Pergamon

Biochemical Systematics and Ecology 30 (2002) 413–424

biochemical systematics and ecology

www.elsevier.com/locate/biochemsyseco

# Identification of factors determining kavalactone content and chemotype in Kava (*Piper methysticum* Forst. f.)

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Received 13 October 2000; accepted 22 June 2001

**Abstract**

This study presents results of field experiments conducted to identify factors determining kavalactone content and chemotype in *Piper methysticum*. The following factors have been studied: (1) the geographical direction of the roots on the plant, (2) the geographical location of the plant, (3) its age, and (4) its organ (roots, stumps, or basal stems). Overall, 185 samples were analysed by HPLC. It appears that the geographical direction of the roots (North, East, South, West) is not significant. Chemotype and kavalactone content variation among clones of a cultivar grown in a common garden is negligible. There is significant variation among different cultivars originating from the same island. The variation within island is comparable to the variation existing within the whole Vanuatu archipelago. For a given cultivar, chemotype is stable across locations. There are however, chemotype differences between organs. Kavalactone content is always higher in the roots than in the stumps and higher in the stumps than in the basal stems. Experimental data obtained from one cultivar indicate that at the juvenile stage (less than 18 months of growth), kavalactone content is still low but increasing progressively: from 3% of dry matter at 10 months to 8% at 17 months. After two years of vegetative growth, the chemotype appears stable and kavalactone content does not increase but rather fluctuates (±2%). Although seasonal factors might have an effect, it is not possible to observe a significant trend. It is confirmed that chemotype is genetically controlled. However, kavalactone content appears to be greatly determined by the growing conditions, either by the local environment or by the agricultural techniques used by the local farmers. Consequently, the selection of the cultivar, its organ and the geographical area of origin are factors contributing

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PII: S0305-1978(01)00093-X

directly to quality control in *Piper methysticum*. 

*Keywords: Piper methysticum*; Kavalactone content; Chemotype; Organ; Environmental factors: Geographical origin

## 1. Introduction

Ground and macerated roots, stumps or basal stems of kava (*Piper methysticum* Forst. f.) produce a traditional beverage that induces relaxation. Kava is a species whose zone of distribution is exclusively limited to the Pacific Islands. It was farmers in Vanuatu who were the first to select and develop cultivars to improve kavalactone composition responsible for the physiological effects (Lebot et al., 1992). There are about 80 different cultivars in Vanuatu, with different morphological characteristics, compared with 7 in Tonga, 12 in Fiji, 5 in Samoa and 11 in Hawaii. Overall, more than 15 000 ha are now in production in the Pacific and growing at a rate of 20% per year. In 1998, more than 2000 tonnes of dry matter were exported towards the nutraceutical and pharmaceutical markets. Quality of the raw material is, however, very variable and represents the major constraint for the development of new products.

Kava plants are usually harvested after 2–4 years but may be left growing for more than 20 years. The stump and roots become larger over time although soil fertility and genotype are more important factors than plant age in determining yield of the underground organs. The quality of kava, that is the total kavalactone content and its chemotype, can be expected to be affected by several factors. Amongst the most important is the cultivar used to prepare the beverage. It has been demonstrated that different cultivars planted in the same plot the same day produce, after three years of growth, kavalactone contents varying from 4 to 17% and different chemotypes (Lebot and Lévesque, 1989).

The six major kavalactones account for 96% of the lipid extract. These kavalactones are numbered to define cultivars chemotype: 1=demethoxyyangonin (DMY), 2=dihydrokavain (DHK), 3=yangonin (Y), 4=kavain (K), 5=dihydromethysticin (DHM) and 6=methysticin (M). Chemical compositions can be coded by listing in decreasing order of proportion the six major kavalactones in the extract (Lebot and Lévesque, 1996a). Drinkers generally do not appreciate the physiological effects of cultivars containing high percentages of dihydromethysticin; chemotypes with a high percentage of kavain induce the most desirable effects. Farmers confirm that different cultivars uprooted from a common garden have different properties.

