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A Strategic Breeding Approach for Improvement of a Native Greek Chamomile (*Matricaria chamomilla* L.) Population for High-Yield and Optimized Chemical Profile Under Mediterranean Low-Input Conditions

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Abstract

Chamomile (*Matricaria chamomilla* L.) is a popular herb of great economic and medicinal value. Despite its significant potential, there are currently no commercially available varieties specifically adapted to Mediterranean low-input farming systems. The present study aimed to develop a genetically improved breeding population derived from indigenous Greek chamomile germplasm, following a multi-year strategy, based on pedigree selection under low-input conditions. This selection process constituted the first phase of the breeding program, during which selection focused on improving inflorescence dry weight and essential oil quality, particularly with respect to α-bisabolol and chamazulene content. After three cycles of selection, considerable genetic gains were achieved. The realized heritability values exceeded 0.5 for all assessed traits, confirming strong genetic control. In the fourth year, representing the second phase of the breeding program, the breeding population—developed through selection during the first phase—was evaluated alongside the initial population and commercial diploid and tetraploid varieties. The breeding population exhibited significant observed gains compared to the initial population: inflorescence dry weight increased by 12.17%, α-bisabolol content by 71.45%, and chamazulene content by 6.57%. Additionally, the breeding population not only surpassed all evaluated diploid genotypes in essential oil composition, but also displayed a chemotypic shift, indicating successful alignment with tetraploid varieties characterized by high-value chemical profiles. Furthermore, this selection process targeting specific commercial chamomile traits indirectly contributed to improvement in plant height and inflorescence morphology. Overall, these results demonstrate that conventional breeding, when applied effectively to native resources, can enhance both agronomic performance and essential oil profile. The newly developed breeding population provides a strong foundation for future cultivar development tailored to Mediterranean low-input sustainable farming systems.

Keywords: genetic variability; plant selection; heritability; breeding program; medicinal and aromatic plants; essential oil composition; low-input farming system

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1. Introduction

Chamomile (*Matricaria chamomilla* (L.) syn.: *Matricaria recutita* (L.), *Chamomilla recutita* (L.) Rauschert) is a highly valued medicinal aromatic plant, with a strong presence in the global market [1,2]. More than 120 components are found in the essential oil and the extracts of chamomile [3], with applications across the pharmaceutical, cosmetic, fragrance, agrochemical, biopesticide, and food industries [4,5]. Among the various constituents of chamomile essential oil, α-bisabolol and chamazulene are considered the most important [6–8]. Currently, chamomile is included in the Pharmacopeias of 26 countries [9–11]. Over the past two decades, many farmers have been motivated to shift from traditional crops to systematic cultivation of chamomile [12].

In addition to its economic importance, chamomile has drawn considerable attention in recent years for its potential contribution to environmental sustainability. The ongoing climate crisis is one of the most pressing challenges of the 21st century [13]. Thus, the need to implement comprehensive and effective adaptive agricultural strategies is crucial. Such measures should prioritize the promotion of alternative crops, which are not only tolerant to extreme and fluctuating environmental conditions, but also exhibit a low energy footprint and are well-suited to local agroecological environments. Among these strategies, the use of native genetic resources is critical for breeding programs aimed at developing new cultivars that are both climate resilient and regionally adapted.

Chamomile demonstrates agronomical and ecological traits consistent with the above requirements. It can grow in different types of soil, whether alkaline or acidic, while its irrigation and fertilization needs are minimal, as it is considered a low-input crop, capable of thriving in poor soils [4,14]. Numerous studies have reported chamomile's resilience and adaptability to a wide range of edaphoclimatic conditions and abiotic stresses [15–23].

Despite its great importance, the absence of high-quality and high-yield chamomile breeding varieties, adapted to diverse agro-climatic regions and conditions remains a major problem even nowadays [12,24,25]. Chamomile is an annual diploid (2n = 2x = 18) [14], mainly cross-pollinated species; however, the exact percentage of cross- or self-pollination is not determined and could differ within the varieties [26]. Most of the cultivated chamomile varieties are diploid, but there are also some tetraploid ones mainly developed through colchicine treatments [4]. Many researchers point out that most of the varieties are actually landraces or populations, making them quite heterogeneous, with a significant level of variability in phenotypic characteristics [27,28].

Regarding the Mediterranean basin, chamomile production largely relies on native chamomile populations, landraces, or, in some cases, on cultivation of foreign varieties that are poorly adapted to local conditions, thus yielding less than their potential if cultivated in environments for which they were originally bred. Salamon et al. [29] emphasize the importance of chamomile populations to the overall harvested chamomile production in Albania. Fahmi [30] describes chamomile as a crop in Egypt, pointing out that there are no specific varieties available for cultivation. Franke et al. [31] mention the importance of harvesting native populations in chamomile production in Turkey. In agreement with these findings, Tsivelika et al. [32] point out the lack of commercially available chamomile varieties, well-adapted to Greek environmental conditions. Most breeding efforts in chamomile have traditionally focused on landrace selection or polyploidization, leaving a gap for systematic multi-year breeding approaches that exploit native genetic resources under realistic, low-input conditions.

