Scientia Horticulturae 122 (2009) 17–25

Contents lists available at ScienceDirect

Scientia Horticulturae

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

Sensing of tomato plant response to hypoxia in the root environment

Hans-Peter Kläring [a,*], Manuela Zude [b]

[a] Leibniz Institute of Vegetable and Ornamental Crops Großbeeren/Erfurt e.V., Theodor-Echtermeyer-Weg 1, 14979 Großbeeren, Germany
[b] Leibniz Institute for Agricultural Engineering Potsdam-Bornim e.V., Max-Eyth-Allee 100, 14469 Potsdam-Bornim, Germany

ARTICLE INFO

Article history:
Received 12 December 2008
Received in revised form 25 March 2009
Accepted 30 March 2009

Keywords:
Anoxia
Carotenoids
Chlorophyll fluorescence
Colour
Diffuse reflectance spectra
Dissolved oxygen
Non-invasive measurement
Photosynthesis

ABSTRACT

A severe drawback in hydroponic production systems and irrigated field cultivation arises due to the risk of hypoxia, provoked by water logging in the root environment. The effects of hypoxia become temporarily visible when plants are irreversibly damaged. For this reason, non-invasive methods are required for detecting hypoxia in good time. In five experiments, tomato plants at two stages of development were grown in containers in aerated nutrient solution. Aeration was interrupted to trigger hypoxic conditions in the root environment. Whereas young plants were able to adapt to hypoxia in the root environment and survived, mature plants wilted two days after aeration interruption and died rapidly. A decrease in leaf photosynthesis, leaf transpiration rates and efficiency of the photosystem II was observed in older plants, while leaf diffuse reflectance changed slowly. On the other hand, if young plants were able to adapt to hypoxia in the root environment and survived, no clear reduction of leaf photosynthesis and the efficiency of the photosystem II arose, although the dry matter growth was decreased by 50%. Changes in leaf colour and reflectance spectra occurred. The latter indicated changes in the profile of the carotenoids. The ratio of intensities at 550 and 455 nm in particular provided a sensitive and diagnostic parameter for hypoxia in the root zone of adapted plants which, nevertheless, displayed severe growth limitation.

1. Introduction

Cultivated plant species for greenhouses production were mainly selected for their high yield in optimum growing conditions. In the selection process, we can assume that tolerances to many stress factors were reduced, since such mechanisms often require extra energy and thus potentially decrease the assimilate availability for the harvest organs. This also concerns sensitivity to hypoxia in the root environment (Crawford and Braendle, 1996).

Irrigation strategies do not only have to avoid water deficiency – they must also prevent hypoxic conditions from occurring in the root environment. Both water and oxygen concentrations of soil or substrate depend on irrigation control. Increasing the rate or frequency of irrigation may increase the water content of the substrate or soil, thus decreasing oxygen availability in the root environment. On the other hand, it increases the availability of water to the plants and decreases the osmotic potential by leaching the substrate or soil, avoiding accumulations of salt. Unfavourable growing conditions – hypoxia and osmotic stress – visually result in similar plant responses. Particularly under hypoxic conditions, symptoms often become visible when plants are already severely damaged. There appears to be a lack of feasible methods for the timely detection of critical hypoxia levels in the root environment affecting the plants, preferably by non-invasive sensing. Oxygen concentrations in the root environment can be measured by a variety of methods; however, the influence of the apparent oxygen concentration on plants depends on exogenous variables such as temperature as well as endogenous factors. The limiting effect of anoxia in the root environment on photosynthesis was shown for several crops, including tomato (Bradford, 1983) and many investigations focussed on the mode of action (Vartapetian and Jackson, 1997; Gibbs and Greenway, 2003), but a decrease in the photosynthesis rate was also observed in several different stress conditions, rendering it no good for practical decision-making (Zude and Kläring, 2009). The present study was aimed at approaching non-invasive sensing methods that show potential for plant monitoring, particularly to detect oxygen deficiency in the root environment of tomato plants. Readings of the leaf apparent photosynthesis rate, chlorophyll fluorescence kinetic, leaf colour and leaf diffuse reflectance were evaluated.

2. Material and methods

2.1. Plant material and treatments

Five experiments (Table 1) were carried out on tomato (*Solanum lycopersicum* cv. 'Vanessa' in experiments 1 and 5; cv. 'Liberto' in

* Corresponding author. Tel.: +49 33701 78351; fax: +49 33701 55391.
E-mail address: klaring@igzev.de (H.-P. Kläring).

0304-4238/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.scienta.2009.03.029

Table 1
Duration of the treatments, number of unfolded leaves at treatment start, number of replications per treatment, non-invasive sensing methods tested and mean environmental conditions.

Experiment	Period (d)	Number of leaves	Replications	Sensing method[a]	PAR (mol m^{-2} d^{-1})	Temperature (°C)	rH (%)
1	14	6	6	PH, CF, R	15.1	22.0	70
2	14	4	14	CF, R	15.0	21.8	71
3	14	4	14	CF, C	17.2	23.5	64
4	2	8	12	CF, R	23.4	23.1	52
5	3	28	4	PH, R	29.2	28.6	61

[a] Non-invasive sensing methods were tested for: PH – apparent leaf photosynthesis rate, CF – chlorophyll fluorescence kinetic, R – leaf diffuse reflectance, and C – leaf colour.

experiments 2–4). In experiments 1–4 seedlings were planted in 2-l containers in an aerated nutrient solution. The containers were covered with black-and-white plastic foil (with the white side facing the outside) to prevent algae growth. The plant stems were adjusted in polystyrene discs, floating on the surface of the solution, and later mechanically supported by strings hanging from a wire. In experiment 5, mature plants were grown in 15-l containers in an aerated nutrient solution covered with black-and-white plastic foil. Plants were trained using strings hanging from a wire.