Kavalactone content decreases progressively from roots to leaves (Duve and Prasad, 1981) and there is significant variation in chemical composition according to the organ analysed (Smith, 1983; Smith et al., 1984; Lebot et al., 1999). Dihydromethysticin (DHM) and dihydrokavain (DHK) are the major components of the leaves but kavain (K) and methysticin (M) are in major proportions in the roots (Smith et al., 1984). It has been shown that the chemotype is genetically controlled (Lebot

and Lévesque, 1996b). However, according to traditional knowledge, the age of the plant and the growing environment are also important factors determining the kavalactone content. Farmers claim that the physiological effect gets more pronounced when the plant gets older.

In fact, little is known about factors determining the kavalactone content and chemotype in a particular organ, within the same plant and/or between clones. This paper presents results of experiments attempting to elucidate the effect of: (1) the geographical direction of the roots on the plant, (2) the geographical location of the plant, (3) its age, and (4) the organ (roots, stumps, stems).

## 2. Materials and methods

### *2.1. Field experiments*

Experiments 1 and 5 were conducted in Valeteruru, Santo Island, on the VARTC research station (Vanuatu Agricultural Research and Training Centre). Experiments 2 and 3 were conducted in six different islands of Vanuatu: Gaua, Vanua Lava, Santo, Pentecost, Malekula and Tanna and experiment 4 was conducted at Tagabe Agricultural Station on Efate island (Fig. 1).

#### *2.1.1. Experiment 1, comparing kavalactone content and chemotype of the roots according to their geographical direction on the plant*

Five clones originating from a single mother plant (cultivar *Malogro*) were planted in a line, spaced every 2 meters, on February 5th 1996. The five plants were harvested after 3 years, 6 months and 20 days on August 25th 1999. At harvest, partitioning was conducted according to the geographical direction: North, East, South and West. Four root samples were collected on each plant between 20 and 40 cm depth. Overall, 20 root samples were analysed.

#### *2.1.2. Experiment 2, comparing kavalactone content and chemotype between distinct cultivars within the same island and between islands*

Cultivars aged between 3 and 4 years were harvested in seven distant environments. In these seven distinct areas, the most cultivated cultivars, identified under their local names in vernacular languages, were collected and in some cases the same cultivar was found to be grown in different environments. Six cultivars were uprooted on the island of Gaua and six on Vanua Lava; 11 cultivars were harvested in central Santo island and 12 in south-west Santo; 10 cultivars were harvested on Pentecost; 6 cultivars were harvested on the island of Malekula, and 10 on the island of Tanna in the southern part of Vanuatu. Overall 61 cultivars were uprooted between latitude 13°45′S and 19°30′S (Fig. 1) and 61 root samples were analysed.

#### *2.1.3. Experiment 3, comparing kavalactone content and chemotype between plants of cultivar "Borogu" grown in different locations*

Root samples were collected from 13 plants of 3 to 4 years of age, in 10 different locations, and were compared.

