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Water uptake and proline index as indicators of predisposition in tomato plants to *Phytophthora nicotianae* infection as influenced by abiotic stresses

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Abstract

To identify quantitative indicators of predisposition and stress development in tomato (*Lycopersicon esculentum* Mill. cv. Counter) plants infected by *Phytophthora nicotianae* Breda de Haan, we examined plants grown under stress-inducing nutrient conditions exposed to different levels of radiation and infection. The plant's response was investigated by analysing plant growth, proline content and disease development. From all plant growth parameters investigated, only leaf surface area and total fresh mass showed a response to radiation and infection, and both indicators were strongly correlated. However, because of the destructive character of these indicators, non- or minimal destructive indicators were sought. To this end, here we report that water uptake per plant (WP) and water uptake per plant and day (WPD) and proline content of leaves represent useful tools to assess plant health status during growth.

Keywords: *Lycopersicon esculentum* Mill; *Phytophthora nicotianae* Breda de Haan; ELISA; Hydroponics; Oomycetes; Proline; Water uptake; Water use efficiency; Leaf area; Fresh mass

1. Introduction

Infection by *Phytophthora nicotianae* Breda de Haan causes stem and root rot in many important crop species [1–3]. While pathogen-produced enzymes and toxins have not been convincingly shown to be important for oomycete-produced disease development [4], fungus carbohydrate polymers (mycolaminarans) may be involved in the expression of certain disease symptoms, especially wilting and water shortage due to occlusions formed in the xylem vessels [1,5–9]. The chances of pathogenic infection depend primarily on the predisposition of the plant. It has been reported that high temperature, oxygen deficiency, ethylene and carbon dioxide production in the rhizosphere, wet stem base, and exudation of carbohydrates and amino acids from roots could improve the conditions for fungal infection [2,3,10–17]. However, to date, the predisposition of plants to infections has not been quantified.

Water shortage in above-ground plant parts, a common symptom in many *Phytophthora* diseases [5], may result from loss of roots rotted by the fungus or from stomatal dysfunction leading to increased transpiration rates (TRs) [18]. Furthermore, translocation of fungus-produced polysaccharides to the shoot causing vascular dysfunction, wilting and necrosis [19,20] may ultimately result in decreased biomass production, accelerated ripeness of fruits or seeds, and subsequently low yields [21–23].

Nutrient solutions containing moderate-to-high nutrient concentrations from about 3.5 to 5 up to 9 or even more $d\,Sm^{-1}$ have frequently been supplied to improve fruit quality, especially the taste, of hydroponically grown tomato plants. Since this practice may increase susceptibility of tomato plants to *P. nicotianae* infection, the abiotic and biotic stress levels should be monitored, in order to adapt the nutrient concentration according to the overall growth conditions and the health status of plants.

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In this study, plants were grown in solutions containing nutrients at a concentration equivalent to the stress threshold level electrical conductivity (EC) 5.0 dS m^{-1} at which additional stress caused, e.g., by exposure to a high-light intensity or infection with a pathogen like *P. nicotianae*, is reflected by an increase in proline content of leaves. In a previous study, proline content was shown to be a suitable indicator for quantifying stress imposed on plants before and during disease development [24]. Moreover, proline is the most noticeable amino acid known for accumulation in a wide range of plant species as a response to water deficiency conditions caused by abiotic stress factors such as salinity and high-light intensity or by biotic stressors such as pathogens [25–31]. Recently, the proline content of tomato leaves was shown to be strongly related to the relative water content of leaves [32] and their osmotic and water potential [unpublished results], and thus is a useful indicator of the plant's water status.

Water uptake, TR and water use efficiency (WUE) are probably further indicators suitable to quantify the stress level caused by both abiotic and biotic stress factors affecting the water economy of plants [33–35]. To assess the suitability of the aforementioned parameters for monitoring stress, and hence the plant's predisposition to fungal infection, tomato plants grown under stress-inducing increased nutrient levels were exposed to different inoculum densities of *P. nicotianae* under two different light intensities. Our investigation was aimed at quantifying predisposition and subsequent disease progression in infected tomato plants under different abiotic stress conditions.