The nutrient solution was based on recommendations for tomato production in hydroponic growing systems (De Kreij et al., 1997), with an air supply to each container. In experiment 1, plants from each treatment were distributed randomly in two growth chambers and were grown at a photosynthetic active photon flux density (PPFD) of 300 μmol m^{-2} s^{-1} for 14 h d^{-1}, with an air temperature of 22 ± 0.2 °C and 70 ± 2% relative humidity. In the other experiments, the plants were randomly distributed in a greenhouse – the small containers in experiments 2–4 on a table and the larger containers in experiment 5 on the floor.

After between three and ten days, when the plants were established in the environment, aeration was interrupted in stress-treatment to trigger hypoxic conditions in the root zone. Additionally in experiment 1, aeration was interrupted for one week and subsequently re-established. Treatments were scheduled for two weeks, if the plants had not already faded away by then.

All of the plants in experiments 1–3 were destructively measured after completion of the experiments. Additionally, samples were removed from experiment 1 before treatments commenced and after re-aeration. The shoot and root mass were measured before and after drying the samples in a ventilated oven at 80 °C for two days.

Non-invasive readings were recorded throughout the experimental phase (Table 1). Unless otherwise stated, the measurements in experiments 1–4 were carried out on the mid part of one lateral leaflet with the already expanded leaf blade of the highest leaf, which completely filled the leaf chamber. Leaf diffuse reflectance measurements required an enhanced leaf area and could therefore not be performed on these young plants at the start of experiments 1–3. The leaf number was the same for all treatments. In experiment 5, the second leaflet of the leaves below the second and third truss (counted from the top) was used.

The oxygen concentration in the nutrient solution was measured using an electrochemical oxygen meter (GMH 3630, Greisinger, Regenstauf, Germany).

2.2. Gas exchange analyses

Leaf apparent photosynthesis and transpiration rates were measured using a portable infrared gas analyser with leaf chamber (LI-6400XT, Licor Inc., USA). Conditions in the leaf chamber were adjusted to the same levels as in the growth chambers (experiment 1: 300 μmol m^{-2} s^{-1} PPFD, 400 μmol mol^{-1} CO_2 concentration, 22 °C, approximately 70% relative humidity) or close to the conditions in the greenhouse (experiment 5: 1000 μmol m^{-2} s^{-1} PPFD, 400 μmol mol^{-1} CO_2 concentration, 25 °C, approximately 60% relative humidity). After closing the leaf chamber, data were recorded every 10 s for 2 min. The steady state appeared rapidly and the average of the last four measurements was used for the data analysis.

2.3. Optical readings

Chlorophyll fluorescence was measured using a pulse–amplitude modulated system (Mini-Pam, Walz, Effeltrich, Germany) after 10 min of dark adaptation (Krause and Weis, 1984, 1991). Readings of light saturation curves were taken to analyse the yield (y = variable fluorescence/maximum fluorescence) and maximum electron transport rate ($ETR = y \times \mathrm{PPFD} \times 0.84 \times 0.5$).

A portable, hand-held spectrophotometer device (Pigment Analyzer PA-1101, CP, Falkensee, Germany) equipped with photodiode arrays was applied to record the leaf spectra in the UV and visible wavelength range from 190 to 720 nm (MMS1 UV/VIS, Carl Zeiss, Jena, Germany) or in the visible and near infrared range from 320 to 1120 nm (MMS1 NIR enh., Carl Zeiss, Germany), providing a spectral resolution of 2.2 nm in experiments 1 and 5 and 3.3 nm in experiments 2 and 3. In the present study, an integrated light cup equipped with light-emitting diodes, capturing the entire wavelength range recorded, served as the light source. Spectralon (20% certified, Labsphere Ltd., North Sutton, USA) was used as the white reference for the calibration. The leaf raw spectra were used to calculate the indices (Table 2).

The leaf colour was measured using a portable spectrophotometer (CM-508d, Minolta Camera Co. Ltd., Osaka, Japan)

Table 2
Indices applied for analysing the leaf spectra recorded in diffuse reflectance geometry using the intensity (I) values at specified wavelengths.

Index	Equation	Indicator for	Literature
PRI	$(I531 - I570)/(I531 + I570)$	Carotenoids	Gamon et al. (1997); Peñuelas et al. (1998)
Car-ratio	$I550/I455$	Carotenoids	Zude and Kläring (2009)
Red-edge	$I''(\lambda_{650-710}) = 0$	Chlorophyll	Lichtenthaler et al. (1996)
NChlI	$(I718 - I660)/(I718 + I660)$	Chlorophyll	Adapted from Richardson et al. (2002)
RVSI	$((I714 + I752)/2)/I733$	Chlorophyll	Merton (1999)
RII	$\int_{705}^{750} \left(\frac{Iy}{I705-1}\right) \mathrm{d}y$	Chlorophyll	Richardson et al. (2002); Gitelson and Merzlyak (1994)
Chlorophyll ratio	$I698/I760$	Chlorophyll	Carter (1994); Moran and Moran (1998)
Lichtenthaler's index	$I750/I550$	Chlorophyll	Lichtenthaler et al. (1996)
PSRI	$(I678 - I500)/I750$	Chlorophyll to carotenoids ratio	Merzlyak et al. (1999)
SIPI	$(I800 - I445)/(I800 - I680)$	Chlorophyll to carotenoids ratio	Peñuelas et al. (1995)