Considering these challenges and the limited availability of high-performing chamomile cultivars adapted to Mediterranean environments, this study addresses the above gap by developing a new genetically upgraded population, based on native Greek chamomile germplasm, using a multi-year breeding strategy. The breeding program was implemented

in two distinct phases. The first phase was carried out over three years, using a field-based pedigree selection strategy designed to enhance inflorescence yield and optimize the composition of essential oil, focusing on the constituents α-bisabolol and chamazulene. During the second phase, the selected germplasm was evaluated under dense planting conditions and compared with commercial varieties. The ultimate goal of this research was to generate a stable and well-adapted new cultivar suitable for low-input and organic farming systems for the Mediterranean region.

2. Materials and Methods

2.1. Plant Material and Experimental Procedure

All experiments were conducted at the Institute of Plant Breeding and Genetic Resources (IPGRB), Thermi, Thessaloniki, Greece, over a four-year period (2016–2019). As starting material, a native chamomile population from Pella, Central Macedonia, Greece—initial population (IP)—known for its promising characteristics and superior essential oil quality, was used [32].

A two-phase breeding program was designed (Figure 1) to enhance both agronomic performance and essential oil quality under Mediterranean, low-input conditions. The first phase (Phase I) focused on developing improved chamomile breeding material—breeding population (BP)—through a multi-year selection process, while the second phase (Phase II) involved evaluating the BP under real farming conditions.

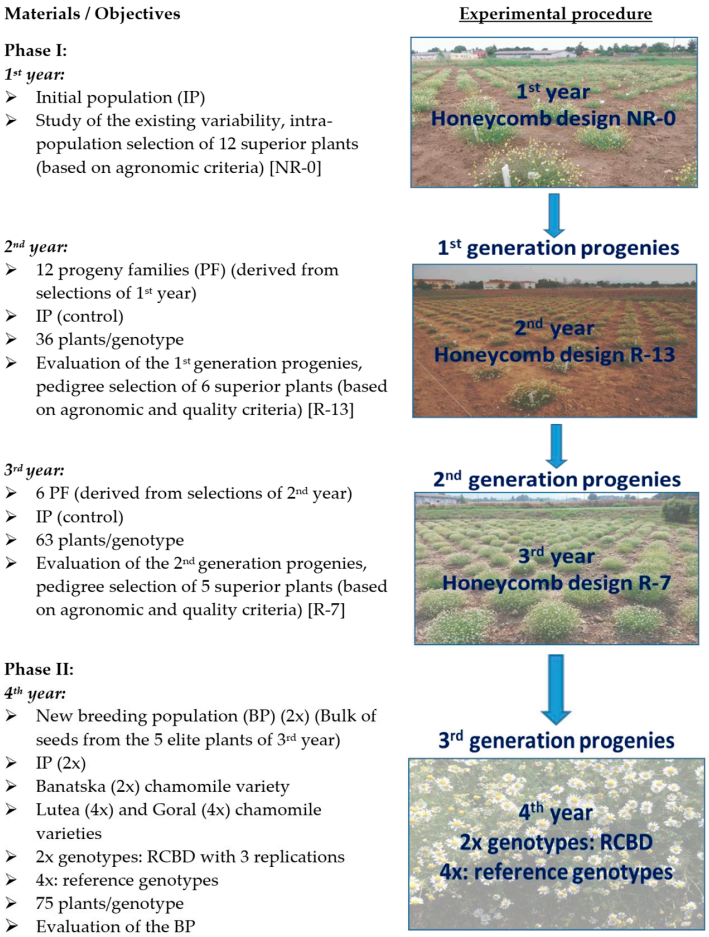


Figure 1. Overview of Phase I and Phase II of the chamomile-breeding program. Phase I: Sequential pedigree selection using the honeycomb design. Phase II: Field evaluation of the selected genotypes under dense (farmer) planting conditions. (IP: initial population, PF: progeny families, BP: breeding population).

2.1.1. First Phase of Breeding Program (Phase I)

A three-year consecutive pedigree selection program was conducted in open-field conditions, following the model of honeycomb design (Figure 2) and sequential selection for main agronomic and phytochemical traits of chamomile. The honeycomb design is based on the principle of wide plant spacing, which minimizes interplant competition and enables precise phenotypic evaluation of individual genotypes. Each plant is uniformly surrounded by individuals from all other families of the experiment, facilitating accurate inter-family comparisons (Supplementary Figure S1) [33–36].

