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ANAEROBIOSIS AND ETHANE PRODUCTION IN *PHASEOLUS VULGARIS*

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Key Word Index —*Phaseolus vulgaris*; Leguminosae; biosynthesis; hydrocarbon; ethane; linolenic acid; anaerobiosis.

When tissues of *P. vulgaris* were vacuum-infiltrated with water, stored under nitrogen for 22hr, and transferred to air, ethane production increased significantly [1]. It was proposed that during anaerobiosis there may have been an accumulation of an ethane precursor which was rapidly decomposed when the tissue was returned to air. Neither the nature of the ethane precursor nor the effect of anaerobiosis on the amount of precursor in the tissue was determined. The precursor of ethane in homogenates of *P. vulgaris* was recently determined to be linolenic acid [2]. The present study was initiated to determine the effect of anaerobiosis on linolenic acid levels in tissues of *P. vulgaris*.

There was no appreciable change in fatty acid content, especially linolenic acid, of tissue which had been vacuum-infiltrated with water and incubated in nitrogen as compared to incubation in air or oxygen (Table 1). However, when tissue incubated in nitrogen was exposed to air for 30 min prior to extracting for fatty acids, there was a pronounced decrease in the amount of linolenic acid, and only this acid, in the tissue (Table 1). This decrease in linolenic acid occurred at the same time that ethane production was the highest [1]. These results are consistent with the view that linolenic acid is the precursor of ethane [2–4]. If one assumes that ethane and propanol are produced in equal proportions from the decomposition of hydroperoxidized linolenic acid, as has been proposed [4], then ethane should be produced in a 0.5M ratio to the amount of linolenic acid lost. The linolenic acid in these experiments declined from 2.81 μg/g fr. wt of tissue to 1.25 μg/g fr. wt, or a net loss of 5.5nmol of linolenic acid/g fr. wt/0.5hr. During this same time there were 2.48nmol of ethane produced by the tissues, which is nearly equal to that predicted by the model [4].

Table 1. Fatty acid composition of apical buds vacuum-infiltrated with H_2O and sealed in air, oxygen or nitrogen for 22 hr

Atmosphere	Fatty acid (% of total acids)*					
	16:0	18:0	18:1	18:2	18:3	Other
Air	19	6	4	31	37	3
O_2	24	7	5	29	32	3
N_2	21	5	7	32	34	1
N_2 22 hr, air 0.5 hr	25	6	11	34	21	3

*Average of 3 replications. 16:0 palmitic; 18:0, stearic; 18:1, oleic; 18:2, linoleic; 18:3, linolenic.

It is evident from Table 1 that there is no accumulation of a precursor during anaerobiosis as was previously proposed [1], but rather that linolenic acid was merely being made more accessible to the enzymes which lead to ethane production. The breakdown of cellular membranes with accompanying leakage of inorganic ions from both plant and animal tissues exposed to anaerobic conditions has been established [5, 6] suggesting that lack of oxygen to provide energy essential for cellular maintainance results in their disruption. Consequently, the cellular environment of the anaerobically treated tissue was similar to that of homogenates [1], frozen tissue [7], or freshly sliced tissue [8] and ethane was liberated when the tissue was again exposed to air. The ease with which ethane or other volatile hydrocarbons [9] can be monitored and the close correlation between production of these gases and lipid peroxidation suggest this is a sensitive *in vivo* monitor of membrane damage in organelles [8], tissues [1, 8], whole plants [10, 11] or animals [3, 12, 13].

EXPERIMENTAL

Seeds of *P. vulgaris* cv Harvester were planted in vermiculite and grown to the primary leaf stage in a controlled climate chamber (30°, 8 hr photoperiod). When the seedlings were 7 days old, apical bud explants [1] were excised and divided into groups of 15. The explants were placed in a test-tube containing 10 ml of deionized H_2O and the tissue vacuum-infiltrated for 2 min with a water aspirator. The vacuum was slowly released over a 2 min period. The tissue was blotted, placed in a test-tube, flushed with either air, N_2 or O_2, and sealed with a rubber stopper. The sealed tubes were incubated in the dark for 22 hr at 28°. After incubation the lipids were extracted from the tissues and saponified by the method of ref. [14]. The saponified lipids were methylated and analysed by GLC as described [2]. Ethane production was determined by GC using an Al_2O_3 column [1].

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LEAF WAX *n*-ALKANE CONSTITUENTS OF THE GENUS *KHAYA*

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Key Word Index—*Khaya* species; Meliaceae; *n*-alkanes; leaf waxes; age of leaves, taxonomy.

INTRODUCTION

The genus *Khaya* (Meliaceae), which is the most important source of mahogany both in Africa and Madagasca, has been found difficult to classify taxonomically, especially in East Africa and the Congo. *Khaya madagascariensis* and *Khaya nyasica* have leaves which are similar both in shape and size. *Khaya anthotheca* and *Khaya ivorensis* are not easily distinguishable in the herbarium [1] and the timber cannot be distinguished microscopically.

In our previous work [2], it was found that the *n*-alkane composition of leaf waxes varied with the age of leaves and that for the *n*-alkane constituents to be useful in taxonomy, variation with leaf age of the *n*-alkane composition of particular aged leaves should be compared. The present work which was carried out on eight species, examined how the variation, with leaf age, in the hydrocarbon constituents of the leaf waxes could be used as a taxonomic character.

RESULTS AND DISCUSSION

Examination of the variation, with leaf age, in the composition of *n*-alkanes in the leaf waxes of *K. ivorensis* and *K. grandifoliola* Uganda showed that for *K. ivorensis* the *n*-C_{29} alkane was the most abundant between the ages of three and six weeks while from the seventh week the *n*-C_{31} alkane took over as the most abundant. In the case of *K. grandifoliola* Uganda, this change took place at the fourteenth week. For convenience, and using the results of the weekly analysis of the leaf waxes of *K. grandifoliola* Uganda and *K. ivorensis* as a guide, the leaf waxes of the other five *Khaya* species involved in this study were analysed at two different ages – six and nineteen weeks.

From the results shown on Table 1, the trees were classified into three groups. *Khaya senegalensis* was the only member of group I as it was either a permanent *n*-C_{29} major or had its change over age above nineteen weeks. Both *K. anthotheca* species, which were either permanently C_{31} majors or had their change over ages below six weeks, were in group II. All the others: *K. nyasica* Malawi; *K. ivorensis*; *K. nyasica* Amani; *K. grandifoliola* Uganda and *K. madagascariensis* were all in group III, in that they all had their change over ages between six and nineteen weeks. The two *K. anthotheca* sp. differed only in the fact that the plants were initially collected for planting at Ibadan from Ghana and Uganda respectively. This study leads to no distinction between the western and eastern varieties of the plant. The leaf alkane content of leaves of known ages can

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