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Essential Oil Variability in Natural Hahadjerine Population of *Cupressus dupreziana* in Tassili n' Ajjer (Algeria)

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Abstract

Essential oils extracted from dried leaves of *Cupressus dupreziana* A. Camus, an endemic species in the Tassili n'Ajjer (Sahara Central Algeria), were analysed by gas chomatography coupled to mass spectrometry (GC-MS). Terpinoid analyses were performed on 13 trees in the natural population of Hahadjerine in order to determine the intra-population variability. 39 trepenoids were identified; the averages of the principal components were trans-totarol (24.4%), Manoyl oxide (21.2%), α-pinene (15%) and Δ^3-carene (11.3%). The terpenoid markers used made it possible to determine the individual patterns of chemotypic variability. This variability confirms that genetic factors are not solely responsible for the decrease in the numbers of this species.

Keywords: *Cupressaceae*; *Cupressus dupreziana*; Genetic variability; Tassili n'Ajjer; Terpenoïds

Introduction

Tassili n'Ajjer is an ecosystems significant and fragile, situated in the south arid zones of Algeria (Sahara central). The Tassili n'Ajjer (National Park) protected area covers a total area of 72.000 km^2. The climate is hyper-arid, characterised by extreme meteorological variability and uncertainty. Mean annual rainfall ranges from 20mm to 100mm, with marked variations across years and seasons. Precipitation may be absent for several years at a given location. The absolute temperatures may range from -7°C to 50°C. The biodiversity inventory is far from complete and data on the distribution and status of most taxa require urgent updating.

The *Cupressus*, family Cupressaceae, is represented in Algeria by one widely endemic species found in the south of the country, namely in Tassili n'Ajjer. *C. dupreziana* A. Camus is a rare plant, which occurs in very dry regions, and is listed by the International Union for the Conservation of Nature and Natural Resources as an endangered species [1]. The climatic conditions, as well as human activities, seem to be the main factors reducing the diversity of this species.

A critical botanical review of this species has been made by Barry et al. [2]. Phytochemical investigations of *C. dupreziana* have been carried by [3-7]. The study on seven French cultivars of *Cupressus* gives an interesting essential oil composition including α-pinene, Δ^3-carene, sabinene, limonene and α-cadinol [8].

The dominant compound of *C. sempervirens* essential oil is the α-pinene [9]. The dominance of α-pinene, in *C. sempervirens*, is confirmed in addition to the sabinene and terpinene-4ol [10]. The α-pinene and Δ^3-carene are the major components of *C. dupreziana* [11-12]. The analysis of the samples of botanical garden of Algiers shows that *C. dupreziana* and *C. sempervirens* have the same major compounds, α-pinene and Δ^3-carene [11]. Several genetic studies on the species are currently being conducted [13-16].

The aims of study were linked to provide data that have always been rare; provide information on the chemical composition and search for variability within populations; fill gaps on natural populations of this species, because most previous studies have used botanical gardens samples.

Materials and Methods

Plant material

The aerial parts of *C. dupreziana* were collected from the Tassili n'Ajjer in Hahadjerine locality in april 2009. A voucher specimen is deposited in the Herbarium of the Sciences Faculty of Ferhat Abbas University (Algeria). Leaves and branches were dried at room temperature for 7 days, and used for analyses. The study is based on the analysis of a random sample of green branchlets of 13 trees from Hahadjerine population in Tassili n'Ajjer (Figure 1).

Essential oil analysis

The essential oils were extracted by hydrodistillation of dried plant material using a Clevenger-type apparatus for 3 h. The oils were stored in sealed glass vials at 4-5°C prior to analysis. Yield based on dry weight of the sample was calculated. The essential oil were analysed on a Hewlett-Packard gas chromatograph Model 5890, coupled to a Hewlett-Packard MS model 5871, equipped with a DB5 MS column (30m X 0.25mm; 0.25µm), programming from 50°C (5 min) to 300°C at 5°C/mn, 5 min hold. Helium as carrier gas (1,0ml/min); injection in split mode (1: 30); injector and detector temperature, 250 and 280°C respectively. The MS working in electron impact mode at 70 eV; electron multiplier, 2500 V; ion source temperature, 180°C; mass spectra data were acquired in the scan mode in *m/z* range 33-450.

