



Chemical and genetic diversity of two Mediterranean subspecies of *Teucrium polium* L.

Nassim Djabou^{a,b}, Alain Muselli^{a,*}, Hocine Allali^b, Mohammed El Amine Dib^b, Boufeldja Tabti^b, Laurent Varesi^c, Jean Costa^a

^a Université de Corse, UMR CNRS 6134, Laboratoire Chimie des Produits Naturels, Campus Grimaldi, BP 52, 20250 Corte, France

^b Université de Tlemcen, Laboratoire des Substances Naturelles et Bioactives, BP 119, 13000 Tlemcen, Algeria

^c Université de Corse, UMR-CNRS 6134 SPE, Laboratoire de Génétique Moléculaire, 20250 Corte, France

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ABSTRACT

Chemical and genetic diversity of *Teucrium polium* L. subsp. *polium* from western Algeria and *T. polium* L. subsp. *capitatum* from Corsica were investigated. Diversity within and among the two populations of subspecies was assessed according to the chemical composition of their essential oils and the genetic diversity. Chemical analysis was performed using a combination of capillary GC-RI and GC/MS after fractionation using column chromatography. Genetic structures were mapped using three polymorphic genetic markers: two chloroplast markers (*RPL32-TRNL* and *TRNL-F*) and ribosomal nuclear markers (ITS region). The statistical analysis showed that both subspecies were clearly distinguished by these chemical and genetic markers. The oil chemical compositions differed qualitatively and quantitatively between the subspecies. Both collective oils were dominated by hydrocarbon compounds however the Algerian sample oils exhibited higher amounts of hydrocarbon sesquiterpenes than those of Corsica (31.2 g/100 g vs. 4.4 g/100 g) while the latter displayed higher amounts of hydrocarbon monoterpenes than the first (59.3 g/100 g vs. 34.3 g/100 g). Neighbor-joining, Maximum likelihood and Bayesian trees constructed from chloroplast markers and nuclear ITS region sequences showed the existence of two groups associated with taxonomic and chemical characteristics. The study indicated that variation in the essential oil composition within subspecies depends on genetic background. The samples of subsp. *capitatum* from Corsica are a homogeneous group, in contrast to samples of subsp. *polium* from Algeria which were clustered in two groups. Chemical and genetic diversity of Algerian populations could be explained by geographical isolation of the populations. In addition, the morphological polymorphism observed throughout the colour of flowers could be explained by environmental parameters as well as the soil pH.

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1. Introduction

Teucrium is a polymorphic and cosmopolitan genus of perennial plants, the largest of the Lamiaceae family in the Mediterranean area, which comprise more than 300 species (Tutin et al., 1972). From a taxonomical point of view, the genus *Teucrium* has been divided into nine sections identifiable through the calyx shape and the inflorescence structure (Navarro and El Oualidi, 2000). Among them, *Polium* section, which includes about half of the species, has been subdivided into four subsections: *Polium*, *Simplicipilosa*, *Pumilium* and *Rotundifolia*. *Polium* section was considered as complex section owing to considerable morphological variations within and between populations and the instability of morphological characters. So it includes many poorly differentiated taxa with

probable recent origin through hybridization followed by polyploidization (El Oualidi et al., 1999, 2002). In North Algeria, 12 subspecies of *Teucrium polium* were described, among them *T. polium* subsp. *polium* was the most common and was widespread in Tell mountains (Baba Aissa, 2000; Quezel and Santa, 1963). In Corsica, only the subspecies *capitatum* was reported (Jeanmonod and Gamisans, 2007).

From a botanical point of view, an unusual feature of *Teucrium* genus compared with other members of Lamiaceae is that the flowers completely lack the upper lip of the corolla (De Martino et al., 2010). *T. polium* is a perennial and out-crossing species with prostrate stems, white to bright pink flowers, crenate leaves, and ramified indumentums. It shows a high variation both in morphological and ploidal levels (El Oualidi et al., 2002; Navarro and El Oualidi, 2000; Tutin et al., 1972). *T. polium* subsp. *polium* is most present in mountains of west Mediterranean region, with white to bright pink flowers, lateral corolla-lobes can be glabrous or hairy

* Corresponding author.

E-mail address: muselli@univ-corse.fr (A. Muselli).

with leaves less than 15 mm and flowering stems 10–25 cm, contrarily to subsp. *capitatum* who its lateral corolla-lobes are always hairy with white or grey hairs present on stems (Tutin et al., 1972). In north Algeria, we noted the presence of *T. polium* subsp. *polium* with two flower colors; specimens with pink flowers were localized in littoral and mountain regions while specimens with white flowers were only widespread in mountain regions (Baba Aissa, 2000; Quezel and Santa, 1963). *T. polium* subsp. *capitatum* grows wild in southern Europe, central and south-west Asia and North Africa. This plant is scarcely found in continental France, but grows in stony ground in Corsica. It is a perennial, pubescent, aromatic plant, 20–50 cm high, with green-grayish leaves and white flowers, which appear from June to August.

