J For Res (2014) 19:301–304
DOI 10.1007/s10310-013-0410-5

SHORT COMMUNICATION

Participation of ascorbic acid in the dormancy establishment of poplar lateral branch buds

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Received: 12 September 2012 / Accepted: 19 April 2013 / Published online: 6 July 2013

Abstract This work studied the participation of ascorbic acid (AA) in the dormancy establishment of apical buds of poplar (*Populus deltoides* Bartram ex Marshall cv. Catfish 2) lateral branches. AA content was highest during the active growth period and decreased to the lowest content during bud dormancy. The accumulation of the oxidised form, dehydroascorbate, was similar in either growth or dormant bud stages. The supplementation of buds with AA at the end of the summer delayed bud set. Similarly, the supplementation of buds with gibberellic acid extended the growth period and kept a high AA content in the apoplast. These results suggest that a decrease in the accumulation of reduced AA might be needed for the dormancy establishment in apical buds of poplar lateral branches.

Keywords Ascorbic acid · Bud · Dormancy · Poplar

Introduction

Ascorbic acid (AA) is a simple metabolite found in large amounts in plant tissues (Smirnoff and Wheeler 2000), with a central role in the antioxidant defense (Foyer and Noctor 2011). Reactive oxygen species (ROS) produced by plant cells are generated as the result of aerobic metabolism and the interaction of photosynthetic pigments with light. ROS can oxidise proteins, unsaturated fatty acids, or deoxyribonucleic acid, impairing cell functions. AA reacts with ROS, producing a stable radical (monodehydroascorbate) that is safely detoxified (Davey et al. 2000). In addition, AA participates in a great number of processes during plant development such as division and expansion of cells. Young and active growing tissues containing high levels of AA present several cells in the mitotic phase. Some evidence demonstrates that supplementations with AA accelerate cell replication in root primordia of *Allium* spp., *Pisum* spp., and *Lupinus* spp. (Citterio et al. 1994; De Cabo et al. 1996; Arrigoni et al. 1997), due to a rise in the number of cells passing from the G1 stage to the S phase (Navas and Gomez-Diaz 1995). AA is also involved in tobacco cell proliferation, and it is postulated that an increased accumulation of its oxidized form, dehydroascorbate (DHA), can arrest the cell cycle in the G1 phase (Davey et al. 2000). Tabata et al. (2001) observed that tobacco cell lines with decreased AA content have lower growth rates than wild-type cell lines. In the cell wall, AA is used in processes related to growth and development, as well as in stress responses (Foyer and Noctor 2011).

AA synthesis and accumulation are tightly regulated by different plant hormones such as ethylene or gibberellic acid (GA; Bartoli et al. 2012 and references therein) and by environmental factors like the amount and quality of light (Bartoli et al. 2006, 2009).

Trees have the ability to suspend growth in response to changes in the environment (Hovarth et al. 2003). Dormancy is considered as a non-growing development stage of plant organs with incapacity to restore growth under a favorable ambient (Olsen 2010 and references therein). Progressive photoperiod reduction has been known to

Electronic supplementary material The online version of this article (doi:10.1007/s10310-013-0410-5) contains supplementary material, which is available to authorized users.

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govern growth cessation establishing bud dormancy of many trees in temperate climates (Olsen 2010). Leaves perceive photoperiod and emit a signal to the apex, where inactivity is installed (Rohde and Bhalerao 2007).

The knowledge of the factors influencing the induction and the release of bud dormancy may provide more information for the manipulation of the growing cycle, allowing the potential increment of biomass yield. The aim of this work was to study the participation of AA in the dormancy establishment of the apical buds of lateral poplar branches.

Materials and methods

Plant material

Lateral branches of *Populus deltoides* Bartram ex Marshall cv. Catfish 2 were propagated during the winter and used for the experiments during the following cycle of growth and dormancy. Lateral branches were taken from the "Vivero Darwin", La Plata, Argentina (Agriculture Ministry of the Province of Buenos Aires) (34°50′S, 58°09′W). Plants were grown in 10-L pots containing soil. Each set of plants was used during one growth/dormant period and data were obtained from three independent experiments conducted during three consecutive years. Three buds were taken from different plants at each sample time of the year. Although a small variation of temperature from year to year may affect plant growth, samples were taken when different bud development stages were achieved.

The experiments were placed in the botanical garden of the Institute of Plant Physiology, National University of La Plata (34°54′45″S, 57°55′52″W), receiving plants' chilling requirements to restore growth after the winter.

