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DOCTORAL THESIS

MORPHOLOGICAL AND CHEMICAL DIVERSITY OF DIFFERENT CHAMOMILE (*MATRICARIA RECUTITA* L.) POPULATIONS OF THE GREAT HUNGARIAN PLAIN

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Literature background and the aims of the study

Chamomile (*Matricaria recutita* L.) is one of the most important medicinal plants in Hungary. It has been used for centuries in the Carpathian basin, and its usage still has great importance even nowadays. Beside its traditional application forms (internally the decoction is useful against inflammations in the throat and in the stomach, externally it is a well-know remedy against phtalmitis, and skin problems) it is also used in the homeopathy, in several cosmetics; there is an increasing demand on chamomile also in the fields of domestic chemistry and food industry as well. Additionally, its therapeutic indications are extended continuously due to the improved research techniques.

Beside its importance as a medicinal plant, chamomile has great economic significance as well, because the dried chamomile flowers are one of the most important exported products of our country. According to the publication of Erdei (1959) about the Hungarian medicinal plant export "among the exported products chamomile flowers are the most important ones; in good conditions 40-50 % of the world demand can be satisfied by the Hungarian plant material". Moreover "in the foreign countries Hungarian chamomile is the definition of high quality" and "its prize is much higher than the average of the world markets". Unfortunately this situation has completely changed since the 1990's; owing to the decreased competitiveness of the drug so called "hungaricum". While in 1999 the exported drug quantity was 120.000 kg, in 2001 this amount decreased until 11.000 kg (AMC, 2002). The main reason was the occurrence and spreading of cheap East European, Egyptian, and Argentine plant material of cultivation in the European market. Further problems are the more strict quality requirements; thus the demand on the Hungarian chamomile was affected negatively in the last few years.

Higher ratio of the exported, Hungarian chamomile is still coming from collection (wild growing populations of the Great Hungarian Plain), cultivation has less importance. However, the quality parameters of the collected populations have been hardly revealed. Although at the beginning of the 1960's Máthé and his co-workers made a survey on wild-growing chamomile populations, only coenological, morpho-phenological characteristics and the pro-chamazulene and α-bisabolol content of the plants were analysed. Other, important properties, such as the essential oil content and its full composition, total flavonoid content and the mucilage content were not included in their work. Some of these characteristics have still not been analysed at all, others have been partly revealed (Marczal, 1982, Sztefanov, 2005). Additionally, since the 1960's the vegetation and climatic conditions have changed in many ways. Therefore, initiation of a new research work became actual focusing on the main natural habitats in the Great Hungarian Plain. Thus, our aim was to determinate the most important of this plant species by applying modern analytical techniques; using this data the effectiveness of the collection, the quality of the final product and the export possibilities can be increased significantly.

Although chamomile is traditionally a collected medicinal plant its cultivation is becoming more important not only in Hungary, but also world-wide. Beside the neighbour, European countries, cultivars with great productivity, that also meet with the requirements of the modern cultivation techniques, are selected in Asia and South-America as well. However, in Hungary an officially recorded chamomile cultivar is still missing even if it would be quite necessary based on the market requirements (the previously accepted cultivars such as 'Soroksári 40' and 'Budakalászi 2', because of their unfavourable essential oil composition, have been deleted from the National List of Varieties). Additionally, production of high quality raw material called "hungaricum" can help to maintain our competitiveness on the international market as well.

Our aims were the following:

- Analysis and description of the morphological (pant height, diameter and structure of the inflorescence) and chemical (essential oil amount and composition, total flavonoid content, total phenolic content, swelling index and antioxidant capacity) characteristics of wild growing chamomile populations of the Great Hungarian Plain, especially collected in the Tiszántúl Region.
- Determination of the main morphological and chemical diversity among the naturally occurring chamomile populations. Additionally our aim was to describe individual diversity as well by making investigations on the progenies of self-pollinated and cross pollinated mother plants, too.
- Exploration of the correlation between the ecological, climatic, geographical conditions and the analysed characteristics.
- Based on our results giving advices referring to the collection (determination of the collection territories).
- Selection of lines and tribes characterised by excellent productivity and similar quality parameters that of the original wild-growing populations found in the Great Hungarian Plain.
- Improvement of technical knowledge which can increase the effectiveness of the selection work carried out on chamomile.

Based on the expected results our further aim is to give technical help and advices to the Group of the Chamomile Collectors and Processors in the Great Hungarian Plain.

