Modelling plant morphometric parameters as predictors for successful cultivation of some medicinal Agastache species

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Abstract

Researches carrying evidence for various uses and bioactive principles of Agastache spp. are justifying the upscaling into cultivation of these medicinal species. But, hindrances in their cultivation exist due to the insufficient documentation of their biology under field conditions. Because productivity of these medicinal species (herba) is ensured by the combined contribution of plant agronomic traits, these are related to the feasibility of the crop and therefore, can be used as predictors for successful cultivation. The aim of this study was to evaluate comparatively four valuable Agastache species (A. mexicana, A. scrophulariifolia, A. foeniculum) and one cultivar (A. rugosa ‘After Eight’), in order to identify the favourability for cultivation in local conditions (Romania). Based on the structural indicators of plant morphology (plant height, shoot number, leaf number, leaf length and width, inflorescence length, verticillaster number and flower number), registered over the span of two years, were explored relationships and similarities as well as their implications in previsioning the phenotypic potential. The results showed that studied species acclimatized successfully and all agronomic parameters studied increased in values in the second year. The average plant height in second year (2020) was 109.8 cm and average inflorescences length 9.6 cm. Stable positive correlations between inflorescence length with plant height and shoot number were observed, while differences among species became pronounced as plants become established, evidenced by clearer distinction in the second year. Phenotypic potential in the absence of inputs enables the feasibility assessment for medicinal plants introduced for cultivation in new regions.

Keywords: agronomic trait; correlation; field conditions; herba Agastache
Introduction

The importance of medicinal plants today extends beyond the traditional uses because these represent trade commodities that cover the demand of diverse industry and market sectors (Schippmann et al., 2002). Worldwide the trade in medicinal plants, herbal drugs and raw materials experiences a growth rate of 15% per year, while about 25% of modern medicines are derived from plants (Ahmad and Ahmad, 2019). At global level it is estimated that production of medicinal and aromatic plants exceeds 300 million tons, but it is difficult to assess how many are actually traded. In Europe there are over 30 thousand companies dealing in this sector, and an area exceeding 200 thousand hectares cultivated Europe-wide, with countries such as France, Poland, Spain, Bulgaria, Croatia and Czech Republic as top players. However, current area cultivated with medicinal plants covers only a small fraction of the industry needs (Argyropoulos, 2019). The number of plant species used for medicinal purposes world-wide might exceed 50,000, but in Europe about 2,000 plant species are in trade for medicinal use (Schippmann et al., 2006).

There is an increasing demand for medicinal plants for the production of plant-based medicines and drugs, various plant-based health products, food supplements, cosmetics and plant protection products (Li and Weng, 2017; Ahmad and Ahmad, 2019; Argyropoulos, 2019). Major markets are located in developed countries, with Europe as main import region due to the large demand for herbal raw materials that end up in various processing or food sectors (Schippmann et al., 2002; Argyropoulos, 2019). Medicinal plants used worldwide are collected from the wild, grown in home gardens or cultivated either as sole cropping or in intercropping systems. There is preference for cultivated material, because most herbal companies, mass-market and pharmaceutical companies opt for cultivated raw material (Schippmann et al., 2002). Also, research has shown that among committed consumers there is a preference for quality, organically-grown plants, ecological certification and traceability (Cadar et al., 2021). Growers can consider extending their range of cultivated species to take advantage of the industry and market opportunities. This can be achieved by extending the cultivation of medicinal species and introducing novel ones into cultivation (Schippmann et al., 2002, 2006; Argyropoulos, 2019).

Agastache is a genus from the family Lamiaceae that includes aromatic species native to North America and East Asia. Plants have prevailingly ovate or deltoid-ovate leaf shapes with serrate or crenate margins. The inflorescence is comprised by small flowers arranged in verticillasters (Lint and Epling, 1945; WFO, 2021). They exhibit a hemicyryptophyte life-form in conditions of Romania (Vârban et al., 2021). The genus does not occur naturally in Europe, but species from this genus are promising medicinal plants from which herba is harvested during flowering and can be valorised as green or dried biomass as well as for essential oil production (Duda et al., 2013; Muntean et al., 2016). Numerous studies regarding the importance of these medicinal species justifies their cultivation: for ornamental purposes (Lord, 2003; Marchioni et al., 2021), as melliferous plant (Anand et al., 2018; Anand et al., 2019a; Anand et al., 2019b), for foods and drinks (Fuentes-Granados et al., 1998; Carović-Stanko et al., 2016; Quattrocchi, 2016; Najar et al., 2019; Marchioni et al., 2021), for valuable essential oil (Ivanonv et al., 2019; Gonzalez-Ramirez et al., 2021) and for source of specific bioactive compounds (Zielińska and Matkowski, 2014; Quattrocchi, 2016; Hwang et al., 2021). But while biochemical properties have received attention (Kovalenko et al., 2019; Najar et al., 2019; Yeo et al., 2021; Najafi et al., 2022) there is a lack of information that could consolidate technological packages to enable the successful cultivation of species from this genus (Palma-Tenango et al., 2021).

