Asian Journal of Plant Sciences 8 (1): 59-63, 2009
ISSN 1682-3974

Foliar Sesquiterpene Variations in Natural Populations of *Cupressus dupreziana* in Tassili N'Ajjer (Algeria)

M. Ramdani and T. Lograda
Laboratory of Natural Resources Valorisation, Ferhat Abbas University,
19000 Setif, Algeria

Abstract: *Cupressus dupreziana* A. Camus (Cupressaceae) is an endemic species in the Tassili n'Ajjer (Algerian Central Sahara). Terpenoid analysis were carried out on 160 trees of 12 natural populations with a view to determining the intra-specific variability. Eighteen sesquiterpenes were detected, including germacrene-D, which was found to be particularly abundant. The terpenoid markers used made it possible to determine the individual patterns of chemotypic variability. The variability of the sesquiterpene composition confirms that genetic factors were not responsible for the decrease in the numbers of this species. The main reason for this decrease is probably the desertification of the Tassili n'Ajjer.

Key words: Cupressaceae, *Cupressus dupreziana*, sesquiterpenoids, genetic variability, Tassili n'Ajjer

INTRODUCTION

Cupressus dupreziana is an endemic species growing on the Tassili n'Ajjers plateau. Although the plateau is hyper arid, the sub arid microclimates pertaining in some places have enabled relics of Mediterranean flora to survive. Mean annual rainfall is 30 mm and mean annual temperature 20.3°C at an altitude of 1100 m (Benhouhou *et al*., 2005). The plateau tends to be highly exposed and arid, although there are some patches with high humidity levels (gueltas).

Several aspects of *Cupressus* have been studied from the phytochemical and genetic points of view (Holeman *et al*., 1990; Cool *et al*., 1998; Cowan *et al*., 2001; Rushforth *et al*., 2003; Chéraif *et al*., 2007). Particular attention has been paid to sesquiterpene (Kim *et al*., 1994; Rafii *et al*., 1992; Cool, 2005; Piovetti and Diara, 1977; Piovetti *et al*., 1980, 1981; Pichot *et al*., 2008). Very little information is available so far, however, about the natural populations of *Cupressus dupreziana* growing in this region. Most studies on Tassili cypress have dealt with specimens of trees planted in botanical gardens. Biochemical studies have deals only with the isolation and identification of terpenoid composites and lipid components (Piovetti *et al*., 1981, 1982; Pauly *et al*., 1983). Studies on the essential oils have shown the presence of 13 sesquiterpenoids, most of which are thought by the researchers to be artefacts (Piovetti and Diara, 1977). A critical review of the botanical studies has been published by Barry *et al*. (1970) and several genetic studies on the species are currently conducted by El-Maataoui *et al*. (1998), Pichot *et al*. (1998), Pichot and El-Maataoui (2000), Pichot *et al*. (2000, 2001) and Ramdani *et al*. (2007).

The terpene composition gives some important information about the variability of the population's *in-situ*. Terpene variability is determined by genetic as well as ecological factors (Hanover, 1966; Squillace, 1976; Loveless and Hamrick, 1984; Yazdani *et al*., 1985; Baradat and Yazdani, 1988).

The availability of better GC techniques and multivariate statistical procedures led us to re-examine the foliage terpenoid contents of native stands from Tassili n'Ajjer, as well as to attempt to obtain a better picture of their chemistry and genetic relationships.

MATERIALS AND METHODS

Plant material: The plants were authenticated by the staff of the Laboratory of Natural Resource Valorization by comparison with herbarium specimens. Voucher specimens are deposited in the herbarium of the Biology Institute of the University of Ferhat Abbas, Algeria.

The present study is based on the analysis of a random sample of green branchlets of 160 trees from populations from twelve sites in Tassili n'Ajjer, as show in Fig. 1.