AA and GA treatments

Three batches of plants consisting of three plants each were used in three independent experiments performed in consecutive years. The apex and the five topmost leaves of elongating branches were sprayed with 10 ml of the following solutions: 50 mM of AA, 50 μM of GA, or water as control. All solutions included 0.5 % (v/v) Tween 20 as a surfactant; this compound does not affect AA contents or bud dormancy progress. The treatments were made in the first week of March (about the end of summer) and the AA contents were determined 14 days after treatments, when control buds stopped growing.

AA determination

Apical buds without scales were used for AA measurements. The contents of reduced AA and its oxidized form, DHA, were determined with a HPLC system as described in Bartoli et al. (2006). The redox state of AA was calculated as: oxidized form % = [(oxidized form)/(reduced + oxidized)$^{-1}$] × 100. AA content was also measured in the extracellular compartment 48 h after AA and GA treatments. Buds were vacuum-infiltrated at −70 kPa with 66 mM K–phosphate buffer (pH 4.5) containing 100 mM KCl and 2.5 mM EDTA (three infiltration periods, 1 min each) (Turcsányi et al. 2000). Apoplast fluids were obtained by centrifugation of buds at 1,500*g* and 4 °C for 1 h. The content and redox state of AA in the apoplast were measured in three independent experiments performed in the same year.

Dry weight was obtained after placing buds at 70 °C for 48 h.

Environmental conditions

Data of photoperiod were taken from the Experimental Station "Julio A. Hirschhorn" located in La Plata, Argentina (34°55′S, 57°57′W) (Asborno and Pardi 2004–2007). The length of the day was estimated at latitude 35°S.

Statistical analysis

Data are presented as the average of three independent experiments and analyzed by means of one-way ANOVA. The means were compared by the LSD test at a significance level of 0.05, 0.01, or 0.001.

Results and discussion

AA content and redox state during the active growth–dormancy cycle of apical buds of poplar lateral branches

Active growth and dormancy periods of apical buds of lateral branches were extended for approximately 200 and 165 days, respectively (Fig. 1). AA decreased to the lowest content during dormancy and reached the highest content during active growth (Fig. 1). In contrast, the amount of DHA did not change during the year (Fig. 1). However, the AA redox state reached about 28 % at dormancy in contrast to 3.7 % reached at active growing stage. Changes in the contents and the redox state of many soluble compounds are key signals between stress perception and physiological responses of plant cells under changing environments (Foyer and Noctor 2011). High AA content and reduced redox state in plant meristems are linked to active cell division and other cell processes connected with an active growth (Tabata et al. 2001). Similarly, low AA concentration and highly oxidised redox state might be important signals leading to growth arrest adapting the plant to resist the cold season.

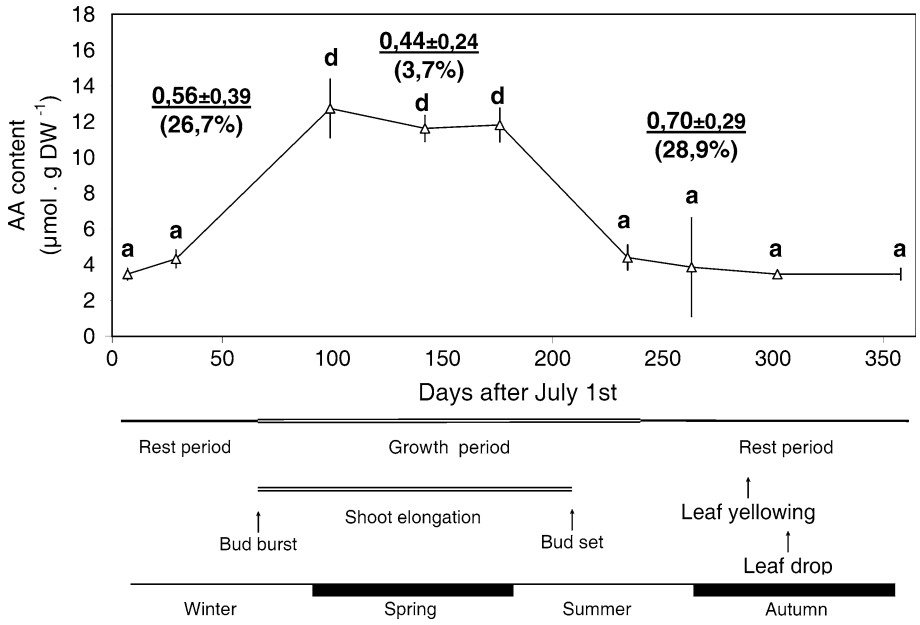

Fig. 1 Reduced ascorbic acid (AA) contents measured in apical buds of lateral branches of poplar trees along the year. The *numbers* indicate the dehydroascorbate content and their correspondent AA redox states between *parentheses* at three times of the growth-dormancy cycle. Data with *same letters* represent a statistically homogenous group (ANOVA, $P < 0.001$)

The photoperiod might be an environmental signal involved in the modification of the AA content during summer. From December to January, there is a decrease of only 9 min; meanwhile, from January to February, there is a reduction of 54 min in the day length reaching a critical photoperiod triggering dormancy. This modification in the length of the day may cause the reduction in the accumulation of AA at the end of the summer (Fig. 1).