Agencies advise for moderation in regards with inputs for medicinal crops (EMEA, 2006). Particularly, the potential accumulation of nitrites and nitrates in medicinal plants is one of the most concerning (Mohamed, 2012; Özcan and Akbulut, 2008; Nchu et al., 2017), and can occur due to excessive N fertilization (Nchu et al., 2017). Therefore, it is preferable that productivity of medicinal plants is not highly reliant on application of fertilizers or stimulators to enhance yield. But in these conditions, feasibility of the crop becomes dependent of the natural phenotypic expression and as a consequence the precise knowledge about the phenotypic potential of the plants has high agronomic relevance.
An outlook on morphometric specifications for *Agastache* species from the existing body of literature (Table 1), reveals that in many studies the measurements were performed on plants grown under controlled conditions and in most cases the plants were not observed until advanced stages of growth (Khorsandi et al., 2010; Do et al., 2020; Jahani et al., 2018; Kim et al., 2018; Lam, et al., 2020a; Lam et al., 2020b). At the same time, some studies focused only on some reproductive traits of plants (Jang et al., 2015; Rudik, 2016). The studies that cover the whole range of morphometric parameters representing key agronomic traits linked to productivity of these species, are few (Ok and Chae, 1998; Lee et al., 1999; Sheahan, 2012; Melnychuk and Rakhmetov, 2016; Carrillo-Galván et al., 2020). As a consequence, the information obtained is insufficient for the optimization of field cultivation of *Agastache* species.

### Table 1. Review of morphometric specifications for *Agastache* species studied

| Spp. | Conditions | Details of the context | Morphometric | Sources |
|------|------------|------------------------|--------------|---------|
|      |            |                        | Veg. | Repr. |                     |
| A. foeniculum | G | Comparative screening under hydroponic culture | x | - | (Do et al., 2020) |
| | G | Effect of ploidy level on morphometric traits | x | x | (Talebi et al., 2017) |
| | G | Influence of foliar stimulants/fertilizers on plant growth | x | - | (Jahani et al., 2018) |
| | F | Acclimatization in Kremenets Botanical Garden | x | x | (Melnychuk and Rakhmetov, 2016) |
| | I | Direct in vitro plant regeneration | x | - | (Hosseini and Moharrami, 2014) |
| | G | Salinity and plant growth | x | - | (Khorsandi et al., 2010) |
| | F | Description - Gardening encyclopedia | x | - | (Lord, 2003) |
| | F | Description - Botany and taxonomic keys | x | x | (Lint and Epling, 1945) |
| | F | Genotypes at different stages of domestication | x | x | (Carrillo-Galván et al., 2020) |
| A. mexicana | I | In vitro regeneration | x | - | (Carmona-Castro et al., 2019) |
| | G | Effect of ploidy level on morphometric traits | x | x | (Talebi et al., 2017) |
| | G | Influence of foliar stimulants/fertilizers on plant growth | x | - | (Jahani et al., 2018) |
| | F | Acclimatization in Kremenets Botanical Garden | x | x | (Melnychuk and Rakhmetov, 2016) |
| | F | Description - Gardening encyclopedia | x | x | (Lord, 2003) |
| | F | Description - Botany and taxonomic keys | x | x | (Lint and Epling, 1945) |
| | F | Comparative inflorescence morphology | - | x | (Rudik, 2016) |
| | F | Floral dimorphism in South Korean natural populations | - | x | (Jang et al., 2015) |
| | I | In vitro propagation of shoots | x | - | (Zielińska et al., 2011) |
| | F | Influence of N fertilization on plant growth | x | x | (Ohk et al., 2000) |
| | F | Description - Gardening encyclopedia | x | - | (Lord, 2003) |
In previous work we demonstrated that a first step for defining suitability for cultivation is to determine the phenology in local conditions (Vârban et al., 2021). This is essential particularly for perennial species from genus Agastache, since depending of their place of origin might or might not be hardy in local climate. Results have shown that Agastache species studied resisted over winter in Cluj-Napoca, Romania. Moreover, phenology revealed that the succession of phenophases follows similar trend, allowing their cultivation at least in Transylvania region (Vârban et al., 2021). The current study concerned with answering the next question that growers might have: what is the phenotypic potential of these species? The answer of this question might allow growers to choose those species that they consider best for their needs.