The site characteristics of the *C. dupreziana* sampled are given in Table 1. The green branchlets of natural populations of *C. dupreziana* and from a planted stand in

Corresponding Author: M. Ramdani, Laboratory of Natural Resources Valorization, Ferhat Abbas University, 19000 Setif, Algeria Fax: 213 36 91 51 22

Table 1: Description of *Cupressus dupreziana* populations sampled

Populations	Longitude (E)	Latitude (N)	Elevation (m)	No. of trees	Ecology
Ineledje	9° 28'	24° 58'	1600	09	Humid gritty Oueds
Tidedje	9° 23'	24° 59'	1600	09	
Tassendjout	9° 27'	24° 29'	1600	09	
Tamrit	9° 38'	24° 39'	1600	20	Gritty or craggy Oueds, rare gueltas
Tifetest	9° 44'	24° 32'	1600	10	
Tichwinet	9° 46'	24° 25'	1600	05	
Ingharouhane	9° 47'	24° 29'	1550	30	
Amazar	9° 49'	24° 28'	1560	30	Rocky and dry Oueds
Tinharwida	9° 47'	24° 28'	1520	13	
Tintarout	9° 24'	24° 58'	1650	05	Dry gritty or craggy Oueds
Tarout	9° 25'	24° 58'	1620	05	
Djanet	9° 30'	24° 33'	1100	15	Humid gritty soils

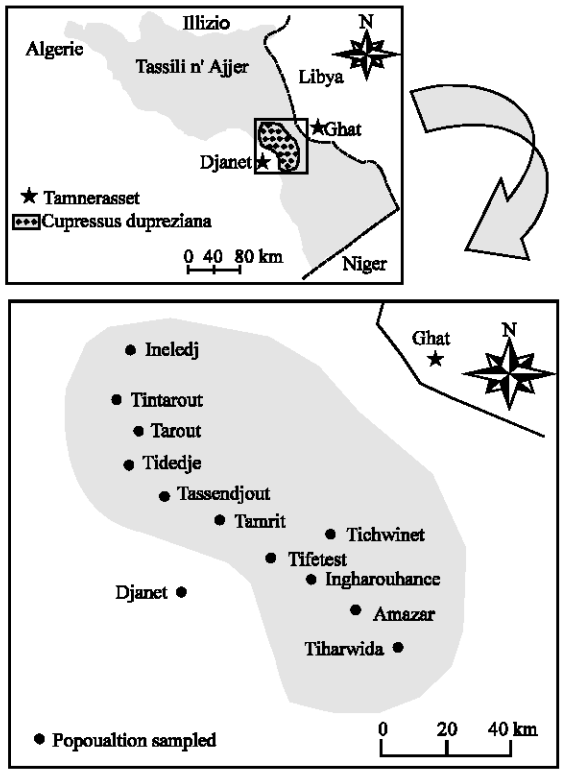

Fig. 1: Distribution of *Cupressus dupreziana* and sampling sites in Tassili n'Ajjer (central Sahara of Algeria)

Djanet were collected in April 2005. The air dried materials were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus.

G-C analysis: The oils were analyzed by GC on a Hewlett-Packard 5890 GC series II, equipped with FID, fitted with a SE-54 capillary column (25 m×0.25 mm; 0.25 μm film thickness). The method of analysis is largely explained by Ramdani *et al.* (2007).

Statistical analysis: The following sesquiterpenes were obtained in sufficiently large quantities to be able to perform statistical analysis (Baradat and Yazdani, 1988): germacrene-D, γ-cadinene, β-caryophyllene, α-humulene, γ-muurolene, δ-cadinene, 4-α-hydroxy germacrene, cedrol, δ-cadinol, β-bourberene and α-muurolene. Data were first subjected to Principal Components Analysis (PCA) to examine the relationships among the sesquiterpene components and identify the possible structure within and among populations. 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 analysis were carried out using STATISTICA 7 software.

RESULTS AND DISCUSSION

Despite the limited resources available and the difficulty of sampling the widely scattered populations of *Cupressus dupreziana* in Tassili n'Ajjers, this preliminary study included 12 natural populations of each of the most accessible individuals and a planted stand growing in the Djanet gardens. The identified sesquiterpenoids accounted for 85% on average of the total sesquiterpenoid weight analysed.

