Planta 150, 347–348 (1980)

Planta

Short Communication

Abscisic Acid and Related Compounds in Phloem Exudate of *Yucca flaccida* Haw. and Coconut (*Cocos nucifera* L.)

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Abstract. Phloem sap collected from *Yucca* and coconut inflorescence stalks was shown to contain abscisic acid (ABA) and trace amounts of 2-*trans* ABA. In coconut sap, two compounds probably derived from ABA with mass spectra consistent with their being dihydrophaseic acid and either hydroxyphaseic acid or oxo-dihydrophaseic acid were also found to be present.

Key words: Abscisic acid – *Cocos* – Phloem exudate – *Yucca*.

In recent years there have been a number of studies on the movement of endogenous abscisic acid (ABA) in phloem exudate (Hoad 1967, 1973a, b, 1978; Zeevaart 1977). In *Lupinus albus* L. and *Ricinus communis* L., ABA has been shown to be present in phloem exudate and its concentration may be considerably higher than in leaf tissue, indicating active loading into the sieve tube (Hoad 1978). Water stress also increases the level of ABA in these tissues and it enhances the amounts of ABA moving from mature leaves to sinks. In our studies, no other compounds related to ABA, except small amounts of 2-*trans*-ABA, were ever detected in sieve-tube sap by GLC(EC). However, Zeevaart (1977) has indicated that phaseic acid and dihydrophaseic acid are present in the phloem exudate of *R. communis*. In this present work the phloem sap of two species of monocotyledons was examined for ABA and ABA metabolites.

Phloem sap was collected from *Yucca flaccida* Haw inflorescence stalks as described by Van Die and Tammes (1975) and from fruit stalks of coconut (*Cocos nucifera* L.), Hodge (1963). Care was taken to avoid degradation of the sap by microorganisms and the samples were freeze dried as rapidly as possible after collection.

The *Yucca* sap was reconstituted using distilled water (pH 3.0) and partitioned against ethyl acetate (x4). The coconut sap was treated similarly except that it was partitioned against diethyl ether (x4) followed by ethyl acetate (x4). After partitioning, the remaining water phase was adjusted to pH 11.0 with KOH and heated for 1 h at 60° C to hydrolyze bound ABA and ABA metabolites. The hydrolyzed compounds were then extracted from the water phase (pH 3.0) with ethyl acetate (x4).

After drying over KOH, aliquots of each fraction were methylated using ethereal diazomethane and analyzed by GLC(EC) using methods described previously (Hoad 1978). Quantitative data were obtained by comparing peak heights of Me-ABA in exudate with those of peaks obtained after injecting known amounts of Me-ABA into the GLC. The TMSi ethers of the methyl esters were then prepared prior to analysis by GC-MS (Gaskin and MacMillan 1978).

Analysis of the methylated ethyl acetate fraction of *Yucca* phloem sap by GLC(EC) gave traces showing that compounds with retention times of Me-ABA and Me-2-*trans*-ABA were present. Their identity was confirmed by GC-MS of the TMSi ethers of the methyl esters, and no other known metabolites of ABA were shown to be present. The concentration of ABA in the sap was approximately 195 ng ml^{-1}.

Preliminary GLC (EC) analyses of the methylated acidic ether fractions of coconut sap indicated the presence of Me-ABA and Me-2-*trans*-ABA, and in the methylated acidic-ethyl acetate fraction a number of more polar compounds. The data obtained from GC-MS confirmed that ABA and 2-*trans*-ABA were present. The mass spectra of the more polar compounds were consistent with their being dihydrophaseic acid (Table 1) and either hydroxyphaseic acid or oxo-dihydrophaseic acid (Table 2). Both compounds have previously been shown to be present in pear seeds (Martin et al. 1977). The concentration of ABA in the coconut sap was approximately 90 ng ml^{-1}, but no firm data could be obtained on the concentration of the ABA metabolites. In the hydrolyzed bound

Abbreviations: ABA = abscisic acid; TMSi = trimethylsilyl; GLC(EC) = gas chromatography (electron capture); GC-MS = gas chromatography = mass spectrometry

0032-0935/80/0150/0347/$01.00

Table 1. MS of Me-DPA-TMSi

368 (M^+; 1), 353 (M^+–15; 2), 246 (1), 189 (1), 159 (72), 125 (13), 122 (5), 121 (3), 117 (100), 75 (18), 73 (65), 43 (71)

Table 2. MS of presumed hydroxy-PA or oxo-DPA, MeTMSi

382 (M^+; 1). 367 (M^+–15; 2), 268 (1), 228 (1), 196 (2), 159 (42), 125 (16), 117 (64), 75 (35), 73 (100), 69 (6), 43 (70)

fractions of both species, neither ABA nor metabolites of ABA could be detected by GLC(EC).