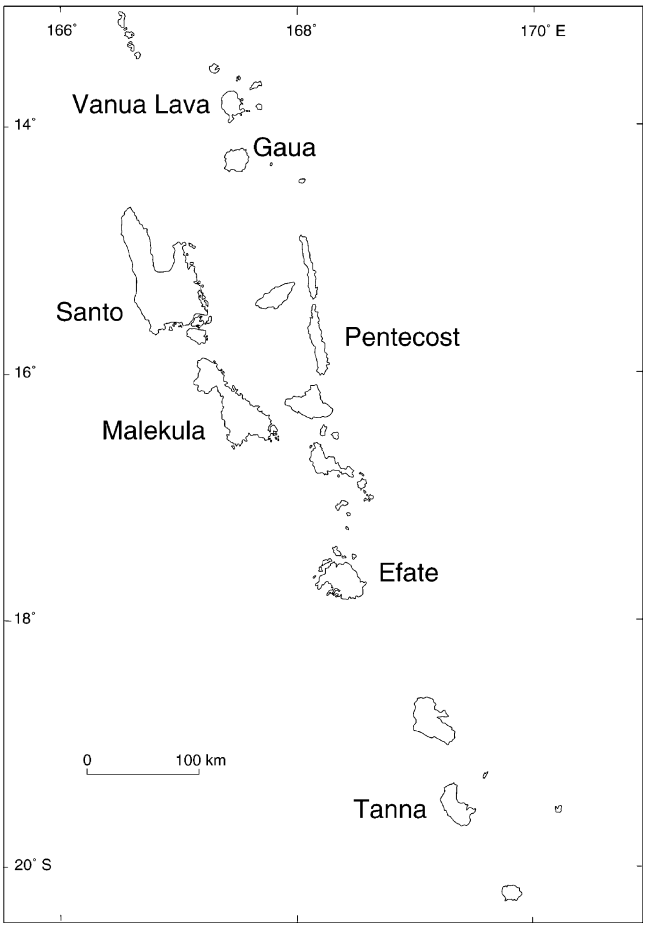


Fig. 1. Vanuatu.

*2.1.4. Experiment 4, comparing kavalactone content and chemotype at the juvenile stage*

To assess the variation before maturity, five lines were planted of clones from a single mother plant (cultivar *Borogu*) on July 21st 1998 at Tagabe Agricultural Station on the island of Efate. The first five plants were harvested after 10 months of growth, and then every month until they reached 17 months. Overall, 40 root samples were analysed.

*2.1.5. Experiment 5, comparing kavalactone content and chemotype at maturity*

Clones of a single mother plant (cultivar *Tudei*) were planted in a line, spaced every two meters, on August 8th 1996. After 27 months of growth (November 12th 1998) a plant was uprooted every month for eleven months. The 17th and last plant was uprooted after the 51st month of growth. After uprooting, plants were divided

into roots, stumps and basal stems. Their fresh and dry weights were recorded. Overall, 51 samples were analysed.

### 2.2. Kavalactone analysis

Overall, 185 samples were analysed for their kavalactone contents and chemotype. All samples were oven dried and ground into fine powder with a laboratory hammer mill. A homogenised powdered kava sample (10 g) was extracted with 200 ml of methanol for 6–8 h or until visually all kavalactone had been extracted. Soxhlet extraction apparatus was used for the extraction process. The methanolic extract thus obtained was made to an exact volume of 250 ml and diluted by a factor of 5, 10 μl of which was injected onto a HPLC. HPLC analysis was performed employing a system equipped with a Waters 510 pump, Waters U6K injector, Perkin Elmers Diode array 235 C detector and Waters 746 data module integrator. The chromatographic separation was carried out using a normal phase Nucleosil 50 column (250×4.6 mm), 5 μm particle size. An isocratic mobile phase of hexane:1,4-dioane:-methanol (85:13:2) was used with a flow rate of 1.5 $cm^3$ $min^{-1}$. The absorption spectra were recorded from 365 nm to 190 nm. Quantification was carried out at a single wavelength of 245 nm. For standards, a composite of the six major kavalactone (2 mg each dissolved in 5 ml of methanol) was used. For calculations of percentage kavalactone on a dry weight basis, the moisture content of the dry plant material was determined by drying ca. 5 g of sample in an oven at 105°C for 5 h (Singh, 1999). The six major kavalactones were quantified: DMY=demethoxyyangonin (1), DHK=dihydrokavain (2), Y=yangonin (3), K=kavain (4), DHM=dihydromethysticin (5), M=methysticin (6). Chemotypes were coded in decreasing order of the proportion of these kavalactones in the extract. The experimental error was estimated to be approximately ±0.5%. All analyses were conducted at the Institute of Applied Sciences of the University of the South Pacific in Suva, Fiji.

## 3. Results

### 3.1. Geographical direction of the roots on the plant

Table 1 presents the results of analyses conducted on five plants aged 3.5 years, planted and uprooted the same day (experiment no. 1). These results indicate that variation between plants (clones) of the same cultivar, grown within the same plot, is negligible. Their respective kavalactone content means are 14.2, 13.2, 14.9, 13.8, 12.5% and their chemotype is consistently 246351. These results also indicate that variation between the four geographical directions (North, East, South, West) is not significant considering experimental error. Chemotype and kavalactone contents are consistent between plants (means are 13.5, 13.8, 13.9 and 13.7%) and independent of the geographical direction of the root sample analysed. Consequently, root sampling appeared to be reliable for a given cultivar, independent of the clone and of the geographical direction of the roots on the plant.

