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Relationship between chemotypic and genetic diversity of natural populations of *Artemisia herba-alba* Asso growing wild in Tunisia

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ABSTRACT

A total of 80 individuals collected from eight populations growing wild in different geographic zones were considered to assess the intraspecific variability of essential oil composition, genetic diversity and population structure of *Artemisia herba-alba*.

The essential oil composition varied significantly between populations. Essential oil profiles were classified into four chemotypes (trans-sabinyl acetate, α-thujone/trans-sabinyl acetate, camphor and α-thujone/camphor/β-thujone). Despite significant correlation between the amount of some essential oil compounds and a set of climatic data, the global chemical divergence among populations was not related to their bioclimatic and geographic appurtenances.

A high level of genetic diversity within populations was revealed either with RAPD and ISSR markers ($Na = 1.67$, $PPL = 66.5\%$, $H = 0.26$, $I = 0.38$ and $Na = 1.7$, $PPL = 69.8\%$, $H = 0.26$, $I = 0.38$, respectively). The level of genetic diversity varied across populations and chemotypes. Populations from the α-thujone/trans-sabinyl acetate chemotype exhibited the highest genetic diversity as revealed by the RAPD markers. However, populations from α-thujone/camphor/β-thujone chemotype showed the important genetic variation determined by ISSR markers. A significant genetic differentiation among populations and among chemotypes was detected.

The combined analysis showed a significant correlation ($r = 0.484$, $p = .032$) between the chemical and molecular markers. The PCA, performed on percentages of major oil compounds and the frequencies of polymorphic RAPD and ISSR bands, divided populations according to their chemotypic classification.

Taking into consideration the current situation of *A. herba-alba* populations and their endangered habitats, these results are of value in order to ensure the in-situ and ex-situ conservation of this medicinal species.

1. Introduction

Artemisia herba-alba Asso. (*Asteraceae* family), a greenish-silver perennial shrub, occurs mainly in arid areas of the Middle East, North Africa, Spain and North Western Himalayas (Wang, 2004). The species is characterized by their rigid and erect stems (30–50 cm in height). Leaves are grey, alternate, 2–3 pinnatisected, ovate, covered with glandular hairs. Flowers are yellow and hermaphrodite. The flowering period extends from September to December. *A. herba-alba* is an outcrossed species, and pollination is mostly anemogamous. Fruits are small achenes mainly dispersed by wind and water. The species is mostly diploid ($2n = 2x = 18$), and may be tetraploid ($2n = 4x = 36$) (Ferchichi, 1997).

A. herba-alba is widely used in folk medicine against several diseases, such as diabetes, bronchitis, diarrhea and neuralgias (Tahraoui et al., 2007; Mohamed et al., 2010). The aerial part extracts were found to possess antioxidant and anticholinesterase activities. Apigenin derivatives and caffeoylquinic acids could be responsible of these activities (Younsi et al., 2016). The antioxidant, antibacterial, antileshmanial, anthelmintic, antispasmodic and anticholinesterase activities of *A. herba-alba* essential oils have

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been also reported (Mighri et al., 2010; Abu-Darwish et al., 2015; Younsi et al., 2016).

In Tunisia, *A. herba-alba* grows spontaneously in different geographic area and bioclimatic zones (from the upper semi-arid to the saharian), on calcareous, marneous, sandy clay and gypseous soils (Ferchichi, 1997; Nabli, 1989). At present, the species is relatively abundant only in the Southern regions. Nevertheless, it persists in degraded populations with low sample size in the Centre and the Northern West of the country. The destruction of natural habitats under anthropic pressure (i.e. urbanisation, overharvesting and overgrazing) decreases continuously the population size, reduces individual fitness, increases the random genetic drift and inbreeding and by consequence can lead to the decline of genetic diversity, the perturbation of population's dynamics, and the reduction of their adaptation to environmental changes.

The majority of studies performed on *A. herba-alba* have been focused on their secondary metabolites, and various bioactive compounds such as flavonoids, sesquiterpene lactones and essential oils have been isolated (Zouari et al., 2010; Khlifi et al., 2013; Bourgou et al., 2016). Despite ongoing research on the species, no published report is yet available on the genetic variation within and among *A. herba-alba* populations based jointly on combined data such as morphological, chemical and molecular markers.

Patterns of genetic diversity within and among populations are often used as an indicator of species responses to evolutionary forces operating within current and past environments. Among the diverse molecular markers successfully used to assess the genetic variability and population structure of plant species, RAPD (random amplified polymorphic DNA) and ISSR (inter-simple sequence repeats) markers are currently applied (Rahali et al., 2016; Yang et al., 2016; Baruah et al., 2017).

Despite their dominant inheritance, RAPD and ISSR involve a large number of loci and cover a large part of the genome. These DNA-based markers are highly sensitive to detect genomic polymorphism and do not require previous knowledge of the genome (Liu et al., 2015; Tiwari et al., 2016). Furthermore, RAPD and ISSR markers, used separately or combined, have proved to be efficient and inexpensive approaches to assess the genetic diversity within and among populations of several *Artemisia* species such as *A. annua* (Kumar et al., 2011), *A. capillaris* (Shafie et al., 2011), *A. herba-alba*, *A. monosperma* and *A. judaica* (Badr et al., 2012a, 2012b) and *A. dracunculus* (Karimi et al., 2015).

