Biochemical Systematics and Ecology 80 (2018) 21–28

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Phenotypic variation of wild Chamomile (*Matricaria chamomilla* L.) populations and their evaluation for medicinally important essential oil

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ARTICLE INFO

Keywords:
Chamomile
Biodiversity
Essential oil
α-Bisabolol
Chamazulene

ABSTRACT

In this study the existing phenotypic and chemotypic variability of native to Greece chamomile populations was investigated, aiming to select the appropriate ones for the production of high quality essential oil. For this purpose, eleven native chamomile populations were studied concerning the main constituents of the essential oil (*α*-bisabolol and chamazulene), and a pre-breeding evaluation experiment was initiated, setting up as main target the essential oil composition and agronomical traits. The two most remarkable populations were further assessed, in comparison to five commercial chamomile varieties, through a honeycomb arrangement in the field. The results showed that, the native populations were classified in lower levels concerning morphological and agronomical traits; however they exhibited high stability for the agronomical characteristics evaluated, in comparison with those of the commercial varieties. Furthermore, the essential oil of the selected native germplasm revealed values up to 32.6% for *α*-bisabolol and 15.3% for chamazulene, approaching or even exceeding the corresponding values of most of the commercial varieties. According to these results it can be concluded that a promising native germplasm can be exploited for further breeding purposes, to produce a new cultivar with medicinally important essential oil composition.

1. Introduction

Matricaria chamomilla (L.) (syn. *Chamomilla recutita* (L.) Rauschert, fam. Asteraceae) commonly known as German chamomile, is an annual diploid (2n = 2x = 18) herbaceous flowering plant (Heywood and Harborne, 1977), well known in folk medicine and used in various herbal remedies for centuries. The drug is obtained from the dried flower heads and is used for the preparation of herbal teas, while their infusions and essential oils have aromatic, flavoring and coloring properties. Chamomile inflorescences and their extracts are used in several products, including food and beverages, cosmetics and cosmeceuticals, as insect and pest repellant, in veterinary medicine etc. A plethora of biological activities has been described for chamomile extracts and essential oils i.e. anti-inflammatory, antiulcerogenic, against gastrointestinal disorders, antimicrobial, antiseptic, antispasmodic, sedative, immunomodulatory, wound healing etc. (WHO Monograph; Gupta et al., 2010). The essential oil is obtained by steam distillation or solvent extraction of the inflorescences in yields varying between 0.24 and 1.90% (Salamon et al., 2010a), depending on the ploidy level, the genotype, the geographical origin, the environmental conditions, the agricultural practices, the stage of capitulum development, etc. (Das, 1999; Formisano et al., 2015; Franz, 1983, 1980; Franz et al., 1985; Salamon, 1994; Salamon et al., 2010a). According to the European Pharmacopoeia (2008) chamomile should contain no less than 4 ml kg^{-1} of blue essential oil. The main bioactive compounds of the essential oil are *α*-bisabolol and *α*-bisabolol oxides A and B, chamazulene and spiroetheres (Gupta et al., 2010; McKay and Blumberg, 2006; Orav et al., 2010; Ristic and Đokic, 1996), with *α*-bisabolol and chamazulene being the most important ones (Schilcher, 1987). Schilcher (1973) cited by Lawrence (1987) has classified chamomile's essential oil in four chemotypes on the basis of the most abundant compounds; Type A: *α*-bisabolol oxide B > *α*-bisabolol oxide A > *α*-bisabolol, Type B: *α*-bisabolol oxide A > *α*-bisabolol oxide B > *α*-bisabolol, Type C: *α*-bisabolol > *α*-bisabolol oxide B > *α*-bisabolol oxide A, Type D: *α*-bisabolol oxide B, *α*-bisabolol oxide A and *α*-bisabolol in equal amounts. The four types contain all chamazulene. Later on, Franz et al. (1985)

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https://doi.org/10.1016/j.bse.2018.06.001
Received 4 April 2018; Received in revised form 30 May 2018; Accepted 8 June 2018

defined the chemotypes in three categories; 1) rich in bisabolone oxide and absence of chamazulene, 2) rich in chamazulene and bisabolol oxides and 3) rich in chamazulene and α-bisabolol. The third type is indicated as the most desirable one (Das, 2015).

Chamomile mainly grows indigenously in the regions of northern and central Europe, and particularly in eastern European countries and also in Mediterranean, N. Africa and the United States. Due to the increased demand for chamomile based products and the high quality standards of the herbal industry, chamomile cultivation has been expanded in several countries and the development of varieties with desired chemical and technological traits has been persistently of major importance.

More than 45 chamomile varieties, diploid (2n = 2x = 18) or tetraploid (2n = 4x = 36) ones, are known worldwide (Das, 2015). Tetraploid varieties (created after chromosomes reduplication using colchicine treatment) differentiated from diploids in several biochemical and morphological traits. The comparative assessment among varieties with different ploidy level, was the goal for many researchers finding higher; drug and essential oil yields, bisaboloids and chamazulene, plant height, inflorescence size and weight and dry seed weight in tetraploid varieties compared to the diploid ones (Franz and Isaac, 1986; Peneva et al., 1989; Repcak et al., 1993; Salamon, 1994). Therefore, there is a view that cultivation of tetraploid varieties is more effective and preferred than diploids to avoid natural crossing between the cultivated and wild plants (Das, 2015).