Table 1
Variation according to the geographical direction of the roots on the plant (five clones of cultivar *Malogro*, experiment 1)

| Plant | North | | East | | South | | West | | Plant Mean | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| No. | Chemotype | KL % | Chemotype | KL % | Chemotype | KL % | Chemotype | KL % | KL % | CV % | Chemotype |
| 1 | 426351 | 13.3 | 246351 | 13.9 | 246351 | 15.3 | 246351 | 14.4 | 14.2 | 5.9 | 246351 |
| 2 | 246351 | 13.2 | 246351 | 12.7 | 246351 | 14.6 | 246351 | 12.4 | 13.2 | 7.4 | 246351 |
| 3 | 246351 | 15.4 | 246351 | 14.9 | 246351 | 13.4 | 246351 | 16.0 | 14.9 | 7.5 | 246351 |
| 4 | 246351 | 13.3 | 246531 | 14.7 | 246351 | 13.0 | 426351 | 14.2 | 13.8 | 5.7 | 246351 |
| 5 | 246351 | 12.5 | 246351 | 12.9 | 246351 | 13.0 | 246531 | 11.4 | 12.5 | 5.9 | 246351 |
| Mean | 246351 | 13.5 | 246351 | 13.8 | 246351 | 13.9 | 246351 | 13.7 | 13.7 | | |

### *3.2. Geographical location of the plant*

Local cultivars aged between 3 and 4 years were sampled and compared for the kavalactone content and chemotype of their roots. Results of analyses conducted on 61 different cultivars originating from five different islands and six distinct geographical regions are presented in Table 2. There is significant variation between cultivars originating from the same island for both kavalactone content and chemotype. Cultivars grown on the island of Vanua Lava, produce an average kavalactone

Table 2
Cultivars variation, within and between islands (experiment 2)

| Island | Gaua | Vanua Lava | Central Santo | S. West Santo | Pentecost | Malekula | Tanna | Vanuatu |
|---|---|---|---|---|---|---|---|---|
| No. cultivars | 6 | 6 | 11 | 12 | 10 | 6 | 10 | 61 |
| KL% min. | 3.7 | 4.5 | 8.3 | 8.9 | 4.9 | 3.8 | 5.8 | 3.7 |
| KL% max. | 12 | 8.6 | 17.5 | 16.2 | 12 | 7.2 | 14.4 | 17.5 |
| KL% mean | 7.8 | 6.3 | 12.9 | 10.7 | 8.8 | 5.2 | 10.8 | 9.5 |
| CV% | 36.8 | 23.7 | 26.7 | 20.1 | 26.4 | 25.1 | 28.5 | 36.3 |
| Chemotypes | 246531 | 243165 | 153264 | 243651 | 246531 | 256431 | 234615 | |
| | 254361 | 243165 | 243165 | 245631 | 246531 | 256431 | 235641 | |
| | 256431 | 245613 | 245361 | 245631 | 246531 | 256431 | 243615 | |
| | 423165 | 423165 | 243561 | 245361 | 423615 | 423516 | 243615 | |
| | 423615 | 423615 | 243651 | 245361 | 423615 | 423561 | 243615 | |
| | 521364 | 452136 | 423615 | 245361 | 423615 | 423651 | 243165 | |
| | | | 423615 | 421365 | 426351 | | 254361 | |
| | | | 423615 | 423615 | 432165 | | 423615 | |
| | | | 423165 | 423561 | 431265 | | 423651 | |
| | | | 423651 | 423561 | 512364 | | 423651 | |
| | | | 423651 | 423561 | | | | |
| | | | | 423561 | | | | |

content (6.3%) that is half the average content of cultivars found in Central Santo (12.9%). Variation within island is, however, comparable to the variation existing within the Vanuatu archipelago (CV=36.3%). The island of Gaua, for example, presents the highest coefficient of variation of the mean (CV%) between six cultivars for their kavalactone content (36.8%). The south west Santo region presents the lowest CV with 20.1% of variation of the mean for 12 cultivars analysed. In all of the six islands surveyed, some cultivars can yield more than twice the kavalactone content of others at the same age (KL% min. and KL% max. values). Overall, the most variable kavalactone are DHM (2), DMY (3) and K (4). It appears that within island, some cultivars present identical chemotypes. For example, two cultivars present chemotype 243165 in Vanua Lava, three cultivars present 423615 in Central Santo, three cultivars present 245361 in S.W. Santo, three cultivars present 423615 in Pentecost, three cultivars present 256431 in Malekula and three present 243615 in Tanna. Chemotype 423615, with high kavain (4) and low dihydromethysticin (5) contents is highly appreciated among drinkers for daily consumption and is found in each of the five islands surveyed. It is also observed, however, that chemotypes with high dihydromethysticin (5) which are not suitable for daily consumption, are also found in the five islands surveyed. None of the islands appear to produce chemotypes with peculiar characteristics.