The compounds assayed by GC in the different essential oils were identified by comparing their retention indices with those of reference

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compounds in the literature and confirmed by GC-MS by comparison of their mass spectra with those of reference substances [17-19].

Statistical analysis

16 terpenoïds were obtained in sufficiently large quantities to be able to perform statistical analysis [20]. Data were first subjected to Principal Components Analysis (PCA) to examine the relationships among the terpenes compounds and identify the possible structure of the population. Cluster analysis (UPGMA) was carried out on the original variables and on the Manhattan distance matrix to seek for hierarchical associations among the populations. Statistical analyses were carried out using STATISTICA 9 software.

Results

This study included the natural Hahadjerine population, with 13 trees, in Tassili n'Ajjer. The average oil yield of the different trees was found to be 0.3%. The composition of the oils from the trees of Hahadjerine population differed only quantitatively. The general chemical profiles of the tested oils and the percentage content of the individual compounds are summarized in Table 1.

39 compounds were separated by GC-MS and characterized, with varied concentrations, particularly trans-totarol (19.1-33.5%), Manoyl oxide (14.1-26%), α-pinene (12.4-19.7%) and Δ^3-carene (8-17.7%). The average of the remaining compounds is low, such as germacrene-D (5.3%), Ferruginol (5.2%), cis-totarol (4.5%), isopimaradiene (2%) and 2-hydroxy-12-methoxy-19 -norpodocarpa-4,8,11,13-tetra-3-one (1.3%). Analyses revealed the presence of intra-population variability. The components identified (α-pinene, Δ^3-carene, germacrene-D, manoyl oxyde, cis and trans-totarol and ferruginol) show significant terpinoids variability (Figure 2). The compounds found in the Hahadjerine cypress generally resembled those previously reported to occur in some of cypress species [22-25]. In particular, α-pinene and Δ^3-carene were present in high relative levels. The only exception was the tree 11 which contains a low rate of Δ^3-carene, similar rate observed in several North American species of Cupressus [26].

The principal component analysis (PCA) performed on the correlations between the 16 variables presented three axes comprising

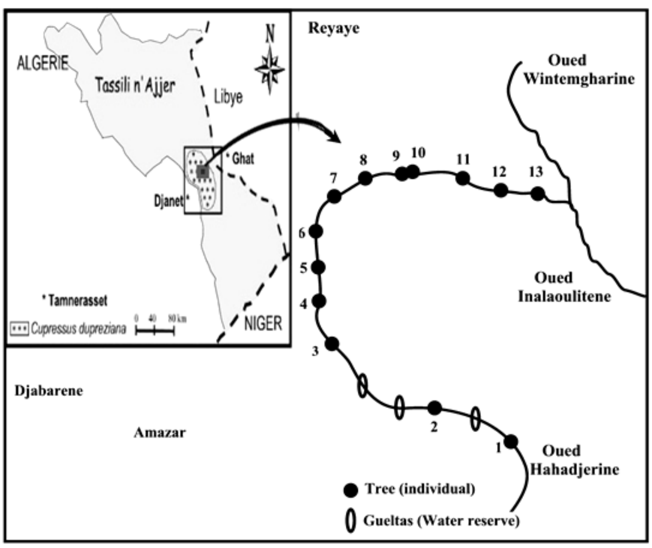

Figure 1: Location of individuals of *Cupressus dupreziana* in the Hahadjerine Wadi of Tassili n'Ajjer.