The aim of the research was to evaluate comparatively the phenotypic potential of some valuable Agastache species by assessing plant morphometric characteristics under field conditions. All the characters studied are of agronomic interest, determining the production and quality of the biological product obtained. For defining the morphometric parameters with highest precision, in the experimental field were not performed interventions (irrigation, fertilization, growth stimulators etc.). The research attempts to offer proof of concept for facile analysis of agronomic traits for predicting favourability of cultivation of some promising medicinal species. The results could complete a gap from literature by providing specifications regarding phenotypic expression of four Agastache species (A. foeniculum, A. rugosa, A. mexicana, A. scrophulariifolia) and a cultivar (A. rugosa ‘After Eight’) under field conditions. Two objectives were defined: evaluation of morphometric parameters with agronomic importance and study of the relationships between them; assessment of similarity between species based on projection of the morphometric traits.

Materials and Methods

Description of the study site

The experiment took place in the years 2019 and 2020 in the Agro-Botanical Garden of the University of Agricultural Sciences and Veterinary Medicine (UASVM) from Cluj-Napoca, Romania and located at latitude 46°45′36″ N and longitude 23°34′24″ E, elevation 380-430 m (Index Seminum - Hortus Agrobotanicus, 2021).

The local climate is temperate-continental with oceanic influence (Criveanu, 2001) and Dfb (warm humid continental) according to Köppen-Geiger classification (https://en.climate-data.org/). According to
climatic conditions from the experimental interval registered by the UASVM Cluj-Napoca weather station, in the year 2020 precipitations were more abundant, and were associated with lower average temperatures over summer months, compared with year 2019 (Figure 1).

Figure 1. Climatic conditions during plant development in the field, according to weather station of UASVM Cluj-Napoca, Romania

In the Agro-Botanical Garden, the soil has a loam-clay texture, pH 6.72, and low humus content (1.35%). The supply with macronutrients is good: Nitrogen 0.461%, Potassium 312 ppm, and Phosphorus 68 ppm (Vârban et al., 2021).

Biological material
In the experiment were studied accessions of four species of *Agastache* and one cultivar obtained from Botanisches Institut und Botanischer Garten from Universität Gesamthochschule Essen, Germany (Table 2). Voucher specimens are stored at the Agro-Botanical Garden Herbarium from UASVM Cluj-Napoca, 30078–30082 voucher numbers. These species have not been cultivated before in local conditions, with the exception of *A. foeniculum*.

Table 2. The *Agastache* sp. accessions from the study

| Species/Cultivar          | International Plant Exchange Number | Native range                                      |
|---------------------------|-------------------------------------|---------------------------------------------------|
| *A. foeniculum* (Pursh) Kuntze | XX-0-CLA-4152                      | throughout North America                          |
| *A. scrophulariifolia* (Willd.) Kuntze | XX-0-CLA-4155                      | throughout North America                          |
| *A. mexicana* (Kunth) Lint et Epling | XX-0-CLA-4153                      | southern part of North America (Mexico)           |
| *A. rugosa* (Fisch. et C.A.Mey.) Kuntze | XX-0-CLA-4154                      | throughout East Asia                              |
| *A. rugosa* ‘After Eight’ | XX-0-CLA-1950                      | -                                                 |

Experimental procedures
The cultivation has been described in our previous work (Vârban et al., 2021) and therefore only briefly reminded here. The field crop was started in June 2019 by planting seedlings obtained in greenhouse during the spring. These species are perennials, and once planted the species were monitored for two consecutive years. There were no plant losses during winter, all plants resisted over winter. After establishment of the crop, in this experiment there were no interventions such as irrigation, fertilization, growth stimulators or application of plant protection products.

The experimental plot had 1000 m$^2$ and organized in a subdivided plots design.
Sampling design

The parameters were quantified at phenophase stage 7 on BBCH scale, in both of the experimental years (Varban et al., 2021). This coincides with the moment when plants were flowering and the growth of the shoot has stopped. During this phenophase, plants are also usually harvested for herba. The observations were conducted concomitantly with phenology (Varban et al., 2021), during the same experimental years: 2019 and 2020. Determination of morphometric traits completes the assessment for favourability of cultivation, as a second step after phenological assessment (Varban et al., 2021).

Determinations were conducted for ten plants of each species in four replicates each year, randomly selected (in total 200 plants were analysed each year).

The agronomic traits determined were:
- Vegetative traits: plant height (cm) (PH), shoot number per plant (SN), leaf length (cm) (LL), leaf width (cm) (LW), leaf number per shoot (LN);
- Reproductive traits: inflorescence length (cm) (IL), verticillaster number per inflorescence (VN), flower number per verticillaster (FN).

A number of ten determinations for each agronomic trait were obtained from every plant analysed. The leaf number per shoot was determined by counting all normally and fully developed leaves. To determine the size of the leaves, leaves located at different levels on the plant (base, middle and upper third of the shoots) were harvested and measured with the ruler. The inflorescence length was determined by measuring the inflorescence with the ruler. The ten inflorescences from each plant analysed were taken from different levels on the plant. The number of flowers per verticillaster was determined by counting the flowers in each verticillaster of the inflorescence.

