Scientia Horticulturae 169 (2014) 179–188

Contents lists available at ScienceDirect

Scientia Horticulturae

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

Influence of furostanol glycosides treatments on strawberry (*Fragaria* × *ananassa* Duch.) growth and photosynthetic characteristics under drought condition

Raluca-Petronela Caulet[a], Gica Gradinariu[a], Dorina Iurea[b], Aliona Morariu[a,*]

[a] *University of Agricultural Sciences and Veterinary Medicine Iaşi, Romania*
[b] *Biological Research Institute, Iaşi, Romania*

ARTICLE INFO

Article history:
Received 24 July 2013
Received in revised form 22 February 2014
Accepted 24 February 2014
Available online 16 March 2014

Keywords:
Furostanol glycosides
Strawberry
Drought
Photosynthesis
Chlorophyll fluorescence

ABSTRACT

Water availability is one of the major limiting factors for plant growth and productivity. In recent years, efforts to search for specific and practical approaches to improve the adaptability of plants to this environmental condition have been made. In this study we have examined the influence of two furostanol glycoside application on the capacity for acclimation to drought of two strawberry (*Fragaria* × *ananassa* Duch.) cultivars, Real and Magic, grown in greenhouse conditions. Two regimes of irrigation were used: well-watered (80% WHC) and drought stress (50% WHC) for one month. Two furostanol glycoside aqueous solutions (G1 and G2) were applied by foliar spraying during late afternoon at 3 days intervals on 5 mature plants for each variant. Changes in plant growth and photosynthesis (gas exchange and chlorophyll fluorescence measurements) were studied.

In drought conditions, glycoside application induced a decrease in leaf size and increase in roots length, resulting in significant increases of root/shoot ratio. The two glycoside treatments alleviated the effect of drought and improved photosynthetic rate (A) and water use efficiency (A/E) in both cultivars. In addition, G1 treatment reduced the severity of drought effect by increasing the stomata responsiveness thus maintaining a higher relative water content (RWC), while G2 treatment led to a lower decrease of stomatal conductance (g_s) and transpiration rate (E) and a significant decrease in internal CO_2 (C_i) concentration compared to untreated plants subjected to stress. The chlorophyll fluorescence measurements indicate that furostanol glycoside treatments mitigated the reduction of photosystem II efficiency (ΦPS II) and electron transport rate (ETR) caused by water stress, compared to untreated plants. Moreover, under drought stress, treated plants recorded higher values of non-photochemical processes (NPQ) than untreated ones. One can safely assume that glycoside treatments induced an enhancement of non-photochemical processes as well as an alleviation of the drought-induced alteration in PS II by increasing the efficiency of light utilization and dissipation of excitation energy in the PSII antennae.

1. Introduction

Drought is one of the most commonly limiting environmental factors affecting plant growth and productivity. In response to water deficit, plants developed drought avoidance and drought tolerance strategies (Chaves et al., 2003). Morphological adaptations in response to water stress involve reduction of leaf area for minimization of water loss (Singer et al., 2003) and increase of investments in the root system (Jackson et al., 2000). The latter occurs as a consequence of altering assimilates partitioning by directing a supplementary amount of phytoassimilants to the roots for increasing water and nutrient uptake (Starck et al., 1995).

The strawberry is a plant with a large demand for water, due to a shallow root system, large leaf area and fruits with a high content of water. It is well known that plant roots perform many essential functions, including water and nutrient uptake, storage of reserves, synthesis of specific compounds, etc. (López-Bucio et al., 2003). The size and the architecture of the root system determine

Abbreviations: WHC, water holding capacity; G1, glycoside 1; G2, glycoside 2; A, photosynthetic rate; E, transpiration rate; g_s, stomatal conductance; WUE = (A/E), water use efficiency; C_i, internal CO_2; RWC, relative water content; PSI, photosystem I; PSII, photosystem II; F_v/F_m, maximal quantum yield of PSII efficiency; ΦPSII, quantum efficiency of PSII; ETR, electron transport rate; NPQ, non-photochemical process; PAR, photosynthetic active radiation; RuBP, ribulose-biphosphate; ROS, reactive oxygen species.

* Corresponding author. Tel.: +40 232407370.
E-mail address: alionamorariuu@yahoo.co.uk (A. Morariu).

http://dx.doi.org/10.1016/j.scienta.2014.02.031
0304-4238/

the plant's ability to access water and nutrients, factors that limit growth and, thus, yield in many agricultural ecosystems (Lynch, 1995; Price et al., 1997). Therefore, an enhancement of processes developed in roots is one of the most important agronomic traits, having as final goal the decrease of costs related to irrigation and fertilization, increasing in this way economic efficiency and crops profitability.

During the last decade, treatments with biostimulants based on humic acids (Neri et al., 2002) or chitosan (Górnik et al., 2008), have been found to be efficient in strawberry and grapevine roots' growth stimulation, both in well watered and drought conditions. Positive results in alleviation of drought effects have been obtained at pot-grown strawberry plants treated with ABA (Jensen et al., 2009) and paclobutrazol (Navarro et al., 2007), both of them acting as antitranspirants, promoting stomatal closure and inducing the activation of drought avoidance mechanisms. Moreover, another class of compounds has been proved to enhance drought tolerance mechanisms by improving osmoregulation. For example, Ambiol (a synthetic antioxidant compound) had a significant positive effect in alleviating drought symptoms by manitol accumulation (Roussos et al., 2010). Similar results have been previously obtained in plants treated with seaweed extracts (Blunden et al., 1997).

