS m^{-1}), e 13 porta-enxertos de citros, correspondentes ao limoeiro 'Cravo Santa Cruz', três Citrandarins, a tangerineira 'Sunki Tropical' e oito híbridos de citros, todos enxertados com a limeira ácida 'Tahiti', no delineamento em blocos casualizados com quatro repetições. Foi avaliada, aos 70 dias após o transplante, a fluorescência da clorofila 'a'. O quenching fotoquímico indica início do estresse iônico em plantas de citros sob irrigação com águas salinas; a limeira ácida 'Tahiti' enxertada nos genótipos TSKC x (LCR x TR) - 059, limoeiro 'Cravo Santa Cruz', Citrandarin 'Riverside', Citrandarin 'San Diego' e TSKC x TRBK - 007 têm maior ativação de mecanismos de proteção por meio da fluorescência transiente.

Palavras-chave: *Citrus* spp., fluorescência, eficiência quântica, águas salinas

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Introduction

Salinity is one of the abiotic factors that mostly cause problems to crops, especially in arid and semi-arid regions, which have a negative water balance (Gheyi et al., 2016). Under salinity conditions in soil and water, there is a reduction in the growth and production of crops, especially citrus plants that are considered sensitive to salinity (Maas, 1993).

The effect of salinity on citrus plants depends on canopy/ rootstock combination (Simpson et al., 2014; Brito et al., 2014; 2015); if crowns are grafted with compatible and tolerant rootstocks, it would be possible to cultivate them in semi-arid regions using water with electrical conductivity greater than 1.1 dS m^{-1} (Grieve et al., 2007; Prior et al., 2007; Fernandes et al., 2011; Barbosa et al., 2017); however, these researchers have not reported any significant reduction in the growth and production of crops with the use of water of up to 2.4 dS m^{-1} in less sensitive combinations.

Several strategies can be used to identify tolerant genotypes. Evaluation of physiological behavior through chlorophyll fluorescence (Sá et al., 2018) could help in the identification of damage in chloroplast tissues, especially via determining the photochemical quantum yield.

Khoshbakht et al. (2018) studied the influence of polyamine application on growth, gas exchange, and chlorophyll fluorescence of Bakraii mandarin under salt stress in a rootstock formation. They reported that salinity reduced the growth of plants and that it was attributed to the increase in non-quenching photochemical and reduction in photochemical quenching caused by ionic disturbances.

Therefore, the study of chlorophyll fluorescence, especially photochemical and non-photochemical quenching in plants, may help to identify ionic stresses in citrus plants.

Thus, the aim of this study was to assess chlorophyll fluorescence and photochemical and non-photochemical quenching in 'Tahiti' acid lime grafted with new citrus genotypes under irrigation with three types of water at the pre-flowering stage of the first crop season.

Materials and Methods

The research was conducted at the experimental farm of Campus do Sertão, at the Federal University of Sergipe, Brazil, which is located in the city of Nossa Senhora da Glória (10°12'18" of latitude S and 37°19'39" of longitude W and altitude of 294 m), in the high backlands of Sergipe, where hot and dry semi-arid climate is predominant, with a mean precipitation of 750 mm and an annual mean temperature of 24 °C.

During the experimental evaluation period, which ranged from December 27, 2018 to March 7, 2019, the mean temperature ranged from 24.19 to 29.03 °C, with a minimum of 20.62 and a maximum of 37.96 °C (Figure 1A).

The mean relative air humidity (Figure 1B) ranged from 79 to 95.12%, with a minimum of 29.88% and a maximum of 97.73%. In the evaluated period, there was an accumulated precipitation of 30.49 mm, and the highest precipitation rate was recorded in February, about 12 mm. The mean daily evapotranspiration was 5.41 mm d^{-1}, with the lowest value being 2.73 and the highest being 6.92 mm d^{-1}.

