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THE DISTRIBUTION OF MONOTERPENE HYDROCARBONS IN AIRBORNE VOLATILES FROM PSEUDOSTEM OF EIGHT CULTIVATED BANANA CULTIVARS IN EAST AFRICA

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ABSTRACT. The GC-MS analysis of activated charcoal trapped air-borne volatiles from eight different banana cultivars, revealed qualitative and quantitative differences in their monoterpene content. Eighteen monoterpene hydrocarbons were found in airborne volatiles from the eight cultivars. Tricyclene, α-pinene, camphene, sabinene, β-pinene, β-myrcene, α-phellandrene, limonene, ocimene and α-dimethylstyrene are the major monoterpenes which are universally found in all cultivars. The other seven monoterpenes are present in low relative amounts. The results indicate a potential for the use of the air-borne volatile composition in chemosystematic identification or classification of bananas.

INTRODUCTION

Banana is one of the most important food crops widely cultivated in the tropics. In some these regions, banana is traditional staple food which is available throughout the year. The different banana cultivars have found different uses in different regions of the tropics. Apart from the cooking and dessert types, there are varieties specifically used for brewing or flour making. Some banana cultivars are spread across the tropics whereas others are endemic to particular regions. The use of regional names coupled with somatic mutations have made comparison of cultivars in different banana growing areas difficult. Further difficulties in such comparisons arise from environmental, climatic and soil variations [1]. Taxonomy of bananas is currently based on morphological characteristics which may change with climatic or geographical factors and soil fertility. Banana taxonomy therefore requires a reliable analytical method which is independent of climatic, geographical factors or soil fertility variations.

In some banana growing areas, the production has declined due to insect pests [2], nematodes [3], soil condition and plantation management [3]. One of the major insect pests of banana is the banana weevil, *Cosmopolites sordidus* (Germar), a native of Malaysia but now spread in all the banana growing areas [3]. Some banana cultivars are apparently less susceptible to weevil attack than others [3,4]. Resistance of crop plants to insect attack has been correlated to the absence of kairomones [5] or the presence of volatile repellents [6], feeding deterrents [7], metabolic inhibitors [8], toxic chemicals [9] or to biophysical factors [10]. Knowledge of the bases of resistance and the biological activity of the allelochemicals involved may facilitate banana breeding programs aimed at developing new varieties which combine resistance with other desirable traits.

The composition of the air-borne volatiles emanating from the pseudostem of a susceptible banana cultivar [11] and the orientational behaviour of the weevils towards the natural blend [12] was recently reported by us. We hereby report both the qualitative and quantitative differences found in the monoterpenoid hydrocarbon air-borne volatiles from eight banana cultivars of different genomic constitution and varying degree of susceptibility to the banana weevil and discuss their possible use in banana taxonomy.

RESULTS AND DISCUSSION

Monoterpene composition of mature tissues in *Picea mariana* and *P. sitchensis* has been shown to exhibit only minor seasonal variation [13]. It was therefore found desirable to collect volatiles from mature banana pseudostem tissue to minimize such changes in their monoterpenoid composition and also to avoid destroying banana plants since the plants take a long time (15-18 months) to flower. GC and GC-MS analysis of the volatiles from the eight banana cultivars revealed that they all contained monoterpenes, oxygenated terpenoids and sesquiterpenes. No two profiles were found to be qualitatively and/or quantitatively similar. The oxygenated terpenoids were found to represent a very small fraction of the total volatile composition (1-5% depending on the cultivar). The monoterpenes constituted between 60 and 95% of the total volatiles depending on the cultivar (Table 1). The rest (4-40%) was found to be due to sesquiterpenes. In general the aromatic, aliphatic bicyclic and tricyclic monoterpene hydrocarbons were found to be present in high relative abundances in all the banana cultivars studied. However, the p-menthane type monoterpene hydrocarbons except limonene and α-phellandrene were found to be selectively distributed in the various banana cultivars and more so in low relative abundances hence could be used to identify some banana cultivars.