The studied species and method of determination for some parameters are illustrated in Figure 2.

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Method of quantifying some quantitative traits (A-B) in some Agastache species/cultivar: (C) A. scrophulariifolia; (D) A. rugosa; (E) A. foeniculum; (F) A. rugosa 'After Eight'; (G) A. mexicana

Statistical procedures

The tests applied were chosen in accordance with the objectives of the study.

For the comparative assessment of agronomic traits, were applied ANOVA test followed by Tukey’s (HSD) honestly significant differences test using RStudio version 1.4.1106 (https://www.rstudio.com) for R statistical software (https://www.r-project.org). For these tests were used the packages “agricolae” (Mendiburu, 2020) and “broom” (Robinson et al., 2021).

Relationships between agronomic traits were studied by using Pearson correlations. The correlations coefficients were obtained using package “Hmisc” (Harrel and Dupont, 2021) in R (https://www.r-
Previsioning of the interrelationships between parameters was achieved by comparing models fitted by maximum likelihood with Akaike's Information Criterion (AIC) within the "vegan" package for R (Oksanen et al., 2020), and verified with "mass" package (Ripley et al., 2021). The resulted regressions were verified and completed with formulas from package "caret" (Kuhn et al., 2021).

Projection of similarity between the studied species based on morphometric parameters was achieved through Principal Component Analysis (PCA) using PAST software version 4.05 (Hammer, 2021).

Results

Comparative evaluation of agronomic traits

Analysis of variance revealed that all agronomic traits were highly influenced by species/cultivar ($p < 0.001$) (Table 3).

Between the two experimental years, all of the eight agronomic traits studied increased in value. All species exceeded one meter in height in the second year. The cultivar *A. rugosa* 'After Eight' had in both years the tallest plants with longest inflorescence.

### Table 3. Agronomic traits of *Agastache* sp. studied in Cluj-Napoca in the experimental years (mean ± SE)

| Year | Species      | Plant height (cm) | Shoot number | Leaf length (cm) | Leaf width (cm) | Inflorescence length (cm) | Verticillaster number | Flower number |
|------|--------------|-------------------|--------------|------------------|-----------------|---------------------------|-----------------------|--------------|
| 2019 | A. rugosa 'After Eight' | 85.0 ± 0.85 | 6.7 ± 0.13 | 6.0 ± 0.22 | 4.0 ± 0.21 | 24.0 ± 1.04 | 11.2 ± 0.38 | 10.6 ± 0.36 |
|      | A. foeniculum | 70.0 ± 1.08 | 4.5 ± 0.22 | 6.5 ± 0.15 | 3.4 ± 0.16 | 23.1 ± 0.88 | 7.8 ± 0.25 | 8.5 ± 0.36 |
|      | A. mexicana  | 65.0 ± 1.03 | 5.6 ± 0.25 | 6.0 ± 0.18 | 3.5 ± 0.10 | 27.3 ± 1.00 | 7.0 ± 0.29 | 12.0 ± 0.36 |
|      | A. rugosa    | 65.3 ± 0.63 | 6.3 ± 0.16 | 6.0 ± 0.04 | 4.6 ± 0.02 | 19.8 ± 0.34 | 8.0 ± 0.29 | 9.0 ± 0.36 |
|      | A. scrophulariifolia | 73.6 ± 0.74 | 4.0 ± 0.11 | 7.5 ± 0.05 | 5.0 ± 0.04 | 25.0 ± 0.92 | 7.5 ± 0.09 | 8.5 ± 0.12 |
| F test |             | 58.86 | 34.71 | 41.24 | 22.37 | 10.95 | 45.05 | 17.73 |
| p value |             | $p < 0.001$ | $p < 0.001$ | $p < 0.001$ | $p < 0.001$ | $p < 0.001$ | $p < 0.001$ | $p < 0.001$ |
| 2020 | A. rugosa 'After Eight' | 119.6 ± 0.60 | 12.4 ± 0.19 | 7.0 ± 0.18 | 4.8 ± 0.11 | 46.4 ± 0.39 | 13.2 ± 0.27 | 13.2 ± 0.19 |
|      | A. foeniculum | 104.0 ± 1.74 | 9.4 ± 0.30 | 5.2 ± 0.16 | 4.0 ± 0.12 | 44.3 ± 0.93 | 8.8 ± 0.19 | 12.0 ± 0.39 |
|      | A. mexicana  | 103.5 ± 0.85 | 9.7 ± 0.25 | 6.8 ± 0.15 | 4.8 ± 0.12 | 54.1 ± 0.75 | 8.3 ± 0.20 | 15.2 ± 0.34 |
|      | A. rugosa    | 107.0 ± 0.35 | 13.7 ± 0.04 | 6.8 ± 0.04 | 5.6 ± 0.04 | 37.6 ± 0.38 | 8.7 ± 0.05 | 13.3 ± 0.26 |
|      | A. scrophulariifolia | 115.0 ± 1.12 | 8.3 ± 0.22 | 7.6 ± 0.05 | 5.7 ± 0.04 | 40.0 ± 0.40 | 9.3 ± 0.16 | 10.6 ± 0.20 |
| F test |             | 46.67 | 72.00 | 44.51 | 52.52 | 109.38 | 118.81 | 35.96 |
| p value |             | $p < 0.001$ | $p < 0.001$ | $p < 0.001$ | $p < 0.001$ | $p < 0.001$ | $p < 0.001$ | $p < 0.001$ |

