Abuhashem *et al.*
Beni-Suef Univ J Basic Appl Sci (2023) 12:70
https://doi.org/10.1186/s43088-023-00408-2

Beni-Suef University Journal of Basic and Applied Sciences

RESEARCH **Open Access**

Exploring the morphological and genetic diversity of Egyptian basil landraces (*Ocimum* sp.) for future breeding strategies

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Abstract

Background Basil (*Ocimum* sp.) exhibits significant morphological and genetic diversity. This variation provides an opportunity to identify novel traits that can be used in breeding programs to improve the productivity, quality, and disease resistance of basil plants. The integration of morphological and genetic data for basil varieties can improve our understanding of biodiversity, conservation, and breeding programs.

Results In 2019, we conducted a comprehensive investigation on 25 basil landraces collected from 12 Egyptian governorates to explore their morphological and genetic diversity. Morphological characterization revealed variability in plant vigor, flower color, cyme shape, and leaf shape, indicating distinct growth patterns and potential for specific applications. Productivity and biochemical evaluation demonstrated significant variation in yield and oil distillation, identifying top-performing landraces L5, L11, and L24 that can be used in breeding programs to optimize performance and reduce environmental impact. The genetic diversity of the landraces was investigated using 11 SCoT primers revealed high levels of genetic diversity among landraces, with a wide range of polymorphism variation. The phylogenetic analysis identified two distinct clades, providing valuable insights into genetic diversity and relationships between different landraces. An interesting observation was made in the study, whereby L6 and L13, collected from Fayoum and Port-Said, respectively, were found to be the closest landraces. Following closely were L7 and L14, which were also collected from the same governorates. These findings have significant implications for the conservation of these landraces, as they may have evolved from similar species.

Conclusions This study sheds light on the genetic diversity and relationships among 25 Egyptian basil landraces. Using SCoT markers, a high level of polymorphism was detected, indicating significant variation in the genetic makeup of the landraces. The study also revealed interesting observations regarding the relationships among the landraces, with some landraces appearing to have evolved from similar species. The phylogenetic analysis provided insights into the genetic relationships among the landraces, which can guide conservation efforts and breeding programs. Overall, this study provides valuable information for researchers, breeders, and farmers involved in the conservation, breeding, and utilization of basil genetic resources in Egypt.

Keywords Basil, *Ocimum* sp., Plant diversity, SCoT, Phylogeny, Plant conservation, Breeding

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1 Background

Basil, *Ocimum* sp., is a genus of the Lamiaceae family that has a highly valued culinary herb with a long history of use in traditional medicine. The herb is native to tropical regions of Central Africa and Southeast Asia, and it is recently cultivated and used all over the world [1]. Basil contains various bioactive compounds including flavonoids, phenolic acids, and essential oils [2, 3], which are responsible for its characteristic flavor, aroma, and medicinal properties. In addition, these compounds possess a range of beneficial effects, including antioxidant [1], anti-inflammatory [4], antimicrobial [5], and anticancer activities [6]. The extract of the herb has been traditionally used to treat a variety of diseases, such as digestive disorders, respiratory infections, and skin problems [7, 8]. Recent studies have also shown promising results for the potential use of basil in the prevention and treatment of chronic diseases such as cancer [9], diabetes [10], and cardiovascular disease [11].

