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Diurnal fluctuations of endogenous IAA content in aralia leaves

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Abstract

Diurnal variations in endogenous IAA levels in *Fatsia japonica* leaves, maintaining constant other external factors such as temperature and relative humidity, were studied. Plants were cultivated in a growth chamber (20 °C, 75 % RH, 16 h photoperiod, 400 μmol m^{-2} s^{-1} PAR). IAA analyses were carried out by analytical IP-HPLC with on-line spectrofluorimetry. Rhythmic variation of endogenous IAA levels was found. At the onset of the light period the IAA concentration dropped very rapidly from 1070 pmol g^{-1} (fr.m.) to 144 pmol g^{-1} (fr.m.). This concentration was nearly constant throughout the entire light period. During the subsequent dark period the IAA levels increased again to about 1000 pmol g^{-1} (fr.m.) at the end of the dark phase. These results were not confirmed in open field conditions where many other external factors probably influence the endogenous IAA content.

Introduction

Light and growth regulators interact in many developmental responses. The regulation of IAA-pool-sizes by biosynthetic and catabolic processes is an integral part of the understanding of the hormone action (Sandberg *et al.* 1990).

Light treatments often result in decreased levels of extractable auxin in tissues. Furthermore, in many systems light treatment also results in decreasing IAA-oxidase activity, which could be expected to result in an increased, rather than a decreased, level of auxin. Further the auxin content of oat mesocotyl increases in red light, although growth is strongly inhibited. Therefore, the general relationship between light and auxin is not clear (Hart 1988). Sandberg *et al.* (1990) working with *Nicotiana tabacum* observed that 30 - 40 % of the IAA pool was located in the chloroplast, while the remainder was found in the cytosol.

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Moreover, light enhanced the rate of IAA catabolism in the chloroplast fraction (Sandberg *et al.* 1983, Brown *et al.* 1986).

Further studies are needed to ascertain the effects of light on variations in the IAA content in various plant organs, and parallel variations in other metabolic processes such as photosynthetic and respiratory pathways. Therefore we decided to study the daily variations in endogenous IAA levels in *Fatsia japonica* leaves. This plant is of economic importance in the Mediterranean area and the effect of light on leaf structure and some physiological parameters such as photosynthesis have been studied by several authors in growth chamber conditions (Araus *et al.* 1986, Vidal *et al.* 1990). Likewise leaf abscisic acid levels have also been studied (Carrasquer *et al.* 1990), but there are no studies comparing the behaviour of this plant in the growth chamber conditions and outdoors.

Material and methods

Plant material and growth conditions: *Fatsia japonica* Decne and Plank plants were grown either in a controlled growth chamber (at 16 h photoperiod, 400 μmol m^{-1} s^{-1}, air temperature 20 °C and 75 % relative humidity, or in in field conditions under a shadehouse in the experimental fields of the Faculty of Biology at Barcelona University. Samples were taken in June and September 1989. Daily photosynthetic photon flux density was approximately 400 μmol m^{-2} s^{-1} and day/night temperature was 25/17 °C. In both experiments, plants were grown in 17 cm diameter plastic pots with a substrate of peat-perlite (1:1, v/v), and were fertilized weekly with Hoagland solution. Fully expanded leaves from the second upper node were harvested every 4 h during 24 h.

Extraction and analysis of IAA: After determination of fresh mass and area, leaves were frozen in liquid nitrogen and stored at -20 °C until IAA analysis, essentially according to Van Onckelen *et al.* (1988). Tissues (1 g of fresh mass per sample) were homogenized in 9 ml MeOH containing 340 Bq 3-(5(n)-^{3}H) indolylacetic acid (777 GBq $mmol^{-1}$, *Amersham*) for recovery measurements. After extraction and centrifugation 15 min at 28 000 *g*, the extract was adjusted to 50 % methanol and purified using two sep-pak C_{18} columns (*Waters Ltd.*). The IAA present in the effluent was retained on an anion-exchange column (*Sephadex-DEAE A25 Pharmacia*, 2 cm^3, washed with 0.01 M ammonium formate) and eluted with 6 % formic acid. The IAA in this fraction was further retained on a Bond-elute C_{18} column (*Sophar Biochem.*) and eluted with 5 ml of diethylether. The lower aqueous phase was removed, whereas the upper ether phase was vacuum dried.

Further purification of the IAA-fraction was performed by preparative reversed phase ion-suppresion (IS) HPLC (*Perkin Elmer* Series 2 column: Rosil C_{18} 3μm, 100/4.6 mm, R.S.L.; 40 % MeOH, 60 % H_2O , 0.5 % HAc; 0.5 cm^3 min^{-1}).

The IAA content was quantified after analytical reversed phase ion paring (IP) HPLC (60/40 v/v 0.01 M tetra-butyl ammonium hydroxide (TBHA) in a 0.001 M phosphate buffer (pH 6.6) / MeOH; 0.5 cm^3 min^{-1}) by on-line *Shimadzu RF 530*

fluorescence detector (λem=360 nm and λex=285 nm). Results were calculated based on the principles of isotope dilutions and expressed as pmol IAA g^{-1}(fr.m.). The values are means of 3 individual measurements of each sample.

