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Phytochemical and physiological changes in the leaves of St. John's wort plants under a water stress condition

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Abstract

Water stress is known to increase the secondary metabolites concentration in plant tissues and severe water stress conditions may cause oxidative stress due to the formation of reactive oxygen species and photoinhibitory damage. Current study was undertaken to evaluate the changes in the physiological status especially the photosynthetic efficiency and the biochemical profile of the leaf tissues of St. John's wort (*Hypericum perforatum*) plants exposed to water stress. The net photosynthetic rates of the leaves of plants grown under a water stress condition were significantly low compared with those of the control. The maximal quantum efficiency of PSII photochemistry (ϕ_p^{max}) of the dark adopted leaves was similar for both wilted and recovered plants although these values were significantly low compared with those of the control. The leaf tissues of the plants under a water stress condition had a significantly higher capacity to detoxify oxygen radicals with an about 2.5-fold increase over the antioxidant potential of the leaves of non-treated (control) or recovered plants. In the leaf tissues of plants grown under a water stress condition, both hypericin and pseudohypericin concentrations reduced with time and on Day 12 of the treatment, the concentration was significantly lower than that of the control; in contrast, the hyperforin concentration increased significantly and the value was nearly double after 12 days of the treatment. Under a water stress condition, the hyperforin concentration was about 70-fold higher than the total hypericins concentration in the leaf tissues; in case of control it was only 10–12 times higher. The results also indicate that the major secondary metabolite, hyperforin concentration of 62-day-old plants was three- to four-fold higher than the previously reported values for 1–2-year-old field-grown plants.

Keywords: Controlled environment; Hypericin; Hyperforin; Photosynthetic efficiency; Pseudohypericin; Secondary metabolite; St. John's wort; Water stress

1. Introduction

Water stress is one of the most important environmental stresses that can regulate plant growth and development, limit plant production, alter the physiological and biochemical properties of plants. Water stress is known to increase the amount of secondary metabolites in plants, for example, artemisinin in *Artemisia* (Charles et al., 1993) and betulinic acid, quercetin and rutin in *Hypericum brasiliense* (de Abreu and Mazzafera, 2005). Accumulation of secondary metabolites is known as a defense mechanism of plants and plants can respond and adapt the water stress by altering their cellular metabolism to invoke various defence mechanisms (Gulen and Eris, 2004). The survival of plants under such a stressful condition depends on the plant's ability to perceive the stimulus, generate and transmit the signals, and to initiate various physiological and chemical changes (Bohnert and Jensen, 1996; Shinozaki and Yamaguchi-Shinozaki, 1997). Severe water stress conditions may cause oxidative stress due to the formation of reactive oxygen species and photoinhibitory damage (Asada, 1996). In the chloroplasts of the plant cells, protection against oxidative damage is provided by both enzymatic and non-enzymatic antioxidants (Asada, 1999) and thus the antioxidant concentrations may therefore increase under a water stress condition in plants (Eskling et al., 1997) which may also be able to increase the secondary metabolite concentrations in plant tissues.

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The perennial herb St. John's wort (*Hypericum perforatum*) has been used for decades to treat depressive disorders and biochemical, behavioural, pharmacological, as well as clinical data clearly indicate the antidepressant profile of St. John's wort extracts (Alan and Miller, 1998; Müller, 2003). The St. John's wort extract contains a large number of secondary metabolites including hypericin, pseudohypericin and hyperforin.

Current study was attempted to evaluate the changes in the physiological status especially the photosynthetic efficiency and the biochemical profile of the leaf tissues of St. John's wort plants exposed to water stress. The hypothesis of the current study was that water stress can alter the secondary metabolite concentrations in St. John's wort. The objective of the current study was to increase the secondary metabolite concentrations in St. John's wort and correlate its alteration with the changes in the physiological process of plants under stress conditions. Plants were grown under controlled environments with artificial light and CO_2 enrichment and 50-day-old plants were subjected for 12 days to water stress before harvested.

