Photosynthetic Gas Exchange and Chlorophyll Fluorescence as Drought Tolerance Indicators in Citrus Rootstocks under Water Stress and Recovery

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ABSTRACT

Water stress induced by consecutive years of drought constitutes a major constraint for citric culture in Fars province in Iran. The aim of this study was rapid and accurate screening of citrus seedling rootstocks (grown under greenhouse conditions) in terms of drought tolerance and ability to recovery, using chlorophyll fluorescence technique combined with measuring the leaf gas exchange parameters. Eight month-old seedlings of Mexican lime, sour orange, Volkameriana and Rangpur lime plants were submitted to a cycle of drought for 14 days/ rewatering for 3 days and control plants were maintained in an optimal soil water condition during the whole experimental period. At the end of stress and recovery periods, measurements were made using fully developed leaves. According to Fv/Fm ratio index, treated Volkameriana plants had no significant photoinhibitory damage to PS II. Rewatered Mexican lime and Rangpur lime plants had ability to fully recover the significantly reduced fluorescence index, but in sour oranges, full recovery did not happened. Also, our results indicated that the rate of net photosynthesis (A), transpiration (E) and stomatal conductance (gs) significantly reduced under drought stress, only Volkameriana and Mexican lime plants fully recovered from stress. Increased Ci in concomitant with stomata closure means a reduction in CO2 photoassimilation and is a symptom of photoinhibition (in addition to decreased Fv/Fm ratio, maximum quantum yield of PS II). So, it seemed that at the end of drought period, except for Volkameriana, stomatal limitation combined with PS II photodestruction were responsible to significant reductions in net photosynthesis in treated plants. In other words, in stressed Volkameriana plants only stomatal closure caused the reduction in photosynthesis. Also it was demonstrated that in rewatered sour orange and Rangpur lime plants damage imposed to PS II and lack of recovery in stomatal conductance were major obstructions in photosynthesis recovery, respectively. Finally, it was concluded that Volkameriana seedlings were more tolerant and had better recovery ability and Mexican lime plants had profound ability to recover from imposed negative effects of water stress, too.

Key words: Chlorophyll fluorescence, Citrus rootstocks, Drought stress, Photosynthesis, Recovery

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INTRODUCTION

Drought consist the most important environmental restriction to plant growth and production (Patakas, 2012). Drought stress is known to change a range of physiological processes such as photosynthesis, stomatal conductance and transpiration rate (Ow et al., 2011). It is well known that photosynthetic systems in higher plants are most sensitive to water stress (Falk et al., 1996) and drought situations can, directly or indirectly, influence the CO2 assimilation rate of plants and as a consequence alter chlorophyll fluorescence kinetics. (Kalaji et al., 2014). At physiological temperatures, fluorescence is predominantly emitted from Photosystem II (PS II) (Deg'Ignocenti et al., 2008). Plant physiologists and horticulturists use chlorophyll a fluorescence analysis technique as one of the most rapid and powerful methods giving information about the state of PS II. It can indicate the extent to which
PS II is using the absorbed energy and the extent to which it is being injured by excess light (Maxwell and Johnson, 2000; Rong-hua et al., 2006). Nowadays, \( F_{v}/F_{m} \) ratio is Chlorophyll fluorescence parameter widely used as trustworthy indicator and biomarker of photoinhibition (Maxwell and Johnson, 2000). In other words, the maximum quantum efficiency of photosystem II is calculated as \( F_{v}/F_{m} = (F_{m} - F_{o})/F_{m} \), where \( F_{m} \) and \( F_{o} \) are maximal and minimal fluorescence of dark-adapted leaves, respectively and \( F_{v} \) is variable fluorescence (Pérez-Pérez et al., 2007). Of course, the most clever and graceful applications of chlorophyll fluorescence technique, do not use this alone, but combine it with other techniques, in particular, gas exchange measurements, to obtain a full view of the response of plants to their environment (Maxwell and Johnson, 2000).

