Plant Science 175 (2008) 547–555

Contents lists available at ScienceDirect

Plant Science

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

Effects of chilling temperatures and short photoperiod on PSII function, sugar concentrations and xylem sap ABA concentrations in two *Hydrangea* species

Majken Pagter [a,*], Fulai Liu [b], Christian R. Jensen [b], Karen K. Petersen [a]

[a] Department of Horticulture, Faculty of Agricultural Sciences, University of Aarhus, Kirstinebjergvej 10, DK-5792 Aarslev, Denmark
[b] Department of Agricultural Sciences, Faculty of Life Sciences, University of Copenhagen, Hoejbakkegaard Allé 9, DK-2630 Taastrup, Denmark

ARTICLE INFO

Article history:
Received 10 March 2008
Received in revised form 9 June 2008
Accepted 16 June 2008
Available online 26 June 2008

Keywords:
Abscisic acid
Chlorophyll fluorescence
Hexose
Freezing tolerance
Performance index
Sucrose

ABSTRACT

Cold injuries are frequently seen in *Hydrangea macrophylla* but not in *Hydrangea paniculata*. This may be ascribed to different levels of hardiness in the non-acclimated and the acclimated state, and to differences in responses to short day (SD) and low temperature (LT) and hence in the ability to cold acclimate. In this study *H. macrophylla* ssp. *macrophylla* (Thunb.) Ser. 'Blaumeise' and *H. paniculata* Sieb. 'Kyushu' were exposed to short photoperiod (10-h) and 4 °C in controlled conditions for 25 days, with measurements and samplings carried out at regular intervals. Chlorophyll fluorescence measurements revealed significant alterations in O–J–I–P fluorescence kinetics and decreases in the photochemical efficiency of photosystem II in stressed plants, followed by less chlorophyll contents. Perturbations of the photosynthetic apparatus were relatively greater in *H. macrophylla* than in *H. paniculata*. Likewise, induction of a transient increase in xylem sap abscisic acid concentrations ($[ABA]_{xylem}$) and accumulation of soluble sugars in leaves and stems were different in the two species. Stem cold hardiness in the non-acclimated state did not differ between *H. macrophylla* and *H. paniculata*, indicating equal sensitivity to sudden temperature drops in the growing season. Despite adaptive responses induced by the treatment neither species developed increased stem cold hardiness, suggesting that cold acclimation in *Hydrangea* may require exposure to temperatures below ca. 4 °C.

1. Introduction

Two major components of successful performance of woody perennials in temperate climates are hardiness in the non-acclimated state ('constitutive' level of hardiness) and capacity to cold acclimate. Cold acclimation is induced by shortening photoperiod and declining temperature, and involves several physiological and biochemical changes, whereby plants become increasingly tolerant to subsequent freezing temperatures [1,2]. The level of hardiness in the non-acclimated state is essential in the autumn and early spring, when frost incidents may occur while plants are in a growth stage. Cold acclimation is essential to successfully withstand the first fall frost episodes. Although chilling temperatures (~12–0 °C) are implicated in induction of cold acclimation their occurrence are not strictly associated with the annual cycle of cold hardiness, but may occur throughout most of the year.

Cold hardiness in the non-acclimated state and responses to SD and LT have been shown to vary among species, ecotypes and sexes indicating genetic variation for constitutive cold hardiness and cold acclimation ability [3–7]. Adaptive responses to SD and LT include alterations in tissue concentrations of abscisic acid (ABA), induced losses of photochemical efficiency and altered carbohydrate metabolism. ABA is a well-known stress-inducible phytohormone and growth inhibitor, which increases in chilling sensitive and chilling tolerant annual plants [8,9] and in overwintering woody plants [5,10] exposed to SD and/or LT conditions, indicating an important role for ABA in the acquisition of chilling tolerance and/or cold acclimation.

