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# Burst potential characterisation by capacity for nucleotide accumulation in *Rhododendron catawbiense* apical buds

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## Abstract

*Rhododendron catawbiense* cv. Album propagated *in vitro* were transferred *ex vitro* and grown in a greenhouse, under long or short days. Under long days, the rhythmic growth led to an acrotonous development. In contrast, under short days, the upper buds were unable to burst, allowing basitony. In both photoperiodic conditions, the apical buds were sampled at different stages of the experiment. Growth capacities of the isolated buds were estimated by measuring their abilities to increase and diversify their non-adenylic nucleotide pool (NTP) after supplying adenosine as a precursor. Under long days, during the growth pause, the apical buds were able to increase and diversify their NTP pool. Under short days, adenosine was used to produce important quantities of ATP, while NTP pool increase became weaker. Nevertheless, during this long growth pause, apical bud tissues retained capacities to increase their NTP pool until the basal shoots developed.

*Additional key words*: day length, growth pause, morphogenesis, non-adenylic nucleotide pool.

## Introduction

Endogenous rhythmic growth has been observed in many woody plants under temperate, tropical and subtropical climates. Plants are made of growth units whose arrangement gives them a particular shape. In *Rhododendron catawbiense*, an evergreen woody plant, each growth unit is made up of an axis bearing elliptic petiolated alternate leaves (five to seven) gathered in close succession in its distal part (Fustec 1993). When placed under long days, plants show rhythmic growth: active growth periods followed by resting periods that last four to six weeks (Beaujard *et al.* 1991, Fustec 1993).

In young plants of *R. catawbiense* from *in vitro* culture, day length affects the growth pause delay and has a consequence on plant morphogenesis (Beaujard *et al.* 1991, Fustec 1993). Under long days (16 h), growth pauses are followed by the development of the apical bud: plants show a strictly monopodial development. In contrast, under short days (8 h), the vegetative apical buds increase in volume but never burst. The growth pause then lasts several months and leads to the development of basal shoots in about ten weeks (Beaujard *et al.* 1991, Fustec 1993). Long growth pause observed can be compared to a dormancy of the distal buds. *Rhododendron catawbiense* is an interesting model to study mechanisms responsible from short or long growth pauses of the distal buds in relation to morphogenesis.

Different kinds of dormancies have been described in woody plants (Lang *et al.* 1987, Champagnat 1989, Borchert 1991, Crabbé and Barnola 1996, Vince-Prue 1997). For some several deciduous woody species, growth potentialities of the buds have been characterised by a nucleotide test (Lavarenne *et al.* 1986, Barnola *et al.* 1986, Balandier *et al.* 1993, Bonhomme *et al.* 1999). This technique, was performed to study dormancy in Jerusalem artichoke tubers (Gendraud 1977), and has also been used in apple tree embryos (Thévenot *et al.* 1989). In using the nucleotide test to *R. catawbiense* cv. Album apical buds in both short and long growth pauses, we asked whether energetic metabolism could be related to this evergreen woody plant morphogenesis.

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*Abbreviations*: AMP - adenosine monophosphate; ATP - adenosine triphosphate; GTP - guanosine triphosphate; IBA - indole butyric acid; NTP - non-adenylic nucleoside triphosphate.

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## Materials and methods

**Plants:** The *Rhododendron catawbiense* Michaux cv. Album plants were obtained from head-cuttings produced *in vitro* (Labaume *et al.* 1989). In mid-February, 300 explants were prepared and immersed in a buffer solution (10 mM ammonium phosphate, 80 g dm$^{-3}$ sucrose, 5 mg dm$^{-3}$ IBA, pH 6) for 24 h. Then, these explants were placed in a greenhouse (21 ± 2 °C) in rooting trays filled with moist perlite on culture tables. The explants were watered and sprayed with deionised water by mist-system under a plastic tunnel (relative humidity > 80 %). When the first adventitious roots appeared, the young plants received a nutrient solution: (macroelements in mmol dm$^{-3}$) 4 $NO_3$, 2 Cl, 5 Ca, 2.41 P, 2.25 K, 3 Mg, 6 S, 1 Na, 3 $NH_4$, (microelements in µmol dm$^{-3}$) 41.2 B, 0.94 Cu, 21.5 EDTA-Fe, 23.3 Mn, 0.56 Mo, 6.48 Zn, pH 6.5.