They are common compounds, such as germacrene-D (68.4%), γ-cadinene (5.0%), β-caryophyllene (4.3%), α-humulene (3.5%), γ-muurolene (3.3%), δ-cadinene (3.1%), 4-α-hydroxy germacrene (2.3%), cedrol (2.1), δ-cadinol (1.5%), β-bourberene (1.1%) and α-muurolene (1.1%). Further analysis revealed that 11 sesquiterpenoids with lower rate (<1%) (Table 2), indicating that with and between populations variability. Terpenoids markers allowed detecting the presence of chemotypic variations in most of the sesquiterpenoids except for β-bourberene, α-humulene and germacrene-D.

Results of principal components analysis: Principal Components Analysis (PCA) performed on the correlations between the 11 variables yielded three eigenvectors accounting for 86% of the total variation presents in the original data. The first two of these

Table 2: Means of foliar sesquiterpenes of *Cupressus dupreziana* in percent of total sesquiterpenes

	Populations											
Compounds	Tifetest	Tinabou	Amazar	Ingharouhane	Tichwinet	Ineledje	Tintarout	Tassendjout	Tarout	Tamrit	Tidedje	Djanet
α-copaene	0.4	0.5	0.5	0.5	0.4	0.5	0.3	0.4	0.3	0.4	1.3	1.3
β-bourbonene	0.7	0.9	0.8	0.8	0.6	1.0	0.3	0.9	0.5	0.8	3.8	2.4
β-cubebene+elemene	0.4	0.4	0.4	0.3	0.3	0.4	0.3	0.3	0.3	0.2	0.2	0.3
β-funibrene	0.8	0.8	0.5	0.6	0.4	0.8	0.2	0.6	0.2	0.8	0.2	1.1
β-caryophyllene	4.6	5.0	4.7	4.5	4.5	4.7	4.2	4.5	4.3	4.4	3.5	3.3
β-copaene	0.5	0.7	0.5	0.4	0.3	0.4	0.2	0.4	0.3	0.3	0.2	0.2
α-humilene	4.0	4.2	4.0	4.0	4.0	3.7	3.7	3.8	3.7	3.8	0.4	2.7
γ-muurolene	2.7	3.8	4.4	3.1	2.8	4.8	1.9	3.8	2.8	3.0	3.1	3.2
Germacrene-D	69.3	64.7	65.2	70.0	72.3	61.3	77.5	67.7	74.5	71.0	67.5	59.4
γ-amorphene	0.6	0.8	0.9	0.6	0.5	1.2	0.7	1.1	0.5	0.5	0.7	0.7
α-muurolene	1.4	1.6	1.6	1.4	1.4	0.8	0.3	0.6	0.4	1.3	1.1	1.4
δ-amorphene	0.6	0.7	0.5	0.5	0.4	1.8	1.2	1.6	1.4	0.6	1.1	0.6
γ-cadinene	3.1	3.8	3.7	2.8	2.4	4.2	1.4	3.2	1.9	3.1	3.9	3.2
δ-cadinene	4.7	6.0	6.6	4.8	4.7	7.0	3.1	5.8	4.3	4.5	4.3	4.8
4 α-hydroxy germacradiene	2.5	2.1	2.2	2.9	3.0	2.2	2.2	1.8	1.9	2.4	3.2	1.8
Caryophyllene epoxyde	1.0	1.1	0.8	0.9	0.7	0.9	0.2	0.6	0.3	0.7	1.1	0.9
Cedrol	2.0	1.9	1.1	1.3	1.0	1.5	0.3	1.3	0.4	1.7	2.8	11.1
δ-cadinol	0.8	1.1	1.3	0.7	0.4	2.9	2.1	1.6	2.2	0.7	1.8	1.8