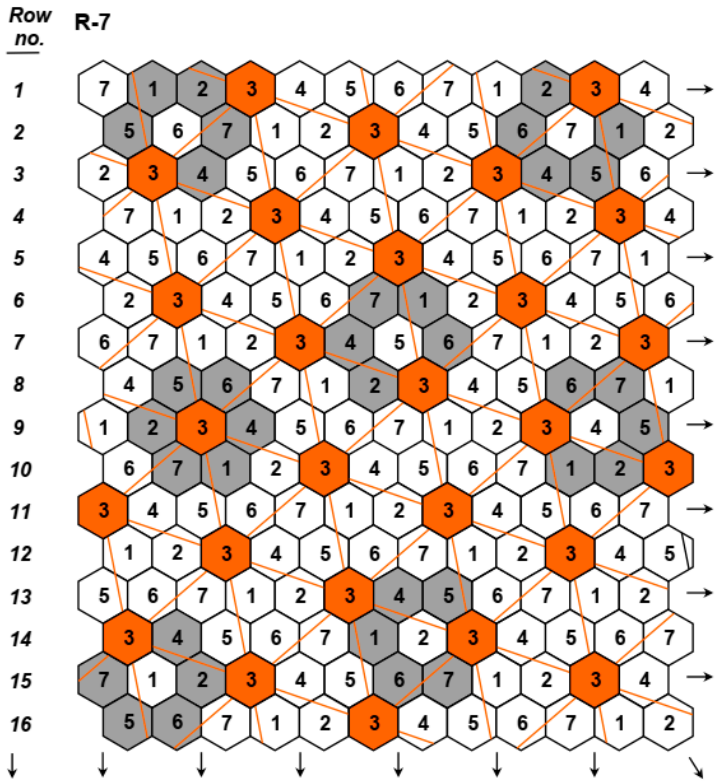


Figure 2. Schematic representation of honeycomb design and plant layout in the field. Illustration of R-7 design (reproduced from Fasoula [36]). Each plant is uniformly surrounded by individuals from the 6 other families in the experiment, allowing for accurate inter-family comparisons [33,36].

Specifically, in the first year, a total of 160 plants of the IP were established in the field using a non-replicated NR-0 honeycomb design at an ultra-low density of 1.2 plants m^{-2}. Each plant was evaluated individually based on agronomic characteristics. Following intra-population selection, 12 superior plants were identified, producing the progeny families (PF) for the next year.

In the second year, these 12 PF, along with individual plants from the IP—serving as control—were evaluated using an R-13 replicated honeycomb design. A total of 468 plants—36 plants per genotype—were grown under the same spatial arrangement as in the first year. Based on comprehensive assessment of agronomic and quality traits, 6 high-performing plants were selected, producing the PF for the next year.

In the third year, the 6 PF and individual plants from the IP—used as control—were planted in an open field using an R-7 replicated honeycomb design. 63 plants per genotype were grown. Selection was again performed based on key agronomic and quality related criteria.

This three-year sequential selection breeding approach (Phase I), employing the honeycomb design and focusing on principal traits, led to the identification of 5 advanced chamomile genotypes, with superior yield and quality characteristics (Figure 1).

2.1.2. Second Phase of Breeding Program (Phase II)

Following the first phase pedigree selection under honeycomb design, seeds from the 5 elite plants were bulked to create a new BP. This newly formed population was evaluated for commercial trait performance under dense (farmer) planting conditions.

In the fourth year (Phase II), a randomized complete block design (RCBD) with three replications was established to compare three diploid genotypes: the existing IP, the newly developed BP and the commercial variety Banatska. Commercial tetraploid varieties (Lutea, Goral) were also cultivated in an adjacent field, under similar conditions, and were included as reference materials (Figure 1). According to the literature, tetraploid varieties outperform diploid ones in morphological, agronomic and quality traits, generally exhibiting higher values and yield [37–41]. Therefore, it was considered appropriate to evaluate the diploid genotypes among themselves under experimental conditions, while using the tetraploid ones as reference materials. The commercial varieties were selected based on their adaptability to environments similar to those of Greece, on their ploidy level and/or on proven superiority in key traits, such as high content of α-bisabolol and chamazulene in their essential oil [31,32,42–44].

The plants of all genotypes were spaced at 30 × 30 cm—representing dense planting conditions and reflecting the standard spacing commonly used in commercial chamomile crops—an arrangement shown to maximize crop and essential oil yield [45–49]. Each plot measured 1.50 × 1.50 m and consisted of 5 rows with 5 plants per row. Thus, each genotype comprised a total of 75 plants across the three replications.

2.2. Environmental Conditions and Cultivation Treatments

The climate type was the typical Mediterranean climate, classified as Csa: Temperate climate with dry-hot summer in the Köppen climate system (Supplementary Table S1) [50]. The soil type was loamy, with differences in the composition through the years (Supplementary Table S2). The cultivation treatments followed the organic farming principles. No herbicides or fertilizers were used, the weed control was performed manually and through mechanical inter-row cultivation, pest management relied on natural enemies, and irrigation applied was limited (120–180 mm).

2.3. Agronomic and Morphological Characteristics

Agronomic performance was assessed by measuring the inflorescences dry weight, which is the primary commercial yield component of chamomile. The drying conditions were shade, low humidity and room temperature (20–25 °C).