At the beginning of May, plants were pricked out in moist perlite in small plastic containers (600 cm$^3$). The substrate was covered with a 0.5 cm thick waterproofed layer (*Mursain*®) which prevented microorganisms development.

**Acclimatization period:** Plants were grown in greenhouse without plastic tunnel under 16-h photoperiod (long day - LD; irradiance 800 µmol m$^{-2}$ s$^{-1}$, natural light was completed during evenings and mornings by low pressure Na vapour lamps). Plants received a complete mineral solution by subirrigation: (macroelements in mmol dm$^{-3}$) 10 $NO_3$, 4.82 P, 8 S, 1 Cl, 4 K, 2 Na, 10 Ca, 6 Mg, 3 $NH_4$, (microelements in µmol dm$^{-3}$) 82.4 B, 1.88 Cu, 43 EDTA-Fe, 43.6 Mn, 1.12 Mo, 12.96 Zn, pH 6.5. Plants were maintained in these conditions until they have formed two growth units (six to eight weeks).

**Photoperiodic treatments:** After culture establishment, 200 plants were selected based on their homogeneous development. In order to modify the growth potential of the distal buds, two treatments were applied:

*1*) LD/N+ (LD and complete nutrient solution during 10 weeks) which allowed a strictly monopodial development (Fig. 1). Apical buds from 24 plants were sampled at the beginning of the growth pause (week 0, morphologically characterised by the appearance of the first protective apical bud scale). Twenty-four apical buds were removed at the beginning of week 2 (increasing apical bud volume) and 24 at the beginning of week 4 (apical bud-burst). Twelve plants were maintained in culture for 10 weeks without removal to control their monopodial development.

*2*) SD/N-/N+ (short days - SD, 8-h photoperiod at the beginning of the growth pause). This treatment was applied to obtain basitonous plants (Fig. 1). Plants did not receive nitrogen compounds during the first six weeks The following nutrient solution was used: (macroelements mmol dm$^{-3}$) 4.82 P, 6.5 S, 3.75 Cl, 3 K, 7.5 Ca, 4.5 Mg, same microelements and subirrigation frequency. After week 6, plants received a complete mineral solution with nitrogen (N+). The apical buds from 24 plants were removed three, six, and ten weeks after the beginning of the SD treatment. At week 22 the remaining basitonous plants used as control were sampled (*n* = 24).