2. Materials and methods

2.1. Plant materials experimental design and treatments

St. John's wort (*H. perforatum*, cv. 'Topas') seeds were obtained from Murakami Seed Co. Ltd. (Ibaraki, Japan). The seeds were washed and placed on top of moist potting soil in a multicell tray covered with plastic wrap. Germination was conducted over a 10-day period at 27 °C with a 16-h photoperiod, a light intensity or photosynthetic photon flux (PPF) of about 100–120 $\mu mol\ m^{-2}\ s^{-1}$, and periodic watering with tap water (pH 6.0). Following germination, plants (about 1.0 cm high) were transferred to pots (9 cm in diameter, one plant per pot) containing 200 g of soil mixture (Yanmar Agricultural Equipment Co. Ltd., Osaka, Japan) mixed with 20 g of fertilizer (MAGAMP, N:P:K, 6:6:6, Hyponex Corp. Ltd., Osaka, Japan). The plants were watered (150 ml per plant) every other day with tap water (pH 5.9) and grown in a controlled environment growth room at 27/24 °C with a 16 h photo and 8 h dark conditions. The maximum PPF at the leaf level was about 250 $\mu mol\ m^{-2}\ s^{-1}$, the relative humidity was 60% and CO_2 concentration was 1000 $\mu mol\ mol^{-1}$. On Day 50 of transplanting, two groups of 30 plants were randomly selected and assigned to the (a) water stress condition and (b) control (untreated) under the similar environmental conditions as described above. For water stress condition, plants were irrigated every 3 days (50 ml per plant) and for the control, everyday (150 ml per plant). The choice of 50 ml of water to create a water stress condition was made after testing plants over a range of amount of irrigated water (25, 50, 100 and 150 ml per plant). The optimum amount for water stress was 50 ml per plant; below this there was a severe stress condition and plants did not survive, while above it there were insignificant alterations of morphological, biochemical and physiological properties compared with that of the control.

Plants were harvested every 3 days of the treatment to assess the hypericin, pseudohypericin and hyperforin concentrations of the leaf tissues. Antioxidant potential was measured after 12 days treatment of water stress. Net photosynthetic rate and chlorophyll fluorescence were also measured.

In a separate experiment, effects of water stress conditions on the photosynthetic efficiency and biochemical profiles of the leaf tissues of 50-day-old St. John's wort plants were further evaluated. Net photosynthetic rates, photosynthetic efficiency, secondary metabolite concentrations (hypericin, pseudohypericin and hyperforin) and antioxidant potential of the leaf tissues were measured every 24 h from Day 9 until Day 12 of the treatment. The water potential of the leaves were also measured every 24 h.

2.2. Measurement of net photosynthetic rates, quantum yield and water potential (Ψ) of the leaf tissues

Net photosynthetic rates (P_n) per leaf area of the fifth uppermost, fully expanded leaf of the plant were measured every 3 days using a portable photosynthesis system (LICOR-6400®, LI-COR Inc., USA) with 6400-15 Arabidopsis chamber. For water stress treatments, net photosynthetic rates were measured before irrigation as well as after 3–4 h of irrigation (recovered). Fiber-optic based chlorophyll fluorimeter (WALZ, Heinz Walz GmbH, Effeltrich, Germany) was used to analyze the photochemical activity of the leaves on Day 12. In dark adapted samples (2 h), the maximal quantum yield of photochemistry through PSII (ϕ_p^{max}) was calculated from $(F_M - F_O)/F_M$ ratio (Kitajima and Butler, 1975). The actual quantum yield (ϕ_p) of PSII photochemistry in light adapted leaves was calculated from the steady-state level of chlorophyll fluorescence (F_s) and maximal florescence level: $\phi_p = (F_M - F_s)/F_M$ (Havaux et al., 1991).

Water potential of the whole leaves was measured early morning (just before ending the dark period) by using a pressure chamber (Tru Psi, Model WP3, USA).

2.3. Determination of hypericin, pseudohypericin, hyperforin concentrations and antioxidant potential

Fifth uppermost, fully expanded leaf with total fresh mass of about 100 mg were collected, frozen immediately in liquid N_2 and stored at −80 °C for the analyses of hypericin, pseudohypericin and hyperforin concentrations. The extraction, isolation and chemical analysis methods for the metabolites were as described by Zobayed et al. (in press).

Antioxidant potential of the fifth or sixth uppermost leaf was determined by modifying the method developed by