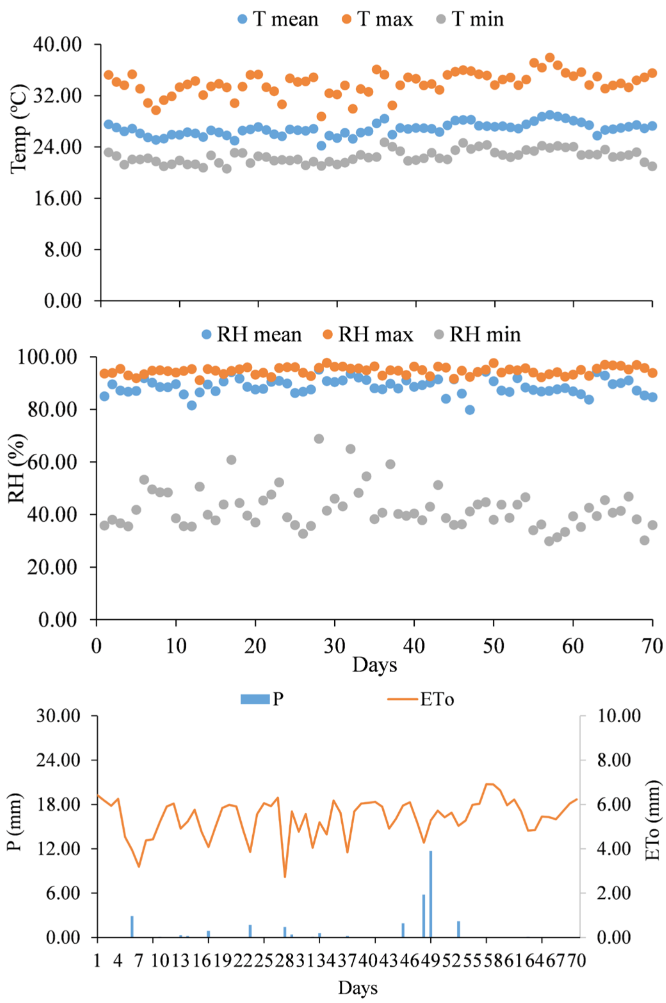


Figure 1. Maximum (Tmax), mean (Tmean), and minimum (Tmin) air temperatures (A); maximum (RHmax), mean (RHmean), and minimum (RHmin) relative air humidity (B); and reference precipitation (P) and evapotranspiration (ETo) (C) observed during the experimental period in an agrometeorological station near the experimental area

A split-plot randomized block experimental design, with four replicates, was used in evaluating 13 rootstocks (genotypes) in the main plot, as described in Table 1. The rootstocks were all from the citrus genotype breeding program (PMG-Citros) from Embrapa Mandioca and Fruticultura, using the 'Tahiti' acid lime as the crown.

Table 1. List of rootstocks (genotypes) grafted with the 'Tahiti' acid lime [*Citrus* x *latifolia* (Yu Tanaka) Tanaka]

No.	Genotype	No.	Genotype
1	'Cravo Santa Cruz' Lemon tree	8	TSKC × CTTR - 012
2	'Indio' Citrandarin	9	TSKFL × CTTR - 013
3	'Riverside' Citrandarin	10	HTR - 069
4	'San Diego' Citrandarin	11	TSKC × (LCR × TR) - 040
5	'Sunki Tropical' Mandarin	12	TSKC × (LCR × TR) - 059
6	TSKC × TRBK - 007	13	TSKC × CTARG - 019
7	TSKFL × TRBK - 030		

HTR - 069 - Trifoliate hybrid of 'Pera' orange (*Citrus × sinensis* L.) with citrange [*C. × sinensis* L.) Osbeck × *Poncirus trifoliata* (L.) Raf.] 'Yuma'; LCR - 'Cravo' lemon tree (*C. limonia* Osbeck); TSKC - 'Sunki' Common Selection Mandarin {common selection [*C. sunki* (Hayata) hort. ex Tanaka]}; TR - *P. trifoliata*; TSKFL - 'Sunki seleção da Flórida' Mandarin; TRBK - *Poncirus trifoliata* 'Beneke'; CTARG - 'Argentina' Citrange; CTTR - 'Troyer' Citrange

The subplot consisted of three electrical conductivities for irrigation water (0.14, 2.4, and 4.8 dS m^{-1}) one from the São Francisco River and two from tubular wells (2.4 and 4.8 dS m^{-1}). The experimental unit consisted of a plant. The application started at 30 days after transplantation (DAT) of the seedlings in lysimeters and lasted the entire pre-flowering period.