Material and Method

Diversity of wild growing chamomile populations collected in the Great Hungarian Plain

50 natural habitats of chamomile were involved in our study native to the Great Hungarian Plain at the beginning of 2009 (Table 1). We focused on the seven, most important counties from the collection's point of view (*South part of the Great Hungarian Plain*: Békés and Csongrád, *North part of the Great Hungarian Plain*: Jász-Nagykun-Szolnok and Hajdú-Bihar, *North Hungary*: Heves and Borsod-Abaúj-Zemplén, *Central Hungary*: Pest region), since the most frequent occurrence of this plant species is connected to the above mentioned areas. The greatest abundance of chamomile was observed mainly in the Eastern part of the Great Hungarian Plain, in Békés and Hajdú-Bihar counties, near to the Tisza Lake and in the Southern parts of Heves and Borsod-Abaúj-Zemplén counties. In all cases we made morphological measurements and we cut the inflorescence for the chemical analysis. Seeds were also collected in each population and were taken into our gene bank for the long term gene-reservation. Climatic conditions were evaluated by using the data of meteorological stations, near to the analysed chamomile populations.

Table 1. Codes, places and times of collection, habitat type of the analysed chamomile populations

Code	Collection place	Collection time (2009)	Habitat type	Code	Collection place	Collection time (2009)	Habitat type
1	Dévaványa	02.05.	ruderal field^{2}	**26**	Kertészsziget	13.05.	arable
2	Körösladány1	02.05.	ruderal field^{1}	**27**	Füzesgyarmat 1	13.05.	arable
3	Körösladány2	02.05.	arable	**28**	Füzesgyarmat 2	13.05.	arable
4	Szeghalom	02.05.	ruderal field^{2}	**29**	Darvas	13.05.	ruderal field^{1}
5	Vésztő	02.05.	ruderal field^{1}	**30**	Zsáka	13.05.	ruderal field^{2}
6	Zsadány	02.05.	arable	**31**	Bakonszeg	13.05.	ruderal field^{2}
7	Sarkadkeresztúr	02.05.	ruderal field^{2}	**32**	Nagyrábé	13.05.	ruderal field^{1}
8	Méhkerék	02.05.	ruderal field^{2+}	**33**	Biharnagybajom	13.05.	arable
9	Szabadkígyós	02.05.	natural association	**34**	Sárrétudvari	13.05.	ruderal field^{1}
10	Poroszló 1	03.05.	ruderal field^{1}	**35**	Mezőcsát	15.05.	ruderal field^{1}
11	Poroszló 2	03.05.	ruderal field^{2}	**36**	Ároktő	15.05.	ruderal field^{1}
12	Egyek	03.05.	ruderal field^{2}	**37**	Tiszakeszi	15.05.	ruderal field^{1}
13	Nagyiván	03.05.	ruderal field^{2+}	**38**	Gelej	15.05.	ruderal field^{2}
14	Hajduszovát	03.05.	arable^{+}	**39**	Szentistván	15.05.	ruderal field^{2}
15	Kisköre	03.05.	arable	**40**	Négyes	15.05.	ruderal field^{1}
16	Hevesvezekény	03.05.	arable	**41**	Tiszavalk	15.05.	arable^{+}
17	Heves	03.05.	arable^{+}	**42**	Borsodivánka	15.05.	arable
18	Gátér	12.05.	arable^{+}	**43**	Poroszló 3	15.05.	ruderal field^{1}
19	Sándorfalva	12.05.	arable^{+}	**44**	Sarud	15.05.	ruderal field^{1}
20	Szeged	12.05.	ruderal field^{2}	**45**	Kömlő	15.05.	arable
21	Rákos (Makó)	12.05.	arable^{+}	**46**	Jászberény	15.05.	arable
22	Tótkomlós	12.05.	arable	**47**	Kőröstetétlen	16.05.	arable^{+}
23	Nagymágocs	12.05.	arable	**48**	Jászkarajenő	16.05.	arable^{+}
24	Karcag	13.05.	ruderal field^{1}	**49**	Újszilvás	16.05.	arable^{+}
25	Bucsa	13.05.	ruderal field^{1}	**50**	Tápiógyörgye	16.05.	arable^{+}

Ruderal field^{1} =with many cosmopolitan plant species; ruderal field^{2} =mainly species from the *Poaceae* family are determinant (grassland); arable= field under cultivation or lea-land; $^{+}$ = on saline soil.