Stress-induced perturbations in the photosynthetic apparatus measured during LT conditions by means of chlorophyll (Chl) *a* fluorescence has frequently been reported, also as a rapid screening method to asses chilling tolerance [11–13]. Alterations in Chl *a* fluorescence have mainly been studied in evergreen woody plants and herbaceous winter annuals, but the maximum quantum efficiency of photosystem II (F_v/F_m) has also been shown to decrease in deciduous leaves of woody perennials subjected to environmentally controlled chilling temperatures [14]. Most studies have used F_v/F_m, with a theoretical value of 0.83 in non-stressed plants, to monitor the physiological status of the photosynthetic apparatus, but F_v/F_m is just one of a number of parameters which can be derived from the Chl *a* fluorescence transient. Based on the fast rise in O–J–I–P fluorescence Strasser

* Corresponding author. Tel.: +45 89993388; fax: +45 89993496.
E-mail address: majken.pagter@agrsci.dk (M. Pagter).

0168-9452/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.plantsci.2008.06.006

and Strasser [15] developed a so-called JIP test, which calculates a range of different parameters related to energy and electron fluxes in photosystem II (PSII), including the performance index (PI_{ABS}).

Exposure of plants to LT often results in accumulation of carbohydrates, as the photosynthetic energy capture is reduced to a lesser degree than the metabolic utilization processes [16]. Soluble sugars accumulating as a response to LT can play multiple roles. They can act as an immediate energy source upon stress removal and as osmoprotectants stabilizing cellular membranes and maintaining turgor [2,17]. In cold acclimating plants sucrose, glucose and fructose are probably the most studied soluble carbohydrates in the early course of cold hardening, whereas the raffinose family of oligosaccharides (raffinose and stachyose) has been associated with later, season-long freezing tolerance in several woody plants [18–20].

Hydrangea's are very popular ornamentals, widely used and commercially important in landscape gardening. Especially the species *H. macrophylla* ssp. *macrophylla* (Thunb.) Ser. is popular due to its attractive flower heads. In Denmark and other countries of the temperate zone it is a common problem that current year shoots of *H. macrophylla* are frost killed or injured during winter. As flower buds of most *H. macrophylla* varieties are formed during the fall and over-winter on dormant stems, flowering will only occur the following year if terminal and/or lateral flower buds are present and undamaged. Cold injuries may be ascribed to early fall frost, low minimum temperatures mid-winter or frost episodes in late winter and spring, since *H. macrophylla* is considered to acclimate late in fall and de-acclimate early in spring and in a number of cultivars maximum stem hardiness is limited to −18 °C–(−24 °C) [21]. *H. paniculata* Sieb. is hardly ever cold injured in Denmark and its maximum cold hardiness is considerably greater than *H. macrophylla*'s. Freezing tolerance of well-hardened stems of *Hydrangea paniculata* 'Grandiflora' clones of different origin, determined as the temperature representing 50% injury (LT_{50}), is −36 to −37 °C [22].

In addition to greater mid-winter hardiness, we hypothesized that the frequent cold injuries encountered in *H. macrophylla* but not in *H. paniculata* may be ascribed to different levels of cold hardiness in the non-acclimated state and more pronounced physiological adaptations to SD and LT. In *H. paniculata* we expected greater alterations in ABA and faster and/or greater accumulation of soluble carbohydrates and other protective substances, and in *H. macrophylla* we expected greater alternations in Chl *a* fluorescence of deciduous leaves in response to SD and LT. Hence, the present study was conducted to (1) determine the constitutive level of cold hardiness in *H. macrophylla* and *H. paniculata* and to identify physiological responses of the two species to a period of short days and low, non-freezing temperatures, and (2) determine whether differences in adaptive responses to SD and LT conditions may be related to differences in the capacity to cold acclimate in *H. paniculata* compared with *H. macrophylla*.