Knowledge on the genetic and phenotypic diversity in terms of traits of interest is significant for plant breeding. Previous studies have reported great variation among different chamomile populations, their progenies, diploid and tetraploid varieties, between varieties of the same ploidy level, concerning certain morphological characteristics, such as plant growth, weight and size of inflorescence, ramification of branches etc. (Franz et al., 1985; Aiello et al., 2004; Gosztola et al., 2008; Solouki et al., 2008). Nevertheless, the morphological characteristics are mostly influenced by the environmental conditions, are not stable and in case of diploid plants, they cannot be estimated beforehand (Salamon, 2009). Artificial selection and recurrent selection from elite populations, is therefore the effective method of chamomile breeding, while the essential oil composition is crucial parameter and key character for selection (Das, 2015). Several authors have reported the variability of the essential oil content and its bioactive compounds, mainly α-bisabolol, α-bisabolol oxides A and B, bisabolone oxide, chamazulene and spiroethers, in chamomile populations of different origins and commercial varieties, as well (Raal et al., 2003; Salamon, 2004; Sashidhara et al., 2006; Jalalil et al., 2008; Salamon et al., 2010a, 2010b; Faehnrich et al., 2014; Rezaeih et al., 2015). Franke and Schilcher (2007) mention some of the main breeding targets for chamomile which are related to morphological (e.g.; large inflorescence, uniform plant height), agronomical (e.g. high yield), qualitative (e.g. high α-bisabolol and chamazulene content) characteristics and traits for resistance to biotic and abiotic conditions.

Among the high demanded medicinal species in the global trade chamomile possesses a particularly high level of interest (Solouki et al., 2008; Salamon et al., 2010a), and is known also as the “star” of medicinal plants (Upadhyay et al., 2016). Hence, the need of finding new chamomile genetic sources with remarkable characteristics and using them for different breeding targets is highlighted. Otto et al. (2015) point out the fact that recent breeding efforts for chamomile are limited, comparing to other crop plants. There is a demand therefore for searching new chamomile genetic resources with superior characteristics and valorizing them for different breeding targets. Taviani et al. (2002) refer that the diversity of wild chamomile populations has a future potential economic value if it is exploited, effectively. In addition, Okoń et al. (2013) suggested that is feasible to obtain chamomile plants with higher composition in certain essential oil substances, through the detection of new sources of diversity among wild native populations and inducing their desirable traits into commercial varieties.

The honeycomb methodology has been applied for crop improvement (Vlachostergios et al., 2011), and has been proposed as a procedure to develop cultivars that entirely meet the needs of sustainable agriculture. Additionally, it has major principles that distinguish this method from other conventional breeding schemes and experimentation designs that include the large interplant distance, which is used to minimize stress and eliminate genotype's competition, following a systematic entry arrangement to cope with the soil heterogeneity (Fasoula and Tokatlidis, 2012). Selection in the absence of competition optimizes heritability and response to selection, by allowing application of high selection pressure, eliminating the confounding effects of the negative relationship between yielding and competitive ability and maximizing the phenotypic expression and differentiation (Fasoula and Fasoula, 2002). The honeycomb designs exclude interplant competition and soil heterogeneity resulting in the reliable estimation of the crop yield potential (CYP) and its three independent genetic components. They are defined as (i) the yield potential per plant, estimated by the mean yield $\bar{x}$, (ii) the stability, estimated by the standardized mean yield $\bar{x}/s$, where $\bar{x}$ and s represent the mean and the standard deviation of the entry where each plant belongs, and (iii) the adaptability or responsiveness to inputs, estimated by the standardized selection differential $\frac{\bar{x}_{sel}-\bar{x}}{s}$ (Fasoula and Fasoula, 2000). In addition, honeycomb designs compare different entries by allocating each entry systematically and uniformly according to a hexagonal arrangement in the entire field.

Despite the rich biodiversity of Greece in Medicinal and Aromatic plants (MAPs), little efforts have been made towards the exploitation of native chamomile genetic diversity, for domestic cultivation, using elite genotypes and for further breeding programs. As a result, foreign chamomile varieties, having high productivity are mostly used for cultivation in the country. Consequently, as future prospect a Greek wild native chamomile population could be domesticated and become a stable, competitive reproductive material, eliminating problems such as variability not only in terms of morphological and agronomical characteristics but also of valuable bioactive compounds. This strategy, exploring the natural biodiversity for breeding and/or selection purposes, has been adopted by several researchers. Gosztola et al. (2006) have studied wild *Chamomile* populations from Hungary in order to define the natural habitats and populations, suitable to produce high quality drug, and select genotypes for breeding new varieties. Otto et al. (2017) concluded that most of the high-performed tetraploid chamomile varieties are genetically highly similar, and this arises the demand to further broaden chamomile's diversity, by including different germplasm in the future breeding process.

Taking into account all the aforementioned, the aim of the present work was to investigate the native to Greece chamomile chemo- and phenotypic diversity, aiming to select superior genotypes with high quality and essential oil potential, well adapted to the climatic conditions of Greece that would be suitable starting material for further breeding purposes. Furthermore, through the use of a pre-breeding evaluation honeycomb scheme, a future attempt of our study is to test and create new beneficial variability, beyond the assessment and exploitation of the existing one, in order to be used for different breeding targets.

2. Materials and methods

2.1. Assessment of wild populations and selection of starting material - first experimental season

Eleven native to Greece chamomile populations were investigated, in terms of the most valuable essential oil constituents. Chamomile inflorescences were collected during full blooming, in May 2015, from wild grown plants, the majority of them from areas of N. Greece (Table 1). Additionally, chamomile inflorescences were collected at the same period, from one, native to Greece population (ch.19/2009),