Individual variability tested by the progenies

Basic plant material was collected in May (2006 and 2007), from 16 wild growing chamomile populations of the Great Hungarian Plain. In each case seeds were taken from 10-10 cross pollinated individuals. All samples were stored individually in a cooled place until the seed sowing (2008). During the spring of 2008 the propagated progenies (all together 160) were grown in a glass-house and were transplanted into the open field at the beginning of April in the Experimental and Research Farm of the Corvinus University of Budapest, in Soroksár.

Evaluation of the selected lines

According to the previous results 5 wild-growing populations were selected based on their advantageous chemical characteristics (high essential oil, α-bisabolol and chamazulene content). Using their collected seeds in 2007 we made cultivated populations in our experimental field, where 9-11 individuals characterised by good growing-vigour of each population was separated by veil-foil. After self-pollination we collected the seeds separately. In 2008 new cultures were made by using the seeds of 50 self-pollinated progenies and 5 original seed batches of the mother plants (as control) as written in the previous chapter. In the doctoral thesis morphological and chemical features of the I_1 progeny, grown in 2008, were evaluated and presented.

Methods

In our work we determined the morphological and chemical characteristics, as well as the drug production of the evaluated chamomile populations (Table 2). The morphological properties were evaluated by applying more replications, however, in the case of the chemical features the number of the measurements was limited by the drug mass.

Table 2. The analysed characteristics and the numbers of replications

Analysed characteristic	1. Wild-growing chamomile diversity of the Great Hungarian Plain	2.Individual variability tested by the progenies	3. Evaluation of the selected lines
Morphological characteristics			
Plant height	20x	20x	20x
Flower diameter	25x	25x	25x
Discus diameter	25x	25x	25x
Chemical properties			
E.o. content	3x	without replication	without replication
E.o. composition	3x	without replication	without replication
Swelling index	3x	3x	3x
Total flav. content	2x	2x	2x
Total phenolic content	3x	—	—
Total antiox. capacity	3x	—	—
Drug mass	—	without replication	

For the analysis of the chemical properties representative amount of samples, with maximum 1 cm stem part, were collected in full flowering stage. The flowers were dried in natural way on trays; then the material was stored on a dry place, protected from moisture, until the chemical measurements.

Determination of the **essential oil amount** was carried out by hydrodistillation using a Clevenger type apparatus where 20 g drug was distilled by 500 ml water for 3 hours. Since the essential oil is easily sticking on the wall of the cooling part of the apparatus, it was washed by hexane. The amount of the essential oil was measured after the evaporation of the solvent, and was expressed as ml/100 g dry material.

Essential oil composition was analysed by gas chromatograph (GC 6890 N) equipped with mass spectrometer (MS 5975), Agilent Technologies. Capillary columns: HP-5 MS (5 % phenyl, 95 % dimethyl polysiloxane, lenght: 30 m, film thickness: 0.25 μm, id. 0.25 mm). The instrument was programmed as follows: initial temperature 60 °C, then by rate of 3 °C/min up to 204 °C; the final temperature was kept for 5 min; injector temperature: 250 °C, carrier gas: helium (constant flow rate: 1ml/min); split ratio: 30:1. Ionization energy was 70 eV. The mass spectra and linear retention indices (LRI) were compared with those of commercial (NIST) and home-made library mass spectra built up from data obtained from pure compounds.

According to our methodological development we came to the conclusion that the separation of α-bisabolol and bisabolon-oxide-A was not sufficient. Therefore in the PhD Thesis the α-bisabolol content involves the ratio of bisabolon-oxid-A as well (in approximately 0.1-2.0 %).

For the characterisation of the mucilage content the **swelling index** (SI) was determined according to the general and specified descriptions (for *Althaeae folium*) of the VIII. Pharmacopoeia Hungarica, by using 0.2 dry, powdered drug.

Analysis of **the total flavonoid content** was carried out following the descripitons of the VIII. Pharmacopoeia Hungarica, specified for *Crataegi folium cum flore*, with some modifications (we applied half amounts of the necessary chemicals as it was described).

In our work we also determined the total phenolic content and total antioxidant capacity of the aqueous (0.25 g powdered drug was infused with 25 ml 100 °C distilled water and filtered after 24 hours) and alcoholic extracts (0.25 g powdered drug was extracted by 25 ml ethanol (20 %) and was filtered after 72 hours) of chamomile. The extracts were stored in freezer until the experiments.

Total phenolic content (TPC) was analysed by the modified method of Singleton and Rossi (1965), where the solutions were taken into a water-bath (50 °C) for 5 minutes accelerating the colouring reaction. The absorbance was measured at 760 nm; gallic acid (0.3 M) was used as chemical standard for calibration. The results were compared to the dry material of the extracts and were expressed as mg gallic acid equivalents per mg dry weight (mg GAE/mg d.w.).