With the foreseen increase in erratic precipitation across important cropping areas in the world, the severity and duration of extreme drought stress are probably to increase, putting extra pressure to meet the mankind demand for food (Impa et al., 2012). Nowadays, there are signs of more frequent and more severe droughts in Iran, due to global climate change, population growth, and increased water resource demands for agriculture, industries, and municipal uses (Salami et al., 2009). Water stress induced by consecutive years of drought constitutes a major constraint for citrus culture in Fars province in Iran. Sour orange (Citrus aurantium L.) is the oldest common citrus rootstock in Iran. Also, despite the lack of comprehensive scientific investigations on horticultural characteristics of Mexican lime (Citrus aurantifolia Swingle cv. Mexican Lime) as a rootstock, its seed availability for propagation and some characteristics as good crop load and vigorous habit of grafted cultivars as scion made it favorite and popular in Fars province. On the other hand, Rangpur lime (Citrus limonia Osbeck) and Volkameriana (Citrus volkameriana Ten & Pasq) are not common rootstocks for this region and are used only for performing researches aimed at achieving and introducing trustable rootstocks for citrus culture industry in south regions of Iran (as Fars province). The objective of nearly all of these researches is obtaining the graft combinations suitable for growing in calcareous soils of these regions.

Water stress tolerance is seen in almost all plant species but its extent varies from species to species (Chaitanya et al., 2003). These variations in response to stress among genotypes offer opportunities for selecting plants with preferred optimal characteristics (Niu et al., 2008). Effects of water stress on plants alone have been well documented in scientific literature. However, researches considering the combined plant reactions to drought and rewatering are relatively limited (Xu et al., 2010).

So, the aim of this study was rapid and accurate screening of four mentioned rootstocks in terms of drought tolerance and ability to recovery, using chlorophyll fluorescence technique combined with measuring the leaf gas exchange parameters.

**MATERIALS AND METHODS**

The experiment was performed on 8-month-old citrus seedlings of Mexican lime (Citrus aurantifolia Swingle cv. Mexican Lime), sour orange (Citrus aurantium L.), Volkameriana (Citrus volkameriana Ten & Pasq) and Rangpur lime (Citrus limonia Osbeck) plants in 2013. Experimental seedlings were obtained from seeds collected from Darab Citrus Research Station in Fars Province, Iran. Plants were grown under greenhouse conditions, in 25-cm diameter pots (4.2-L capacity) filled with a mixture of leaf mold, sand, and soil (1:1:1, v/v/v) and a gravel layer at the bottom. The field capacity of the medium used for potting was calculated according to the procedure described by Richards (1949). Potted plants were irrigated to field capacity level, until the beginning of the experiment. During this period, all of horticultural practices such as fertilization and pests control performed uniformly for all of growing plants. The maximum and minimum temperatures during the experimental period were 16 and 32°C (±2°C), respectively, and mean relative humidity was 67%. After 8 months, plants were selected for uniformity of size and health. The research was performed in a factorial plan based on completely randomized design with four replicates (four plants per replicate of each treatment). The beginning of experiment was in concomitant with summer flashes of plant growth in September, when 16 plants of each kind of seedlings were submitted to a cycle of drought for 14 days (until the majority of experimental plants showed a strong loss of photosynthetic rate) and rewatering for 3 days and control plants were maintained in an optimal soil water condition by irrigation to field capacity during the whole experimental period. At the end of both the stress (day 14) and recovery (day 17) periods, measurements were made using one fully developed leaf per plant. For chlorophyll fluorescence evaluation, an additional assessment at day 7 (middle of drought stress) was performed. Chlorophyll \( a \) fluorescence was measured after 20 min of leaf acclimation to dark under leaf clips, using a OS-30p hand held portable modulated chlorophyll fluorometer (Opti-Sciences, Inc., Hudson, NH, USA), following the manufacturer's instruction. Net photosynthetic rates (\( A \)), stomatal conductance (\( gs \)), transpiration rate (\( E \)) and sub stomatal CO\(_2\) concentration (\( Ci \)) were assessed using a LCI portable photosynthesis system (ADC BioScientific Ltd., Hoddesdon, Herts, UK). All measurements were made in the morning from 09.00 to 10.00 h. Data were expressed as means ± standard deviation (SD) of four replicates. Using SAS 9.1.3 service pack 4 software (SAS institute, Cary, NC, USA) differences among means were determined by the least significant difference (LSD) test.