These results confirm those obtained previously with *L. albus* and *R. communis* (Hoad 1973a, 1978), namely that phloem exudate contains ABA and that it moves from 'sources' to 'sinks', whether these are vegetative growing points or reproductive structures. These data indicate that it is likely that the sieve-tube sap of most, if not all, species of higher plants contains ABA and that its movement throughout the plant is largely governed by the extent of photosynthate movement to areas of utilization.

It has previously been suggested that export of ABA from mature leaves in the phloem could be part of the mechanism enabling the proper functioning of the leaves to continue (Hoad 1978). The possibility also arises that ABA imported into fruits could have a role in seed development (Browning 1980). Of the plants we have examined so far, it is only in coconut that metabolites of ABA have been found in phloem exudate. Previous work led us to believe that the mechanism for loading these compounds into the sieve-tube was specific for ABA and excluded metabolites such as phaseic acid and dihydrophaseic acid which have been found in leaf tissues. It seems unlikely that coconut phloem sap contains the enzyme systems for converting ABA into the metabolites found here and we conclude that they move directly from their site of synthesis into the sieve-tube. The absence of phaseic acid, the likely intermediate between ABA and dihydrophaseic acid, is an anomaly which can only be resolved by further studies.

Thanks are due to Dr. C.R. Vonk, Centre for Agrobiological Research Wageningen, The Netherlands for supplying *Yucca* sap, to Dr. U. Pethiyagoda, Coconut Research Institute, Lunuwila, Sri Lanka for supplying coconut sap, and to Philippa Hughes-Games for valuable technical assistance. P.G. also thanks the Agricultural Research Council for financial assistance.

References

Browning, G. (1980) Endogenous *cis, trans*-abscisic acid and pea seed development: Evidence for a role in seed growth and changes induced by temperature. J. Exp. Bot. **31**, 185–197

Die, J. van, Tammes, P.M.L. (1975) Phloem exudation from monocotyledonous axes. In: Transport in plants I, Phloem translocation, pp. 196–222, Zimmermann, M.H., Milburn, J.A., eds. Springer, Berlin Heidelberg New York

Gaskin, P., MacMillan, J. (1978) GC and GC-MS techniques for gibberelins. In: Isolation of plant growth substances, Hillman, J. ed. Soc. Exp. Biol. Semin. Ser. **4**, 79–95

Hoad, G.V. (1967) (+)-abscisin II, ((+)-dormin) in phloem exudate of willow. Life Sci. **6**, 1113–1118

Hoad, G.V. (1973a) Effect of moisture stress on abscisic acid levels in *Ricinus communis* L. with particular reference to phloem exudate. Planta **113**, 367–372

Hoad, G.V. (1973b) Hormones in the phloem of higher plants. In: Transactions of the 3rd Symposium on Accumulation and Translocation of Nutrients and Regulators in Plant Organisms, Warsaw, Antoszewski, R., ed., pp. 17–30

Hoad, G.V. (1978) Effect of water stress on abscisic acid levels in white lupin (*Lupinus albus* L.) fruit, leaves and phloem exudate. Planta **142**, 287–290

Hodge, W.H. (1963) Toddy collection in Ceylon. Principles (J. Palm Soc.) **7**, 70–79

Martin, G.C., Dennis, F.G. Jr., Gaskin, P., MacMillan, J. (1977) Identification of gibberellins A_{17}, A_{25}, A_{45}, abscisic acid, phaseic acid and dihydrophaseic acid in seeds of *Pyrus communis*. Phytochemistry, **16**, 605–607

Zeevaart, J.A.D. (1977) Sites of abscisic acid synthesis and metabolism in *Ricinus communis* L. Plant Physiol. **59**, 788–791

Received 18 May; accepted 27 August 1980