Measurement of the **total antioxidant capacity** (TAC) was carried out by the modified method of Benzie and Strain (1996). The absorbance of the solutions was measured by spectro-photometer on 596 nm. Ascorbic acid was used as chemical standard for the calibration. The results were compared to the dry material of the extracts and were expressed as mg ascorbic acid equivalents per mg dry weight (mg AAE/mg d.w.).

The drug mass of the cultivated populations was measured in a field-block experiment. In full flowering period whole inflorescence (with maximum 1 cm of stem part) was collected by hand in each block. The mass of the naturally dried flowers collected from the same block was measured by digital balance. The results were corrected by the numbers of the individuals in all blocks and were given as g/ m^2.

Statistical analysis

Means, standard deviations and coefficient of variation (CV %) were given by correlation -, cluster, and one-way variance analysis carried out by STATISTICA 9.0. and Microsoft Excel 2003. During the homogeneity survey in the case of each characteristic the groups were formed based on the following parameters: rather homogeneous: CV%<10.0%, homogeneous: CV%=10.0-20.0%, heterogeneous: CV%>20.0%. Referring to the correlation analysis weak correlation was defined when $r(x,y) \leq 0.4$, the correlation was regarded as medium strong when $0.4 < r(x,y) \leq 0.6$, and strong connection was determined when $r(x,y) > 0.6$. Homogeneity of the deviations was checked by Levene-test in each case. When the t-test on α-level gave significance the basic hypothesis was rejected and robust statistics were done carried out by Brown-Forsythe test. The results were analysed on 95 % confidence level in all cases.

Results and Conclusions

1. Diversity of wild growing chamomile populations collected in the Great Hungarian Plain

The plant height of the analysed wild chamomile populations varied between 5 and 68 cm, the diameter of the inflorescence were between12 and 22 mm, the diameter of the discus ranged from 5.4 to 7.5 mm, while the length of the ray flowers varied from 6.8 to 14.2 mm. The area percentage of the discus inside of the whole inflorescence was between 34 and 44. In the evaluated wild growing populations the essential oil amount ranged from 0.30 to 0.88 g/100 g d.w. The area percentage of α-bisabolol varied between 6.8 and 71.3%, the amount of bisabolol-oxide A was between 0.00-56.5%, the percentage of bisabolol-oxide B was between 2.1 – 22.0, the chamazulene content was detected in the amount of 5.4-19.7 area percent, the β-farnesene area percent varied between 1.0-6.3, and finally the cis-spiroether area percent was between 3.9-23.3. The above mentioned essential oil compounds were found in all samples with the exception of the bisabolol-oxide A, that could not be detected in one case.

Determination of the typical essential oil composition was done according to the Schilcher chemotaxonomic-system with some modifications and improvements. Four groups (A-D) have been made based on the ratio of the α-bisabolol and its oxides called as chemovarieties. Inside of each chemovariety four chemoforms were made ($A_{1\text{-}4}$, $B_{1\text{-}4}$, $C_{1\text{-}4}$, $D_{1\text{-}4}$) according to the ratio of those compounds (chamazulene, cis-spiroether) having important medical effect (Table 3).

Table 3. Characterisation of chemoforms grouped inside the chemovarieties

Chemoform	Chamazulene content	Cis-spiroether content
1	15%<	15%<
2		15%>
3	15%>	15%<
4		15%>

Based on this modified and improved taxonomic system most of the analysed populations (21) were classified in the A group (A_1: 0, A_2: 2, A_3: 10, A_4: 9), 18 populations were put into the group C (C_1: 1, C_2: 1, C_3: 4, C_4: 12) and 11 populations were belonging to the group D (D_1: 2, D_2: 0, D_3: 5, D_4: 4). None of the analysed wild chamomile populations could be taken into the B chemovariety. Besides the above mentioned essential oil compounds minor constituents were identified as well, such as trans-spiroether, germacrene-D, alloaromadendrene, bicyclo-germacrene, nerolidol, α-eudesmol, epi-α-bisabolol, spathulenol and γ-elemen.

The swelling index (SI) of the evaluated plant samples were between 15.8 and 80.8, their total flavonoid content was between 0.94 and 2.28 %. The total phenol content (TPC) and the total antioxidant capacity (TAC) of the water and ethanol extracts of the samples were analysed, too. The

TPC values varied from 33.7 to 62.5 mg/g d.w. in the water extracts, while by applying 20% ethanol as a solvent the values were between 30.6 and 110.4 mg/g d.w. (in both cases 45-60 mg/g d.w. were measured as average amount). The TAC values of the water extracts varied from 5.6-95.3 mg/g d.w., while in the ethanol extracts the values were between 3.7-125.1 mg/g d.w. (in both cases the average amounts were between 10-60 mg/g d.w.). According to our results the ethanol extracts were characterised by higher amounts of soluble phenolic compounds, while referring to the TAC values the water extracts produced better results in most of the cases.