*Notes (legend): Different letters denote significant differences (Tukey HSD significance test)
By comparison, \textit{A. rugosa} maintained in both years lowest leaf number per shoot. \textit{A. scrophulariifolia} presented in both years the widest and longest leaves. \textit{A. mexicana} presented in both years the shortest plants, but highest leaf number, highest number of verticillasters per inflorescence and highest flower number per verticilaster in both years. \textit{A. foeniculum} did not present any top performance in either of the two years compared to the others species, but leaves maintained smaller in both years since both average length and width remained smallest compared to the other species (Table S1).

Pairwise comparison revealed that between \textit{A. rugosa 'After Eight'} and \textit{A. rugosa}, five out of eight agronomic traits in both years presented significantly different values. Between \textit{A. mexicana} and \textit{A. foeniculum} there were no significant differences between average number of verticilaster and average flower number per verticillaster in either of the two years (Table 3).

**Correlations between agronomic traits**

Based on correlation coefficients between the eight agronomic traits studied, was observed that in both years existed significantly positive correlations between inflorescence length and the following variables: plant height and shoot number (Figure 3). Also, the correlation between leaf length and leaf width maintains significant in both years. However, some changes occurred between years. Although the correlation between flower number per verticillasters and verticillasters number per inflorescence was significantly positive in both years, the strength of the relationship increased in the second year. Similarly, the positive correlation between leaf number and flower number increases in the second year (Table S2).

**Previsioning interrelationships between parameters**

In 2020 the crop was considered acclimatized and it was attempted a previsioning of the possible plant architecture based on measured parameters. There were generated scenarios simulating the interdependence between agronomic traits and their variations. Thus, the model explores estimators of the relationship between predictor morphometric variables and the expected response. The interrelationships between the parameters of the studied species were analysed based on the AIC coefficient, being extracted the regression equations (Table 4).
According to the model, for *A. rugosa* 'After Eight', regarding the inflorescence’s length at 14.14 cm is added significantly 0.27 cm for each leaf that the plant develops, and 0.38 cm for each shoot. The calibration model was negatively set by the plants size and significantly positive increased by the flower number. In the case of *A. foeniculum* flower number, the basis established for calculating the was 24.79 flowers per verticillaster, to which was added a significantly 0.11 for each cm of the observed height, respectively 0.56 for each shoot developed. The width of the leaves decreases this parameter by 1.22 for every 1 cm. The inflorescences length has a high stability, the basic value being 7.28 cm, completed with 0.16 cm for each plant shoot (Table 4).

The height of *A. mexicana* plants is a trait than influences both flower number and inflorescence length. When analysing the flower number, the size was significantly influenced by plant height (0.28/1 cm), while the inflorescence length strongly reduces this parameter due to the lower number of verticillasters (-0.13/1 verticillasters).

**Table 4.** The model assessment (AIC) for *Agastache* flower number and inflorescence length and their interrelationships with the other analysed morphological parameters in the second vegetation year (2020)

| Flower number           | Indicator | Intercept | PH | SN | LL | LW | LN | VN | FN |
|-------------------------|-----------|-----------|----|----|----|----|----|----|----|
| *A. rugosa* ‘After Eight’ | $R^2$     | 34.60     |    |    |    |    |    |    |    |
|                         | $p$ value | <0.001    |    |    |    |    |    |    |    |
| *A. foeniculum*         | $R^2$     | 24.79     | 0.11| 0.56| -1.22|    |    |    |    |
|                         | $p$ value | 0.001     | 0.041| 0.065| 0.112|    |    |    |    |
| *A. mexicana*           | $R^2$     | 12.80     | 0.28|    |    |    |    |    |    |
|                         | $p$ value | 0.369     | 0.043|    |    |    |    |    |    |
| *A. rugosa*             | $R^2$     | 1.67      | 0.03| 0.05| 0.41|    |    |    |    |
|                         | $p$ value | 0.461     | 0.138| 0.029| 0.013|    |    |    |    |
| *A. scrophulariifolia*  | $R^2$     | -7.55     | -0.43| 2.31| 0.41|    |    |    |    |
|                         | $p$ value | 0.525     | 0.141| 0.086| 0.014|    |    |    |    |
| All                     | $R^2$     | 21.52     | -0.09| 0.82| -1.54| 0.37| 0.94|    |    |
|                         | $p$ value | 5.93      | 0.041| 0.14| 0.34| 0.474| 0.06| 0.171|    |