In Egypt, Basil is an important plant that has been used for thousands of years [11]. The herb has been used in traditional Egyptian cuisine for both culinary and medicinal purposes. Archaeological evidence suggests that holy basil (*Ocimum sanctum*) was used in ancient Egyptian culture as a food flavoring and for medicinal purposes [11]. In addition, sweet basil (*Ocimum basilicum*) was widely grown in Egypt and played an important role in the country's cuisine. In Egypt, basil plants have gained recognition as agronomic crops due to their significant cultivation for essential oil production. The thriving demand for basil in local markets, coupled with its remarkable adaptability to the Egyptian environment, has contributed to its widespread cultivation in large-scale. Basil cultivation succeeds in several Egyptian places. The French basil is the most common variety; however, the cultivated area of basil is 1603 Fdan, with an average production of 5.891 tons/fdan. Basil crops spread to the governorates of Fayoum, Minya, Giza, New Valley, and Nubariya [12]. Moreover, there are other species of basil that are grown in Egypt [13], such as lemon basil (*Ocimum citriodorum*), Thai basil (*O. basilicum* var. thyrsiflora) and African basil (*Ocimum gratissimum* and *Ocimum americanum*). Each species of basil has its unique flavor, aroma, and medicinal properties, which make them valuable to the Egyptian cuisine.

Studying the diversity of basil is a complex and multidisciplinary process that involves various scientific disciplines, such as morphology, chemical contents, genetics, and genomics. The identification of unique morphological traits and genetic markers is a fundamental aspect of plant breeding, especially for basil plants [14]. Morphological traits are often used as indicators of plant health and productivity, as they can be easily observed and quantified. The use of morphological traits in breeding programs can be particularly effective in the early stages of breeding, as they allow breeders to identify plants with desirable traits, such as morphological traits such as leaf shape, leaf size, plant height, and flower color can be selected for breeding into new varieties [15]. Genetic markers, on the other hand, provide a more detailed and precise method of tracking genetic variation within a basil plant population [16]. The use of genetic markers in breeding programs has become increasingly popular in recent years, as advances in molecular biology have enabled the development of high-throughput genotyping platforms that can rapidly screen large numbers of individuals for specific genetic markers. These genetic markers can be used to track the inheritance of specific traits, such as disease resistance [17] or yield improvement [18], and can be used to develop new varieties with desirable traits. By identifying and using unique genetic resources, breeders can develop new varieties that are better adapted to changing environmental conditions and have increased productivity, quality, and marketability.

SCoT (Start Codon Targeted) markers are a type of molecular marker that target the start codon region of protein-coding genes. The SCoT markers amplify DNA fragments that contain both conserved and variable regions, allowing for the detection of genetic variation both within and between plant species. SCoT markers have been successfully used to distinguish between closely related basil plant species, as well as between different cultivars of the same species [19]. This high resolution makes SCoT markers particularly useful for studying the genetic relationships between different basil varieties and populations. By comparing the SCoT profiles of different basil plants, we can identify genetic clusters and determine the degree of relatedness between different varieties of *Ocimum* [20]. This information can be used to guide breeding programs, conservation efforts, and the development of new varieties with desired traits.

We aim in this investigation to assess the morphological and genetic diversity of various basil landraces collected from different Egyptian governorates to evaluate the level and distribution of genetic diversity among them. This study provides a comprehensive understanding of the morphological and genetic diversity of Egyptian basil landraces that can help in identifying potential breeding targets or developing conservation strategies.

2 Methods

2.1 Collection of basil landraces

Twenty-five various landraces of *Ocimum* sp. were collected from 12 geographical locations (governorates) within Egypt, including Sharqia, Fayoum, Cairo, Giza, Port Said, Dakahlia, Ismailia, Qalyubia, Menoufia, Assiut, Sohag, and Beheira. The selection of the basil landraces for this study was based on their morphological diversity, specially plant vigor and cyme shape, length, and color. Out of the twenty-five basil landraces, nine were identified at the species level. However, for the remaining landraces, we did not receive any data regarding their specific species identification.

2.2 Experimental design

The current investigation was conducted over the growing seasons of 2019 and 2020 at the El-Kasaseen Research Station in Ismailia governorate that belongs to Horticulture Research Institute, Agriculture Research Center, Egypt. For the morphological evaluation, the experimental design followed a randomized complete block design with three replications. On March 25th of both seasons, 25 seeds of each basil landrace were sowed in a tray, then transferred into the field after 35 days of planting. The fertilization system utilized in the study was organically and excluded chemical nutrients.