The examined wild populations were defined heterogeneous referring to the plant-height ($CV_{\%}$=47.5%), essential oil content and composition ($CV_{\%}$=22.0-124.5%), swelling-index ($CV_{\%}$=41.2%), total flavonoid content ($CV_{\%}$=22.2%), TPC values of the alcoholic extracts ($CV_{\%}$=35.2%) and TAC values of the aqueous extracts ($CV_{\%}$=63.8-77.1%). However, based on most of the morphological characteristics (diameter of the inflorescence and the discus, ratio of the discus and the ray flowers inside of the inflorescence, length of the ray flowers) as well as on the TPC content of the aqueous extracts the plants showed moderate heterogeneity ($CV_{\%}$=6.5-16.2%).

For the optimization of the collection, assuring high quality drug, possible correlations were looking for and evaluated between the ecological, climatic, geographical conditions and the morphological and chemical characteristics of the plant material. Clear connection between the evaluated features and the natural habitats could not be detected, since we found in all territories (in ruderal fields and in cultivated places as well) plants characterised by good and weak results, too. Outstanding results were found in only one case; a dwarf chamomile population protected from human impact, growing on saline soil contained extremely high α-bisabolol (71.3%), its TPC and TAC values in the ethanol extracts were also very good. On the other side, referring to the remaining characteristics it did not reach the average levels.

Significant correlation between the meteorological conditions and the evaluated characteristics was found in two cases: between the spring total heat units and the SI values medium strong connection could be seen (r=0.56), while between the total heat units and the total flavonoid content strong negative correlation was found (r= (-0.63)). Based on these findings it can be ascertained that the raising temperature has a positive effect on the mucilage accumulation, however, it can result in a decreasing level of the total flavonoid content.

Based on the data referring to the geographical origin and the main characteristics of the plants we came to the conclusion that referring to the α-bisabolol and bisabolol-oxid A ratios strong correlations can be seen. While in Pest region, and in the North part of the Great Hungarian Plain (surroundings of the Tisza Lake) the A-chemovariety is dominant, the South, South-East territories (Csongrád, Békés, Hajdú-Bihar and Jász-Nagykun-Szolnok counties) were characterised by mainly the C-chemovar. populations.

The correlation between all evaluated features was also determined. Between the total flavonoid content - diameter of the inflorescence; area percentages of the α-bisabolol - bisabolol-oxid A strong connections were found (r = 0.63 and r = (-0.87)). Medium strong, reverse correlation was between the essential oil and α-bisabolol content (r = (-0.55)) in accordance with the findings of Marczal (1982). The TAC values of the water and ethanol extracts showed also a strong connection (r=0.62) meaning that the main compounds having an antioxidant effect solved in both solvents. Moreover, the TPC and TAC values in the ethanol extracts were also connected very strong to each other (r=0.91), thus the TAC values of the ethanol extracts were mainly (in 83 %) due to the measured TPC. On the other hand in the water extracts clear correlation cannot be seen between the TPC and TAC values (r = 0.31), therefore we assume that in this case the TAC is due to other, water-soluble, non phenolic compounds as well.

2. Individual variability tested by the progenies

In the case of the individual genetic diversity we came to the conclusion that the progenies of the free flowering plants were rather homogeneous ($CV_{\%}$<10%) based on the diameter of the inflorescence and the discus, ratio of the ray flowers. With relevance to the plant height, ratio of the discus in the inflorescence and size of the ray flowers they were also homogeneous ($CV_{\%}$=10-20%). Those families consisted of populations of the same origin showed the same variability, therefore, it can be ascertained that the chamomile populations are characterised by relatively low individual genetic diversity referring to the morphological features; however, some differences can be seen between the populations.

Referring to the chemical characteristics based on the SI and the total-flavonoid content the 16 progenies of the free flowering plants were homogeneous, however, with relevance to the essential content, composition and the drug yields they were rather different ($CV_{\%}$>20%). Because of the above mentioned variability of the progenies with the same origin great genetic diversity of the mother plants and significant polymorphism of the basic, wild-growing populations can be assumed. Therefore, huge differences can occur between the populations as well.