**RESULTS**

**Chlorophyll \( a \) fluorescence**

Both at the middle and the end of drought period, except for Volkameriana, the reduction in maximum quantum efficiency of photosystem II (\( F_{v}/F_{m} \) index) in all of treated seedlings was significant. At the end of recovery period (day 17), except for sour orange, this ratio in all of treated plants was the same as controls. Sour orange seedlings had no full recovery (Fig 1).

**Net photosynthetic rates (\( A \))**

Drought stressed plants had significantly lower photosynthetic rate than control ones, but at the end of
recovery period, only treated plants of Volkameriana and Mexican lime had photosynthetic rate similar to their controls (Fig 2).

Transpiration rate (E)
Water stress significantly reduced the transpiration rate of treated plants. After rewatering, all of treated seedlings were statistically recovered from stress, except for Rangpur lime plants that had significantly lower values than controls (Fig 3).

Stomatal conductance (gs)
As shown in Fig 4, all of treated plants had significant decrease in the rate of stomatal conductance. Except for Rangpur lime, there were no significant differences between treated and control plants at the end of recovery period.

Sub stomatal CO₂ concentration (Ci)
At the end of drought period, except for Volkameriana, all stressed plants significantly had higher internal CO₂ concentrations. At day 17, except for Rangpur lime, other treated plants had higher values compared to the controls that the rate of increase was significant for sour orange. On the other hand, in rewatered Volkameriana a significantly reduced Ci value was recorded (Fig 5).

DISCUSSION
The Fv/Fm ratio for a normally functioning leaf varies between 0.75 and 0.85 and a decrease in this ratio is indicative of photoinhibitory damage (DeEll et al. 1999). In our experiment, about Volkameriana no significant negative effect from stress on maximum quantum efficiency of photosystem II (Fv/Fm ratio) was observed. But, other treated rootstocks had significantly lower ratio (below the normal range) compared to their controls. However, after rewatering period, only sour orange treated plants showed no full recovery. Former, Ow et al. (2011) suggested that genetic differences exist in the reaction of the photosynthetic apparatus to drought, and that in drought tolerant species the photosynthetic processes have developed various mechanisms to safeguard against water stress. The underlying mechanisms to drought stress adaptability and tolerance are beyond the scope of this study, but according to analysis of fluorescence data, it was concluded that Volkameriana seedlings were more drought tolerant and Mexican lime and Rangpur lime plants had ability to full recovery after rewatering, too.

In general, our results indicated that the rate of net photosynthesis (A), transpiration (E) and stomatal conductance (gs) significantly reduced under drought stress condition, but the ability to recovery was different between experimental plants. Rewatered sour oranges had transpiration rate and stomatal conductance same as the control plants, but they could not recover the photosynthetic rate as controls. In contrast with Mexican lime and Volkameriana, Rangpur lime seedlings had no ability to completely recover all of mentioned traits after rewatering. Despite the significant reduction in stomatal conductance in all of treated plants, they had significantly
higher internal CO₂ concentrations (Ci) at the end of drought period (except for Volkameriana). This trend was kept at the end of rewatering period, except for Rangpur lime that in which significant reduction was recorded. As mentioned above, sour orange plants did not recovered the lime that in which significant reduction was recorded. As kept at the end of rewatering period, except for Rangpur lime plants could be happened.

Conclusions
Considering the evaluated parameters, it was concluded with certainty that Volkameriana seedlings were the most tolerant plants among all of drought treated plants and also, Mexican lime plants had profound ability to recovery from negative effects of water stress. Our data indicated that sour orange and Rangpur lime plants had no ability to fully recover, but it was likely that if rewatering period was more extended, complete recovery in Rangpur lime plants could be happened.

Fig. 5: Sub stomatal CO₂ concentration (Ci) in leaves of four citrus seedling rootstocks under drought stress and after recovery period. S, R, C and D: end of drought period, end of recovery period, control and stressed plants, respectively. Data were expressed as means ± SD of four replicates. Bars with the same letter are not significantly different at the 0.05 level according to LSD test.

REFERENCES