Plants physiological responses to water deficit are related to holding a positive turgor pressure of the mesophyll cells by control of stomatal aperture in order to decrease transpiration rate (Bosabalidis and Kofidis, 2002), or by osmotic adjustment that sustained turgor maintenance through accumulation of osmolyte (glycinebetaine, sugars, sugar alcohols, proline, manitol, etc.) (Serraj and Sinclair, 2002), as well as through reduction of tissue elasticity (Savé et al., 1993) that allows a higher degree of stomatal aperture.

In drought conditions, the decrease of the stomatal conductance acts as a limitative factor for photosynthesis by limiting CO_2 diffusion in the mesophyll (Cornic and Massacci, 1996). A low level of internal CO_2 (C_i) leads to inhibition of Rubisco or ATP synthesis (Lawlor and Cornic, 2002) and slows down the oxidation of NADPH, disturbing electron transport between PS II and PS I, which may cause photoinhibition at PS II reaction centres (Flexas and Medrano, 2002), therefore light energy is in excess and can produce damages to the structures of the photosynthesis apparatus. Plants adapt to this situation by developing a system through which they can dissipate this energy through the xanthophyll cycle (Demmig-Adams and Adams, 1996). Therefore, several studies have been focused on maintaining the efficiency of the specific light use in water stress conditions. Good results in reducing of the drought effect, by increasing excitation energy dissipation as heat, have been reported by Hu et al., 2013 in an experiment with brassinosteroids treatments in pepper. The encouraging effects obtained until now promote further research in the domain of discovering new classes of compounds with adaptive effect to stress conditions especially in the field of ecological agriculture.

Furostanol glycosides are a large group of steroid compounds, of plant origin, with exhibited anti-fungal (Liu et al., 2003) and nematicidal activities (Vasil'eva et al., 2005). It has been reported that furostanol glycosides extracted from *Dioscorea deltoidea* induce activation of nonspecific defensive responses as enhancement of xanthophyll pigments biosynthesis and increases of antioxidant enzyme activity, which improved the resistance of plants to biotic stress (Vasil'eva et al., 2005). Furostanol glycosides extracted from seeds of *Lycopersicon* sp. and *Capsicum* sp., had stimulative effect on vegetative growth and moreover, have been shown to increase the photosynthetic pigment content of grapevine and apple trees (Munteanu et al., 2008). Our preliminary experiments show that furostanol glycosides extracted from *Lycopersicon* sp. and *Digitalis* sp. stimulated root system development and influenced photosynthetic processes and increased water use efficiency, fact that suggests the activation of some adaptive mechanisms towards water deficit conditions (Caulet et al., 2013).

Therefore, the present study aims at evaluating the influence of treatments with furostanol glycosides on strawberries' growth and development in water stress conditions. Changes in photosynthesis were studied both in terms of CO_2 assimilation rate as well as functionality of the photosynthetic apparatus, as assessed by *chlorophyll a* fluorescence measurements.

2. Materials and methods

2.1. Plant material and experimental design

The experiment was conducted at the Faculty of Horticulture of Iasi, Romania, during November 2012–March 2013. Two strawberry cultivars (*Fragaria* × *ananassa* Duch.), Real and Magic were used in this experiment. In order to have the most uniform biological material we decided to use young daughter plants (runners) with 2 leaves and 5–7 roots (initial plant biomass was 2.10–2.54 g). Plants were grown in greenhouse conditions, in 6 L plastic pots filled with a mixture of soil:sand:litter in (1:1:1, v/v/v). Photosynthetic active radiation (PAR) was 400–500 μmol $m^2\ s^{-1}$, relative humidity 52%, temperature 22–23 °C and CO_2 concentration 380 μmol mol^{-1}.

After two weeks of adaptation to greenhouse conditions, plants were divided into two groups, well-watered and drought-stressed. Well-watered plants were maintained at 80% WHC (Water holding capacity) while drought stress was imposed by withholding water from plants, until soil humidity reached 50% WHC; these conditions have been maintained for 4 weeks. Each pot was weighed daily to maintain the desired water levels in soil by adding appropriate volumes of water.

2.2. Preparation of furostanol glycoside and treatment

The two furostanol glycosides, provided by the Biological Research Institute from Iasi, were extracted and purified from seeds of *Lycopersicon* sp. – G1 (*M* = 1082) and leaves of *Digitalis* sp. – G2 (*M* = 1230). The dry plant material was crushed and extracted with 60% EtOH (70–80 °C). After concentration under reduced pressure, the ethanol extract was partitioned between water and n-butanol. Chromatographic separations of the organic phase on Sephadex G-50 and silica gel L 540 M gave G1 and G2 compounds respectively that were detected with Ehrlich reagent.

The G1 and G2 compounds were eluted with 70% EtOH and were further subjected to resin"Dowex-8" and "Amberlite IR-120B to neutralize the acid solution and then evaporated to dryness to give crude glycoside. Determination of structures of the isolated constituents was performed using spectral analysis of IR, ^{1}H and ^{13}C NMR spectra including 2D NMR spectroscopic techniques (COSY, HETCOR and COLOC).

Foliar treatments with furostanol glycosides were applied with aqueous solutions in concentration of 0.03 mM, which have already been proved to be the optimum for these plants in our preliminary experiments. Treatments were applied on mature plants in late afternoon, with 3 d intervals, using a hand-held sprayer. In order to avoid interferences with different moisture levels, "untreated" plants were sprayed with distilled water. 10 plants have been used for each variant.

2.3. Growth parameters

At the end of the experimental period, newly developed leaves, leaf length, total leaf area, root number/plant and their length, were determined and measured.