In the 16, evaluated populations similar splitting patterns were observed in most of the cases, however huge differences could be seen with relevance to the essential oil content, swelling index and the total flavonoid content. Regarding every analysed characteristic most of the populations showed similar diversity with the exception of the 10 and 11 signed populations which were more homogeneous, and the 5 and 14 signed families which showed more heterogeneity.

During our work we found several populations that can be regarded perspective based on their advantageous chemical properties for further breeding work; especially in the families signed by 10, 14 and 16 we detected progenies characterised by good essential oil parameters (content and composition).

3. Evaluation of the selected lines

In our experiment on the I_1 progeny of the self-pollinated plants, comparison of the mother plants and the offspring populations was carried out in the same year, in the same ecological-climatic conditions, referring to the morphological and chemical characteristics.

During the evaluation of the self-pollinated I_1 progeny significant improvement was detected referring to certain characteristics (compared to the mother populations the ratio of the ray flowers, the essential oil-and total flavonoid content increased). In the case of other analysed properties half of the progenies gave better results, while in the rest a declining was detected (for example with relevance to the plant height, size of the ray flowers, ratio of α-bisabolol and bisabolol oxide A in the essential oil, drug yield). And finally, in some cases the I_1 progeny produced much lower results than the mother population (for example: diameter of the flowers and the discus, ratio of the discus inside of the inflorescence, ratio of bisabolol oxide B and cis-spiroether in the essential oil, swelling index).

By analysing the progenies most of them (with the exception of only 2) could be taken in the same chemovariety with the mother plants, thus the main essential oil compound remained the same – α-bisabolol. In 79 % the chemoforms (C_4) typical of the mother plants were also characteristic to the progenies, in this case different results were found in 8 cases.

Regarding every analysed characteristic the progenies of self-pollinated plants showed similar diversity, the average $CV_{\%}$ values varied between 32.5 and 37.3 %. The most significant improvement was observed in the progeny signed by K/12 in almost all cases, especially referring to those parameters which can be regarded important for the selection work. Highest declining of the chemical properties was found in the K/15 progeny.

The progenies of the spontaneously free flowering (160) and the self-pollinated mother plants (50) were compared as well. As a result of the self-pollination greater heterogeneity was detected referring to the plant-height and the inflorescence's diameter, however, in the case of the inflorescence'structure the progenies were more homogeneous than the offspring of the free flowering mother plants. Progenies of the self-pollinated plants were characterised by more homogeneous essential oil composition (same ratios of α-bisabolol, chamazulene, β-farnesene, cis-spiroether) preserving the basic chemovarieties and chemoforms of the mother plants, however, based on their SI, essential oil amount and ratio of the bisabolol-oxid compounds, greater heterogeneity was found compared to the offspring of the cross pollinated populations. Referring to the flavonoid content and the drug mass the same variability was detected in each case.

Scientifically new results and technical recommendations

- It was the first time when relatively huge number (50) of wild-growing chamomile populations of the Great Hungarian Plain was evaluated in a complex way focusing on the morphological and chemical properties, revealing also the possible correlations between the production-biological characteristics and the ecological conditions. Owing to the high number of the evaluated populations, great diversity of the analysed characteristics and the applied up to date techniques our research work can be regarded new and special. As a result of our work valuable data have become available about the quality parameters of the collected populations of the Great Hungarian Plain and about the diversity of Hungarian chamomile populations.
  - We concluded that the wild growing populations were heterogeneous ($CV_{\%}$=22.0-77.1 %) with the exception of some morphological (diameter of the inflorescence and discus, ratio of the discus inside of the inflorescence, length of the ray flowers) and chemical (TPC in the aqueous extract) properties ($CV_{\%}$=10.5-16.2 %).
  - It was first time when the mucilage content given by the SI, the total flavonoid content, TPC and the TAC values of chamomile were analysed. We found that higher TPC values were detected in the alcoholic extracts, while stronger antioxidant activity was measured in the aqueous extracts. We proved that the TAC values of the alcoholic extracts were in correlation with the soluble phenolic compounds (r=0.91), however, the TAC and TPC values of the aqueous extracts did not show significant connection (r = 0.31).
  - We modified and improved the chemotaxonomic-system of Schilcher . Based on the ratio of α-bisabolol and its oxides to each other, 4 groups were formed (A-D) called chemovarieties instead of the previously used chemoforms. In each chemovariety 4 chemoforms were defined ($A_{1\text{-}4}$, $B_{1\text{-}4}$, $C_{1\text{-}4}$, $D_{1\text{-}4}$) based on the ratio of those essential oil compounds possessing valuable medical effect (chamazulene, cis-spiroether). Therefore, a more precise taxonomical analysis could have been carried out.
  - Based on the correlation analysis between the measured characteristics and the ecological parameters we concluded that evident connection could not be detected, because good and weak results were found both in ruderal fields and arables.
  - We proved the first time, that medium strong, positive correlation was between the heat-units of spring and the swelling index (r = 0.56), and strong, negative correlation was between the heat-units and the total flavonoid content (r = -0.63). Based on these results we concluded that the increasing temperature influenced positively the mucilage accumulation, however it had a reverse effect on the flavonoid content of chamomile.