| Inflorescence length    | Indicator | Intercept | PH | SN | LL | LW | LN | VN | FN |
|-------------------------|-----------|-----------|----|----|----|----|----|----|----|
| *A. rugosa* ‘After Eight’ | $R^2$     | 14.14     | -0.11| 0.38|    | 0.27| -0.13|    |
|                         | $p$ value | 0.115     | 0.102| 0.080| 0.016| 0.040|    |    |
| *A. foeniculum*         | $R^2$     | 7.28      | 0.16|    |    |    |    |    |    |
|                         | $p$ value | <0.001    | 0.102|    |    |    |    |    |    |
| *A. mexicana*           | $R^2$     | 4.89      | 0.05|    |    |    | -0.13|    |    |
|                         | $p$ value | 0.244     | 0.159|    |    |    | 0.161|    |    |
| *A. rugosa*             | $R^2$     | 2.19      | 0.53| 6.06| -0.35| 0.40|    |    |    |
|                         | $p$ value | 0.874     | 0.015| 0.004| 0.069| 0.149|    |    |    |
| *A. scrophulariifolia*  | $R^2$     | 3.25      | 0.15|    |    |    |    |    |    |
|                         | $p$ value | 0.179     |    |    |    | 0.0156|    |    |
| All                     | $R^2$     | -3.71     | 0.120| 0.33| -0.720| 0.07| -0.080|    |    |
|                         | $p$ value | 0.07      | <0.001| <0.001| <0.001| <0.001| <0.001| 0.001|    |

*Notes (legend):* plant height (PH), shoot number (SN), leaf length (LL), LW (leaf width), leaf number (LN), verticillasters number (VN), flower number (FN).

Examination of coefficients for *A. rugosa* indicated a large number of parameters involved in determining the flower number and their length. The flower number has a low calculation base (1.67), but is significantly increased by 0.05 units/1 shoot, respectively 0.41 units/1 cm of leaf length. The inflorescences length is significantly higher due to the width of the leaves, 6.06 cm/1 cm wide, significantly supported by the
shoot number and the number of verticillasters. Instead, the pattern is negatively correlated by the leaf number (not significant), with a reduction in inflorescence length of 0.35 cm/leaf.

*A. scrophulariifolia* had a high number of parameters influencing the flower number. Increase both in leaf length and leaf number being associated with increased flower number. Contrary, the number of shoots have a reducing effect for the inflorescence length. At an inflorescence length of 3.25 cm as base, is added 0.15 cm/1 developed leaf.

**Projection of studied species based on the morphometric parameters**

Projection based on the agronomic traits analysed, resulted in the clustering of species that enables visual identification of similarity or divergence among them (Figure 4). The first two components obtained explain over 70% of the variance observed in both years (Table S3). The cluster of data points corresponding to each species is packed closer together in the second year compared to the first, fact that might indicate to the stabilization of all species. The species *A. foeniculum* maintained a certain degree of overlapping with other species in both years, but notably with *A. rugosa*. In the first year, *A. foeniculum* also presented a certain overlapping with *A. scrophulariifolia* but this similarity did not maintain in the second year. The cultivar *A. rugosa* ‘After Eight’ clusters on plant height vector in both years, because it maintained the tallest during the experiment. The PCA analysis indicates that the analysed species expressed their phenotypic particularities during the studied interval, but differences become pronounced as plants become established and, evidenced by their clearer distinction in the second year.

![Figure 4](image)

**Figure 4.** Principal component analysis of *Agastache* species displaying the least distance spanning tree, where vectors represent the eight agronomic traits studied in: (A) 2019, (B) 2020; ellipse 95% confidence interval; component values (PC1, PC2) indicate percentage of explained variance

**Discussion**

In the current context, the growers of medicinal crops are presented with the opportunity to extend their range of cultivated species, but they face difficulties at implementing the cultivation phase. These arise from the lack of informed knowledge to generate technological packages. Thus, agronomists must engage into defining suitability for cultivation and providing a consistent basis of knowledge and recommendations for local growers to enable them to overcome challenges related to extending their range of cultivated medicinal species.

Although the literature describes five stages of transition from wild harvesting to possible cultivation, the fifth stage called “cultivation phase” representing the stage when formal cultivation systems are designed
and instituted (Schippmann et al., 2002) might benefit from a broadened and detailed consideration. This stage could be enlarged, to address also challenges related to species proposed for cultivation in other geographical areas, where their behaviour is known. Establishing best technological approaches, agronomic calendar of agricultural activities in relation with the behaviour and development of the species either new in cultivation or new to the area, requires a methodical approach. The stages of knowledge that could be considered when extending the cultivation of species from other geographical regions are summarized in Figure 5.