The tolerant homogeneous sweet dessert triploid (AAA), *nyoro* in Kikuyu but also known as dwarf cavendish was found to have all the monoterpenes except β-phellandrene and pseudolimonene (Table 1). The presence of β-terpinene is characteristic of its volatile profile. Volatiles from the susceptible sweet dessert diploids (AA) *muraru* (Kikuyu) and *njuru* (Kikuyu) were found to have a similar pattern, in addition to the absence of β-terpinene. The monoterpene profile from the susceptible heterogeneous sweet dessert triploid (AAB) banana cultivar, *githungu* in Kikuyu but also known as *pome* or *prata,* was also found to be closely related to the two diploids (AA) qualitatively except for the absence of γ-terpinene in this cultivar. However, quantitative differences between these cultivars were noted for the individual monoterpenes in their volatile profiles. The resistant heterogeneous multipurpose triploid (ABB) cultivar, *mbuu* in Kikuyu but also known as *pisang awak* in Malaysia revealed a completely different monoterpene volatile profile from any other cultivar. The exclusive presence of β-phellandrene and pseudolimonene in this cultivar is characteristic of its volatile profile. The presence of both pseudolimonene and β-phellandrene in the monoterpene volatile profile is therefore indicative of this cultivar. The tolerant heterogeneous sweet dessert diploid (AB) cultivar, *wangae* in Kikuyu but also known as *ney poovan* in India, exhibited a completely different monoterpene volatile profile from the rest of the cultivars. The absence of α-phellandrene was found to be unique to this cultivar.

The airborne volatile from two susceptible cooking cultivars, *githumo* and *mitahato* (AAA-EA) which are indigenous to East Africa, were found to be closely related to that of *nyoro* (AAA) except for the absence of β-terpinene in both. *Githumo* was found to have trace amount of α-terpinene which was completely lacking in *mitahato*. Similarly, *mitahato* was found to have α-dimethylstyrene which was found to be absent in *githumo*.

From the data presented here six out of eight banana cultivars can be identified from qualitative comparison of their monoterpene volatile profiles. In summary the heterogeneous triploids can be identified by the absence of γ-terpinene in *githungu* (AAB) and in *mbuu* (AAB) by the exclusive presence of β-phellandrene and pseudolimonene. The diploids can be identified by the absence of α-phellandrene in *wangae* (AB). The homogeneous triploid (AAA) cultivar *nyoro* can be identified by the presence of β-terpinene. The East African highland triploid cultivar *mitahato* can be identified by the absence of α-terpinene whereas *githumo* can be identified by the absence of α-dimethylstyrene. The remaining two cultivars *muraru* and *njuru* have all the monoterpene hydrocarbons as in *nyoro* volatiles except for the absence of β-terpinene. These two cultivars can not be easily differentiated from each other by their volatile profiles on qualitative comparison of the components. However quantitatively, *muraru* has twice (9.8%) as much of α-pinene as *njuru* (5.0%).

Table 1. Relative amounts (%) of monoterpene hydrocarbons found in eight banana cultivars.

Cultivars Class	Nyoro AAA	Muraru AA	Njuru AA	Githungu AAB	Mbuu ABB	Wangae AB	Githumo AAA-EA	Mitahato AAA-EA
Tricyclene	4.3	4.3	1.4	0.5	0.1	7.5	0.1	0.2
α-Pinene	18.3	9.8	5.0	14.4	62.6	60.4	33.8	46.0
Camphene	0.6	0.3	0.2	0.3	1.7	0.9	0.7	0.9
Unidentified	0.4	0.1	t	0.2	0.1	-	0.2	0.2
Sabinene	1.7	6.2	1.7	0.2	0.5	6.2	0.5	1.2
β-Pinenene	1.9	1.5	0.9	0.9	0.9	2.1	12.5	15.7
β-Myrcene	11.3	11.5	11.7	9.1	5.1	4.8	5.7	4.7
Unidentified	0.5	0.4	0.3	0.5	t	-	0.1	0.2
Pseudolimonene	-	-	-	-	2.6	-	-	-
α-Phellandrene	0.5	0.4	0.3	0.5	0.3	-	0.4	0.2
β-Terpinene	0.2	-	-	-	-	-	-	-
α-Terpinene	1.0	0.6	0.3	0.3	-	1.0	t	-
Cymene	2.2	3.2	0.8	2.3	1.8	0.7	1.8	2.7
Limonene	5.8	3.1	1.7	1.5	19.3	4.4	5.7	7.6
β-Phellandrene	-	-	-	-	0.2	-	-	-
Ocimene	18.8	25.4	35.8	10.5	t	2.2	3.2	2.3
γ-Terpinene	0.1	0.1	0.1	-	t	t	0.1	t
α-Dimethylstyrene	0.7	0.4	0.8	1.2	t	t	-	0.2