- Our results were not in accordance with the findings of Máthé and Tyihák (1962), who concluded that the accumulation of α-bisabolol and pro-chamazulene was decreasing from North-West to South-East in Hungary. According to our data, wild growing populations rich in α-bisabolol (C-chemoveriety) were found especially in the southern counties of the Great Hungarian Plain (Csongrád, Békés, Hajdú-Bihar and Jász-Nagykun-Szolnok). Clear correlation between the chamazulene content and the geographic origin was not found. The contradictory data can be explained by the different analytic methods, or by a genetic drift which could have happened in the last 50 years. These findings are in accordance with the results of Sztefanov (2005).
- The seeds of the analysed 50 wild-growing populations were taken into our gene-bank, giving the necessary descriptions (germination capacity, thousand seed weight) as well for the long term gene reservation.

Summarizing our results it can be ascertained that wild-growing chamomile populations with advantageous essential oil characteristics can be found in the South-Eastern, Eastern part of the Great Hungarian Plain – in Csongrád, Békés, Hajdú-Bihar and Jász-Nagykun-Szolnok counties. With relevance to the other active compounds selection of the populations has to be based on a pre-analysis of these substances, since clear correlation cannot be proven between the quality parameters and the ecological-, geographical conditions.

➢ It was the first time when individual diversity of chamomile populations was evaluated by testing progenies. These progenies were originated from 10-10 free flowering and 9-11 self-pollinated individuals of 16 and 5 wild growing populations; the offspring was evaluated in the same climatic and ecological conditions with relevance to the morphological and chemical properties to characterise the individual genetic diversity of the populations.
  - We found that the individual genetic diversity of the wild-growing chamomile populations was quite weak based on the morphological characteristics; however, referring to the chemical properties, they showed stronger heterogeneity. The weakest heterogeneity was found in the mucilage content (given by the SI) and in the total flavonoid content; while the strongest heterogeneity was described in the case of the essential oil and drug mass properties. However, this diversity can be different between the populations. in the rate of the diversity differences can be seen between the populations.
  - The progenies of the self-pollinated mother plants were more homogeneous than those of the spontaneously free flowering ones referring to the structure of the inflorescence; however, their individual diversity increased based on the plant-height and the size of the inflorescence. The progenies became more homogeneous in the case of the essential oil's

composition (α-bisabolol, chamazulene, β-farnesene, cis-spiroether), chemovariety and chemoform; however, depending on the SI, essential oil content, and the ratio of bisabolol-oxides they showed greater heterogeneity. Significant different was not detected either in the total flavonoid content or the drug yields. Therefore we assume that the spontaneously occurring chamomile plants in the Great Hungarian Plain are probably heterozygote.

- Besides the evaluation of the wild-growing populations our further aims were to improve the genetic background and to expand the theoretic knowledge on chamomile for the breeding-selection work as well; in practice our results can help to develop new cultivars as well.
  - We demonstrated that the individual chemical diversity in the chamomile wild populations can assure an excellent background for the selection-breeding work. It can be also ascertained that the individual variability of some characteristics inside of the population can be decreased by self-pollination; therefore the effectiveness of breeding can be improved.
  - Although in the case of several characteristics the progenies produced lower amounts than the mother plants, after the evaluation of the progeny signed I_1 we found that in some cases the progenies can contain much higher level of the active compounds (the essential oil amount of 5 lines was more, than 1%, in 4 lines the ratio of the α-bisabolol content in the essential oil exceeded 80%, in 3 lines the ratio of the chamazulene in the essential oil was more than 20%, the SI of 2 lines was more, than 89). In the case of the essential oil-, and total flavonoid content also significant increase was observed in the progenies.
  - For further breeding work 8 lines, and 12 populations of the families signed 10, 14 and 16 can be perspective based on their essential oil content and composition, therefore, their seeds were taken into our gene-bank.

