BIOLOGIA PLANTARUM 38 (4): 489-494, 1996

Abscisic acid content in the root hemiparasite *Melampyrum arvense* L. before and after attachment to the host plant

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Abstract

The content of abscisic acid (ABA) in abaxial leaf epidermis of the host (*Capsella bursa pastoris*) and the unattached hemiparasite *Melampyrum arvense* showed diurnal changes. ABA content increased during the light period and declined rapidly upon the darkening of leaves. In an attached hemiparasite the content of ABA in the epidermis was maintained at an almost constant level irrespective of the diurnal cycle. As compared with the maximum level in the host, at the end of the light phase the content of ABA in abaxial epidermis constituted about 70 % and 164 % in the unattached and attached hemiparasite, respectively. No significant changes in ABA content were recorded in adaxial epidermis. In all the samples abaxial/adaxial epidermis ABA content ratio was about 3.6:1 in light phase. In darkness this ratio decreased to about 1.1:1 in the host and the unattached hemiparasite and did not show significant change after attachment. ABA content ratio in mesophyll was 1:0.7:1.5 for the host, the unattached, and attached hemiparasite, respectively. In comparison with the host the concentration of ABA in xylem sap of the hemiparasite constituted about 31 % and 152 % for the unattached and attached *M. arvense*, respectively.

Additional key words: guard cells, xylem sap.

Introduction

In intact plants grown in natural conditions leaf ABA concentration changes during the day partly because of diurnal fluctuations of leaf water status (Henson *et al.* 1982) but also because of diurnal variations in the import and export of ABA to and from the leaves (Brenner *et al.*1986). Besides water and solutes (Musselman 1980, Ehleringer *et al.* 1985) plant parasites also receive a part of hormones (Lechowski and Białczyk 1996) from the host via transpiration stream. It is generally accepted that stomatal behaviour can be modified by ABA originating from mesophyll of leaf or arriving from the root in the xylem stream (Davies and Zhang 1991). Stomata of

Received 7 March 1996, *accepted* 4 April 1996.
Abbreviations: ABA – abscisic acid; CKs – cytokinins; d.m. – dry mass; GCs – guard cells.

the root hemiparasite are insensitive to exogenous application of ABA to a detached shoot (Shah *et al.* 1987). The level of ABA in plant parasite organs is not known.

The present paper describes experiments whose primary aim was to measure the ABA content in the extracts from the abaxial and adaxial epidermal strips, in bulk mesophyll extract, and in xylem sap of the root hemiparasite *Melampyrum arvense* before and after attachment to the host.

Materials and methods

Plants: Seeds of the hemiparasite *Melampyrum arvense* L. and its host *Capsella bursa pastoris* (L.) Med. were collected from nature. Plants were grown in greenhouse similarly as described earlier (Lechowski 1995).

Collection of xylem sap: Xylem sap was collected using a Scholander pressure bomb (see Lechowski and Białczyk 1996 for details). The samples were centrifuged (1000 *g*; 5 min) to remove insolubles. After freeze-drying in liquid nitrogen the residue was extracted with ice-cold 90 % (v/v) aqueous acetone according to Quarrie and Henson (1982). The samples were stored at -20 °C until analysis.

Extraction of leaf samples: Immediately after the leaves were sampled the main veins were removed. Tissues were freeze-dried in liquid nitrogen for 48 h and stored in a desiccator. Freeze-dried leaf materials were powdered and 50 mg of dry mass was placed in silanized glass test tube for extraction. Samples were extracted in 2 cm^3 ice-cold 90 % aqueous acetone. The crude aqueous acetone extract was centrifuged at 1000 *g* for 5 min. The supernatant was collected and stored at -20 °C until determination.

Extraction of epidermal samples: Epidermal samples were collected after peeling leaves. Abaxial and adaxial epidermal strips were collected separately and freeze-dried in liquid nitrogen. A freeze-dried sample of 15 mg was powdered and extracted in 1 cm^3 of 90 % acetone. After centrifugation (1000 *g* for 5 min) the supernatant was collected and stored at -20 °C.

