Scientia Horticulturae 238 (2018) 75–82

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Drought tolerance of a microcitrangemonia when treated with paclobutrazol and exposed to different water conditions

Eliana Maria Rocha Sousa[a], Luciana Silva Almeida[a], Andressa Rodrigues de Oliveira Sousa[a], Matheus de Carvalho Silva[a], Carlos Alberto da Silva Ledo[b], Alex-Alan Furtado de Almeida[a], Marcio Gilberto Cardoso Costa[a], Mauricio Antônio Coelho Filho[b], Walter dos Santos Soares Filho[b], Abelmon da Silva Gesteira[a,b,*]

[a] Department of Biological Sciences, State University of Santa Cruz (UESC), 45662-900, City of Ilhéus, Bahia, Brazil
[b] Embrapa Cassava & Fruits, 44380-000, Cruz das Almas, Bahia, Brazil

ARTICLE INFO

Keywords:
Hybrid
Citrus
Water deficit
Cultivation

ABSTRACT

The microcitrangemonia {[RPL ('Rangpur' lime) x YMCT ('Yuma' citrange) - 005] x MCP (*Microcitrus papuana*) - 011} (H011) demonstrates characteristics of agronomic interest that may benefit citriculture in different aspects, such as tolerance to water stress, irregularity of rainfall and lack of irrigation, which can cause drought stress conditions. In other varieties, one of the alternatives other than the use of tolerant varieties is the use of substances like paclobutrazol (PBZ). However, for citrus, there is a lack of information regarding the use of PBZ and the response to water deficit. The objective of this work was to evaluate the physiological behavior of H011 when compared to 'Rangpur' lime, both as rootstocks to 'Valencia' sweet orange and as nucellar seedlings, when exposed to different water conditions and to PBZ. The plants were cultivated in the presence and absence of PBZ and exposed to two water regimes (constant irrigation and water deficit), while water, physiological and morphological alterations were evaluated. The plants cultivated in the presence of PBZ in the soil showed greater tolerance to water deficit, and H011 as a nucellar seedling and as rootstock demonstrated greater efficiency in the use of water over time. The strategy adopted by H011 differed from the way of survival adopted by 'Rangpur' lime. Therefore, H011 presents the potential to be used in studies related to water stress tolerance in citrus.

1. Introduction

The dissemination and adaptation of citrus to different environmental conditions are the result of the significant genetic variability existing in *Citrus* (L.) and in related genera (Hynniewta et al., 2014; Shimizu et al., 2016). This diversity enables the development of varieties with desirable horticultural characteristics on a commercial scale, through techniques such as hybridization.

Citrus hybrids can be used as scion varieties as well as rootstocks, depending on the purpose of the hybridizations. In Brazil, this is due to the small diversity of rootstocks in the orchards, also associated with the predominance of 'Rangpur' lime trees (*C. limonia* Osbeck) (Mendes-da-Glória et al., 2000; Oliveira et al., 2008). The difficulty in obtaining a new genotype with coexistence of desirable agronomic traits for citrus rootstocks, through traditional improvement, is one of the main limiting factors for the implantation of new varieties in orchards, such as 'Swingle' citrumelo [*C. paradisi* Macfad. x *Poncirus trifoliata* (L.) Raf.] and 'Carrizo' and 'Troyer' citranges [*C. sinensis* (L.) Osbeck x *P. trifoliata*], which are consolidated as rootstocks and used in different countries (Castle, 2010; Bastos et al., 2014).

It is desirable that hybrids destined for use as rootstocks present characteristics such as a shorter pre-reproductive period, low occurrence of heterozygosity, reduced size and resistance to adverse conditions caused by biotic and/or abiotic stress (Oliveira et al., 2008). The predominance of these characteristics enables the promotion of greater production and longevity of the orchards. Therewith, the Citrus Breeding Program of Embrapa Cassava & Fruits (CBP), in the city of Cruz das Almas in the state of Bahia has an active citrus germplasm bank (CGB), which is the basis of the CBP for the generation of hybrids by controlled pollinations. The choice of the parents was made according to the following criteria: agronomic profile and acclimatization to adverse environmental conditions (Soares Filho et al., 2003).