The seedlings of each citrus genotype grafted with the ‘Tahiti’ acid lime were obtained from the Tamafe® seedling production nursery, which has a partnership with Embrapa Mandioca e Fruticultura, in Cruz das Almas, BA, Brazil, following production recommendations of certified seedlings and using materials derived from asexual reproduction, produced using plastic bags with a capacity of 2 dm^3 filled with commercial substrate (Basaplant®).

The citrus plants were transplanted to lysimeters/60 L pots, and the soil used for filling was obtained from an Oxisol, from an area close to the experiment site. The soil was previously sieved with a 10-mesh sieve.

The irrigations with water corresponded to the three electrical conductivities and started at 30 DAT, when the plants were already adapted to the environment. Irrigations were carried out every 2 days using a drip irrigation system installed in the lysimeters/pot. Irrigation was managed through water balance to meet the mean daily consumption of the plants. An additional fraction, derived by dividing the value of the volume to be applied (mL) by 0.9, was used to obtain a leaching fraction that correspond to 10% to leach part of the salts accumulated in the root zone from the irrigation water (Eq. 1).

$$IV = \frac{(Va - Vd)}{1 - LF} \quad (1)$$

where:

IV - Irrigation volume in the next irrigation event, mL;
Va - volume applied in the previous irrigation event, mL;
Vd - volume drained, mL; and,
LF - coefficient used to obtain a leaching fraction of approximately 10% (0.9).

Before 30 DAT, the plants received water with low electrical conductivity (EC_a) from the São Francisco River. After 30 DAT, other types of water were applied.

Nutritional management followed the recommendations of Mattos Junior et al. (2005) and other common recommendations for weed control and pest and disease prevention and control in the production of citrus seedlings (Mattos Junior et al., 2005).

Chlorophyll ‘a’ fluorescence was assessed 70 DAT using an Opti Science OS5p modulated pulse fluorometer. Initially, the OJIP protocol was used to determine the fluorescence induction variables after adaptation to dark environment for 30 min to ensure that all primary acceptors were oxidized.

Subsequently, evaluations were carried out under lighting conditions using the ‘Yield’ protocol, and an actinic light source was applied with a saturating multi-flash pulse, coupled to a clip for determining the photosynthetically active radiation (PAR-Clip); thus, the following variables were determined: initial fluorescence before the saturation pulse (F’), maximum fluorescence after adaptation to saturating light (Fm’), quantum efficiency of photosystem II (Y_{II}).

Consequently, the minimum fluorescence of the illuminated plant tissue (Fo’) was determined using Eq. 2; the photochemical quenching coefficient, considering the lake model (qL), was determined using Eq. 3; the quantum yield of regulated photochemical quenching (Y_{NPQ}) was determined using Eq. 4; and the quantum yield of unregulated photochemical quenching (Y_{NO}) was determined using Eq. 5.

- Oxborough & Baker (1997)

$$Fo' = \frac{Fo}{\left(\frac{Fm - Fo}{Fm} + \frac{Fo}{Fm'}\right)} \quad (2)$$

- Kramer et al. (2004)

$$qL = \frac{(Fm' - F')}{(Fm' - Fo')}\left(\frac{Fo'}{F'}\right) \quad (3)$$

- Kramer et al. (2004)

$$Y_{NPQ} = \left(\frac{F'}{Fm'}\right) - \left(\frac{F'}{Fm}\right) \quad (4)$$

- Kramer et al. (2004)

