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# Effect of wounding on nucleotide pools in *Bidens pilosa* L.

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## Abstract

Wounding both cotyledons of *Bidens pilosa* (var. *radiatus*) induces the inhibition of hypocotyl growth. The wound signal is transmitted very rapidly from cotyledon to hypocotyl and can be visualized by the change in nucleotide pools. First we have shown that the irradiance of the plant can change the ATP level without plant wounding. Therefore, plants were harvested at the start of the light period. Under these conditions, we have determined in hypocotyl the levels of adenosine triphosphate (ATP), guanosine triphosphate (GTP) and non adenylic triphosphates (NTP), and adenylate energy charge (AEC) after wounding. We have observed a transient (2 min) increase in the ATP level followed by a decrease 5 to 30 min later. A similar result was obtained for the GTP level but with some delay. The GTP level increased in 5 min and then decreased after 60 min. For the NTP level the decrease is effective from 5 to 60 min after wounding. The calculation of AEC has shown that a very tight control in the level of ATP may be involved in response to wounding.

*Additional key words*: ATP, distant response, signal transduction.

## Introduction

In many plant species electrical action potentials and/or variation potentials are generated after various treatment including chemical, electrical and light stimulations (Paszewski *et al.* 1977, Pickard 1973, 1984, Davies *et al.* 1991). Moreover, Ullrich-Eberius *et al.* (1983) showed that electrical potentials depend upon the ATP level and that ATP changes were followed in time by very similar electrical transmembrane potential changes.

In *Bidens pilosa*, Frachisse *et al.* (1985) demonstrated that wounding both cotyledon elicits an action potential followed by a slow wave of depolarization. Later, Julien and Frachisse-Stoiljkovic (1994) showed that $CN^-$ induces a drop of the level of ATP to about 50 % correlated to a depolarization of the membrane. In addition, transduction of external signals is followed by the changes in the

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*Abbreviations*: AdN - adenine nucleotides; AEC - adenylate energy charge; CoA - coenzyme A; FM - fresh mass; GTP - guanosine triphosphate; NTP - non-adenylic triphosphates.

phosphorylation-status of the target proteins. The donor for phosphorylation of the target protein is most often ATP (Budde and Chollet 1988). Very few data are available on the other nucleotide pools but their involvement in the transduction of a wound signal seems to be important since they are implied in the synthesis of nucleic acids.

The adenine nucleotide (AdN) pools could play an essential role in energy metabolism, transport processes and metabolic regulation. In *Bidens pilosa*, they may represent a marker of the wound signal that is transduced from cotyledon to an intact tissue, and that is followed by an inhibition of hypocotyl growth 24 h later (Desbiez *et al.* 1981, Henry-Vian *et al.* 1995a). In the present study, we examined relationship between wounding and nucleotide pools, especially of AdN, GTP and NTP, in the hypocotyl of *Bidens pilosa* to determine the kinetics of the response.

## Materials and methods

**Plant material:** Seeds of *Bidens pilosa* L. (var. *radiatus*) were germinated under controlled environment as described previously (Vian *et al.* 1993). Plants were maintained on a ion-rich culture medium (Desbiez *et al.* 1984) for 5 d (photoperiod 9 h, irradiance of 60 μmol $m^{-2}$ $s^{-1}$, temperature of 21 ± 1 °C) and then they were transferred to deionized water for 24 h. On day 7, hypocotyls were harvested immediately after the light-on (0 h) and 2, 4, 6 or 8 h after the light-on. Plants were wounded immediately after the dark-light transition, and then harvested 2, 5, 30, 60 or 90 min later. Control plants (*i.e.* unwounded) were harvested as the same time.

**Nucleotide extraction and adenylate determination:** Free nucleotides were extracted according to Keppler *et al.* (1970) with some modification. Hypocotyls (200 mg) were ground in a mortar and homogenized with 0.5 $cm^3$ of 0.6 M $HClO_4$. After centrifugation 10 min at 10 000 *g*, the supernatant was neutralized with $KHCO_3$. The precipitate of $KClO_4$ was eliminated by centrifugation 5 min at 10 000 *g*. The supernatant was kept deep-frozen until used. For all the analysis, the reaction mixture contains 0.5 $cm^3$ 20 mM succinate, 0.01 $cm^3$ 20 mM CoA (100 nmol $cm^{-3}$), 0.05 $cm^3$ 0.05 mM ADP and 0.1 $cm^3$ 5 mM glucose and the total volume is adjusted to 1 $cm^3$ by deionized water.