Root analyses conducted on samples collected from 13 different *Borogu* plants grown in ten different locations on two distinct islands (Pentecost and Santo) are presented in Table 3. Chemotype appears to be stable and independent of the growing location (4236 . . or 4263 . .). Kavain is always in higher proportion than dihydrokavain. Kavalactone content however, is not constant and varies from 5.9% in Altakre to 11.1% in Vansasa. In two locations, Lebutsulala and Lalak, two distinct plants of *Borogu* were uprooted for analysis and were compared. The results indicate that the

Table 3
Variation in kavalactone content and chemotype of cultivar *Borogu* grown in different locations (experiment 3)

| Location | Island | DMY 1 | DHK 2 | Y 3 | K 4 | DHM 5 | M 6 | Chemotype | KL % |
|---|---|---|---|---|---|---|---|---|---|
| Vansasa | Pentecost | 10.81 | 20.72 | 15.31 | 28.83 | 10.81 | 13.51 | 423651 | 11.1 |
| Altakre | Pentecost | 10.09 | 22.02 | 13.76 | 30.27 | 10.09 | 13.76 | 423651 | 10.9 |
| Nokovel | Pentecost | 11.54 | 20.19 | 14.42 | 30.77 | 9.61 | 13.46 | 423615 | 10.4 |
| Valeteruru | Santo | 10.00 | 26.00 | 17.00 | 30.00 | 8.00 | 9.00 | 423165 | 10.0 |
| Kona | Santo | 9.78 | 21.74 | 16.30 | 29.35 | 10.87 | 11.95 | 423651 | 9.2 |
| Butmas | Santo | 9.78 | 21.74 | 16.30 | 29.35 | 10.87 | 11.95 | 423651 | 9.2 |
| Gun | Pentecost | 9.52 | 22.62 | 16.67 | 29.76 | 10.71 | 10.71 | 423651 | 8.4 |
| Lebutsulala | Pentecost | 11.11 | 18.52 | 13.58 | 33.33 | 8.64 | 14.81 | 426315 | 8.1 |
| Lebutsulala | Pentecost | 11.11 | 18.52 | 11.11 | 33.33 | 9.88 | 16.05 | 426315 | 8.1 |
| Ilamre | Pentecost | 8.97 | 21.79 | 12.82 | 33.33 | 10.26 | 12.82 | 426351 | 7.8 |
| Lalak | Pentecost | 10.39 | 16.88 | 12.99 | 35.06 | 9.09 | 15.58 | 426315 | 7.7 |
| Lalak | Pentecost | 11.29 | 16.13 | 14.52 | 33.87 | 9.68 | 14.52 | 423615 | 6.2 |
| Altakre | Pentecost | 8.47 | 20.34 | 10.17 | 35.59 | 10.17 | 15.25 | 426351 | 5.9 |

data generated for the six major kavalactones is reliable and that the relative proportions are consistent. Results obtained from this experiment confirm that chemotype is genetically controlled. The quality of cultivar *Borogu* is partly preserved when the cultivar is grown in different conditions, at least for its chemical composition. Kavalactone content, however, appears to be greatly determined by the growing conditions, either by the local environment or by the agricultural techniques used by local farmers.