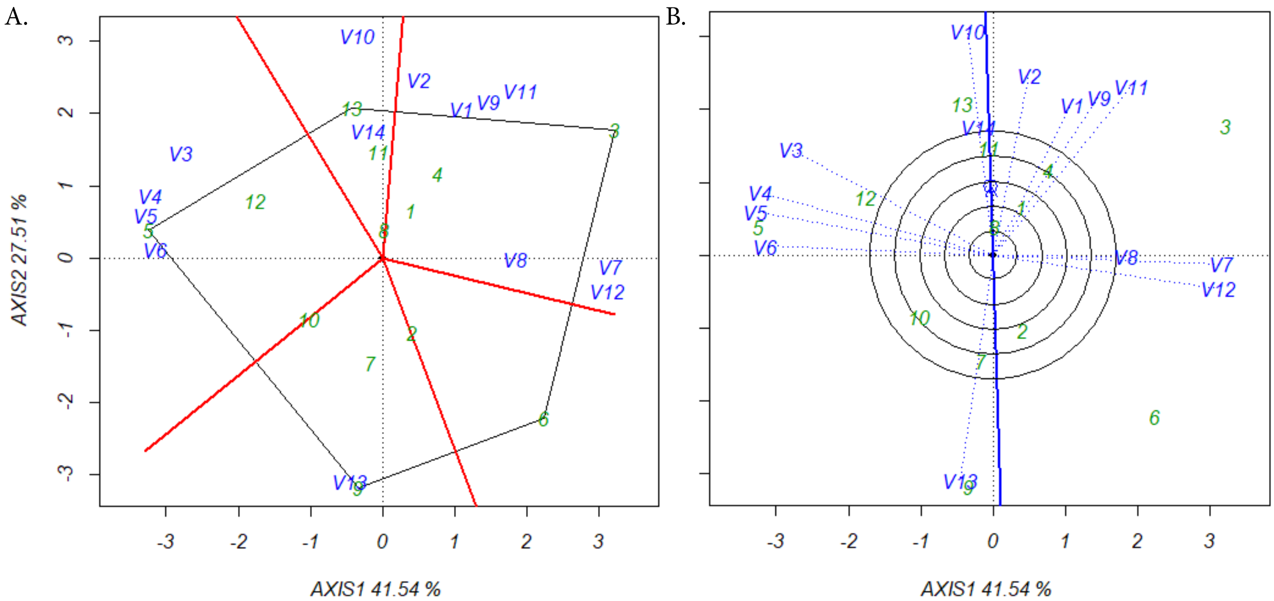
$$Y_{NO} = \frac{F'}{Fm} \quad (5)$$

The data were standardized to obtain zero mean and unit variance, and a principal component analysis (PCA) was performed for each electrical conductivity of the irrigation water, and it resulted in linear combinations of the variables analyzed using the highest eigenvalues in the correlation matrix (Hair et al., 2009). R 3.6® software with the GGEBiplot package were used to create a biplot of groups of genotypes and variables and a biplot of discriminant variables versus number of variables in the principal components (PC1 and PC2) (Setimela et al., 2007).

Results and Discussion

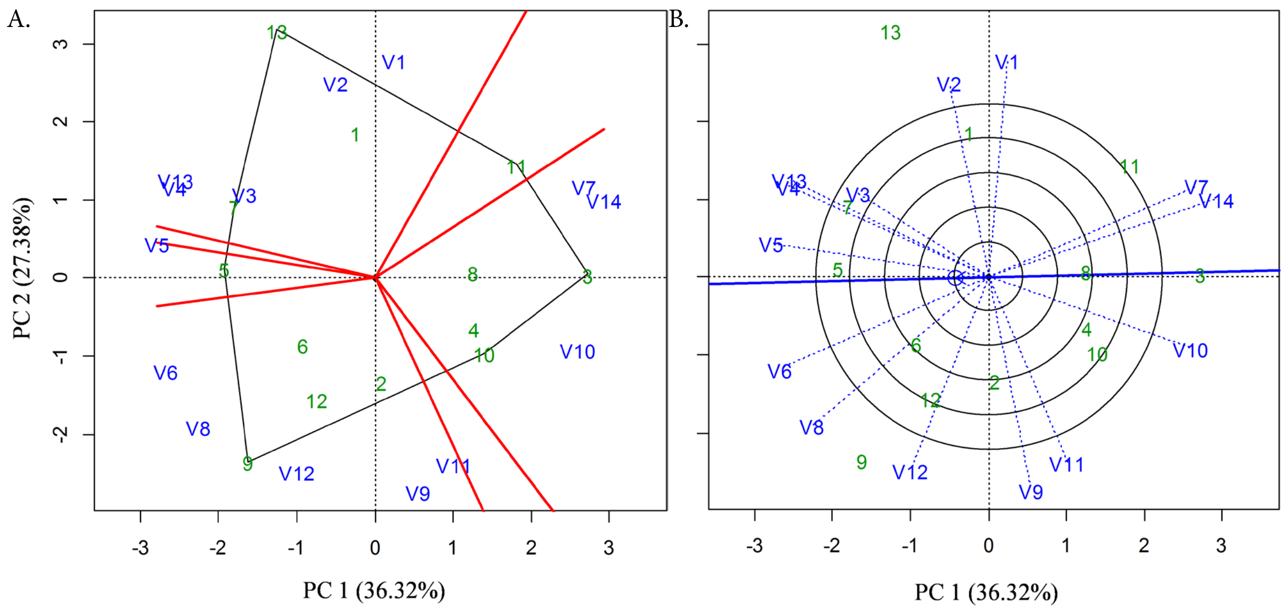
Figure 2 shows that the first two principal components (PCs) related to the chlorophyll ‘a’ fluorescence variables from the combinations of rootstock genotypes grafted with ‘Tahiti’ acid lime plants under irrigation with water of 0.14 dS m^{-1} explain more than 69% of the variance in the data, with PC1 and PC2 accounting for 41.54% and 27.51% of the variance, respectively. Therefore, the fluorescence behavior of the genotypes is associated with the variables that make up the components.