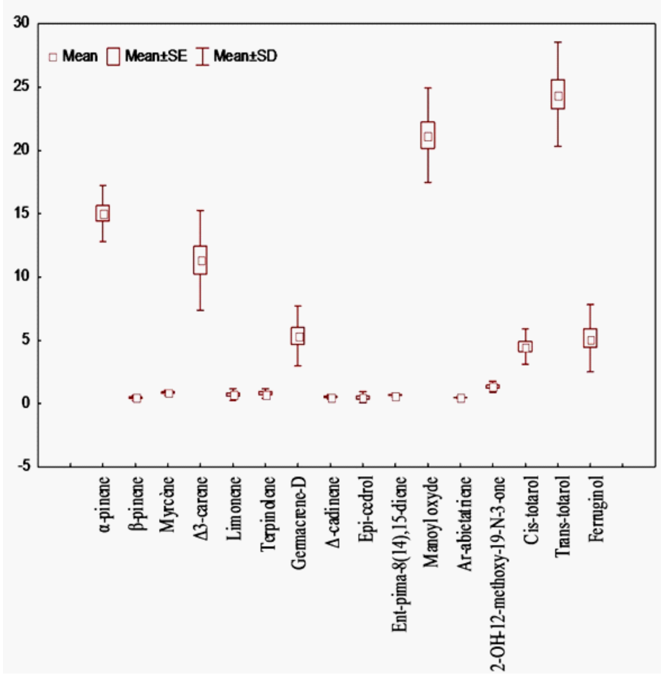

Figure 2: Chemical Variability of main compounds of *Cupressus dupreziana*.

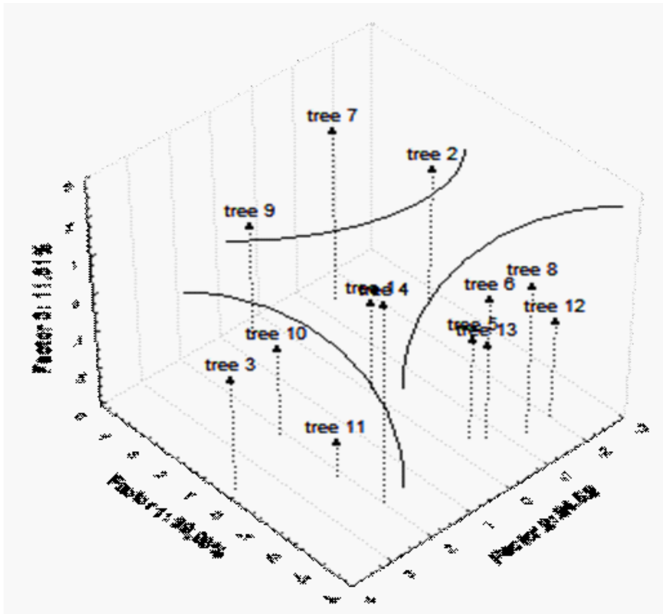

Figure 3: Ordination of the first three principal axes of Hahadjerine population.

76.19% of the total variation present in the original data. This analysis clustered populations in several groups, but the separation of populations is not clear. The ordination of population's means obtained for the three vectors is shown in (Figure 3).

All individuals of this population have showed high α-pinene and trans-totarol levels and low quantitative variations in all their components. Mono and sesquiterpenoids variability reflects the heterogeneity of the genetic structure of Hahadjerine population [21].