Among the hybrids obtained by the CBP, there is {[RPL ('Rangpur' lime) x YMCT ('Yuma' citrange) - 005] x MCP (*Microcitrus papuana*) - 011} (H011), which was developed for testing as a genotype tolerant to drought and presents differentiated characteristics such as a very short

* Corresponding author at: Embrapa Cassava & Fruits, 44380-000, Cruz das Almas, Bahia, Brazil.
E-mail address: abelmon.gesteira@embrapa.br (A.d.S. Gesteira).

https://doi.org/10.1016/j.scienta.2018.04.038
Received 16 November 2017; Received in revised form 18 April 2018; Accepted 19 April 2018
0304-4238/ Published by Elsevier B.V.

juvenile period, constant flowering and concomitant presence of flowers and fruit across all seasons of the year. In addition, it also demonstrates other important characteristics in citrus plants, which include medium size, resistance to the *citrus tristeza virus* (CTV) according to Santos et al., 2015, a large quantity of fruit, a less developed canopy and seeds that are easy to obtain. The characteristics of H011 can be explored in studies related to flowering, ornamental citriculture, rootstock production and resistance to biotic and abiotic stress such as water restriction.

The climatic risks associated with summer events and with the duration of drought periods are one of the great current problems in citriculture on a global scale, which can be aggravated by climate change scenarios (García-Tejero et al., 2012). The effects act directly on the gas exchange of plants such as stomatal conductance (Gs), transpiration rate (E) and photosynthesis rate (A), also influencing plant growth and production patterns. Therefore, cultivation environments that present abiotic stress related to prolonged water deficiency require rootstocks that demonstrate mechanisms that confer drought tolerance and assist in orchard survival and continuity of production (Santana-Vieira et al., 2016; Warschefsky et al., 2016).

It was observed in many works related to flowering and water restriction for other plant species that the use of regulators such as paclobutrazol [(2RS, 3RS) -1- (4-chlorophenyl)-4,4-dimethyl-2-(1 h-1,2,4-triazol- 1-yl) pentan-3-ol] (PBZ) may antedate the plant's reproductive period and/or assist in tolerance to water stress. PBZ interacts with endogenous bioregulators and consequently influences the physiological and morphological aspects of plants (Pal et al., 2016; Souza et al., 2016). In citrus, the use of PBZ is reported in different works (Tsagkarakis et al., 2012; Martinez-Fuentesa et al., 2013) related to flowering and morphology. However, research studies related to different water conditions and rootstocks are still scarce.

The lack of studies involving the use of PBZ in citrus associated with water restriction, the need to test new rootstocks and the fact that H011 presents differentiated and important characteristics justify the present study. Therefore, the general objective of this work was to evaluate the physiological behavior of H011 when compared to 'Rangpur' lime (RPL), both as nucellar seedlings and as rootstocks to 'Valencia' sweet orange (*C. sinensis*), when exposed to different water conditions and to PBZ.

2. Methodology

2.1. Plant material and microsatellite markers for confirmation of nucellar clones

The study was carried out in a protected environment located at *Embrapa Cassava & Fruits,* in the municipality of Cruz das Almas, state of Bahia, Brazil. The plant material used in the experiment included: like rootstocks, H011 and 'Rangpur' lime (RPL); the crown was Valencia (V) orange, H011 and RPL.

The sowing of the rootstocks and 'Valencia' sweet orange was carried out in 75-mL plastic tubes containing substrate composed of decomposed pine shells, and one seed was used per tube. After emergence and initial growth, about 60 days after sowing, the most vigorous nucellar seedlings were visually selected, and their nucellar origin was confirmed by the use of microsatellite markers. At about 20 cm of height, the plants were transplanted into plastic bags (1.5 kg), conducted on a single stem, with weekly irrigation. Fertilization was carried out by the application of Osmocote® fertilizer and urea immediately after transplantation. Irrigation was performed manually, three to five times a week, according to meteorological and substrate moisture monitoring. After the period of adaptation to the culture condition, collections were carried out for the confirmation of the nucellular clones only of the H011 through the use of microsatellite markers. RPL and V the confirmation of the nucellar origin was obtained exclusively by phenotypic analysis, through the observation of the vigor in the growth, characteristics of the leaves as na form, arrangement of the vein and limbus.