Summarizing our results we came to the conclusion that the wild growing chamomile populations are rather variable both inside and between the populations especially referring to the chemical characteristics. Therefore good quality, homogeneous plant material is difficult to be produced by collection, which method is useful only for assuring common quality characteristics. The collection has to be restricted to those populations having appropriate chemical properties. In the bigger collection zones, based on the quality control done parallel with the primary processing, the different quality plant material needs to be handled as individual batch. Further solution can be that one part of the demand on the drug is satisfied by cultivation of those cultivars characterised by "traditional quality".

Publications in relation to the PhD Thesis

Publications without IF:

1. **Gosztola B.**, Szabó K., Sztefanov A., Zámboriné N. É. (2005): Különböző eredetű vadon termő orvosi kamilla (*Matricaria recutita* L.) populációk összehasonlító vizsgálata. Kertgazdaság, 37 (1). 73-81.
2. **Gosztola B.**, Németh É., Sárosi Sz., Szabó K., Kozak A. (2006): Comparative evaluation of chamomile (*Matricaria recutita* L.) populations from different origin. International Journal of Horticultural Science, 12 (1). 91-95.
3. **Gosztola B.**, Varga L., Németh É., Bodor Zs., Sárosi Sz., Szabó K. (2008): Morfológiai tulajdonságok alakulása orvosi kamilla (*Matricaria recutita* L.) szelektált I_1 nemzedékében. Kertgazdaság, 40 (4). 72-78.

Conference papers (Hungarian, full-paper):

1. **Gosztola B.**, Kirinovics K., Németh É., Szabó K. (2009): Kemotípus alakulása orvosi kamilla (*Matricaria recutita* L.) szelektált I_1 utódpopulációiban. XV. Növénynemesítési Tudományos Napok, 2009. márc. 17. MTA, Budapest. Hagyomány és haladás a növénynemesítésben, 145-149.

Conference papers (Hungarian, abstracts):

1. **Gosztola B.**, Németh É., Szabó K., Bodor Zs. (2008): Orvosi kamilla (*Matricaria recutita* L.) utódnemzedékek értékelése morfológiai tulajdonságaik alapján. XIV. Növénynemesítési Tudományos Napok, 2008. márc. 12. MTA, Budapest. Összefoglalók, 127.
2. **Gosztola B.**, Kirinovics K., Németh É. (2009): Morfológiai tulajdonságok, droghozam, illóolaj-tartalom és –összetétel alakulása orvosi kamilla (*Matricaria recutita* L.) szelektált I_1 utódpopulációiban. Lippay János – Ormos Imre – Vas Károly Tudományos Ülésszak, 2009. október 28-30. Budapest. Összefoglalók, 100-101.
3. **Gosztola B.**, Bernhardt B., Sárosi Sz., Bernáth J. (2011): Alföldi vadontermő orvosi kamilla (*Matricaria recutita* L.) populációk illóolaj-tartalma és összetétele. XII. Magyar Gyógynövény Konferencia, 2011. május 5-7. Szeged. Gyógyszerészet, LV. S27-S28.

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1. **Gosztola B.**, Németh É., Kozak A., Sárosi Sz., Szabó K. (2006): Comparative evaluation of hungarian chamomile (*Matricaria recutita* L.) populations. I. International Symposium on Chamomile Research, Development and Production, 7-10. June 2006, Presov, Slovakia. Proceedings of Papers, Acta Horticulturae, 749. 157-162.

International conference papers (abstracts):

1. **Gosztola B.**, Szabó K., Németh É., Bodor Zs., Sárosi Sz., Sztefanov A. (2005): Comparative investigations on wild growing chamomile (*Matricaria recutita* L.) populations of different origin. 36th International Symposium on Essential Oils, 4-7. Sept. 2005, Budapest, Hungary. Book of Abstracts, P-99.
2. **Gosztola B.**, Németh É., Bodor Zs., Szabó K., Kutta G. (2008): Bewertung der Nachkommenschaften von Kamillenpflanzen (*Matricaria recutita* L.) auf Grund morphologischer Merkmale. 18. Bernburger Winterseminar und 5. Fachtagung Arznei- und Gewürzpflanzen, 18-21. Febr. 2008, Bernburg, Deutchland. Book of Abstracts, 27.
3. **Gosztola B.**, Sárosi Sz., Czirbus Z., Nádosi M., Németh É. (2010): Chemical Characterisation of Hungarian Wild Chamomile (*Matricaria recutita* L.) Populations. 41th International Symposium on Essential Oils, 5-8. Sept. 2010, Wroclaw, Poland. Book of Abstracts, PP-A23.