Figure 3 shows the PCA of the chlorophyll fluorescence variables of ‘Tahiti’ plants under irrigation with water of 2.4 dS m^{-1}. Under this condition of abiotic stress, the sum of the first two PCs was 63.7%, with PC1 and PC2 accounting for 36.32% and 27.38% of the variance, respectively.



Genotypes: 1 - ‘Santa Cruz Rangpur’ lime; 2 - ‘Indio’ Citrandarin; 3 - ‘Riverside’ Citrandarin; 4 - ‘San Diego’ Citrandarin; 5 - ‘Sunki Tropical’ Mandarin; 6 - TSKC × TRBK - 007; 7 - TSKFL × TRBK - 030; 8 -TSKC × CTTR - 012; 9 - TSKFL × CTTR - 013; 10 - HTR - 069; 11 - TSKC × (LCR × TR) - 040; 12 - TSKC × (LCR × TR) - 059; 13 - TSKC × CTARG - 019.
Legend of variables: V1-O; V2-J, V3-I, V4-P, V5-FV, V6-FvFm, V7-F0Fm, V8-Fs, V9-Fms, V10-Y, V11-Fos, V12-Y_{no}, V13-Y_{npq} V14 - qL

Figure 2. Dispersion of 13 rootstock genotypes grafted with ‘Tahiti’ acid lime plants under irrigation with water of 0.14 dS m^{-1} 70 days after transplantation based on the scores of the first (PC1) and the second (PC2) principal components. (A) Biplot of groups of genotypes and variables, (B) Biplot of discriminant variables versus number of variables in the principal components (PC1 and PC2)



Genotypes: 1 - ‘Santa Cruz Rangpur’ lime; 2 - ‘Indio’ Citrandarin; 3 - ‘Riverside’ Citrandarin; 4 - ‘San Diego’ Citrandarin; 5 - ‘Sunki Tropical’ Mandarin; 6 - TSKC × TRBK - 007; 7 - TSKFL × TRBK - 030; 8 -TSKC × CTTR - 012; 9 - TSKFL × CTTR - 013; 10 - HTR - 069; 11 - TSKC × (LCR × TR) - 040; 12 - TSKC × (LCR × TR) - 059; 13 - TSKC × CTARG - 019.
Legend of variables: V1-O; V2-J, V3-I, V4-P, V5-FV, V6-FvFm, V7-F0Fm, V8-Fs, V9-Fms, V10-Y, V11-Fos, V12-Y_{no}, V13-Y_{npq} V14 - qL