2.3 Morphological and biochemical evaluation

The collected basil landraces were subjected to rigorous scientific analysis to determine their unique morphological and biochemical parameters. The morphological characteristics of the basil plants were recorded after the growing seasons. These characteristics included plant height (cm), number of branches per plant, plant fresh weight (g), flowered cyme length (cm), number of flowers per cyme, plant dry weight (g), root length (cm), root fresh weight (g), and root dry weight (g).

In addition to the morphological characteristics, a biochemical analysis was performed to mount bail essential oils. The analysis was conducted at the laboratory for medicinal and aromatic plants at the Horticulture Research Institute. To obtain the essential oil, the fresh aerial sections of each basil plant weighting 50 g were harvested from the field and subjected to hydrodistillation for three hours using a Clevenger apparatus, as described by Clevenger [21].

2.4 Molecular analysis of basil plant landraces

2.4.1 DNA isolation

The small fresh leaves of each basil plant were collected for DNA extraction according to a Doyle and Doyle method [22]. In 1.5 ml Eppendorf tube, the leaf tissues were pulverized and incubated for 30 min at 65° C with 700 µl of CTAB. Following this, a chloroform: isoamyl alcohol (24:1 v/v) mixture (500 µl) was added, and the samples were centrifuged at 18,000×*g*. The supernatant was carefully transferred to fresh Eppendorf tubes, about 500 µl of ice-cold isopropanol was added. The mixture was then incubated for two hours in the fridge and centrifuged at 18,000×*g* for 10 min. The pellets were washed with 70% ethanol and dissolved in TE buffer. The DNA quality was checked using gel electrophoresis (1% agarose), and the samples were diluted for use in PCR reactions. For PCR reaction, DNA was diluted to 25 ng/µl in TE buffer.

2.4.2 Polymerase chain reaction using SCoT

Polymerase chain reaction (PCR) was carried out using a reaction volume of 25 µl consisting of 12.5 µl of PCR master mix (comprising 200 mM Tris–HCl pH 8.5, 160 mM $(NH_4)_2SO_4$, 0.1% (v/v), 3.0 mM MgCl2, 0.4 mM of dNTPs, and 1.0 U of Taq DNA polymerase), 1 µM of SCoT primer, 1 µM of the genomic DNA and 10.5 µl of sterile distilled water. The details of the SCoT primers are listed in Table 1. The reaction mixture was subjected to PCR cycling conditions optimized for each specific primer set.

DNA amplification was carried out using a thermocycler involved an initial denaturation step at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 45–57 °C (depending on the ScoT primer sequence) for 90 secs, and extension at 72 °C for 2 min. A final extension was performed at 72 °C for 10 min.

Agarose gel electrophoresis was performed to separate and visualize the amplified PCR products. A 1.5% agarose gel was prepared using 1X TBE buffer and loaded with the PCR products. Electrophoresis was carried out at a constant voltage of 100 V for 1 h. The gels were then stained with ethidium bromide and visualized using a gel documentation system. Only those primers that produced clear and reproducible fingerprints (DNA bands) were considered for further data analysis. For band scaling, 1KB ladder (1Kb DNA Ladder RTU, GeneDireX, Inc) was used. The PCR amplification products were scored as either present (1) or absent (0) for each band marker,

Table 1 List of 11 SCoT primers used in this study

Scot primers ID	Primer sequence
SCoT 3	5′ ACGACATGGCGACCCACA3′
SCoT 10	5′ ACAATGGCTACCACCAGC 3′
SCoT 11	5′ ACAATGGCTACCACTACC3′
SCoT 22	5′ CCATGGCTACCACCGCAC 3′
SCoT 30	5′ CAACAATGGCTACCACCT 3′
SCoT 33	5′ AAGCAATGGCTACCACCA 3′
SCoT 36	5′ CACCATGGCTACCACCAT 3′
SCoT 39	5′ CAACAATGGCTACCACGG 3′
SCoT 43	5′ ACGACATGGCGACCATCG 3′
SCoT 44	5′ ACCATGGCTACCACCGAC 3′
SCoT 48	5′CACCATGGCTACCACCAG 3′

which was used to generate a binary matrix for each sample. The resulting banding patterns were analyzed using SPSS statistical software to generate a dendrogram representing the genetic relationships among the samples.