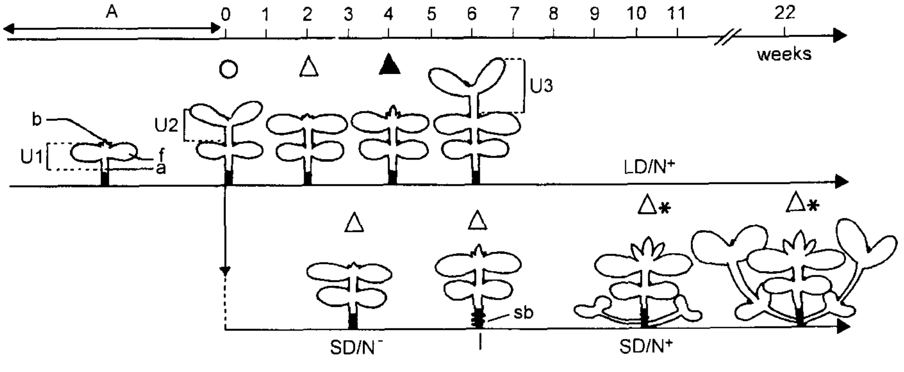

Fig. 1. Morphology of *Rhododendron catawbiense* cv. Album grown under different photoperiodic treatments. A - acclimatization period; *open circle* - appearance of the new apical bud (growth pause beginning); *open triangle* - increase in apical bud volume; *closed triangle* - bud burst; (LD/N+) - long days treatment; (SD/N-/N+) - short days treatment ; U1, U2, U3 - first, second, and third growth units; a - axis; f - alternate leaves at the distal part of the axis; b - bud; sb - swollen basal buds; * - basal shoots development.

**Bud preparation for measurements:** After sampling, the apical part of the bud containing the meristem and the primordia was cut off from the axis part. Half of these explants (meristem and primordia, *n* = 12 for each stage), were placed separately in darkness in a 10 mM adenosine solution for 16 h, in order to increase ATP and NTP pools (Gendraud 1977). The incubating temperature was determined at 12 °C after preliminary tests made on resting buds (Fustec 1993). The remaining explants constituting control samples, were placed separately on distilled water under the same conditions. After incubation, protective bud scales were removed from the explants.

**Nucleotide test:** The biological extracts for nucleotide measurement were prepared for each explant (meristem and primordia) separately according to the method of Keppler *et al.* (1970). Nucleotides were extracted with 0.6 M perchloric acid (0.5 $cm^3$). After centrifugation (2 min at 7 500 *g*), the acid-soluble supernatant was kept for nucleotide measurement and neutralised (solid $KHCO_3$, 10 min). The pellet was rinsed with 95 % alcohol and prepared with 0.5 M NaOH (0.5 $cm^3$ for 15 min at 20 °C). After centrifugation (2 min at 7 500 *g*), the protein extract was measured following Bradford (1976).

Using the luciferin-luciferase technique, the ATP content was determined as reported by Pradet (1967). This method allows indirect measurement of total non-adenylic nucleotides (NTP: GTP, UTP and CTP). ATP content in the extract was measured and then, after incubation with nucleoside diphosphate kinase, the sum (ATP + NTP). The GTP level was calculated after an incubation of the extract with succinyl thiokinase which allowed to measure [(ATP + NTP) - GTP ] (Gendraud 1977, Bon 1988).

For each growth stage: *1*) endogenous nucleotides were measured in explants incubated on water, and *2*) endogenous and produced nucleotides were measured in explants incubated with adenosine. Then, nucleotide accumulation due to the precursor could be calculated.

## Results

**ATP and NTP in acrotonous plants:** Under LD, the buds incubated on water contained ATP and NTP in concentration lower than 18 μmol $g^{-1}$(protein) (Fig. 2*A*). In explants incubated with adenosine, ATP pool did not increase significantly. In contrast, NTP accumulation was important until the second week of the growth pause [to about 40 μmol $g^{-1}$(protein), Fig. 2*B*]. At bud burst, the increased NTP pool dropped to 10 μmol $g^{-1}$(protein).

**ATP and NTP in basitonous plants:** Under SD, the endogenous ATP and NTP concentrations in the apical buds tended to increase slowly (from about 8 to 30 μmol $g^{-1}$(protein), Fig. 3*A*). In explants incubated with adenosine, ATP greatly increased after three weeks to 80 μmol $g^{-1}$(protein) and high content remained at weeks 10 and 22. The NTP pool increased at the beginning of the growth pause and then slightly decreased until the end of the experiment (Fig. 3*B*).