Genetic analyses were carried out using 16 terpinoids including

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Compounds	Trees													Moy
	1	2	3	4	5	6	7	8	9	10	11	12	13	
α-pinene	13.0	8.1	9.7	9.5	11.1	10.6	11.7	9.8	11.7	9.8	14.2	11.2	15.8	11.2
Fenchene	0.2	0.2	0.2	0.1	0.2	0.2	0.4	0.2	0.3	0.2	0.1	0.2	0.2	0.2
Sabinene	0.2	0.0	0.2	0.1	0.1	0.1	0.2	0.1	0.2	0.2	0.1	0.0	0.1	0.1
β-pinene	0.4	0.3	0.4	0.4	0.4	0.4	0.4	0.3	0.4	0.4	0.5	0.0	0.0	0.3
Myrcène	0.7	0.5	0.6	0.6	0.6	0.6	0.9	0.6	0.9	0.6	0.7	0.5	0.5	0.6
Δ^3-carene	8.6	8.6	7.5	6.1	7.1	7.7	13.6	7.9	12.6	7.3	4.1	6.6	10.4	8.3
Para cymene	0.1	0.4	0.1	0.0	0.0	0.0	0.2	0.6	0.2	0.1	0.0	0.0	0.1	0.1
Limonene	0.7	0.1	0.5	0.4	0.4	0.4	1.2	0.1	1.2	0.7	0.4	0.3	0.6	0.5
β-phellandrene	0.1	0.0	0.1	0.1	0.0	0.0	0.2	0.0	0.2	0.1	0.1	0.1	0.2	0.1
Terpinolene	0.5	0.6	0.9	0.6	0.3	0.3	1.1	0.4	1.0	0.7	0.5	0.4	0.5	0.6
Terpinene-4-ol	0.0	0.2	0.2	0.0	0.0	0.0	0.4	0.0	0.4	0.2	0.1	0.1	0.1	0.1
α-terpineol	0.0	0.0	0.1	0.0	0.0	0.0	0.5	0.0	0.5	0.2	0.0	0.0	0.0	0.1
Terpinen-4-yle acetate	0.0	0.1	0.1	0.0	0.0	0.0	0.3	0.0	0.3	0.1	0.0	0.0	0.1	0.1
α-terpenyle acetate	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2	0.0	0.5	0.0	0.0	0.1
β-caryophyllene	0.2	0.1	0.3	0.2	0.3	0.3	0.3	0.2	0.3	0.3	0.0	0.3	0.4	0.2
α-humulene	0.2	0.1	0.3	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.0	0.3	0.3	0.2
γ-muurolene	0.2	0.3	0.1	0.1	0.3	0.3	0.4	0.3	0.3	0.1	0.0	0.3	0.5	0.2
Germacrene-D	2.5	1.7	5.0	4.4	2.9	2.5	3.5	2.3	4.5	4.7	9.9	4.3	4.7	4.1
α-muurolene	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.2	0.2	0.2	0.1
γ-cadinene	0.2	0.2	0.1	0.1	0.3	0.2	0.4	0.2	0.3	0.1	0.1	0.2	0.3	0.2
Δ-cadinene	0.3	0.4	0.2	0.2	0.5	0.4	0.5	0.4	0.4	0.3	0.4	0.5	0.7	0.4
Caryophyllene oxyde	0.1	0.0	0.0	0.0	0.2	0.2	0.2	0.2	0.2	0.1	0.0	0.1	0.1	0.1
Epi-cedrol	0.2	0.4	0.2	0.1	0.0	0.3	1.1	0.3	1.0	0.4	0.1	0.3	0.3	0.4
Torrilenol	0.2	0.2	0.2	0.2	0.3	0.3	0.3	0.3	0.3	0.2	0.1	0.3	0.3	0.2
β-acorenol	0.0	0.1	0.0	0.0	0.0	0.1	0.4	0.1	0.4	0.1	0.0	0.1	0.2	0.1
Epi-α-cadinol	0.0	0.1	0.0	0.0	0.1	0.1	0.2	0.0	0.2	0.0	0.2	0.1	0.2	0.1
Epi-α-muurolol	0.0	0.0	0.1	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.4	0.1	0.1	0.1
α-cadinol	0.0	0.0	0.2	0.2	0.4	0.3	0.2	0.3	0.2	0.2	0.8	0.2	0.3	0.3
Eudesma-4(15),7-dien-3-β-ol	0.1	0.1	0.1	0.0	0.3	0.3	0.2	0.3	0.2	0.1	0.1	0.2	0.2	0.2
Eudesma-4(15),7-dien-1-β-ol	0.2	1.2	0.5	0.1	0.2	0.2	0.1	0.2	0.1	0.1	0.2	0.2	0.2	0.3
Ent-pima-8(14),15-diene	0.4	0.2	0.4	0.7	0.6	0.7	0.5	0.7	0.4	0.4	0.6	0.7	0.5	0.5
Manoyl oxyde	13.2	10.7	10.2	17.7	18.7	20.0	15.6	20.3	12.1	12.2	15.6	21.3	21.0	16.0
Isopimaradiene	1.3	1.0	0.9	1.6	1.8	2.0	1.5	2.2	0.9	0.9	1.5	2.3	1.8	1.5
13-epimanoyl oxyde	0.2	0.1	0.1	0.2	0.2	0.2	0.2	0.3	0.1	0.1	0.2	0.2	0.2	0.2
Ar-abietatriene	0.4	0.2	0.3	0.4	0.4	0.4	0.3	0.5	0.3	0.3	0.4	0.4	0.4	0.4
2-hydroxy-12-methoxy-19-norpodocarpa-4, 8,11,13-tetra-3-one	0.8	0.9	0.7	1.0	1.2	1.4	1.0	1.4	0.6	0.4	1.0	1.7	1.0	1.0
Unk 01	2.7	1.3	5.5	5.2	3.2	3.2	2.6	3.3	3.2	3.6	4.3	3.0	3.0	3.4
Totarol	14.5	10.6	20.5	25.6	22.8	21.7	15.0	19.1	14.3	16.2	21.0	20.8	17.7	18.4
Ferruginol	3.7	0.6	5.6	0.4	6.0	5.6	0.4	4.9	4.1	5.2	5.7	4.7	4.5	3.9
Unk 02	1.4	1.0	2.2	4.3	3.2	3.1	1.7	2.7	1.4	2.1	4.1	3.4	0.0	2.3
Unk 03	2.2	35.0	14.0	5.2	7.0	5.0	6.7	8.5	11.7	24.2	6.2	6.7	0.0	10.2
Total														87.4