2.5 Statistical analyses

To analyze the collected data, statistical analyses were conducted using the SPSS software package. Specifically, the SPSS Statistics for Windows, Version 14.0 was utilized in this study to analyze the collected data.

3 Results

3.1 Morphological diversity of basil landraces

A comprehensive investigation was performed to collect a total of twenty-five various landraces of *Ocimum* sp. from 12 Egyptian governorates (Sharqia, Fayoum, Cairo, Giza, Port Said, Dakahlia, Ismailia, Qalyubia, Menoufia, Assiut, Sohag, and Beheira). Each landrace is uniquely identified by a specific code, which has been assigned for the purpose of accurate identification and record-keeping (Table 2). The selection of the plants for this study was based on their morphological diversity including plant vigor and cyme shape, length, and color. This selection strategy was effective in capturing a representative sample of the genetic and morphological diversity of basil landraces in Egypt.

The 25 landraces were collected from Sharqia ("Landrace" identification code: L1, L2, L3, and L4), Fayoum (L5, L6, and L7), Cairo (L8, L9, and L10), Giza (L11 and L12), Port Said (L13 and L14), Dakahlia (L15 and L16), Ismailia (L17 and L18), Qalyubia (L19 and L20), Menoufia (L21 and L22), Assiut (L23), Sohag (L24), and Beheira (L25) (Fig. 1). The photographs were taken under field conditions to ensure accurate representation of the morphological features of each landrace. The landraces collected for this study encompassed various species. However, many of them could not be identified at the species level. Out of the collected landraces, nine were assigned to four different species, including *O. tenuiflorum* (L1, L4, and L16), *O. basilicum* (L10, L21, and L23), *O. sanctum* (L14, L17), *O. gratissimum* (L19). All distributed in various governorates, including Sharqia, Cairo, Port Said, Dakahlia, Ismailia, Qalyubia, Menoufia, and Assiut (Table 3). We assigned the morphological characteristics including the color of the flowers, the shape of the cymes, and the shape of the leaves to generally characterize each landrace (Table 3). The collected plants revealed a considerable amount of variability of their morphology. This variation was observed in a range of morphological characteristics such as cyme shape, leaf shape, and flower color. The majority of plants had a semi-erect habit, semi-dense branching, and sparse stem pubescence. This primary classification criteria encompassed cyme shape (released or compacted cymes), flower color (white or purple flowers), and leaf shape (narrow or expanded leaves). The presence of such variability indicates that there are likely to be numerous subtypes within *Ocimum* sp., which could potentially have distinct physiological properties. This information is essential for proper identification and classification of species of *Ocimum* and for understanding their diversity. Overall, this investigation provides a comprehensive picture of the diversity of *Ocimum* sp. in Egypt, which can be used for various purposes including plant breeding and conservation efforts.

Table 2 The geographical locations of 25 landraces used to evaluate the morphological and genetic diversity of basil plants in Egypt

Geographical location ID	Governorates name	Landrace code
1	Sharqia	1, 2, 3, 4
2	Fayoum	5, 6, 7
3	Cairo	8, 9, 10
4	Giza	11, 12
5	Port Said	13, 14
6	Dakahlia	15, 16
7	Ismailia	17, 18
8	Qalyubia	19, 20
9	Menoufia	21, 22
10	Assiut	23
11	Sohag	24
12	Beheira	25

3.2 Morphological, economical, and biochemical parameters used to assess basil landraces

In two seasons in 2019 and 2020, a randomized complete block design with three replications was employed to conduct the morphological evaluation of investigated basil landraces. This resulted in a total of six replicates per landrace, providing sufficient data for an accurate evaluation of each landrace. After collecting and analyzing the data, the average values for each morphological trait were calculated from the six replicates from two seasons for each landrace. These values were then used to determine the relative ranking of each landrace based on its morphological traits, using Duncan's test (Table 4, Fig. 2). Broadly, we categorized the parameters into morphological, economical, and biochemical domains.