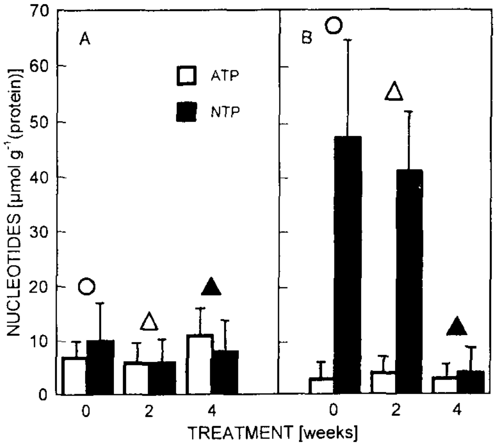

Fig. 2. ATP or NTP contents in the apical buds sampled during growth pause under LD: *A* - endogenous nucleotides, *B* - increase of nucleotide pool after incubation with adenosine; *circle* - appearance of the new apical bud, *open triangle* - bud volume increase, *closed triangle* - bud burst; means ± SD, *n* = 12.

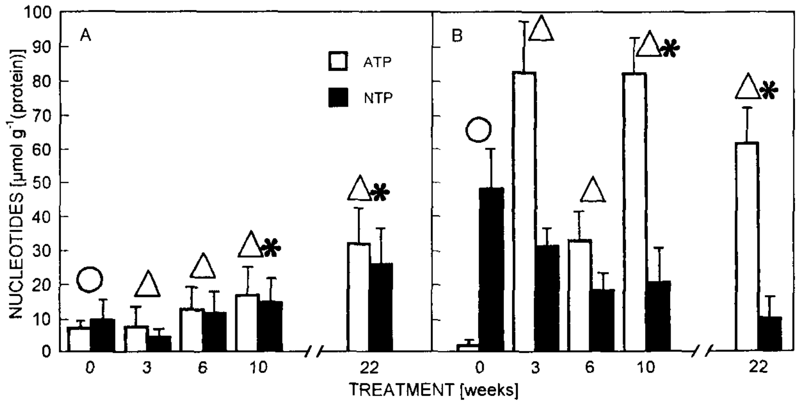

Fig. 3. ATP or NTP contents in the apical buds sampled under SD: *A* - endogenous nucleotides, *B* - increase of nucleotide pool after incubation with adenosine; *circle* - appearance of the new apical bud, *open triangle* - bud volume increase, *closed triangle* - bud burst, * - basal shoot development; means ± SD, *n* = 12.

**GTP proportion in relation to NTP:** Under LD, from the beginning of the growth pause, endogenous NTP were represented by GTP in increasing quantity (from 30 to 57 %, Fig. 4*A*). In the same way, the proportion of GTP in explants placed in an adenosine solution increased at the bud burst (Fig. 4*B*). Under SD, the proportion of endogenous GTP varied widely (Fig. 4*C*). After incubation on water, the values reached 80 % at week 3 and at the end of the culture. The GTP proportion found in explants incubated with adenosine increased markedly until the sixth week (from 18 to 82 %), before reaching lower values at weeks 10 and 22 (15 and 40 %, respectively, Fig. 4*D*).

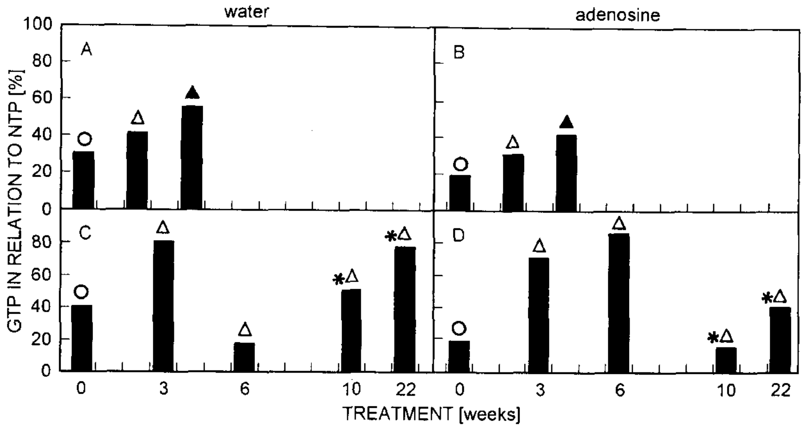

Fig. 4. GTP in relation to NTP in the apical buds: *A* - endogenous GTP under LD, *B* - GTP increase with adenosine under LD, *C* - endogenous GTP under SD, *D* - GTP increase with adenosine under SD; *circle* - appearance of the new apical bud, *open triangle* - bud volume increase, *closed triangle* - bud burst, * - basal shoot development; means ± SD, *n* = 12.