By applying adequate cultivation technology, the plant growth and development can be optimized to achieve biologic uniformity or high level of active principles (Muntean et al., 2016; Schippmann et al., 2002). In addition, cultivation can ensure a steady flow of raw material including from non-native species through a short-value chain (Schippmann et al., 2002). Further selection and breeding efforts can give rise to highly productive genotypes (Wang et al., 2020). Cultivation can also enable accurate planning of harvesting moment and processing of vegetal biomass (Vârban et al., 2021).

![Figure 5. The interlinked stages within cultivation phase for species introduced/extended into cultivation in new areas](image)

In the context of increasing demand of herbal remedies, safety and quality also come into forefront. Thus, at the European level, the Committee on Herbal Medicinal Products indicates in the “Guideline on Good Agricultural and Collection Practice” (GACP) that in the cultivation of medicinal plants the substances for growth and crop protection should be avoided or kept at the minimum while fertilizing agents should be applied sparingly (EMEA, 2006). In addition, World Health Organization announces that it is in the process of implementing the Traditional Medicine Strategy until 2023 (WHO, 20019). These guiding instruments provide principles and directions to be followed that supports a long-term and increasing contribution of medicinal plants to health and well-being.

Although there is an opportunity for growers to extend their range of cultivated species in the current context, the cultivation has to ensure a safe and natural biologic product. This springs up from increased demand of medicinal plants worldwide, that is fuelled by the association of their use with health promoting properties and a healthy lifestyle. Therefore, it is understandable why the final consumer generally prefers products derived from medicinal plants such as herbal medicines, teas, essential oils and extracts in cosmetics that have been grown as natural as possible. One of the most economically important botanic families for medicinal and aromatic purposes is Lamiaceae, that comprises some of the most widely used herbs, therefore the interest for species from this family, such as *Agastache* spp., is high (Hernandez-Leon et al., 2021).

Based on this study, were determined the morphometric characteristics of *Agastache* sp. in local conditions. Overall, the studied species/cultivar displayed promising agronomic traits, and thus with promising potential to be grown and extended in cultivation, either for ornamental or for medicinal purposes. The average values for all parameters were higher in the second year, while the relationship between some traits tightens. The projection revealed a clearer distinction in second year. All these indicate to the acclimatization of the
studied species. Similarly, monitorization of acclimatization process of *A. foeniculum* conducted in Kremenets Botanical Garden from Ukraine between 2013 and 2015, reported also an increase in plant height from first (75.3 cm) to second year (135.7 cm) (Melnnychuk and Rakhmetov, 2016). There is evidence that with age the presence of major compounds also changes. Content of rosmarinic acid, apigenin glucoside, chlorogenic acid were shown to be significantly differed between *A. rugosa* plants of different age (Bielecka et al., 2019).

Plant height of *A. rugosa* in this study situates within the threshold of 60-120 cm mentioned in literature (Lord, 2003). In nine collections of *A. rugosa* from South Korea, grown under field conditions, average stem length was 88.86 cm, lateral branch number 23.93, leaf 9.80 × 6.88 cm, while the inflorescence length was 12.27 cm (Ok and Chae, 1998). By comparison, in our study *A. rugosa* presented taller plants in second year than reported by the authors cited, but the inflorescence and leaf size were smaller. Genotypes collected from seven regions of South Korea presented a plant height of 151.5 cm, leaf size of 8.4 × 6.1 cm, inflorescence length of 13.7 cm (Lee et al., 1999). The parameters obtained in our study are lower for this species, however, one has to consider that East Asia is the native range, and therefore it could be expected that plants present superior values of morphometric parameters there. In a later study was showed that the application of calcium nitrate ensured increased average values of stem and inflorescence length of *A. rugosa*, as well as increased leaf length and width. Under different N application rates, the average shoot length was 75.2-81.5 cm, inflorescence length 10.2-11.1 cm, leaf length 8.3-8.9 cm, leaf width 5.6-6.2 cm. Study also showed that estragole content at blooming remained higher in leaves compared to inflorescence at all N fertilization levels tested or under different N forms (Ohk et al., 2000), outlining the importance of leaves as source of bioactive compounds. Such results come to highlight the existing optimization possibilities by applying adequate technology.

A study conducted in Mexico on the domestication of *A. mexicana*, indicated that encouraged genotypes presented a plant height of 98.80 cm and inflorescence length of 19.73 cm while cultivated *A. mexicana* presented a vegetative height of 90.53 cm and inflorescence length of 12.15 cm (Carrillo-Galván et al., 2020). According to the values obtained in our study, *A. mexicana* presented taller plants in second year than those registered by either encouraged or cultivated genotypes from Mexico, but average inflorescence length had smaller values. This could be attributed to the climate of Romania, that is colder, more humid, while solar radiation lower considering latitudinal position, and all these probably favoured the vegetative development.