3.2.1 Morphological parameters

The morphological features of the 25 basil landraces were evaluated. The parameters used in this evaluation were included the number of branches per plant, plant

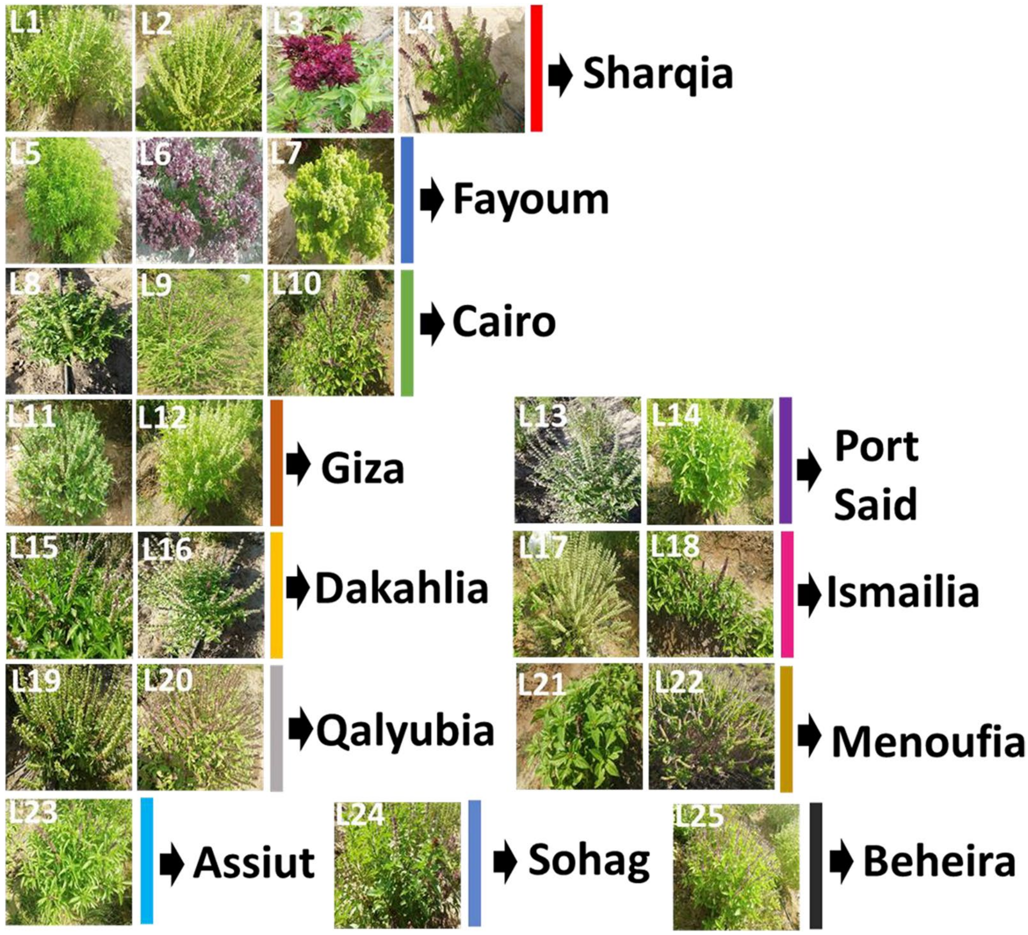

Fig. 1 Morphological diversity of *Ocimum* sp. landraces collected from 12 Egyptian governorates. Photographs of basil landraces were taken to showcase the morphological diversity. The 25 various landraces of *Ocimum* sp. collected from 12 Egyptian governorates, namely Sharqia (L1-L4), Fayoum (L5-L7), Cairo (L8-L10), Giza (L11-L12), Port Said (L13-L14), Dakahlia (L15-L16), Ismailia (L17-L18), Qalyubia (L19-L20), Menoufia (L21-L22), Assiut (L23), Sohag (L24), and Beheira (L25). The photographs show the variation in plant, cyme, and leaf shapes, as well as the color of the flowers among the various landraces