In the second phase of breeding program, morphological characteristics were additionally measured. Three chamomile traits—plant height, head and disc diameter of inflorescences—closely associated with yield and essential oil content [51], were evaluated.

All measurements were conducted at full blooming stage on an individual plant basis, except during the second phase of breeding program, where the inflorescences dry weight was assessed per m^2. Harvesting was carried out at development stage III of inflorescences, when more than 50% of the tubular flowers were open [52].

2.4. Essential Oil Isolation and Analysis

During the first phase of the breeding program, the essential oil isolation and analysis was performed on an individual plant basis, resulting in a limited quantity-per-sample available for analysis. Due to scarce plant material available per sample, the technique of simultaneous distillation extraction (SDE) was applied to obtain the volatile fraction by using the Likens–Nickerson apparatus, since this method has the advantage of obtaining volatile compounds from plant matrices, requiring small amounts [53]. A sample of 2.5 g

of dried inflorescences was used for each extraction and 3 replicates per plant took place. Pentane was used as a solvent and each extraction was carried out for 2.5 h. The volatile fraction was isolated from pentane by rotary evaporator (Heidolph Laborata 4000-Efficient) (Heidolph Instruments GmbH & Co, Schwabach, Germany).

In the second phase of the breeding program, the amount of sample was referring to the dried inflorescences of each genotype per m^2, so the quantities were sufficient to allow the use of a solvent-free isolation technique, making it possible to obtain pure essential oil and accurately measure the essential oil content. Therefore, a Clevenger-type hydro distillation apparatus was used. A sample of 50 g of dried inflorescences was used for each extraction and 3 replicates per genotype took place. The samples were subjected to hydro distillation for 3 h each [32].

All essential oil samples were stored at 4–6 °C until their analysis. The analysis was conducted by means of a GC-MS on a fused silica DB-5 column, using a Gas chromatograph 17A (ver. 3) interfaced with a mass spectrometer Shimadzu QP-5050A (SHIMADZU EUROPA GmbH, Duisburg, Germany) supported by the GC/MS Solution (ver. 1.21 software), using the method described by Sarrou et al. [54]. The conditions of analysis and the procedure for identification of the compounds were the same as the ones described by Tsivelika et al. [32]. All samples were analyzed in triplicate.

2.5. Statistical Analysis

The data were analyzed by using SPSS software (version 16, IBM). An analysis of variance (ANOVA) was applied to the randomized complete block design. All hypothesis tests were evaluated at a significance level of $\alpha \leq 0.05$, and mean comparisons were performed using Duncan's multiple range test. The Shapiro–Wilk test confirmed that the data followed a normal distribution. The ANOVA assumptions of homogeneity of variances and residual normality were verified using Levene's test and inspection of quantile–quantile (QQ) plots, respectively [55]. The honeycomb designs were analyzed by using a specialized program specifically developed for plant selection and analysis of honeycomb trials [56,57]. Breeding parameters were also estimated—selection differential (S), response to selection (R), realized heritability (h^2)—as described by Koutsika-Sotiriou et al. [58], in order to evaluate the genetic gain. For the second phase of breeding program, a descriptive heatmap was generated from GC-MS data. Analysis was performed with MetaboAnalyst platform [59]. Clustering was conducted using Euclidean distance and Ward clustering algorithm.

3. Results

The primary objective of Phase I of the breeding program was the identification and selection of superior individual plants within the IP. These selections were intended to form a new BP capable of optimal adaptation to Mediterranean conditions and competitive performance, equivalent to already existing chamomile varieties. This objective was assessed through sequential selection and further validated during Phase II of the breeding program.

3.1. First Phase of Breeding Program (Phase I)

Throughout Phase I, the IP with high variability (Figure 3) and notable qualitative profile [32] underwent a structured pedigree selection process using the honeycomb design over three consecutive years. In the first year, selections were based on the primary agronomic characteristic of chamomile—inflorescences dry weight—which is the crop's main commercial part. In subsequent years, essential oil composition became an additional selection criterion, with emphasis on two key components, α-bisabolol and chamazulene. This strategy aimed to first isolate high-yielding individuals and then identify among

them those with superior quality. Therefore, from the second year onward, for the plants exhibiting the best agronomic performance each year, a further selection process was carried out based on their qualitative traits, in order to ultimately identify the most promising plants of each year (Tables 1 and 2).