Effect of AA and GA treatments on dormancy entrance

GA treated buds were still actively growing and only a small fraction of those buds receiving AA supplementation arrested their growth when all control branches stopped to grow and showed bud set (Table 1; supplementary material 1). The delay in bud set produced by exogenous GA or AA applications suggests a delay of dormancy entrance of treated apical buds (Table 1).

It has been well demonstrated that exogenous application with GA delays the onset of bud dormancy (Olsen 2010). Short days induce a decrease of GA content in poplar tissues (Eriksson and Moritz 2002) and consequently growth cessation. Additionally, it has been demonstrated that GA stimulates AA formation in plants, since mitochondria isolated from GA-treated leaves have increased capacity to synthesize AA (Millar et al. 2003). In the present work, treatments with GA and AA maintained higher AA bud contents than the treatment with water (Table 1). Surprisingly, AA and GA applications increased DHA contents (Table 1) suggesting that AA recovery capacity from oxidised forms (e.g., activities of DHA reductase, glutathione reductase, and other enzymes) is decreased by environmental changes (i.e., photoperiod and/or temperature). These data also indicate that AA recovery from DHA is not controlled by the exogenous GA application but confirm that this hormone controls AA levels, thus affecting its biosynthesis as mentioned above (Millar et al. 2003). The presence and redox state of AA in the apoplast are considered as important signals related to the plant cell growth (Pignocchi et al. 2006). Here, it was observed that AA content in the apoplast was higher in AA- and GA-treated buds compared with water-treated

Table 1 Effect of 50 mM ascorbic acid (*AA*) and 50 µM gibberellic acid (*GA*) treatments on the dormancy entrance and AA and dehydroascorbate (*DHA*) contents of lateral branch buds

Treatment	Bud set (%)	AA content (µmol g^{-1} DW)	DHA content (µmol g^{-1} DW)
Water	100 ± 0	3.6 ± 0.6	0.70 ± 0.17 (16.3)
AA	8.3 ± 1***	5.9 ± 0.4*	1.70 ± 0.17** (22.3)
GA	0 ± 0***	14.1 ± 1.0***	2.7 ± 0.13** (15.9)

Apical buds of each branch with the five leaves above the apex were sprayed with AA or GA by the end of the summer. Determinations of bud set percentages and AA contents were made 2 weeks after chemical applications. AA redox state is shown between parentheses as oxidized form %. More details are described in "Materials and methods"

*, **, and *** denote statistical differences from water-treated samples (ANOVA, $P < 0.05$, 0.01 and 0.001, respectively)

Table 2 Effect of 50 mM ascorbic acid (*AA*) and 50 μM gibberellic acid (*GA*) treatments on the dormancy entrance and AA and dehydroascorbate (*DHA*) contents in the apoplast fluids obtained from apical buds of poplar lateral branches

Treatment	AA (μmol g^{-1} DW)	DHA (μmol g^{-1} DW)
Water	0.006 ± 0.0005	0.129 ± 0.017 (95)
AA	0.201 ± 0.017**	0.802 ± 0.042** (80)
GA	0.041 ± 0.004*	0.404 ± 0.039* (91)

Growing buds of each branch were sprayed with AA or GA and determinations of AA contents were made 48 h after chemical applications. AA redox state is shown between parentheses as oxidized form %. More details are described in "Materials and methods"

* and ** denote statistical differences from water treated samples (ANOVA, $P < 0.05$ and 0.01, respectively)

buds (Table 2). However, redox state was highly oxidized for all treatments. All these results suggest that modifications in AA content, but not in DHA content, are part of the physiological mechanisms involved in the initiation and establishment of bud dormancy. In addition, the manipulation of AA level might be used to extend poplar growth.

Acknowledgments We are grateful to Forestry Engineer Sebastián Galarco from the "Vivero Darwin" of La Plata (Agriculture Ministry of the Province of Buenos Aires) who brought the plant material and to Agronomist Marcelo Asborno who provided the environmental data of the Agricultural Research Station "Julio A. Hirschhorn" (School of Agronomy and Forestry, National University of La Plata). G.G. is a fellowship student and C.G.B. is a researcher of CONICET (Argentina).

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