For ATP measurement, 0.025 $cm^3$ of neutralized extract were added to the reaction mixture (assay II). An identical assay was made by adding, to the essay II, 0.05 $cm^3$ of hexokinase (100 μg $cm^{-3}$, 140 U $mg^{-1}$), to determine the background (assay I). To quantify the level of ATP produced, 10 nmol $cm^{-3}$ of exogenous ATP was added to the reaction mixture (assay III). In order to determine the level of all NTP, 0.05 $cm^3$ of succinyl thiokinase (250 μg $cm^{-3}$, 10 U $mg^{-1}$), 0.05 $cm^3$ of nucleotidyldiphosphate kinase (NDPK, 250 μg $cm^{-3}$, 80 U $mg^{-1}$) and 0.05 $cm^3$ of hexokinase were added to the reaction mixture (assay IV). The level of NTP, without GTP, was determined by adding only 0.05 $cm^3$ of hexokinase and 0.05 $cm^3$ of NDPK (assay V). Under these experimental conditions we can obtain the level of ATP alone

(assay II - assay I), GTP alone (assay V - assay IV) and other NTP (assay V - assay I).

The level of ADP and AMP was determined by adding to 0.05 $cm^3$ of the neutral extract, 0.5 $cm^3$ of phosphoenol pyruvate (0.15 mM) and either 0.05 $cm^3$ of pyruvate kinase (25 $\mu g\ cm^{-3}$, 200 U $mg^{-1}$) for ADP measurement or 0.05 $cm^3$ of pyruvate kinase and 0.05 $cm^3$ of myokinase (120 $\mu g\ cm^{-3}$, 360 U $mg^{-1}$) for AMP measurement. The resulting ATP content was determined as described above.

For each measurement, 0.1 $cm^3$ of the reagent Luciferin/luciferase was added to 0.2 $cm^3$ of the reaction mixture and the light emission was measured using a *Lumat LB 9501* (Berthold, Germany).

## Results and discussion

**Alterations of the ATP pool with the duration of the light period:** In an attempt to ascertain whether the duration of the light period could influence the ATP pool, we used hypocotyls of plants harvested immediately after the light-on (0) or 2, 4, 6 or 8 h after the light-on (Fig. 1). We have shown that 2 h of light were sufficient to induce a drop in the ATP level of 30 %. Then, between 4 and 6 h the ATP level remained quite similar and 8 h after the light-on it decreased to around 60 % of the initial level. These variations have been previously described in *Bidens pilosa*. Desbiez (1976) demonstrated that the level of ATP, in 20 d-old-plants, was maximal 2 h after the light-on. Wilson *et al.* (1992) have also shown that in bean, photoperiodic changes occurred in ATP pools with a minimal level in the light phase and a maximal level during the dark phase.

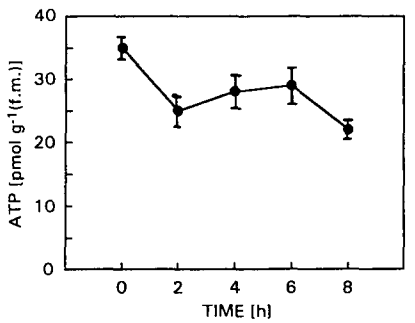


Fig. 1. Effect of irradiance on ATP level in hypocotyls of *Bidens pilosa*, which were harvested at the start of the light-on (0) and 2, 4, 6 and 8 h later.

In *Bidens pilosa*, the high level of ATP observed at the beginning of the light phase could be due to an increase of the chloroplastic and cytosolic ATP that would take place during dark-light transition as described by Hampp *et al.* (1982). Indeed, the level of ATP is increased in 30 s upon illumination and remain high during 5 min (Hampp *et al.* 1982). During the rest of light period, irradiance may decrease the level of ATP by increasing transpiration and decreasing leaf water potential (Turner

and Wellburn 1985). To study the wound effect on the nucleotide pools we have pricked plants immediately after the dark-light transition to be sure that plants have a sufficient pool of energy.

**Effects of wounding on ATP pool:** In order to determine how cotyledons wounding can affect the ATP level at distance we analyzed hypocotyls harvested 2, 5, 30, 60 and 90 min after pricking both cotyledons (Fig. 2A). Wounding leads to a rapid increase in the level of ATP (+20 %) followed by a decrease that was significant after 30 min (-30 %). 60 and 90 min later we could observe a weak increase to around 80 %. These data show that in a short period (2 min) ATP accumulated in the cells at distance from the wounded site. This result could be related in part to an inhibition of the plasmalemma ATPase activity observed 5 min after the treatment and followed by a recovery of its activity in 15 min (data not shown).