### *3.3. Age of the plant*

Table 4 presents the results of roots analyses conducted each time the plants from experiment nos. 4 and 5 were harvested. Cultivar *Borogu* was studied at the juvenile stage during eight months, between the 10th and 17th month of growth. The means of five plants are presented. It appears that at this juvenile stage, kavalactone content is still low but increasing progressively: from 3.84% of dry matter at 10 months to 7.96% at 17 months (experiment no. 4). Cultivar *Tudei* was studied between the

Table 4
Variation according to the age of the plant. Roots of cultivars *Borogu* (experiment no. 4, mean of five plants) and *Tudei* (experiment 5)

| Age | Months | DMY 1 | DHK 2 | Y 3 | K 4 | DHM 5 | M 6 | KL % |
|---|---|---|---|---|---|---|---|---|
| *Borogu* | 10 | 13.04 | 20.74 | 21.80 | 24.16 | 10.41 | 9.84 | 3.84 |
| | 11 | 12.34 | 26.18 | 18.48 | 22.55 | 10.47 | 9.97 | 5.02 |
| | 12 | 11.81 | 28.82 | 15.85 | 24.09 | 9.44 | 9.97 | 4.38 |
| | 13 | 10.13 | 26.13 | 21.16 | 20.79 | 13.16 | 8.62 | 5.52 |
| | 14 | 9.30 | 25.14 | 19.75 | 20.38 | 11.66 | 13.78 | 6.68 |
| | 15 | 8.99 | 28.20 | 20.28 | 18.59 | 11.86 | 12.12 | 6.9 |
| | 16 | 8.99 | 23.46 | 21.42 | 21.99 | 11.66 | 12.49 | 7.54 |
| | 17 | 10.83 | 26.60 | 20.72 | 21.33 | 9.88 | 10.65 | 7.96 |
| *Tudei* | 27 | 7.09 | 25.16 | 16.12 | 22.58 | 13.53 | 15.48 | 15.5 |
| | 28 | 7.38 | 23.48 | 16.77 | 22.81 | 13.42 | 16.10 | 14.9 |
| | 29 | 9.57 | 26.60 | 15.42 | 20.74 | 12.76 | 14.89 | 18.8 |
| | 30 | 13.41 | 25.61 | 17.68 | 17.68 | 12.80 | 12.80 | 16.4 |
| | 31 | 9.40 | 26.49 | 17.09 | 20.51 | 12.82 | 13.67 | 11.7 |
| | 32 | 9.23 | 26.15 | 15.38 | 19.23 | 13.85 | 16.15 | 13.0 |
| | 33 | 11.54 | 23.08 | 18.46 | 20.00 | 11.54 | 15.38 | 13.0 |
| | 34 | 11.27 | 21.83 | 18.31 | 20.42 | 12.68 | 15.49 | 14.2 |
| | 35 | 11.11 | 25.64 | 17.10 | 20.51 | 11.11 | 14.53 | 11.7 |
| | 36 | 9.27 | 20.83 | 16.67 | 23.33 | 13.33 | 16.67 | 12.0 |
| | 37 | 10.00 | 22.14 | 16.43 | 22.14 | 13.57 | 15.71 | 14.0 |
| | 40 | 8.61 | 23.18 | 15.23 | 23.18 | 12.58 | 17.22 | 15.1 |
| | 43 | 9.33 | 22.67 | 15.33 | 21.33 | 14.00 | 17.83 | 15.0 |
| | 45 | 10.26 | 23.08 | 14.10 | 21.79 | 13.46 | 17.31 | 15.6 |
| | 47 | 10.07 | 26.85 | 15.44 | 19.46 | 14.77 | 13.42 | 14.9 |
| | 49 | 9.94 | 27.33 | 15.53 | 17.39 | 15.53 | 14.29 | 16.1 |
| | 51 | 9.40 | 27.52 | 14.77 | 16.11 | 16.78 | 15.44 | 14.9 |

27th and 51st months of growth. Surprisingly, and unlike farmers' claims, kavalactone content does not seem to increase between 27 and 51 months of age but rather to fluctuate around 14%. None of the six major kavalactones appears to significantly increase or decrease, while the plants are ageing. This is proven to be true for only one cultivar, *Tudei*, and for those environmental conditions (Exp. 4). Therefore, generalisation of the results should be cautious.