Accordingly, the aims of the present work, performed on Tunisian *A. herba-alba*, were to assess the level of interpopulational chemotypic variation based on essential oil composition and to study the genetic diversity and structure of populations based on RAPD and ISSR markers. This study was carried out in order to provide relevant information regarding the management and selection of interesting genotypes and chemotypes of high value from this medicinal plant.

2. Results and discussion

2.1. Chemotypic variation among populations

A total of 46 components, accounting for 94–98.5% of the total essential oils, were identified. Oxygenated monoterpenes constituted the highest proportion of the essential oils (73.6–89.7%) followed by sesquiterpene hydrocarbons (5.8–13.3%). At the species level (average overall populations), the essential oil of Tunisian *A. herba alba* was found to be rich in camphor (19.1%), α-thujone (16.1%), trans-sabinyl acetate (12.5%), β-thujone (9%), chrysanthenone (7.6%), germacrene D (5.4%) and 1,8-cineole (5.1%).

The essential oil composition, mainly the percentage of the major compounds, varied significantly ($p < .05$) between populations (Fig. 1). The uppermost amount of α-thujone (35.1%) was detected in the population 4. The highest percentage of β-thujone (19.3%) was observed in population 1. Samples collected from populations 5 and 7 exhibited the highest amounts of camphor (31.3–31.7%). The percentage of chrysanthenone ranged from 0.11% (population 3) to 15.6% (population 5). The most important content of trans-sabinyl acetate (72.8%) was observed in population 3. Essential oil from population 6 showed the highest percentages of chrysanthenyl acetate (14.7%) and germacrene D (8.2%). Population 7 was found to be rich in 1,8-cineole (12%).

The dendrogram, based on Gower's General Similarity Coefficient calculated on all essential oil compounds, showed two main groups (Fig. 2). The first cluster included the population 3 and the second one enclosed the seven others populations. This second cluster of populations was further separated into two subclusters. The first subcluster encompassed the populations 1, 2, 4 and 8, and the second one contained the populations 5, 6 and 7.

These results support the existence of four different chemotypes, i.e., trans-sabinyl acetate (population 3), α-thujone/trans-sabinyl acetate (population 8), camphor (populations 5, 6 and 7) and the chemotype α-thujone/camphor/β-thujone (populations 1, 2 and 4).

The α- and β-thujone chemotype was previously reported in some *A. herba-alba* samples collected from the Southern region of Tunisia (Haouari and Ferchichi, 2009). *A. herba-alba* essential oil from Morocco was marked by the co-existence of camphor, chrysanthenone, α-thujone and β-thujone as major components (Paolini et al., 2010). The chemotypes identified for the Algerian species were mainly camphor/thujones, camphor/thujones/chrysanthenone, camphor/chrysanthenone and 1,8-cineole/chrysanthenyl derivatives (Bezza et al., 2010; Belhattab et al., 2014). The main components of *A. herba-alba* essential oils collected from Jordan were α- and β-thujones (Hudaib and Aburjai, 2006). Essential oils with a high amount of trans-sabinyl acetate were described here for the first time. It should be considered as a new chemotype of *A. herba-alba* growing wild in Tunisia.

The grouping of populations suggests that their chemical divergence was not associated with their bioclimatic and geographic locations. In fact, the Mantel test performed on euclidian and geographic distances between populations did not show a significant correlation between the two matrices ($r = 0.05$, $p = .414$). Furthermore, non significant correlation ($p > .05$) was observed between the bioclimatic data (annual rainfall, Q_2 and altitude) of the studied populations and the amounts of the majority of their major essential oil compounds such as α-thujone, β-thujone, chrysanthenyl acetate, trans sabinyl acetate, germacrene D and 1,8-cineole, which showed significant variations among populations. However, the contents of camphor ($r = 0.65$, $p = .049$), pinocarvone ($r = 0.72$, $p = .035$) and δ-cadinene ($r = 0.69$, $p = .047$) were found to be positively correlated with the annual rainfall. The percentages of pinocarvone and borneol were positively related to the altitude ($0.69 < r < 0.79$, $p < .05$).

The effect of environmental conditions, such as air temperature, water precipitation, sunlight intensity and humidity, on the yield and composition of secondary metabolites has been largely reported on species belonging to the *Asteraceae* family (Bailen et al., 2013; Formisano et al., 2015; Riahi et al., 2015). However, in our study, the divergence among populations belonging to the same bioclimatic zone and the absence of significant correlations between the measured ecological parameters and the amounts of some major essential oil compounds were also observed. So, further studies considering the effect of soil characteristics (i.e. pH, texture, type and concentration of organic nutrients) on the specie's essential oils could be necessary to better explain the chemical differentiation between populations.