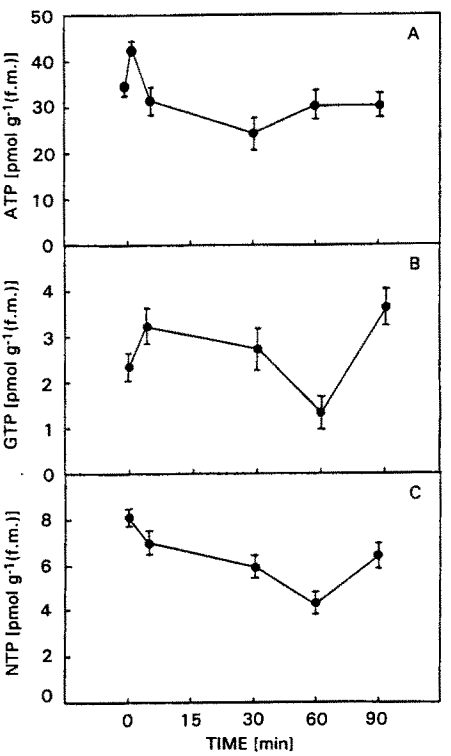


Fig. 2. Effect of cotyledon wounding on ATP (*A*), GTP (*B*) and NTP (*C*) levels in hypocotyls of *Bidens pilosa*, which were harvested 5, 30, 60 and 90 min after wounding. The zero time corresponds to a control hypocotyl (*i.e.* from a non-wounded plant).

The ATP/ADP ratios (Table 1) were calculated for control plants and for plants wounded only for 30 and 60 min since changes observed in the level of ATP were the most significant. Wounding reduced in 30 min the ATP pools, the ATP/ADP

ratios and AEC without markedly reducing total AdN. We suggest that the majority of ATP has been converted to ADP (and possibly to AMP) and this transient mechanism was followed in 60 min by the recovery of a metabolic state similar to that of control plants. Thus a very tight control probably operates to regulate metabolic activities of the cells in response to wounding.

Table 1. The effect of wounding on the adenine nucleotide pools [pmol $g^{-1}$(FM)], ATP/ADP ratios and adenylate energy charge (AEC) [pmol $g^{-1}$(FM)] of hypocotyl of *Bidens pilosa*. AEC is defined as ([ATP]+0.5[ADP])([ATP]+[ADP]+[AMP]). ND - non determine.

| Time [min] | ATP | ADP | AMP | ∑AdN | ATP/ADP | AEC |
|---|---|---|---|---|---|---|
| 0 | 35 | 4 | ND | 39 | 8.7 | 0.94 |
| 30 | 24 | 9 | ND | 33 | 2.7 | 0.86 |
| 60 | 30 | 3 | ND | 33 | 10.0 | 0.96 |

**Effects of wounding on the other nucleotide pools:** We determined the level of GTP in hypocotyls (Fig. 2B) under the same conditions as for ATP. When cotyledons were pricked for 5 min, the level of GTP in hypocotyl increased to about 40 %. Then, it decreased from 30 to 60 min, when the level of GTP reached only of 1.3 pmol $mg^{-1}$. In contrast, a marked increase can be observed 90 min after wounding (+56 %). These changes in the level of GTP could be induced by the activity of GTP-binding proteins, particularly the G-protein that have been identified as part of the signal transduction systems in a wide variety of organisms (Kaufman 1994). In *Bidens pilosa*, the transient increase in GTP pool could be induced by an inhibition of the activity of the G-protein that may occur very rapidly after wounding.

We have also analyzed the level of the other NTP (different from ATP and GTP). The level of the other NTP decreased progressively after wounding reaching 46 % of the initial level after 60 min (Fig. 2C) and after 90 min the level of NTP increased to reach 70 % of the initial level. The drop of the NTP could be related in part to a decrease of UTP as described by Nieman *et al.* (1988) in young leaf of pepper after a salt stress.

To conclude, our results show that in *Bidens pilosa*, the nucleotide pool depends of two parameters, the duration of the light period and the wound treatment. Furthermore, changes in nucleotides levels were observed very rapidly (in 2 min for the ATP level) in the intact tissue suggesting that the wound signal is transmitted very rapidly. These results are consistent with those previously obtained on rapid gene expression (Henry-Vian *et al.* 1995a) or on rapid modifications of *in vitro* protein synthesis (Henry-Vian *et al.* 1995b). It is likely that ATP and the others nucleotides play an important role in these mechanisms.

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