### *3.4. Organ of the plant*

The partitioning of the roots, stumps and basal stems indicate that kavalactone content is decreasing from the underground organs to the upper parts of the plant. Results from experiment 3 are presented in Table 5. They indicate that differences between the different organs are maintained independently of the age of the plant. Kavalactone content is always higher in the roots than in the stumps and higher in the stumps than in the basal stems. Differences between stumps and basal stems are, however, of a smaller proportion than differences between roots and stumps. Kavalactone content variation (CV%) in the three different organs is comparable: from 14.91% in the roots to 20.29% in the basal stems. This indicates that in each of the three organs, kavalactone content can fluctuate according to the period of harvest. The chemotype also appears to be variable according to the organ of the plant. In this particular case, cultivar *Tudei* produces chemotypes in the roots, stumps and basal stems that are respectively 243651, 243561 and 254361. The kavalactones DMY, Y, K and M are in decreasing proportions from the roots to the basal stems while DHK and DHM are in increasing proportions. Based on the value of the coefficients of variation, it appears that DMY is the most variable kavalactone.

Results of kavalactone content analyses obtained from roots, stumps and basal stems are represented in Fig. 2. After two years of age, kavalactone content does not appear to increase in a particular organ (i.e. in the roots). It rather fluctuates in the three organs analysed during this study and does not seem to be stocked in selective cell tissues. In most cases, kavalactone contents are correlated, they decrease in the basal stems when they decrease in the roots and vice versa. Kavalac-

Table 5
Chemotype variation according to the organ and the age (cultivar *Tudei*, experiment no. 5)

| Organ[a] | DMY 1 | DHK 2 | Y 3 | K 4 | DHM 5 | M 6 | Chemotype | KL |
|---|---|---|---|---|---|---|---|---|
| Roots | 9.82 | 24.57 | 16.23 | 20.54 | 13.44 | 15.40 | 243651 | 14.52 |
| CV% | 15.30 | 8.69 | 7.71 | 10.17 | 10.09 | 8.80 | | 12.74 |
| Stumps | 8.18 | 25.76 | 16.29 | 19.31 | 15.97 | 14.48 | 243561 | 11.36 |
| CV% | 18.21 | 7.93 | 9.89 | 7.73 | 8.79 | 7.06 | | 13.54 |
| Basal stems | 7.55 | 30.26 | 13.99 | 16.32 | 19.32 | 12.57 | 254361 | 8.85 |
| CV% | 16.94 | 15.81 | 18.78 | 12.44 | 13.68 | 13.45 | | 20.72 |

[a] Mean of 17 plants harvested from 27 to 51 months.

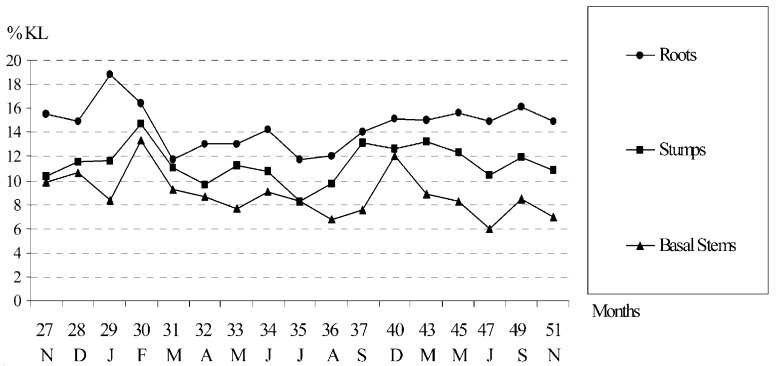


Fig. 2. Kavalactones content variation according to the organ (roots, stumps, basal stems) and age of the plant (experiment 5).

tone content might be correlated to seasonal factors (rainfall, temperature, and/or day length) but more data are needed to confirm this.

## 4. Discussion

The two major determinants of kava quality are the chemotype and the kavalactone content. Factors determining the levels of kavalactone content in the various organs of the kava plant have attracted interest from various researchers but in the absence of controlled experiments, results were preliminary. Using data produced by controlled field experiments, our study could not reveal any significant differences in chemotype or kavalactone content according to the place where the root sample is collected from the kava plant. We have demonstrated that sampling of a given organ is reliable and independent of the geographical direction of the plant. This information allows comparison of samples taken from different plants at different locations, as far as the cultivar and the age of the plant are accurately known. In our study, the results obtained from experiments nos. 1, 4 and 5, which were conducted in controlled environments, allow reliable interpretation of the results obtained from experiments 2 and 3, which were conducted in "on-farm" conditions throughout the archipelago of Vanuatu.