2. Materials and methods

2.1. First trial

Tomato (*Lycopersicon esculentum* Mill. cv. Counter) plants were grown in 2-L containers (3 plants each, 9 replications per treatment) in aerated nutrient solution under controlled conditions in two similar phytotrons (Voetsch Industrietechnik VB1014, Germany; day/night = 16/8 h, air temperature 25 °C/20 °C, relative humidity 70%/90%) with either 600 or 1000 µmol m^{-2} s^{-1} photosynthetic photon flux density (HQI-T 250 W/400 W). On Day 3, the nutrient solution was adjusted from low-to-moderate (the stress threshold) nutrient concentration (corresponding to an EC of 1.5 and 5.0 dS m^{-1}, respectively). On Day 7, plants were inoculated with either 10^4 or 10^6 propagules mL^{-1} *P. nicotianae* Breda de Haan or with nutrient solution only (control). The composition of the nutrient solution supplied prior to the start of the experiment (EC 1.5 dS m^{-1}) was as follows: NO_3-N 11.0, NH_4-N 1.0, P 1.9, K 5.9, Mg 1.0, Ca 2.75, SO_4-S 1.5 mM L^{-1} and Fe 14.3, Mn 9.09, B 18.2, Cu 0.78, Zn 1.85 and Mo 0.52 µM L^{-1}. Stress was quantified by measuring proline content of leaves [24], as well as by water uptake either per plant (WP, mL $plant^{-1}$) or per plant and day (WPD, mL (plant d)$^{-1}$) 8 h and 1, 2, 5, 8 and 12 d after inoculation. Simultaneously, total fresh mass (FM) of plants was determined by weighing and root rot severity was monitored by enzyme-linked immunosorbent assay (ELISA) and assessing visually the progression of the disease (disease severity, %) during the course of the experiment. Leaf surface area (LA), FM and dry mass (DM) and dry matter content (DMC) of root and shoot, root length, surface as well as specific length (m (kg DM)$^{-1}$) and radius (mm) [36], WUE (g DM (kg H_2O)$^{-1}$) and TR (µmol H_2O m^{-2} s^{-1}) were determined at the end of the experiment.

The proline index (PI), as a measure for stress development reflected by the proline content of leaves over the period of the experiment, was calculated by the following equation:

$$\mathrm{PI} = \sum_{i=1}^{n} (P_{i+1} + P_i)/2 \times (t_{i+1} - t_i),$$

where P is the proline content of leaves (absorbance at 546 nm × 1000); i the date of measurement and t_i the time in days.

2.2. Second trial

The phytotron chambers employed were the same as for the first trial. However, in contrast to the first trial, simultaneous simulation of the two light intensities applied occurred in the same chamber to avoid chamber effects. The light in the chamber was adjusted to the high-light level (1000 µmol m^{-2} s^{-1}) and one half of the chamber was shaded to provide a 600 µmol m^{-2} s^{-1} photosynthetic photon flux density. Under each of the two light intensities, 12 containers were planted with 4 plants each. Six of the 12 containers were inoculated with 10^6 propagules mL^{-1} 7 d after planting. Two, 5, 8, 12 and 15 d after inoculation, water uptake, proline content, absorbance of ELISA at 405 nm and plant growth parameters such as FM and DM of root, shoot and leaves as well as LA and shoot length were measured. All other parameters and the composition of the nutrient solution supplied were the same as during the first trial.

Detailed information on solution culture, disease severity, inoculation with *P. nicotianae,* ELISA and determination of proline content of leaves are found in previous publications by the authors or in co-operation with other coworkers [21,22,24,37–40].

2.3. Statistical methods

Both trails were performed under the same environmental conditions with differences in light intensity and inoculum density. The experimental design of the first trial was a complete randomised design with nine replications of three fungal treatments (control and two inoculum densities) carried out in two phytotrons exposed to a photosynthetic photon flux density of either 600 or