Results and discussion

In the onset of light period the IAA content in leaves of *Fatsia japonica* grown under controlled growth chamber conditions dropped from 1070 to about 200 pmol g^{-1} (fr.m.), a value which was maintained throughout the entire light period. During the subsequent dark period the endogenous IAA levels in *F. japonica* leaves increased gradually, similar level as that found by the end of the previous dark period (Fig. 1). These results agree with Paash *et al.* (1991) who observed a circadian rhythm of IAA concentration in *Daucus carota*.

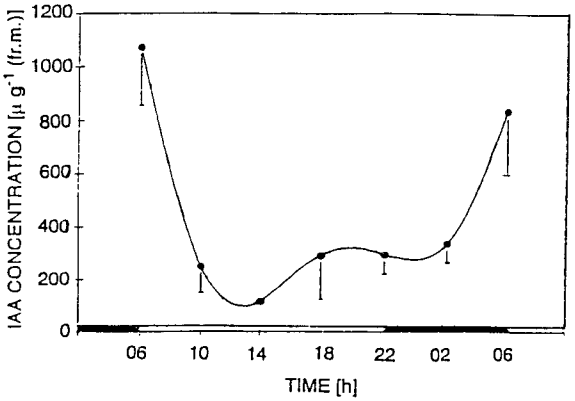

Fig. 1. Daily cycle of endogenous IAA levels [pmol g^{-1} (fr.m.)] in *Fatsia japonica* leaves. Plants cultivated in growth chamber conditions. Each point represents the mean and standard deviation of the analysis of three independent samples.

The observed diurnal fluctuations of the endogenous IAA levels in *F. japonica* leaves may be related to main metabolic pathways as they proceed differently under dark and light. During the dark period an active glycolytic pathway might provide the necessary metabolic precursors to sustain the gradually increasing endogenous IAA levels. During the light period, due to an activated photosynthetic pathway and attenuated glycolysis, a rapid exhaustion of metabolic precursors might cause the observed drop of the endogenous IAA level at the onset of the light period. This effect of light might even be accentuated by a light dependent activation of IAA catabolism (Brown *et al.* 1986). These authors suggested that IAA catabolism took place in the chloroplasts and that the rate of oxidation increased drastically (2400 %) when chloroplast suspensions were incubated in light. Recently, however, Ros

Barcelo *et al.* (1990) warned that the light enhanced oxidation of IAA in chloroplasts should be interpreted with caution.

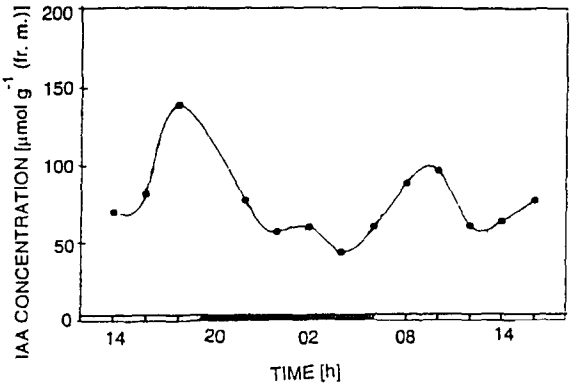

Fig. 2. Daily cycle of endogenous IAA levels [pmol g^{-1} (fr.m.)] in *Fatsia japonica* leaves. Plants cultivated in mediterranean field conditions (September 1989). Temperature, photosynthetic photon flux density and relative humidity were scored at the same time when samples were taken (Table 1).

Table 1. Diurnal fluctuation in temperature [°C], relative humidity [%] and photon flux density [µmol m^{-2} s^{-1}] in field (September 1989).

Time	14	16	18	20	22	00	02	04	06	08	10	12	14	16
Temperature	24.5	24.0	21.4	21.7	19.8	18.0	17.5	18.6	17.0	20.5	21.7	24.0	25.0	22.5
Humidity	54.8	42.2	40.4	46.8	55.6	56.0	58.0	58.0	54.4	44.0	40.4	42.0	45.2	48.4
PAR	400	280	6	-	-	-	-	-	-	230	320	340	380	292

In contrast to controlled growth chamber conditions (with only photon flux density as varying parameter) much less dramatic IAA fluctuations were observed under field conditions. The significantly increased IAA levels measured in this particular experiment at 10.00 [97 pmol g^{-1} (fr.m.)] and at 18.00 [139 pmol g^{-1} fr.m.)] corresponded in both cases with periods of low relative humidity (40.0 %). This was in agreement with results presented elsewhere (Lopez-Carbonell *et al.* 1989) showing IAA accumulation in water stressed *F. japonica* plants.

Under open field conditions no accumulation of IAA was observed at night. This might be due to the heterogenic reactions of the plant material grown under these uncontrolled conditions to the other environmental factors (*e.g.* lower temperatures at night) which might negatively influence the IAA built up, as it was recorded under strictly controlled growth chamber conditions.

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