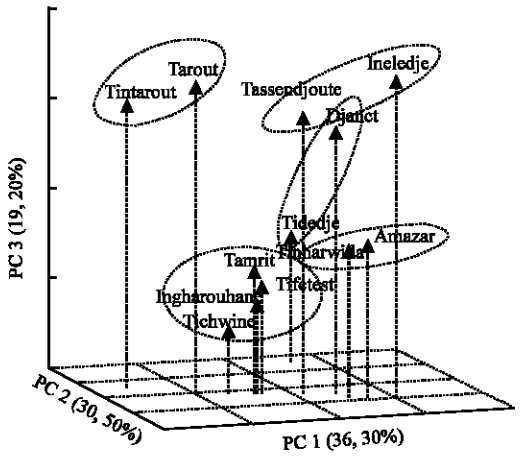

Fig. 2: Ordination of the first three principal axis of *Cupressus dupreziana* populations

eigenvectors explained respectively 36.3 and 30.5% of the total variation. The ordination of population's means obtained for these two vectors is shown in Fig. 2. This analysis clustered populations in several groups.

Samples obtained from the first group which concerns, Tamrit, Tifetest, Ingharouhane and Tichwinet, are characterized by high 4-α-hydroxy germacradiene levels. These populations are found in open craggy or gritty Oueds with some rare gueltas.

While in very dry gritty or craggy Oueds, populations of Tintarout and Tarout are clustered together indicating a specific group characterised by germacrene-D.

The third group that of, Tinharwida and Amazar, with high caryophyllene and α-humulene levels, are localised in dry rocky Oueds. But the fourth groups witch concerns the populations from Inelege and Tassendjoute, growing in humid, gritty soils, are characterized by γ-muurolene and γ-cadinene. Whereas Djanet planted stand population and Tidedje population of the Tassili, were found to be characterized by their bourborene and cedrol contents. All these populations showed high germacrene-D levels and low quantitative variations in all their components. Sesquiterpenoid variability reflects the heterogeneity of the genetic structure of *Cupressus dupreziana* populations.

The compounds found in the Tassili cypress generally resembled those previously reported to occur in some of cypress species (Zavarin *et al*., 1971; Rafii *et al*., 1992; Kim *et al*., 1994; Cool *et al*., 1998; Cool, 2005). In particular, germacrene-D and γ-muurolene were present in high relative levels. The one exception was the single population from Djanet, which has a cedrol content: similar chemotype has been observed in several North American *Cupressus* species (Rafii *et al*., 1992).

Results of cluster analysis: Genetic analysis were carried out using 11 sesquiterpens including 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 is shown in Fig. 3, confirms presence of the same three groups obtained from ACP analysis and emphasizes the distinctiveness of Djanet population.

This distinction is mainly based on the cedrol, germacrene-D, α-muurolene, caryophyllene and α-humulene levels. This component probably reflects the effects of environmental factors on these populations and shows how individuals have responded to ecological pressure (Yani *et al*., 1993).

The Djanet population was found to be very different, showing little similarity with the other

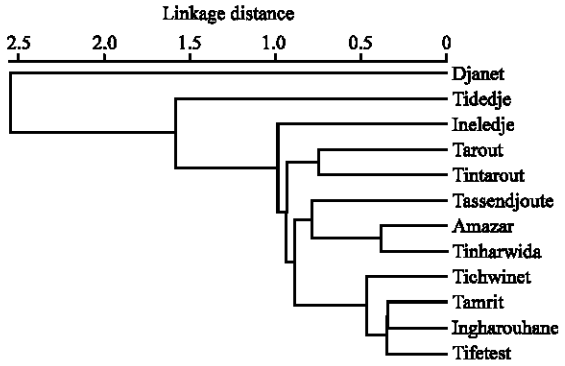

Fig. 3: Dendrogram based Manhattan similarity distance

populations. This population has the highest cedrol levels (11.10%). However, populations from Tassili n'Ajjer tray can be divided into two subgroups. These populations, exposed to difficult ecological conditions, namely: drought, extremely high temperature, poor soil and overgrazing, have particularly a low rate of cedrol (Yani *et al*., 1993). Aggregation of Tassili n'Ajjer populations into small groups is an indication of variability in this species sesqiterpenoids. The diversity of the sesquiterpene contents reflects the existence of considerable genetic variability (Forrest, 1980; Raddi and Sümer, 1999).