The present study demonstrates that vegetative propagation of kava allows the reproduction of a mother plant chemotype when its clones are planted and grown at the same location (experiment no. 1) but also in different locations (experiment no. 3). It is confirmed that chemotype is genetically controlled and that the cultivar is the most important factor determining this important trait (Lebot and Lévesque, 1996b). It has been found that chemical composition is variable according to geographical origin. For example, root samples of kava from Vanuatu contained a high quantity of dihydrokavain and kavain, whereas samples from Fiji contained a majority of methysticin (Lebot and Lévesque, 1989; Singh, 1999). Our study con-

firms that there is significant cultivar variation within and between islands for chemotype (Table 2).

Kavalactone concentration has already been shown to vary with geographic source (Lebot and Lévesque, 1989; Lebot et al., 1999). Some cultivars produce more kavalactone than others do when planted in the same garden. Since chemotype is genetically controlled, it was suspected that geographical variation in kavalactone content was caused by differences between cultivars found on different islands rather than between-island differences in growth due to diverse environmental factors (i.e. soils, rainfalls). Our study confirmed that there is significant cultivar variation within and between islands for kavalactone content, but this content also varies according to location for a given cultivar. This is revealed by comparing the performances of the plants of the same cultivar (*Borogu*) having the same age but grown in different locations (experiment no. 3). It is now possible to conclude that the geographical factor is more important than the genetic factor in determining kavalactone content, and that the growing location appears to play a significant role.

For a given cultivar, grown in a particular location, it appears that kavalactone content will progressively increase during the first 18 months of vegetative growth but will remain fairly constant after two years. It might be possible that there are seasonal factors responsible for the limited variation but more data are needed to confirm this. Experiments 4 and 5 have also shown that percentages of dry matter and overall yield increase over time (data not presented here). Farmers claim that an older kava gives a stronger physiological effect when the beverage is prepared from the fresh roots. They are probably right, however, this does not result from an increase of the total kavalactone content. It is rather related to the increase of the dry matter ratio which produces a less diluted concentration for the same amount of water added to the same volume of fresh roots when the beverage is prepared in a traditional manner.

Two previous studies have found that the concentration of the kavalactones kavain, demethoxyyangonin and yangonin are higher in the roots than in the stems and leaves (Smith, 1983; Smith et al., 1984). Conversely, dihydrokavain, methysticin, tetrahydroyangonin and dihydromethysticin are in lower proportions in the roots than in the stems and the leaves. Our study has shown that kavalactone content and chemotype vary according to the organ of the plant and that these differences are independent of the age of the plant. It is also confirmed that total kavalactone concentrations are highest in the roots and stumps and progressively decrease towards the aerial portions of the plant. We observe a decrease from 3 to 5% from the roots to the stumps and between 2 and 3% from the stumps to the basal stems. It appears that differences in chemotype and kavalactone content between the organs of the plant (roots, stumps and basal stems) are maintained while the plant is ageing (Fig. 2).

The quality of the local beverage, and/or the dry matter exported to the nutraceutical and pharmaceutical markets, are highly variable and this variation is a major constraint to quality control. The selection of the cultivar, its organ and the geographical area of its cultivation are factors contributing to quality control. There is potential for developing a system to protect kava varieties by defining quality in terms of their origin and chemical characteristics. The system could incorporate the

notion of "terroir" and everything that can have an effect on the quality: soil, climate and cultural practices. In order to be able to detail so-called appellations of origin for kava, information is needed on indigenous knowledge, delimitation of the area of cultivation, identification of cultivars specific to particular islands and the determination of the effect of local growing practices on different cultivars, in terms of chemotypes and yields.

## Acknowledgements

Financial support for this study was provided by VARTC, the French Aid Programme in Vanuatu and the French Ministry for Science and Technology which funded a Ph.D. scholarship for Patricia Siméoni. We thank Professor Bill Aalbersberg, Director of the Institute of Pure and Applied Sciences, University of the South Pacific, Suva, and Sarabjeet Singh, graduate student, for carrying out most of the HPLC work.

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