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Monitoring physiological and biochemical responses of two apple cultivars to water supply regimes with non-destructive fluorescence sensors

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ABSTRACT

Worldwide, increased drought stress induced by water shortages will be especially hazardous for maintaining high yields in agricultural and horticultural crops. It is known that not only do different crops have different water requirements, but that these can also vary between cultivars. Most commonly, plant responses to drought stress are monitored on the physiological level, for example via plant water status (leaf water potential, relative water content) or stomatal behavior, photosynthesis and osmotic adjustment. However, analyses of these parameters are time-consuming and destructive. In contrast, novel approaches such as fluorescence sensors seem promising as they are easy to embark in the field, non-destructive and quick to analyze. Therefore, the aim of this study was to investigate whether two apple (*Malus domestica*) cultivars, 'Pinova 10' and 'Gala Galaxy', show different physiological reactions, for example in leaf water potential, relative water content as well as chlorophyll and proline concentration, in response to water stress. In addition, chlorophyll fluorescence parameters were analyzed as a promising stress symptom detection technique. 'Gala Galaxy' showed higher tolerance to water deprivation conditions than 'Pinova 10', indicated especially by increased chlorophyll fluorescence indices on certain measurement days throughout the experimental course. Fluorescence indices related to chlorophyll content (Chl_Index and SFR_R) and nitrogen balance (NBI and NBI_R) showed similar curves. However, leaf chlorophyll analysis performed wet-chemically was not a reliable indicator of water stress in apple trees. Leaf water potential was affected on DAT 38, during complete watering withholding, without significant differences between the cultivars. In summary, fluorescence-based indices, related to chlorophyll content and nitrogen balance, promise to be a useful non-destructive tool to estimate physiological status of young apple trees submitted to water restriction regimes.

1. Introduction

Understanding physiological changes in plant tissues is one of the starting points for the development of non-destructive and non-time-consuming tools to assess current metabolic activities in response to environmental constraints. The fluorescence technique has been researched for several years to address the needs of farmers and producers on timely detection of plant stresses during different growth stages in the field (Gorbe and Calatayud, 2012; Tremblay et al., 2012; Bürling et al., 2013). Promising results have been reported on annual cultures for different abiotic factors, such as nitrogen fertilization and water deficit (Shangguan et al., 2000; Bürling et al., 2011). At the orchard level, the effectiveness of any remedial measures also depends on the early detection and identification of the cause of stress (Kim et al., 2011). Chlorophyll fluorometers based on chlorophyll fluorescence kinetics techniques – such as Imaging-PAM (Heinz Walz GmbH,

Abbreviations: PAM, pulse amplitude modulation; WW, well-watered; WD, water deficit; DAT, days after treatment; UV, ultraviolet; R, red; FR, far-red; Chl_Index, chlorophyll index; Flav_Dx, flavonol index from sensor dualex®4 scientific; NBI, nitrogen balance index; LED, light emitting diodes; RF, red fluorescence; FRF, far-red fluorescence; SFR_R, simple fluorescence ratio with red excitation light; NBI_R, nitrogen balance index with red excitation light; Flav_Mx, flavonol index from sensor multiplex®3; FRT, far-red transmission; RT, red transmission; FRF_R, far-red fluorescence with red excitation light; RFR, red fluorescence with red excitation light; FRF_UV, far-red fluorescence with UV excitation light; FER_UV, fluorescence excitation ratio with red and UV excitation lights; Ψ_{leaf}, leaf water potential, MPa, megapascal; RWC, relative water content, W, leaf fresh weight; TW, Leaf Fully Turgid Weight; DW, Leaf Dry Weight; UV–vis, ultraviolet–visible spectrophotometry; Chl *a*, Chlorophyll *a*; Chl *b*, Chlorophyll *b*; Chl *a*+*b*, total chlorophyll; Vol, volume; DM, dry mass; rpm, rotation per minute; SPSS, statistical package for the social Sciences

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Effeltrich, Germany) and CF 1000 (P.K. Morgan Instruments, Andover, MA, USA) - are highly sensitive research instruments, which give quantitative information on the quantum yield of photosynthetic energy conversion, and are therefore extensively used in studies assessing responses to stress conditions in orchards plants, such as apple (*Malus domestica* Borkh.) trees (Fernandez et al., 1997). However, the necessity of dark-adaptation of leaves before measurements and the time-consuming nature during recording of fluorescence signals from the samples are just some of the drawbacks that lead, in many respects, to impracticability for plant stress assessments in situ.

Instead, portable fluorescence sensors with light emitting diodes would encompass a feasible technology in orchards to timely assess plant disorders and deliver a set of qualitative information about the plant physiological status. This technology has already been applied during crop production to evaluate performance and productivity of cultures growing under different stress conditions (Leufen et al., 2014a, 2013; Peteinatos et al., 2016). Nevertheless, there are very few studies about the employment of portable fluorescence sensors to detect effects of water limitation on apple trees. In this culture, water is one of the most common limiting factors to reach productivity all over the world. Consequently, drought stress is a situation which apple trees have to deal with frequently. The increasing worldwide shortage of water emphasizes the need to develop sparing irrigation systems. In recent years, there has been a wide range of proposed novel approaches to schedule irrigation which are based on sensing the plant response to water deficit directly, as opposed to sensing the soil moisture status (Alizadeh et al., 2011; Šircelj et al., 2007). Fluorescence sensors might also cover these requirements.