2. Materials and methods

2.1. Plant material and treatments

The experiment was carried out using 2-year old vegetatively propagated commercially produced *H. macrophylla* ssp. *macrophylla* (Thunb.) Ser. 'Blaumeise' and *H. paniculata* Sieb. 'Kyushu' plants grown in 3.5-L pots containing sphagnum peat. Prior to the experiment, in the beginning of April, plants were forced in a greenhouse at 24 ± 2 °C day and night with venting at 26 °C and a light:dark cycle of 18 h:6 h. Supplementary light was turned on when the photosynthetic photon flux density (PPF) was less than 70 μmol m^2 s^{-1} and switched off when the radiation exceeded 90 μmol m^2 s^{-1}. When the radiation reached 1600 μmol m^2 s^{-1} curtains were drawn. Plants were ebb and flood irrigated daily with a standard nutrient solution prepared from tap water containing (in mM): 12.2N–0.5P–4.3K–4.0Ca–0.7Mg–1.7SO_4, at EC 1.96 pH 6.0. Micronutrients were added making up 0.1‰ of the nutrient solution.

From 8 June to 15 June batches of plants of uniform size grown in the greenhouse were pre-acclimated in growth chambers at 20 ± 2 °C day and night, 18-h photoperiod and a PPF of 50–150 μmol m^{-2} s^{-1}. In growth chambers plants were drip-irrigated with a standard nutrient solution (Pioner NPK Macro 14-3-24, Brøste, Denmark) prepared from tap water containing (in mM): 10.3N–0.9P–5.9K–2.5Ca–2.2Mg–2.0SO_4 at EC 1.5, pH 5.5. Micronutrients (Pioner Micro, Brøste, Denmark) were added making up 0.125‰ of the nutrient solution. At onset of the experiment, two treatments were applied. One group of plants was exposed to LT (4 °C day:night) and SD (10-h), and the other group of plants was subjected to control temperatures (20 °C day:night) and a long day (18-h) and served as controls. To avoid shock reactions the temperature and the photoperiod in the SD and LT treatment were gradually lowered in the course of 3 days. The experiment lasted 25 days, where day 0 is defined as the day when the temperature and the photoperiod in the SD and LT treatment started to be lowered. Consequently, days 0–3 was the time period where the temperature and photoperiod in the SD and LT treatment were gradually lowered to the predetermined treatment levels. Non-destructive measurements, including shoot elongation growth, chlorophyll fluorescence and chlorophyll content, were performed on days 0, 3, 7, 10, 14, 16 and 21. Destructive sampling for determination of $[ABA]_{xylem}$ took place on days 1, 4, 8, 11, 18 and 22, while harvesting of leaves and stems for determination of soluble carbohydrates only took place on days 1, 11 and 22. Stem freezing tolerance was determined approximately once every week at days-2, 5, 12, 18 and 25. At each time (treatment duration) separately elongation growth was measured on five replicates per treatment and per species. All other measurements and sample collections included five and three replicates per species of stressed and control plants, respectively.

2.2. Shoot elongation growth

Shoot elongation growth was determined with a ruler measuring the distance between the shoot tip and a reference mark made between the tip and the first node.

2.3. Chlorophyll fluorescence and chlorophyll content

Chlorophyll fluorescence measurements were made on three of the third youngest fully expanded leaves per replicate. Fluorescence emissions were measured using a portable chlorophyll fluorometer (Handy-PEA, Hansatech Instruments, King's Lynn, Norfolk, UK). The chlorophyll fluorometer emits light of 650 nm wavelength with an intensity of 2500 μmol m^{-2} s^{-1} for 10 s. Measurements were carried out on plants dark adapted for 30 min to ensure an initial zero photochemical activity and CO_2 fixation state. During light illumination the Chl *a* fluorescence intensity in dark-adapted leaves rises rapidly from an initial minimal level, F_0 (the O step), to the maximal level, F_m (P step). Two intermediary steps designated as J and I usually appear at ca. 2 ms and 30 ms, respectively, hence the notation O–J–I–P for the fast rise of the fluorescence transient. The O–J–I–P transients obtained by the BIOLYZER program [23] were visually examined for the effects of treatment and time and the performance index (PI_{ABS}) was calculated using the JIP test. The PI_{ABS} is a multiparametric expression combining the three main functional steps of photosynthetic activity by a PSII reaction centre complex, i.e. light energy