## Discussion

Non-adenylic nucleotides play an important role as high-energy intermediates in the protein (GTP), sugar (UTP) and lipid metabolisms (CTP; Granner *et al.* 1989). In deciduous woody plant buds (Lavarenne *et al.* 1986), in tubers (Gendraud 1977) and in apple tree embryos (Thévenot *et al.* 1989), the diversification of the NTP and an increase of their pool are necessary for shoot growth.

Under LD, our results revealed low quantities of endogenous ATP and NTP and an important accumulation of NTP in buds incubated in an adenosine solution at the growth pause beginning. However, this NTP accumulation dropped markedly close to bud burst. These results differ from those obtained in the non-dormant Jerusalem artichoke tuber (Gendraud 1977) and in oak tree buds (Barnola *et al.* 1986) where an increase in NTP accumulation was observed before burst. In our current study, despite our preliminary tests, the incubating temperature may be responsible for the results. The temperature (12 °C) adequate for Jerusalem artichoke tuber (Gendraud 1977) could be one or two degrees too high for *R. catawbiense* bursting buds to entirely prevent the use of accumulated NTP during incubation. Experiments carried out to study ash tree growth (Lavarenne *et al.* 1986) or peach tree dormancy release (Balandier *et al.* 1993), have encountered the same problem: NTP pool decrease observed at bud burst could express a more intense activity from enzymes implied in elongation, such as invertases (Gendraud 1977). On the other hand, before burst, the same buds contained and produced a more important quantity of GTP in comparison to the other NTP (as indicated in Fig. 4*A*,*B*).

Under SD, the increasing contents of endogenous ATP and NTP might express a slow down in the growth and thus a lower requirement of nucleotides. In contrast with plants grown under LD, adenosine supply led to a significant ATP accumulation. Though the buds were unable to burst, the capacity of NTP accumulation tended to decrease, but did not entirely disappear after 10 weeks, when basal shoots began to develop. Three to four weeks after the transfer to SD, the biochemical response of *Rhododendron* bud can be compared with that of the Jerusalem artichoke dormant tuber. Apical buds of *Rhododendron* grown under SD at 20 °C increased in volume, but never burst. Similarly, in Jerusalem artichoke dormant tuber at 25 °C, radial growth ('boulage') is possible but not elongation (Gendraud 1977). These observations also show analogies with results obtained by Lavarenne *et al.* (1986) in resting buds of ash tree grown under LD at 25 °C.

Under SD, despite the proportions of GTP in relation to NTP (water or adenosine incubation) varied widely, they often reached higher values than those measured under LD. This excess of GTP involves an UTP and CTP deprivation, which could represent an obstacle to shoot growth. Results obtained in Jerusalem artichoke tuber and peach tree buds suggested that a more intense mitochondrial AMP-desaminase activity may lead to a higher production of non-adenylic compounds, in particular GTP (Le Floc'h 1984, Le Floc'h and Faye 1995). Thus, in *Rhododendron*, the high proportion of GTP might be in relation to the high ATP content measured at the beginning of the growth pause under SD.

Under SD, important modifications of nucleotide metabolism occurred in the apical bud of *R. catawbiense* which allow to start organogenesis in the basal buds and then shoot development. These basal axes will compete with the distal buds. However, apical bud tissues retained some ability to increase their non-adenylic nucleotide pool. It would be interesting to verify if fluctuations of GTP percentages could not be related to a persistence of the endogenous rhythm in the apical bud even when shoot growth is not possible.

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