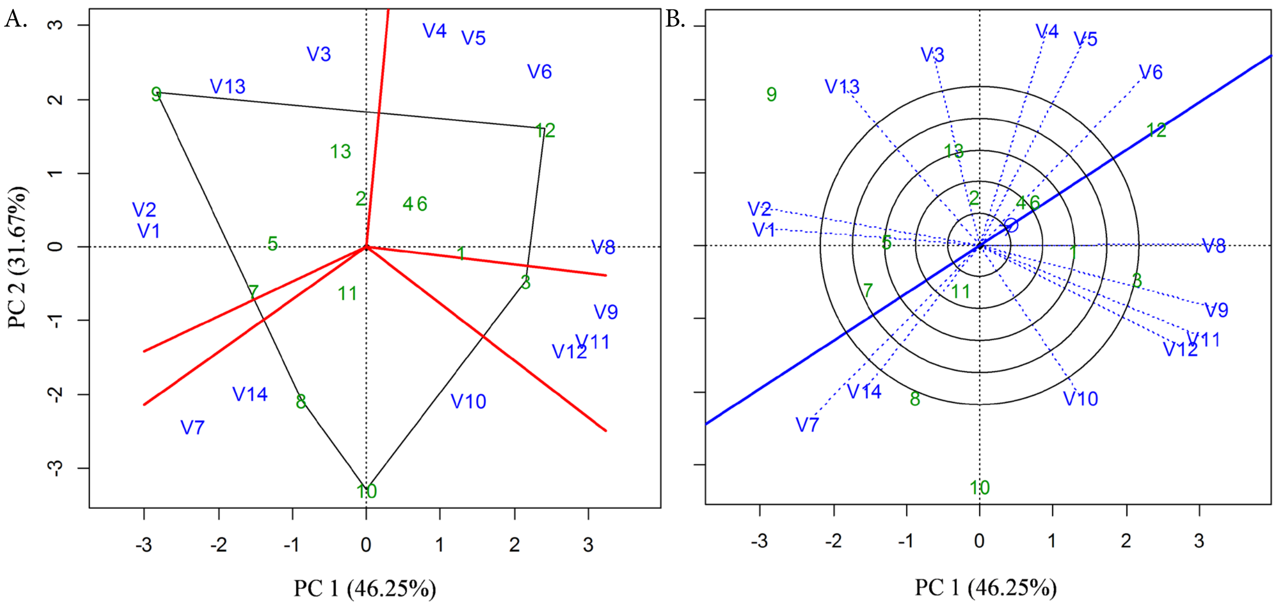
Figure 3. Dispersion of 13 rootstock genotypes grafted with ‘Tahiti’ acid lime plants under irrigation with water of 2.40 dS m^{-1} 70 days after transplantation based on the scores of the first (PC1) and second (PC2) principal components. (A) Biplot of groups of genotypes and variables, (B) Biplot of discriminant variables versus number of variables in the principal components (PC1 and PC2)

The biplot analysis of data on the fluorescence of citrus plants under irrigation with water of 4.80 dS m^{-1} showed that the first two principal components are representative of the results since they correspond to 77.92% of the total data variance (Figure 4).

The first two principal components, mainly under the condition of high water salinity, represented most of the variance in the original data and, thus, can be used to explain the results, as observed by Hongyu et al. (2015).

When studying discriminant variables versus number of variables under the condition of low salinity (0.14 dS m^{-1}) (Figure 2B), the ideal variable was V10 - Y, followed by V2 - ‘J ‘e, V14 -’ qL ‘, V1 -’ O ‘, V9 -’ Fms’, and V11 - ‘Fos’. The combination of these variables with the genotypes 13 and 11 resulted in (TSKC × CTARG - 019) and [TSKC × (LCR × TR) - 040], respectively (Figure 2B).

There is a greater number of plants irrigated with water of 2.40 dS m^{-1} (Figure 3B) in the variables 5 (Fv), 6 (Fv/Fm),



Genotypes: 1 - ‘Santa Cruz Rangpur’ lime; 2 - ‘Indio’ Citrandarin; 3 - ‘Riverside’ Citrandarin; 4 - ‘San Diego’ Citrandarin; 5 - ‘Sunki Tropical’ Mandarin; 6 - TSKC × TRBK - 007; 7 - TSKFL × TRBK - 030; 8 -TSKC × CTTR - 012; 9 - TSKFL × CTTR - 013; 10 - HTR - 069; 11 - TSKC × (LCR × TR) - 040; 12 - TSKC × (LCR × TR) - 059; 13 - TSKC × CTARG - 019.
Legend of variables: V1-O; V2-J, V3-I, V4-P, V5-FV, V6-FvFm, V7-F0Fm, V8-Fs, V9-Fms, V10-Y, V11-Fos, V12-Y_{no}, V13-Y_{npq} V14 - qL