Plant responses to water limitation are usually monitored with traditional physiological parameters which have been proven to be good indicators of drought. The majority of studies on drought responses of apple trees investigated mainly plant water status (water potential, relative water content) and selected physiological responses such as stomatal reactions, photosynthesis, or osmotic adjustment. These studies showed that the physiological and biochemical reactions of apple trees to water stress are quite variable. This variability is associated with cultivar, time of year, previous water stress level, intensity of stress, and environmental conditions (Šircelj et al., 2007). However, based on the previous study of Fernandez et al. (1997), it seems likely that non-destructive fluorescence-based sensors are suitable to assess possible effects of water restriction on young trees. This present study focused on the behavior of two different apple cultivars under different watering levels with non-destructive fluorescence sensors. In detail, we aimed to understand if and how water deficit leads to physiological responses in apple trees and if the cultivars 'Gala Galaxy' and 'Pinova 10' respond differently to the exposed stress conditions.

2. Material and methods

2.1. Plant material and experimental design

The trial was run from mid of April to beginning of June 2017 at the Horticultural Science Department of the Institute of Plant Sciences and Resource Conservation, University of Bonn, Bonn, Germany. For this experiment, two-year-old healthy and vigorous apple trees (*Malus domestica* Borkh.) of two different cultivars ('Gala Galaxy' and 'Pinova 10') were grown in 27 cm Ø plastic pots filled with a mixed substrate composed of black soil, sand and perlite (4:2:1). Plants stood outside during winter and were pruned in March 2017. In April, trees were moved to tunnel-shaped greenhouses covered by transparent polyethylene foils and equipped with an automatic drip irrigation system. The rootstock used for both cultivars was M9. At the first measuring event - eight days after treatment (DAT) application, i. e. start of irrigation controlling in the acclimation phase - leaves were approximately one month old, with a mean size of 15.62 and 21.43 cm^2 for 'Pinova 10' and 'Gala Galaxy', respectively.

All plants, i. e. 32 unities, received a complete water supply so that the substrate reached its water holding field capacity (according to a pre-determined mean value). For this, pots were watered until the substrate was completely saturated and then left to drain for 24 h. The excess water was subtracted from the supplied water amount, resulting in the mean value of 1 L day^{-1} per pot. To determine for how long the irrigation had to be switched on, drippers attached to the automatic irrigation were placed in empty pots and the time it took to fill them up until 1 L was noted (20 min). Watering was performed daily in the early morning (7:00 a.m.).

Four different *phases* of water supply were adopted in the course of the experiment: *Phase I*) acclimation – for thirteen days, DAT 0–13 – *Phase II*) reduction of water supply – for fourteen days, DAT 13–27 - *Phase III*) introduction of complete watering withholding (water deficit – WD) – for fourteen days, DAT 27–41 - and *Phase IV*) recovery – for four days, DAT 41–45, totalizing 45 days of experimental conduction. During acclimation (Phase I), the calculated water holding field capacity was supplied to all plants, corresponding to the variable 'Watering 100%' (W100). For phase II (reduction of water supply), a second watering level was added to the trial, i. e. 1 L day^{-1} per pot was reduced to half the amount, in which 8 plants per cultivar received either the initial watering supply (Watering 100%) or the reduced variable ('Watering 50%' – W50). During Phase III (water deficit), both watering levels (W100 and W50) were subdivided into two parts: well-watered (WW), in which the testing plants continued receiving their respective water supply from the previous phase, and water deficit (WD), where these plants were submitted to a complete watering withholding. Finally, during the recovery period (Phase IV), all 32 trees were re-watered to the initially determined field capacity (Watering 100%).

The experimental set-up was partially randomized, i.e. irrigation levels (W100 and W50) were conducted in two different green houses with both cultivars allocated in them in a randomized pattern. In Phase I, an initial measurement on DAT 8 was done, when all plants of both greenhouses were fully watered (W100). Table 1 summarizes the plant watering and analyzed parameters as well as the applied watering levels and phases.

Table 1
Experimental design and measured parameters. Two-year-old apple tree cultivars 'Pinova 10' and 'Gala Galaxy' tested under two watering regimes Watering 100% and Watering 50% and four watering phases: Phase I: acclimation, phase II: water restriction, phase III: cancelation of water supply, phase IV: recovery.

DAT	Phase	Watering levels					Measured Parameters	
8	I	Acclimation			W100		F. I.	P. P.
14	II	Water	W100		W50		F. I.	P. P.
17		Reduction	W100		W50		F. I.	P. P.
21			W100		W50		F. I.	P. P.
24			W100		W50		F. I.	P. P.
25			W100		W50			P. P.[a]
28	III	Water deficit	W100		W50		F. I.	P. P.
			WW	WD	WW	WD		
30			W100		W50			P. P.[a]
			WW	WD	WW	WD		
31			W100		W50		F. I.	P. P.
			WW	WD	WW	WD		
35			W100		W50		F. I.	P. P.
			WW	WD	WW	WD		
38			W100		W50		F. I.	P. P.[b]
			WW	WD	WW	WD		
42	IV	Recovery			W100		F. I.	P. P.
45					W100		F. I.	P. P.[b]

W100 = Watering 100%, W50 = Watering 50%, WW = Well Watered, WD = Water Deficit, F.I. = Fluorescence Indices, P. P. = Physiological Parameters (proline and chlorophyll concentration, relative water content and leaf water potential).

[a] = only Leaf Relative Water Content (RWC).

[b] = all P.P., including RWC.