A study conducted on some *Agastache* species grown under a hydroponic culture system for 4 weeks, indicated that *A. foeniculum* was outperformed regarding vegetative parameters by some *A. rugosa* cultivars. Thus, while *A. foeniculum* presented a stem length <30 cm in height and lower leaf number (<20), both *A. rugosa* ‘Spike snow’ and *A. rugosa* ‘Spike blue’ presented a stem over 30 height and higher leaf number (>20) after 4 weeks of cultivation in hydroponic system (Do et al., 2020). This suggests that while through selection some cultivars are better suited for hydroponic cultivation, *A. foeniculum* exhibits less satisfactory results in such conditions. Another study conducted in pot conditions in greenhouse, reported for *A. foeniculum* an average plant height on 10th leaf of 118.11 cm for the diploid plants and of 85.98 cm for the tetraploids. Also, average inflorescence length was 5.19 cm in the diploid plants and 16.82 cm in the tetraploid ones (Talebi et al., 2017). Genetic engineering and plant breeding certainly present some interesting possibilities to customize genotypes able to express maximized agronomic traits under either protected or field conditions and can represent a viable approach in optimizing the cultivation of these medicinal species. There is also emerging evidence suggesting that through adequate technology the morphometric traits of *A. foeniculum* can be enhanced. In this sense, a study has showed that foliar application of 2 g/L urea significantly increased the leaf number and plant height of *A. foeniculum* (Jahani et al., 2018).

*A. foeniculum* is the only species from this genus that has been considered for cultivation in local conditions before. A study from 2013 conducted on *A. foeniculum* that took place in Cluj County, indicated a yield of 3.05-3.83 t/ha dry weight. Out of the *herba* production obtained, highest proportion was represented by leaves and branches, together comprising 61.64% of the *herba* production, followed by inflorescence and main stem with a lower contribution (Duda et al., 2013). Although in general the generative characters are to a less extent influenced by environmental conditions and age of the plant, in this study both the vegetative and
generative parameters registered an increasing trend from one year to the next. This might be explained by the relationships exiting between vegetative and generative traits, considering that inflorescences are actually terminal ramifications of the stems.

Since in the current study were assessed the morphometric parameters in the absence of any interventions, the values reported can be considered the natural phenotypic potential of these species/cultivar in local conditions.

Conclusions

Between market opportunities and implementing diversification of medicinal crops, there is a missing link arising from challenges and difficulties faced by growers in up-scaling cultivation of novel or niche medicinal crops in their area. The successful cultivation of medicinal plants is conditioned by the phenotypic expression of given genotypes in certain environmental conditions. Precise knowledge of the phenotypic potential is a starting point for assessing feasibility of medicinal plants.

The present work tried to define the phenotypic potential in local conditions (Cluj-Napoca, Romania) for some medicinal species from genus Agastache that are not native to Europe in order to identify the favourability of their cultivation.

The eight agronomic parameters studied increased in values in the second year as the plants acclimatized and became established. In the second-year after planting in field, the average height was 109.8 cm. The shoot number almost doubled between first and second year, suggesting the productivity of these species. Average inflorescences length was 8.3 cm in the first year (2019) and 9.6 cm in the second year (2020). Among the species/cultivars studied, A. rugosa ‘After Eight’ was characterized by tall plants with longest inflorescence in both years and could be recommended also for ornamental purposes. At the opposite was situated A. mexicana that was characterized by shortest plants and shortest inflorescence in both years, but high number of verticillasters and flowers. A. foeniculum presented smallest leaves in both years, opposite to A. scrophulariifolia which presented largest leaves in both years. In both years existed significantly positive correlations between inflorescence length and the following variables: plant height and shoot number.

The PCA projection indicated that the analysed species expressed their phenotypic particularities during the studied interval, but differences were more pronounced as plants became established, evidenced by their clearer distinction in the second year.

Based on the present research were put in evidence some differences between studied species regarding the agronomic traits studied. These can be considered for targeted improvement in breeding programs of these species in order to achieve genotypes with balanced growth and development and acclimatized to local conditions. Despite differences, all species studied can be considered for widening the range of cultivated medicinal species.

Authors’ Contributions

Conceptualization, RV and DIV; methodology, RV, DIV, AS, AO, VaS and VS; software, AO, IC and VS; validation, RV and VS; formal analysis, RV, DIV, AS, AO, VaS, VS; investigation, RV, DIV, AS, AO, VaS, VS; resources, RV, DIV and AS; data curation, RV; writing – original draft preparation, RV, AS, AO, VaS, IC and VS; writing – review and editing, RV, RVi, ŠG and SV; visualization IC and VS; supervision, RV, RVi, ŠG and SV. All authors have read and agreed to the published version of the manuscript. All authors read and approved the final manuscript.
Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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