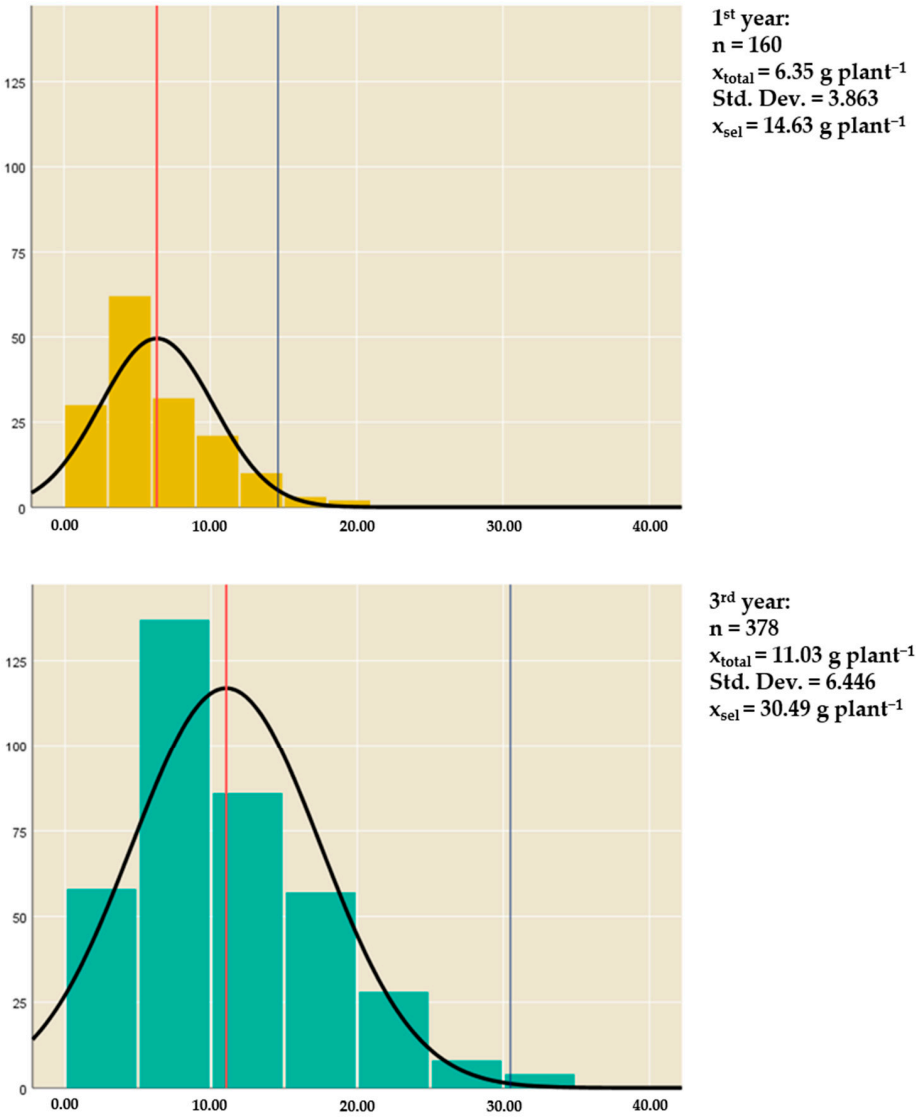


Figure 3. Frequency distribution of individual plant inflorescences dry weight in the initiation—1st year and completion—3rd year of Phase I of the breeding program. (The red line represents the mean value per plant of the total population, while the blue line indicates the mean value per plant of the selected plants in each year).

Table 1. Mean values of the inflorescence yield per plant in 1st year and the final—3rd year—of selection.

Agronomic Characteristics		Phase I of the Breeding Program	
		Initiation—1st Year	Completion—3rd Year
Inflorescences: dry weight plant^{-1} (g plant^{-1})	x_{total} *	6.35	11.03
	$x_{selected}$ **	14.63	30.49
	S ***	8.28	19.46
	R ***		5.70
	h^2 ***		0.568

* x_{total}: Mean value per plant of the total population from which the selections derived; ** $x_{selected}$: Mean value per plant of the selected plants; *** Breeding parameters: (S) selection differential, (R) response to selection, (h^2) realized heritability.

Table 2. Mean values of α-bisabolol and chamazulene (%) per plant in 2nd year and the final—3rd year—of selection.

Quality Characteristics		Phase I of the Breeding Program	
		Initiation—2nd Year	Completion—3rd Year
Essential oil: α-bisabolol (%)	x_{total} *	24.63	31.15
	$x_{selected}$ **	35.45	41.78
	S ***	10.82	10.63
	R ***		6.52
	h^2 ***		0.603
Essential oil: chamazulene (%)	x_{total}	18.13	21.60
	$x_{selected}$	22.37	25.16
	S	4.24	3.56
	R		3.47
	h^2		0.818

* x_{total}: Mean value per plant of the total population from which the selections derived; ** $x_{selected}$: Mean value per plant of the selected plants; *** Breeding parameters: (S) selection differential, (R) response to selection, (h^2) realized heritability.

Regarding inflorescences dry weight per plant, substantial progress was achieved. The population began with a relatively low mean value in the first year (6.35 g plant^{-1}) and through targeted selections nearly doubled in the third year (11.03 g plant^{-1}). A similar trend was observed among the selected individual plants in both the first and third year (14.63 and 30.49 g plant^{-1}, respectively) (Figure 1 and Table 1). The effectiveness of selection is further reflected in the realized heritability (0.568) recorded for this agronomic trait at the completion of Phase I of the breeding program (Table 1). These results are consistent with previous findings indicating that among various agronomic traits, inflorescence head weight is predominantly influenced by genetic factors, rather than environmental conditions [60].