Assay procedure: To test the efficiency of the preparative procedure, DL-[2-^{14}C] ABA (*Amersham*, Searle, Germany; 74 kBq μmol^{-1}) was added to xylem sap, fragments of leaves, and epidermal strips to determine the efficiency of the extraction method at the end of the procedure. ABA analysis was carried out using the modification of techniques described by Quarrie (1978). Samples of the supernatant from the crude aqueous acetone extract were purified by thin-layer chromatography (TLC) using *Merck* silica gel GF_{254} precoated analytical TLC plates. Extracts (0.4 cm^3 for leaves, 0.6 cm^3 for epidermis, 0.8 cm^3 for xylem sap) were loaded onto plates and suspected-ABA elute from two plates was pooled to provide one sample. After methylation with diazomethane the samples were redissolved in 0.1 cm^3 cyclohexane. Amounts of ABA were determined with a gas-liquid chromatography using *Beckman GC–45* equipped with an electron capture detector (model *140 A*,

Analog Technology Corp., USA). The electron capture detector was linear from 10 pg to 100 ng ABA. Samples (1 mm^3) were injected into a glass column (1050 × 4 mm) containing 4 % *SE-30* on 80 - 100 mesh diatomite CO. The glass column was silylated with *Sylon* (*Supelco*, USA) prior to packing, and the supports were silylated with *Silyl 8* (*Pierce Chemical Co.*, USA) after packing. Column, injector and detector temperatures were 215 °C, 235 °C and 280 °C, respectively. Oxygen-free nitrogen was the carrier gas at a flow rate of 40 cm^3 min^{-1} and detector purge rate was 20 cm^3 min^{-1}. The recovery of ABA was regulary checked, being consistently between 80 - 85 %. ABA concentrations have been adjusted to losses during analytical procedure. The ABA in the respective subsamples was quantified on the basis of peak mass compared to ABA standards. ABA content in mesophyll was calculated as a difference in contents between both epidermis and bulk leaf extract.

Results

ABA content in epidermis: Significant diurnal variation in ABA content was detectable in abaxial epidermis of the host and the unattached hemiparasite (Fig. 1*A,B*). In both cases there was a substantial increase in ABA content as the light period progressed. The elevated ABA content in the abaxial epidermis declined

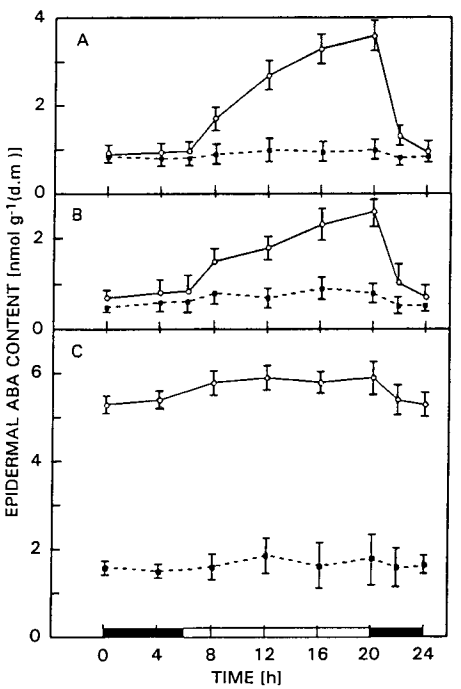

Fig. 1. Diurnal changes of ABA content in abaxial (*open symbols*) and adaxial (*close symbols*) leaf epidermes of *C. bursa pastoris* (the host, *A*) and the hemiparasite *M. arvense* unattached (*B*) and attached (*C*) to the host (n = 6, mean ± S. E.).

rapidly upon the darkening of leaves. In the light phase the ABA content in abaxial epidemis increased about 4.5 and 3.1 times in the host and the unattached hemiparasite, respectively, in comparison with its level at the end of the dark phase. In contrast with this result in the abaxial epidermis of the attached hemiparasite no significant differences were observed in the fluctuation (≤ 10 %) of ABA content (Fig. 1*C*). On the other hand, significant differences in ABA content in the hemiparasite were assessed before and after attachment to the host. By comparing the content of ABA in abaxial epidermis towards the end of the light phase, it was ascertained that in relation to the host it was about 70 % and 164 % in the unattached and attached hemiparasite, respectively. In the investigated cases no significant differences were observed in ABA content in adaxial epidermis during the diurnal cycle. As compared with the host the relative content of ABA in adaxial epidermis of the hemiparasite was comparable to that in the case of abaxial epidermis. The above data show that the association of the hemiparasite with the host did not significantly affect the relative ratios in ABA content in the two epidermes. In all the investigated samples (the host, the unattached, and the attached hemiparasite) the abaxial/adaxial epidermis ABA content ratio in the light phase was similar, approximating to 3.6:1. In the darkness this value was changed in the case of the host and the unattached hemiparasite to 1.1:1. In the attached hemiparasite this ratio was not significantly changed.