From the chemical point of view, the genus *Teucrium* is one of the richest sources of neoclerodane diterpenes: more than 220 diterpenes have been described, many of which are of interest because of their insect-repellent and medicinal properties (Bruno et al., 2003, 2004; Piozzi et al., 2005). It was demonstrated that neo-clerodane diterpenes are useful chemotaxonomic markers (Servettaz et al., 1991). Some neoclerodane diterpenoids with fungicide and insecticide activities (Bruno et al., 2003) and abeo-abietanes diterpenes as protective factors against oxidative stress (Fiorentino et al., 2010) were reported from *T. polium*. Essential oils of *Teucrium* genus have been the subject of many studies. We noted that according to the species studied, essential oil yields ranged from 0.05% to 1.5% and the amounts of main constituents (mono- and sesquiterpene hydrocarbons and oxygenated sesquiterpenes) differed notably (Awadh Ali et al., 2008; Saracoglu et al., 2007). Concerning *T. polium*, 22 studies established the chemical composition of oils extracted by hydrodistillation (Aburjai et al., 2006; Al-Qudah et al., 2011; Antunes et al., 2004; Ashnagar et al., 2007; Bezic et al., 2011; Cakir et al., 1998; Cozzani et al., 2005; De Martino et al., 2010; Eikani et al., 1999; Hammoudi and Hadj Mahammed, 2010; Hassan et al., 1979; Kabouche et al., 2007; Kamel and Sandra, 1994; Kovacevic et al., 2001; Menichini et al., 2009; Moghtader, 2009; Pérez-Alonso et al., 1993; Sarer and Konuklugil, 1987; Stanciu et al., 2006; Tomi and Casanova, 2006; Vahdani et al., 2011; Vokou and Bessiere, 1985). Table 1 reviewed the data of previous chemical investigations with the main components, their abundances and the corresponding references grouped by

same geographical origin. Whatever to the origin of the plant, the main components were hydrocarbon monoterpenes such as limonene, α - and β -pinene; hydrocarbon sesquiterpenes such as β -caryophyllene, γ -muurolene, γ -cadinene and germacrene D; and oxygenated sesquiterpenes such as α - and τ -cadinol, patchouli alcohol, caryophyllene oxide and 8-cedren-13-ol. To our knowledge essential oil from *T. polium* subsp. *polium* was never reported in literature.

Very few studies have been conducted on the genetic structure of *Teucrium* species and to our knowledge, only two studies deals with the genetic diversity of *T. polium*. El Oualidi et al. (1999) tested the utility of ITS sequences analysis for resolving relationships in *Teucrium* sect. *Polium*. The resultant phylogeny analysis showed a low correlation with traditional taxonomic classifications. Boulila et al. (2010) studied the genetic diversity among Tunisian population of *T. polium* based on RAPD markers. The population genetic structure was in accordance with that revealed using chemical variability and bioclimatic characteristics (Adams et al., 2003). Maintenance of a minimum level of intra- and inter-population genetic diversity is important for the genetic fitness and for developing conservation programs (Barret and Kohn, 1991).

In the course of the laboratory characterization of Mediterranean *Teucrium* species (Cozzani et al., 2005; Djabou et al., 2010, 2011, 2012; Muselli et al., 2009), the chemical composition and genetic diversity of *T. polium* L. subsp. *polium* harvested in three areas of Tlemcen province (western Algeria) was investigated for the first time. Owing to the morphological variations of *T. polium*, it was interesting to investigate specimens with white flowers (only localized in the mountain area 1) and specimens with pink flowers (localized in the littoral area 2 and in the mountain area 3) (Fig. 1). In the same time, the chemical composition of Corsican *T. polium* L. subsp. *capitatum* was reinvestigated and its genetic diversity was established for the first time. Corsican samples were considered as out-group for comparison purpose (Fig. 1). Environmental parameters were correlated to the chemical composition of essential oils and genetic diversity. Chemical analysis was performed using a combination of capillary GC-RI and GC/MS after fractionation using column chromatography. Genetic structure was mapped using two chloroplast markers (*RPL32-TRNL* and *TRNL-F*)

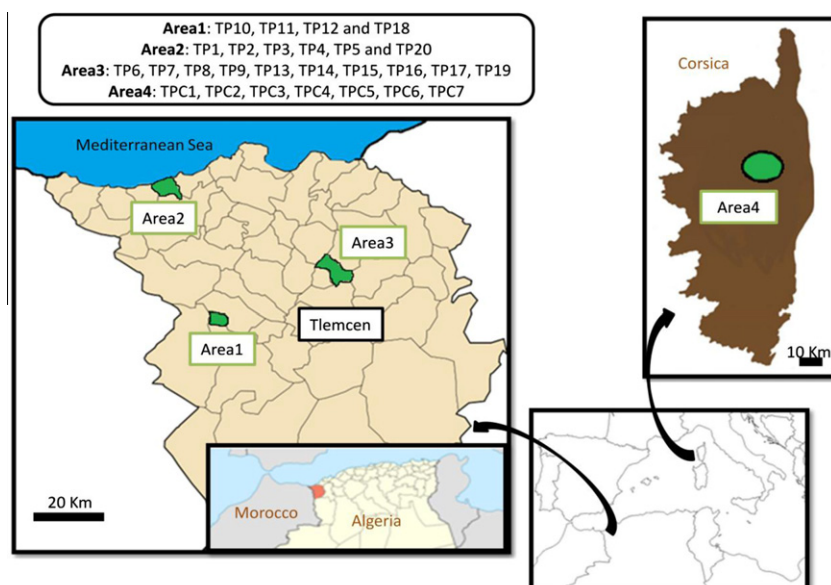


Fig. 1. Geographical distribution of *Teucrium polium* subsp. *polium* (TP1–20) from western Algeria and *Teucrium polium* subsp. *capitatum* (TPC1–7) from Corsica.

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