The evaluation of the two key quality components—α-bisabolol and chamazulene—of chamomile essential oil revealed significant genetic gain at the end of Phase I of the breeding program. Specifically, for both bioactive components, the realized heritability was above 0.5, with 0.603 for α-bisabolol and 0.818 for chamazulene. For α-bisabolol, the mean values in the 3rd year reached 31.15% (x_{total}) and 41.78% ($x_{selected}$) and for chamazulene 21.60% (x_{total}) and 25.16% ($x_{selected}$) (Table 2). These values not only reflect a high qualitative profile, due to high content of chamazulene and α-bisabolol, but also align with studies reporting strong heritability for these two components [7,60].

The above results highlight the effectiveness of the selection process and the breeding strategy implemented. Further evaluation to confirm and assess the consistency of the proposed breeding progress was substantiated in Phase II of the breeding program.

3.2. Second Phase of Breeding Program (Phase II)

In Phase II of the breeding program, a new BP was developed by combining seeds from the 5 superior genotypes selected during Phase I. This population was compared with the IP, a commercial diploid variety Banatska, and two tetraploid varieties—used as reference—Lutea and Goral, under dense planting conditions. The new BP outperformed the IP in both dry weight of inflorescences and two main components of essential oil (α-bisabolol and chamazulene). Indeed, regarding the essential oil quality, the BP not only had the highest percentage of α-bisabolol and chamazulene from all the diploid genotypes, but also approached, or even exceeded, the quality values of the tetraploid ones, which are described in the literature as varieties with high content in α-bisabolol and chamazulene [31,32].

More specifically, in terms of agronomic performance, the BP exhibited improved inflorescences dry weight compared to the IP (63.00 g m^{-2} and 56.17 g m^{-2}, respectively), but was still lower than the diploid variety Banatska (68.43 g m^{-2}). The observed gain for this characteristic was 12.17% between the BP and IP. As expected, all diploid genotypes exhibited lower inflorescences dry weight values compared to the tetraploid ones [32,61,62] (Table 3).

Table 3. Inflorescences dry weight and essential oil content and composition of the genotypes evaluated during Phase II of the breeding program.

Genotype * (Ploidy Level)	Inflorescences		Essential Oil Content and Components				
	Dry Weight (g m^{-2})	Observed Gain (%)	Essential Oil Content (%)	α-Bisabolol (%)	Observed Gain (%)	Chamazulene (%)	Observed Gain (%)
IP (2×)	56.17 ^{c} **	12.17	0.37	27.95 ^{b}	71.45	16.14 ^{b}	6.57
BP (2×)	63.00 ^{b}		0.37	47.93 ^{a}		17.20 ^{a}	
Banatska (2×)	68.43 ^{a}		0.45	7.26 ^{c}		8.20 ^{c}	
Lutea (4×)	86.37		0.61	43.47		21.83	
Goral (4×)	84.49		0.67	26.80		22.03	

* IP: the initial population before the breeding program, BP: the new breeding population derived from the selections after Phase I of the breeding program; ** Data represent the mean values. Values with different letters are statistically different, while values with no letter exhibiting no difference statistically, according to Duncan's test for $\alpha \leq 0.05$.

Regarding quality, the essential oil content remained stable in both populations (IP and BP) (0.37%), and was lower than Banatska (0.45%). However, these three diploid genotypes did not exhibit significant differences among them. Despite the stable essential oil content, the fact that the dry weight of inflorescences per square meter is higher in BP than in the IP could lead to the conclusion that, in terms of essential oil yield per square meter, the BP may outperform the IP. Concerning the tetraploid varieties, researchers outline their superiority over diploid genotypes [32,62], an observation that was confirmed in the present study as well, with essential oil content ranging from 0.61 to 0.67% in tetraploid varieties, compared to 0.37–0.45% in diploid genotypes (Table 3).

With respect to the two main components of chamomile essential oil, the results were more conclusive, confirming that the selection process during Phase I of the breeding program resulted in a desirable observed gain. More specifically, the α-bisabolol content in the BP nearly doubled (47.93%) compared to the IP (27.95%), corresponding to a high observed gain (71.45%). The BP not only outperformed all the diploid genotypes, but also succeeded in surpassing the α-bisabolol content of tetraploid varieties (26.80–43.47%), which, according to the literature, are high α-bisabolol and chamazulene content varieties [31]. In terms of chamazulene concentration, the BP outperformed all diploid genotypes. Specifically, the differences were significant, with the content in the BP being 17.20%, compared to 16.14% in the IP and 8.20% in Banatska. The observed gain between the two populations in this case was 6.57%. The chamazulene content for the varieties Lutea and Goral—classified as high chamazulene chemotypes [31]—was above 20% (21.83% and 22.03%, respectively). However, the BP managed to approach this level more closely than the other two diploid genotypes (Table 3).