ABA content in mesophyll: In the light phase the content of ABA in mesophyll cells constituted about 17 % of that found in abaxial epidermis and about 60 % of that in adaxial epidermis. A more differentiated relative ABA content was assessed in the dark phase. As compared with abaxial epidermis the content of ABA in mesophyll cells constituted about 68 %, 50 %, and 16 % for the host, the unattached and attached hemiparasite, respectively. With respect to adaxial epidermis the content of

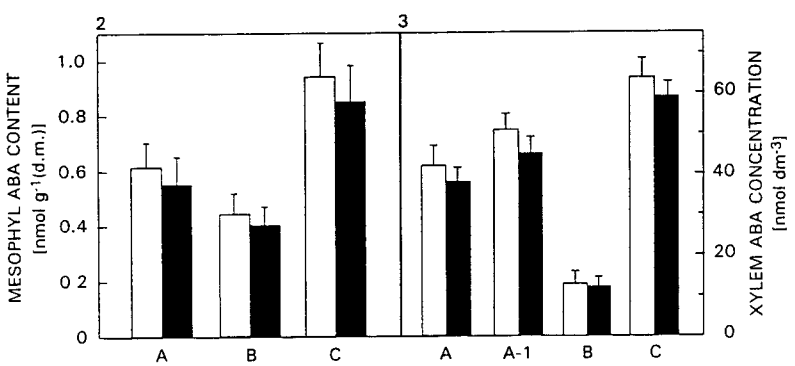

Fig. 2. ABA content in mesophyll cells of *C. bursa pastoris* (the host, *A*) and the hemiparasite *M. arvense* unattached (*B*) and attached (*C*) to the host in the light (*open symbols*) and dark (*close symbols*) phases ($n = 6$, mean ± S. E.).

Fig. 3. Concentration of ABA in xylem sap of *C. bursa pastoris* (the host) grown alone (*A*) or associated with hemiparasite (*A-1*) and the hemiparasite *M. arvense* unattached (*B*) and attached (*C*) to the host in the light (*open symbols*) and dark (*close symbols*) phases ($n = 6$, mean ± S. E.).

ABA in mesophyll cells was about 68 % in the case of the host and the unattached hemiparasite, and about 57 % in the attached *M. arvense*. Differences not exceeding 10 % occurred in the content of ABA in mesophyll cells in the light and dark phases (Fig. 2). If the content of ABA in mesophyll cells of the host was taken as 100 %, this value was about 72 % and 154 % in the unattached and attached hemiparasite, respectively.

ABA concentration in xylem sap: The concentration of ABA in xylem sap did not manifest any significant fluctuations during the diurnal cycle (Fig. 3). This result may be associated with the effect of a sufficiently rich water content in the substrate during the experiment. In the preparasitic stage ABA concentration in xylem sap was about 31 % of that found in xylem sap of the host. After the association with the host the ABA concentration of xylem sap in the hemiparasite increased to about 152 % of that found in xylem sap of the host. Moreover, it was ascertained that after the hemiparasite attachment the concentration of ABA in xylem sap of the host plant also increased by about 25 % compared with the control.

Discussion

Changes in ABA concentration in xylem sap and mesophyll were independent of an exogenous circadian rhythm but connected with water status of plants (Henson *et al.* 1982). The association of the hemiparasite with the host through haustoria influenced the ABA generated in the roots of the host and accumulated in the hemiparasite. There is an evidence that the physiological status of root plays an important role in modulating the concentration of ABA in xylem sap. The very poor development of root system in the preparasitic stage of *M. arvense* was correlated with the low concentration of ABA in xylem sap (about 31 % and 20 % of that in the host and the attached hemiparasite, respectively). Parasitism resulted in increasing of ABA biosynthesis in the host plants and in transport of ABA generated in the root system of the host to hemiparasite. The higher level of ABA in epidermis than in mesophyll was due to its very high content in guard cells (GCs) (Behl and Hartung 1986). GCs do not synthesize ABA and this hormone found in them originates from leaf mesophyll or from the roots.

The transport of chemical signals including ABA from the root to the shoot in the transpiration stream is the mechanism controlling stomatal behaviour (Davies and Zhang 1991). An anomalous pattern of stomatal behaviour is observed in root hemiparasites after attachment to the host. They showed slight changes in aperture in response to environmental factors (Press *et al.* 1987, Shah *et al.* 1987, Lechowski 1995). The lack of stomata response to an increased level of ABA in the attached hemiparasite might be the result of a low osmotic potential of GCs due to high K^+ content (Smith and Stewart 1990, Lechowski 1995) and/or of a simultaneous increase in cytokinins (CKs). The content of zeatin and isopentyladenine in leaves of attached *M. arvense* increased to 10^{-5} and 10^{-6} M, respectively, in comparison to 10^{-7} M and 10^{-8} M, respectively, in the preparasitic stage (Lechowski and Białczyk

1996). CKs may reverse stomatal closure caused by ABA (Blackman and Davies 1984) or cause incomplete dark stomatal closure (Bosselaers 1983) and so increase stomatal conductance (Garrison *et al.* 1984). The occurrence of ABA/CKs interaction suggests that an increase in CKs concentration exported from roots of the host plays an important part in the mediation of ABA effects in the attached hemiparasite.

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