t = trace

We have also conducted comparative behavioural and electrophysiological studies with the volatiles from one triploid *githumo* (AAA) which is susceptible and the diploid *wangae* (AB) which is tolerant [12]. Although responses by weevils in orientational behavioural bioassay appeared stronger to the susceptible (*githumo*) than to the tolerant (*wangae*), the difference was not big enough to match the observed differences in the levels of susceptibility in these two cultivars. This observation suggests that air-borne volatiles are useful in host location by the banana weevil but non-volatile allelochemicals may be involved in arrest, feeding or oviposition site finding stages, hence the differences in cultivar susceptibility. Investigations along this line are in progress. Recent investigations (Ndiege *et al.* in press) have revealed that the major terpenoids hydrocarbons are not useful for the insect but rather the minor oxygenated terpenoids are used in host location.

Although a lot still needs to be done to investigate the use of volatiles emanating from banana pseudostem as a basis for chemosystematic identification of banana cultivars, in view of the data presented here, the approach looks promising. The presence of many banana cultivars spread across the tropical regions, with others being indigenous to particular locations coupled with somatic mutations and common use of local names, demands a strong and unambiguous chemical tool for the identification of banana cultivars. Attempts to develop such a tool can be seen in the efforts of Horry *et al.* [14], Simmonds [15] and Williams *et al.* [16] on the distribution of anthocyanidins in various banana cultivars. We have recently developed a non-destructive micro-analytical method [17] requiring only 10 g of banana tissue which can be used in sampling volatiles. The method can be used for sampling volatiles from all ages of the banana plants since it does not require cutting down the whole plant. Further work on the air-borne volatiles from many other cultivars using this approach is in progress.

EXPERIMENTAL

Plant material. Banana plants of local cultivars known as *nyoro, githumo, mitahato, githungu, muraru, njuru mbuu* and *wangae* in Kikuyu were collected from a commercial plantation in the periphery of the city of Nairobi. Plants from which the fruit had just been harvested were used. The freshly collected banana pseudostems were chopped into small pieces in preparation for the collection of the air-borne volatiles.

Collection of volatiles. The chopped pieces were put into a 5 L three-necked round bottom flask and the central neck closed with a glass stopper. Columns containing cleaned and activated charcoal traps were used as traps and air-cleaners. The trapping was done by pulling air through the activated charcoal air-cleaner into the flask and though another activated charcoal trap using a vacuum pump (cole-parmer air cadet) at a rate of 0.8 L/min for 24 h. Both the traps and air-cleaners were eluted with CH_2Cl_2. The eluates were analyzed by GC-MS after concentration to ca 0.1 mL by passing a gentle stream of N_2 over them. For each banana cultivar the collection was done five times.

Analysis of volatiles. GC was carried out on a Hewlett-Packard 5890 GC on a capillary column (Ultra 1, cross linked methylsilicon, 25 m × 0.32 mm × μm; or CP-wax 51, 30 m × 0.23 mm × 0.21 μm or OV-17, 25 m × 0.23 mm × 0.18 μm) and a FID. Resolution of β-phellandrene and limonene was accomplished on a carbowax 20M (50 m × 0.2 mm × 0.17 μm) capillary column with the GC temperature program of 60° (5 min) to 220° (20 min) at a rate of 5°/min. Authentic samples were bought from Aldrich Chemical Company or from Fluka except for β-phellandrene which was provided by H.D. Pierce of Simon Fraser University, Canada. Comparisons of the GC profiles of the air-cleaner and the trap were done to identify peaks which were due to impurities.

For the identification of the trapped air-borne volatiles by GC-MS (VG-Mass Lab. 12-250), a fused silica capillary column (CP sil 5 CB, 25 m × 0.22 mm × 0.32 μm or ultra 1, methylsilicon 50 m × 0.32 mm × μm) was used. The GC oven temperature was programmed from 40 to 270° with a rise of 5°/min. A computer assisted search in the NBS library of mass spectra was done for the preliminary identification of the compounds from their MS data. To confirm the identity of the compounds their R_t and MS data were compared to those of authentic compounds.

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