To further evaluate chemical profile similarities between all the genotypes—diploid and tetraploid ones—a heatmap with hierarchical clustering was used, and we evaluated not only the essential oil content and its two key components—α-bisabolol and chamazulene—but also other main chamomile essential oil compounds, in order to obtain an accurate and clear understanding of these specific genotypes and the chemotypic associations between them. Notably, the BP clustered closer to the tetraploid varieties, which,

as mentioned above, are classified as high α-bisabolol and chamazulene chemotypes [31]. In contrast, the IP grouped with the diploid variety Banatska (Figure 4). This chemotypic shift in the BP indicates the effectiveness of the breeding strategy in aligning the BP with high-quality essential oil profiles.

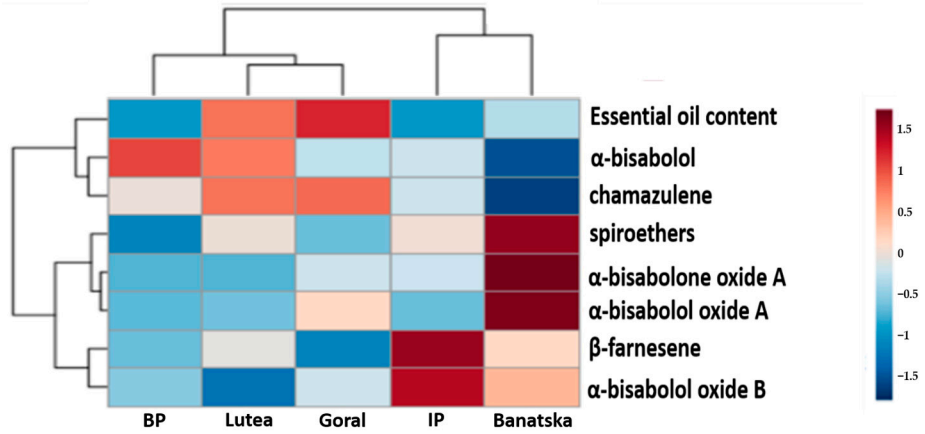


Figure 4. Essential oil content and chemotypic profiles of the genotypes evaluated during Phase II of the breeding program, based on seven main components of chamomile essential oil.

Although during Phase I of the breeding program selection focused on agronomic and quality traits, it was found that this approach indirectly had a positive impact on developing a new upgraded BP for key morphological characteristics (Table 4). In particular, the observed gain regarding plant height was 20.67%, with the BP reaching a mean value of 51.47 cm, with the average plant height being 42.65 cm for the IP. The commercial varieties—diploid and tetraploids—exhibited taller plants (53.82–56.45 cm); nevertheless, the BP succeeded in approximating their height more closely than the IP. Observed gain was also noted in inflorescence morphology. More precisely, the inflorescence head diameter of the BP surpassed the IP (23.13 mm, 19.91 mm, respectively), reaching an observed gain of 16.19%, while the observed gain for the inflorescence disc diameter was 7.32% (6.99 mm for the BP and 6.51 mm for the IP). In particular, concerning the inflorescence head diameter, the BP managed to exceed even the diploid variety Banatska (23.13 mm, 21.07 mm, respectively). As expected, diploid genotypes did not surpass the tetraploid varieties in terms of inflorescence diameter (26.42–27.01 mm for head diameter and 10.65–10.87 mm for disc diameter), which aligns with previous studies about chamomile inflorescence size between diploid and tetraploid genotypes [32,63,64] (Table 4).

Table 4. Plant height and inflorescences morphology—head and disc diameter—of the genotypes evaluated during Phase II of the breeding program.

Genotype * (Ploidy Level)	Plant		Inflorescences			
	Height (cm)	Observed Gain (%)	Head Diameter (mm)	Observed Gain (%)	Disc Diameter (mm)	Observed Gain (%)
IP (2×)	42.65 ^{c} **	20.67	19.91 ^{b}	16.19	6.51 ^{c}	7.32
BP (2×)	51.47 ^{b}		23.13 ^{a}		6.99 ^{b}	
Banatska (2×)	56.45 ^{a}		21.07 ^{b}		7.44 ^{a}	
Lutea (4×)	55.05		27.01		10.65	
Goral (4×)	53.82		26.42		10.87	

* IP: the initial population before the breeding program, BP: the new breeding population derived from the selections after Phase I of the breeding program; ** Data represent the mean values. Values with different letters are statistically different, while values with no letter exhibiting no difference statistically, according to Duncan's test for $\alpha \leq 0.05$.

4. Discussion

Improving the agronomic traits and essential oil quality of native chamomile populations is a viable and environmentally sustainable strategy. This approach is particularly relevant under Mediterranean conditions, which are typically characterized by abiotic stresses and input deficiency. In such environments, regionally adapted germplasm is especially valuable. This aligns with studies highlighting the importance of breeding locally adapted resources under less favorable conditions [44,58,65].