For the nucellar origin confirmation, aproximately eight healthy young leaves were removed from the stem base of each H011 nucellar seedling for extraction of the genetic material. The leaves were homogenized, and the DNA was extracted according to Murray and Thompson's protocol (1980) and stored at ⁻20 °C.

DNA quantification was performed using the ʎ DNA in three different concentrations of 100, 200 and 300 ng/μL. The DNA samples were subjected to 1% agarose gel electrophoresis marked with 1 μg ml ethidium bromide in TAE buffer at 100 V for 25 min. The gel was photographed with the Gel Logic photo documentator. After quantification, the DNA samples were diluted to 5 ng/μL.

Microsatellite markers were amplified from five pairs of primers: OT4S – *Forward* and *Reverse*; Ci06A05b – *Forward* and *Reverse*; miCrCIR01E02 – *Forward* and *Reverse*; CMS14 – *Forward* and *Reverse* and CMS26 – *Forward* and *Reverse* (FROELICHER et al., 2008; CRISTOFANI-YALY et al., 2011; YONG et al., 2006). DNA amplification occurred in a final volume of 25 μL containing 6.0 μg of DNA (5.0 μg/μL), 2.0 μl of Taq DNA polymerase LBM (1U/0.5 μL), 2.5 μl of 10X buffer (500 mM KCl, 100 mM Tris-HCl, pH 8 Invitrogen), 1.25 μL $MgCl_2$ (50 mM), 2.0 μL dNTP (2.5 mM), 1.25 μL of each primer (2 mM) and 8.75 μl of ultrapure water (Milli-Q). The amplification reactions occurred in thermocyclers according to the following schedule: 1 cycle of 94 °C for 3 min, 35 cycles of 94 °C for 30 s, 52 °C for 30 s, 72 °C for 45 s and a final extension of 72 °C for 5 min. The PCR products were visualized on 3% agarose gel marked with ethidium bromide (0.5 ng/ml). The photographic record was done with the Gel Logic photo documentator.

Approximately four months after transplantation and confirmation of the nucellar clones for H011, grafts were performed with a similar scion (LCR and H011) and different (V) of the rootstocks (LCR and H011). Sixty grafts were performed for each scion and rootstock combination, totaling 240 grafts.

2.2. Experimental design

The genotypes H011 and RPL were both evaluated as free standing, as rootstock grafted onto itself and with V scion, composed as follows: (I) H011 nucellar seedling (H011); (II) H011 rootstock and V scion (V/H011); (III) H011 rootstock and H011 scion (H011/H011); (IV) RPL nucellar seedling (RPL); (V) RPL and V scion (V/RPL) and (VI) RPL rootstock and RPL scion (RPL/RPL).

When the plants reached the 10- to 12-leaf stage, 20 individuals from each canopy/rootstock combination, as well as free-standing varieties selected as rootstocks, were selected based on their uniformity and transferred to 45-liter pots containing two quarters of sandy-clayey franc soil, a quarter of sand and a quarter of coconut fiber. The experiment was set up with plants that developed with the addition of PBZ to the substrate and plants that developed with the absence of PBZ in the substrate.

The application of PBZ was carried out using the commercial product Cultar® diluted in water and deposited directly into the soil twice annually, with increasing quantity over time as follows: six months 25 mg PBZ per plant, 12 months 175 mg PBZ per plant, 18 months 225 mg PBZ per plant, and 24 months 250 mg PBZ per plant. During the period of application of the PBZ, the plants were under constant maintenance through fertilization, irrigation, pathogens control and pruning.

2.3. Measurement of physiological parameter: photosynthesis, fluorescence of chlorophyll, and relative water content

After three months from the last application of PBZ to the plants, the conditions of water availability were changed: (I) permanent irrigation of the plants and (II) plants under water deficit (WD) imposed by the