Table 1: Chemical composition of essential oils of Hahadjerine population of *Cupressus dupreziana*.

some compounds that have been shown in other species of *Cupressus* to be under the control of single locus with two alleles. The dendrogram based on UPGMA clustering (Manhattan distance), shows the presence of many groups (Figure 4) that confirms result obtained from ACP analyses.

The first group formed by individuals (1, 13, 2, 7 and 9), rich in myrcene, Δ^3-carene, limonene, terpinolene and manoyl oxyde. The second group is divided into two groups, one formed by individuals (3, 10 and 11) rich in cis-totarol and germacrene-D, while the other includes individuals (4, 5, 6, 8, and 12) characterized by components manoyl oxyde and trans-totarol. Aggregation of Hahadjerine population trees into small groups is an indication of terpenoids variability in this population. The diversity of the terpinoids contents

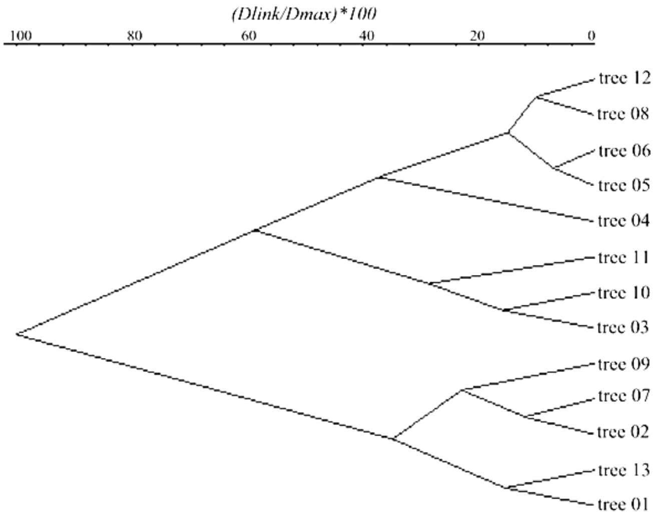

Figure 4: Dendrogram based on Manhattan similarity distance.

reflects the existence of considerable genetic variability [27,28]. The UPGMA analysis of terpene traits confirms this variability, but no clear conclusions can be transmitted between individuals' geographic distribution and genetic structure.

In brief, essential oils analysis carried out on 13 individuals of *C. dupreziana* showed both intra-population variability in their terpenoid content, with abundance of trans-totarol, Manoyl oxide, α-pinene, Δ^3-carene and germacrene-D.

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