Genetic analysis of natural cypress populations, focusing on sesquiterpenoids, would provide a useful complement to morphological, fitness-related trait studies. Data on the genetic variability of species, based on their sesquiterpene contents, combined with information about the ecological requirements and micro evolutionary mechanisms, would be of great interest, since it would provide means of preserving and managing the natural populations.

The present analysis of *Cupressus dupreziana* showed the presence of considerable sesquiterpene variability within the cypress populations studied. The UPGMA analysis of terpene traits confirms this variability, but no clear-cut trends between geographical location and genetic structure can be identified on the basis of these findings.

The populations growing in Tassili n'Ajjer form a large reservoir of genetically diverse material. However, the cypress population growing in Djanet is the most highly differentiated one and has a considerable regenerative capacity. It would therefore be perfectly possible to conserve this species outside its natural territory. In the Tassili n'Ajjer itself, under the present climatic conditions, the preservation and conservation of the existing specimens may be possible, provided the harmful effects of anthropic activities are seriously reduced and controlled.

ACKNOWLEDGMENTS

The researchers was supported by Algerian MESRS. The researchers thank Jessica Blanc for the correction of the English version and OPNT (National Park Office of Tassili) and Tergui's guides (Lahbib, Wandoken and Ahmed Agha).

REFERENCES

Baradat, Ph. and R. Yazdani, 1988. Genetic expression for monoterpenes in clones of *Pinus sylvestris* grow on different sites. Scand. J. For. Res., 3: 25-36.

Barry, J.P., B. Belin, J.C. Celles, D. Dubost, I. Faurel and P. Hethener, 1970. Essai de monographie du *Cupressus dupreziana* A. Camus, cyprès endémique du Tassili des Ajjers (Sahara central). Bul. Soc. Hist. Nat. Afr. Nord, 61: 95-176.

Benhouhou, S., N. Boucheneb, F. Sahli, I. Yaou Adamou, 2005. Floristic and ecological characterisation of the tassili cypress. Secheresse, 16: 61-66.

Chéraif, I., H. Ben Jannet, M. Hammami, M.L. Khouja and Z. Mighri, 2007. Chemical composition and antimicrobial activity of essential oils of *Cupressus arizonica*. Greene Biochem. Syst. Ecol., 35: 813-820.

Cool, L.G., Hu. Zi-Ling and E. Zavarin, 1998. Foliage terpenoids of Chinese *Cupressus* species. Biochem. Syst. Ecol., 26: 899-913.

Cool, L.G., 2005. Sesquiterpenes from *Cupressus macrocarpa* foliage. Phytochemistry, 66: 249-260.

Cowan, S., B. Bartholomew, A.A. Watson, C. Bright, Z. Latif, D.S. Satyajit and J.N. Robert, 2001. Lignans from *Cupressus lusitanica* (Cupressaceae). Biochem. Syst. Ecol., 29: 109-111.

El-Maataoui, M. Pichot, C. Alzubi, C. and S. Grimaud, 1998. Cytological basis for a teteraspory in *Cupressus sempervirens* L. megametogenesis and its implications in genetic studies. Theor. Applied Genet., 96: 776-779.

Forrest, G.I., 1980. Genotypic variation among Native Scots pine populations in Scotland based on monoterpene analysis. Forestry, 53: 101-128.

Hanover, J.W., 1966. Environmental variation in the monoterpenes of *Pinus monticola* Dougl. Phytochemistry, 5: 713-717.

Holeman, M., M. Ait Igri and M. Berrada, 1990. *Cupressus atlantica*: A true species: 1. Hydrodistillation of essential oils of twigs and wood. 2. Petrol extract of wood. Plantes Médicinales et Phytothérapie, 24: 165-174.

Kim, Y.K., L.G. Cool and E. Zavarin, 1994. Cis-Calamenene related sesquiterpenoids from *Cupressus bakari* foliage. Phytochemistry, 36: 961-965.