Figure 4. Dispersion of 13 rootstock genotypes grafted with ‘Tahiti’ acid lime plants under irrigation with water of 2.40 dS m^{-1} 70 days after transplantation based on the scores of the first (PC1) and second (PC2) principal components. (A) Biplot of groups of genotypes and variables, (B) Biplot of discriminant variables versus number of variables in the principal components (PC1 and PC2)

4 (P), 13 (Y_{NPQ}), 3 (I), and 8 (Fs). Although the combination between ‘Tahiti’ and genotype 5 (mandarin ‘Sunki Tropical’) is the ideal combination, combinations with genotypes 6 (TSKC × TRBK - 007), 7 (TSKFL × TRBK - 030), and 12 [TSKC × (LCR × TR) - 059] could exhibit potential.

When applying 4.80 dS m^{-1} water, the variables that best represent the components are arranged in the 1st quadrant (Figure 4B), with the ideal response being observed in variable V6 (FvFm), followed by variables V5 (Fv), V4 (P), V8 (Fs), and V9 (Fms), mainly in the combination between the ‘Tahiti’ acid lime and the genotype 12 [TSKC × (LCR × TR) - 059], followed by genotypes 1 (“Santa Cruz Rangpur’ lime), 3 (‘Riverside’ citrandarin), 4 (‘San Diego’ citrandarin), and 6 (TSKC × TRBK - 007).

Thus, it can be said that the combination of ‘Tahiti’ with these rootstocks is highly efficient, with less electron return and energy loss in the form of heat or light (fluorescence) (Taiz et al., 2017), especially because a high correlation with the FvFm data (V6) is observed in this study (Figure 4B), even when 4.8 dS m^{-1} water was used for irrigation.

Moreover, high quantum efficiency can reduce the photochemical (Y_{NO}) and non-photochemical (Y_{NPQ}) quenching in these genotypes, which according to Ruban (2016), correspond to the energy dissipation by photochemical processes not related to the photochemical phase of photosynthesis, such as the use of adenosine triphosphate (ATP) in the biochemical phase of photosynthesis.

It can be said that the increase in salinity changed the energy flow of the electron transport chain, making the variables Fs, Fms, and Fos highly representative in the first two principal components, indicating that the beginning phase of the stress period was intense since rain increased the water content to approximately 15 mm twenty days before the evaluation (Figure 1).

However, this response was greatly evident in the combination of the ‘Tahiti’ acid lime with genotypes 12, corresponding to TSKC × (LCR × TR) - 059, 1 (‘Santa Cruz Rangpur’ lime), 3 (‘Riverside’ citrandarin), 4 (‘San Diego’ citrandarin), and 6 (TSKC × TRBK - 007) (Figure 4A). Hence, changes in the activity of the photosystems caused by salinity were more evident in these genotypes than in others, thus raising the values of the variables and activating protective mechanisms. This is because, according to Demmig-Adams et al. (2014), photochemical and non-photochemical quenching represent molecular adaptations that constitute the fastest response of the photosynthetic membrane to excess energy, which occurs when a plant is under stress conditions such as salinity.

The restriction in stomatal conductance caused by salinity (Khoshbakht et al., 2015) corresponds to the effect of osmotic stress, which induces ionic disturbances; changes in the photochemical phase of leaves and carbon metabolism occurred in citrus plants up to 70 DAT, indicating the onset of ionic stress.

Although the activation of protective mechanisms may indicate a potential tolerance of the genotypes to salinity, it is still necessary to assess the response of crop yield to the increase in water salinity.

Conclusions

1. Quenching indicates the onset of ionic stress in citrus plants under irrigation with saline water.

2. The ‘Tahiti’ acid lime plants grafted with the genotypes TSKC × (LCR × TR) - 059, “Santa Cruz Rangpur’ lime, ‘Riverside’ citrandarin, ‘San Diego’ citrandarin, and TSKC × TRBK - 007 show high activation of protective mechanisms when transient fluorescence is used.

3. The use of rootstocks with high photochemical activation can guarantee improvement in the yield of ‘Tahiti’ acid lime under moderate water salinity.

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