height (cm), number of flowers per cyme, flowered cyme length (cm), and root length (cm). In general, the vigor of plants in season 2019 was better than 2020 (Table 4, Fig. 2i-p). The superior performance of the plants in the 2019 compared to the 2020 may have been influenced by environmental factors. This highlights the importance of considering external factors when evaluating plant performance.

Number of branches: Of the two seasons, the main average of the number of branches for the 25 landraces was 11.11 varied from 6.21 to 13.71 (B/P) that assigned to Landraces L7 and L2, respectively (Table 4, Fig. 2a).

Plant height: The average of the plant height for the 25 landraces evaluated over two seasons was 59.77. The data indicated that the average plant height varied between 40 and 80 cm across the landraces, with Landraces L7 and L24 exhibiting the lowest and highest average of the plant height, respectively (Table 4, Fig. 2b).

Number of flowers: The number of flowers per each cyme for the 25 basil landraces were recorded, revealing a significant range in values. The data indicated that the number of flowers varied between 18.95 and 72.66 (F/C), with the lowest value observed in L7 and the highest in L18, respectively, (Table 4, Fig. 2d). The overall average was 40.79, with an average of 44.49 for the first season and 37.08 for the second season.

Cyme length: According to the results of the study, there was a significant range in the mean inflorescence length of the evaluated basil landraces. The data indicated that the average inflorescence length varied between 16.48 and 34.23 cm, with the highest value observed in landraces L13 and L7, respectively (Table 4, Fig. 2e). The first season had an average of 26.55 and the second season had an average of 23.35, while an overall average of 25.14.

Root length: According to the results of the study, there was a significant variation in the average of root length

Table 3 The morphological characteristics of investigated basil landraces

Landrace	Origin	Species	Cyme shape	Flower color	Leaf shape
L1	Sharqia	*O. tenuiflorum*	Released	White	Narrow
L2	Sharqia	Unknown	Released	White	Expanded
L3	Sharqia	Unknown	compact	purple	Narrow
L4	Sharqia	*O. tenuiflorum*	Released	purple	Narrow
L5	Fayoum	Unknown	–	–	Expanded
L6	Fayoum	Unknown	compact	purple	Narrow
L7	Fayoum	Unknown	compact	White	Narrow
L8	Cairo	Unknown	Released	White	Expanded
L9	Cairo	Unknown	Released	Purple	Expanded
L10	Cairo	*O. basilicum*	Released	purple	Expanded
L11	Giza	Unknown	Released	White	Expanded
L12	Giza	Unknown	Released	White	Expanded
L13	Port Said	Unknown	Released	White	Narrow
L14	Port Said	*O. sanctum*	Released	White	Expanded
L15	Dakahlia	Unknown	Released	White	Narrow
L16	Dakahlia	*O. tenuiflorum*	Released	White	Narrow
L17	Ismailia	*O. sanctum*	Released	White	Expanded
L18	Ismailia	Unknown	Released	purple	Narrow
L19	Qalyubia	*O. gratissimum*	Released	White	Expanded
L20	Qalyubia	Unknown	Released	Purple	Expanded
L21	Menoufia	*O. basilicum*	Released	Purple	Expanded
L22	Menoufia	Unknown	Released	Purple	Narrow
L23	Assiut	*O. basilicum*	Released	Purple	Expanded
L24	Sohag	Unknown	Released	Purple	Expanded
L25	Beheira	Unknown	Released	purple	Expanded

of the 25 evaluated landraces between the two seasons. The data showed that the average root length per plant ranged from 17.43 to 29.71 cm with Landraces L20 and L18 exhibiting the lowest and highest values, respectively, as presented in (Table 4, Fig. 2f).