Loveless, M.D. and J.L. Hamrick, 1984. Ecological determinants of genetic structure in plant populations. Ann. Rev. Ecol. Syst., 15: 65-95.

Pauly, G., A. Yani, L. Piovetti and C. Bernard-Dagan, 1983. Volatile constituents of the leaves of *Cupressus dupreziana* and *Cupressus sempervirens*. Phytochemistry, 22: 957-959.

Pichot, C., A. Borrut and M. El-Maataoui, 1998. Unexpected DNA content in the end osperm of *Cupressus dupreziana* A. Camus seeds and its implications in the reproductive process. Sex. Plant Reprod., 11: 148-152.

Pichot, C. and M. El-Maataoui, 2000. Unreduced diploid nuclei in *Cupressus dupreziana* A. Camus pollen. Theor. Applied Genet., 101: 574-579.

Pichot, C., B. Fady and I. Hochu, 2000. Lack of mother tree alleles in zymograms of *Cupressus dupreziana* A. Camus embryos. Ann. For. Sci., 57: 17-22.

Pichot, C., M. El-Maataoui, S. Raddi and P. Raddi, 2001. Surrogate mother for endangered *Cupressus*. Nature, 412: 39-39.

Pichot, C., B. Liens, J.L. Nava, I.B. Bachelier and M. El-Maataoui, 2008. Cypress surrogate mother produces haploid progeny from alien pollen. Genetics, 178: 379-383.

Piovetti, L. and A. Diara, 1977. Sesquiterpenes from *Cupressus dupreziana*. Phytochemistry, 16: 103-106.

Piovetti, L., G. Combaut and A. Diara, 1980. Monoterpenes et sesquiterpenes oxygenes du *Cupressus dupreziana*. Phytochemistry, 19: 2117-2120.

Piovetti, L., C. Francisco, G. Pauly, O. Benchabane and A. Diara, 1981. Volatile constituents of *Cupressus dupreziana* and the sesquiterpenes of *Cupressus sempervirens*. Phytochemistry, 20: 1299-1302.

Raddi, S. and S. Sümer, 1999. Genetic diversity in naturel *Cupressus sempervirens* L. Populations in Turkey. Biochem. Syst. Ecol., 27: 799-814.

Rafii, Z., L.G. Cool and E. Zavarin, 1992. Variability of foliar mono-and Sesquiterpenoids of *Cupressus Bakari*. Biochem. Syst. Ecol., 20: 123-131.

Ramdani, M., O. Rached, T. Lograda and A. Aggoun, 2007. Genetic diversity in foliar terpenoids among natural populations of *Cupressus dupreziana* in Tassili n'Ajjer (Algeria). Asian J. Plant Sci., 6: 1211-1216.

Rushforth, K., R.P. Adams, M. Zhong and Ma. X-qiang, 2003. Variation among *Cupressus* species from the eastern hemisphere based on Random Amplified Polymorphic DNAs (RAPDs). Biochem. Syst. Ecol., 31: 17-24.

Squillace, A.E., 1976. Analysis of Monoterpenes of Conifers by Gas-Liquid Chromatography. In: Modern Methods in Forest Genetics, Miksche, J.P. (Ed.). Springer Verlag, New York, pp: 120-157.

Yani, A., G. Pauly, M. Paye, F. Salin and M. Gleizes, 1993. The effect of a long-term water stress on the metabolism and emission of terpene of foliage of *Cupressus sempervirens*. Plant Cell Environ., 16: 975-981.

Yazdani, R., J.E. Nilson and T. Ericsson, 1985. Geographical variation in the relative proportion of monoterpenes in cortical oleoresin of *Pinus sylvestris* in Sweden. Silvae Genet., 34: 201-208.

Zavarin, E., L. Lawrence, M.C. Thomas, 1971. Compositional variations of leaf monoterpenes in *Cupressus macrocarpa*, *C. pygmaea*, *C. goveniana*, *C. abramsiana* and *C. sargentii*. Phytochemistry, 10: 379-393.