Conventional breeding techniques have been widely applied in chamomile [66], particularly for qualitative characteristics, where individual plant selection has proven highly effective [39,67]. Yield stability and quality remain central breeding targets [68]. The honeycomb design adopted in this study facilitated the identification of phenotypically superior individuals by minimizing competition and enhancing differentiation, thereby enabling more precise selection [69]. Pedigree selection further identified genotypes outperforming others in inflorescence yield and essential oil composition. Overall, the implemented strategy improved agronomic performance and essential oil profile, while also contributing to favorable changes in morphological traits.

Sequential plant selection conducted during Phase I resulted in a new BP with significantly higher dry inflorescence weight. This trait is mainly under genetic rather than environmental control [60], which makes simultaneous breeding for inflorescence yield and essential oil feasible [70]. In the present work, although the essential oil content remained similar between the IP and the BP, the positive impact of selection on inflorescences dry weight could raise total oil yield.

Regarding the essential oil composition, powerful genotypic control has been demonstrated [71]. The qualitative profile appears relatively consistent across environments, with main constituents being highly heritable [67,72]. Chamazulene and α-bisabolol depend on genotype [7,22,39,73]. Thus, selecting plants exhibiting suitable genetic variability for these traits is an effective breeding strategy [67]. This view is further supported by the present study, where phenotypic selection within a genetically heterogeneous population proved highly effective in enhancing the content of these key compounds. In particular, α-bisabolol exhibited a notable increase, aligning the chemical profile of the new BP more closely with that of elite tetraploid varieties and clearly distinguishing it from the IP.

Although the overall essential oil content did not differ significantly between the IP and BP, the notable increase, especially in α-bisabolol, made the new breeding material superior. This rise may be linked to a reduction in bisabolol oxides, especially α-bisabolol oxide B. Such inverse trends have been reported in chamomile mutagenesis studies [74] and general surveys [67]. Fejer and Salamon [75] reported that breeding and selection for high α-bisabolol content consistently resulted in progeny with elevated α-bisabolol levels and a simultaneous decrease in bisabolol oxides. Otto et al. [41] also noted strong variation in bisaboloid composition among chamomile genotypes, with plants rich in α-bisabolol, frequently exhibiting low levels of bisabolol oxides. While both α-bisabolol and bisabolol oxides are considered pharmacologically important, α-bisabolol has been extensively studied [76] and is classified by the Food and Drug Administration (FDA) as Generally Recognized as Safe (GRAS) [77,78]. According to Franke and Schilcher [14], a high α-bisabolol content is one of the main breeding targets in chamomile.

It is also important to note that even though the BP exhibited clear agronomic and essential oil profile improvements, its inflorescences dry weight was slightly lower than that of Banatska. Therefore, while the BP may be more attractive for processors seeking high α-bisabolol and chamazulene content, farmers primarily targeting dry inflorescence yield might initially prefer established commercial varieties. However, the balance between yield and oil quality is critical in chamomile cultivation, as it is mostly used for its therapeutic

properties, which are attributed to the components of its essential oil [79]. Therefore, the superior essential oil composition of the BP may compensate for lower inflorescences dry weight in terms of overall product value.

In addition, the chemotypic shift of the BP towards elite tetraploid varieties is particularly important from an industrial perspective, as higher α-bisabolol and chamazulene levels are highly valued in pharmaceutical and cosmetic sectors, reflecting their extensive use, recognized importance and growing industrial application [80–82]. This positions the BP as a promising raw material for high-value essential oil markets, potentially enhancing its adoption despite slightly lower dry inflorescences weight compared to Banatska.

Besides agronomic traits and essential oil profile, the breeding process also led to incidental but beneficial gain in morphological traits. Although plant height and inflorescence size—head and disc diameter—were not specifically targeted, selection had an indirect positive effect on these characteristics. These findings point to positive genetic correlations or pleiotropic effects, which have also been observed in previous research [24,51,83].

5. Conclusions

This study demonstrated that native Greek chamomile, improved by multi-year pedigree selection, exhibited substantial gains in inflorescence yield and essential oil quality. In Mediterranean low-input conditions, the breeding population yielded strongly over the initial population and commercial types, particularly in α-bisabolol and chamazulene. Associated gain in plant height and inflorescence size further ratified the effectiveness of the methodology. These findings provide a solid foundation for the development of high-yielding and vigorous chamomile lines suitable for Mediterranean, low-input sustainable farming systems. To strengthen its prospects, the new breeding material should be tested over years and sites within Mediterranean farming systems, allowing assessment of its yield potential, production stability, and the influence of genotype × environment interactions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture15181915/s1, Figure S1: First phase of the chamomile breeding program: Honeycomb design layout. Each individual plant—positioned at the center of a ring—is evaluated relative to neighboring plants; Table S1: Monthly climatic conditions (maximum/minimum temperature and rainfall) recorded during the chamomile breeding program (2016–2019); Table S2: Soil composition and properties of the experimental field throughout the breeding program. Soil analysis was conducted at a 0–30 cm depth.

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