3.2.2 Productivity parameters

One of the essential considerations in the cultivation of basil plants was their economic value. In this study, the plant fresh weight and dry weight were evaluated to determine their potential yield and economic value. Results of the basil productivity showed differences between investigated landraces in fresh and dry masses.

Plant fresh weight: The average of the fresh weight of the 25 basil landraces were found to vary significantly, ranging from 217 to 580 (g/p). Landrace L8 exhibited the lowest fresh weight yield, while L11 produced the highest fresh weight yield (Table 4, Fig. 2c). The first season had an average of 410.23 and the second season had an average of 278.86, while an overall average was 344.54. These results suggest that there is considerable variation among the different landraces with regard to their fresh weight yield potential.

Plant dry weight: Similarly, the average of plant dry weight for the basil landraces ranged from 80 to 157 (g/p), indicating a significant variation in this parameter. Landrace L14 exhibited the lowest dry weight yield, while L11 showed the highest dry weight (Table 4, Fig. 2g). In terms of average, the second season performed better with 132.84 compared to the first season's overall average of 117.12.

3.2.3 Biochemical parameters

One of the crucial characteristics of basil is its ability to accumulate essential oils, which are highly valued for their medicinal and culinary properties. In this study, the essential oil contents of the 25 basil landraces were evaluated using oil distillation.

Oil distillation: The essential oil contents varied significantly, ranging from 0.11 to 0.68 (ml/50g fresh weight) with total average 0.28. Landrace L5 exhibited the highest essential oil content, while L19 showed the lowest (Table 4, Fig. 2h). These findings are crucial for growers and breeders interested in maximizing the essential oil content of their basil crop. By selecting landraces with higher essential oil content or developing new cultivars

Table 4 Morphological, economical, and biochemical traits of 25 basil landraces evaluated over two seasons in 2019 and 2020

Landrace	No. of branches	Plant height	Plant fresh weight	No. of flowers	Cyme length	Root length	Plant dry weight	Oil distillation
L1	11.17^{abcde}	59.65^{cdefgh}	408.73^{bcd}	38.05^{defghi}	26.63^{b}	27.78^{ab}	130.26^{abcd}	0.53^{b}
L2	13.71^{a}	58.00^{defgh}	375.81^{bcde}	51.66^{abcde}	24.60^{bc}	22.76^{abcdef}	140.00^{abcd}	0.20^{defg}
L3	11.33^{abcd}	57.38^{defgh}	271.93^{cde}	29.06^{efghi}	24.96^{b}	19.01^{def}	101.38^{bcde}	0.18^{efg}
L4	11.05^{abcde}	56.71^{defgh}	305.01^{bcde}	26.06^{fghi}	24.25^{bc}	22.55^{bcdef}	108.96^{abcde}	0.43^{bc}
L5	7.66^{def}	64.43^{bcdef}	451.66^{ab}	23.66^{ghi}	16.73^{d}	19.10^{def}	151.10^{a}	0.68^{a}
L6	10.46^{abcdef}	40.45^{i}	312.56^{bcde}	20.78^{hi}	17.00^{d}	23.88^{abcdef}	126.93^{abcde}	0.33^{cde}
L7	6.21^{f}	40.00^{i}	256.78^{cde}	18.95^{i}	16.48^{d}	22.78^{abcdef}	81.95^{e}	0.36^{cd}
L8	7.60^{ef}	55.11^{fgh}	217.11^{e}	25.45^{fghi}	26.48^{b}	23.33^{abcdef}	135.38^{abcd}	0.25^{defg}
L9	13.11^{abc}	52.73^{gh}	271.65^{cde}	45.83^{bcdefg}	19.33^{cd}	26.56^{abc}	148.55^{abc}	0.20^{defg}
L10	9.58^{cde}	55.05^{fgh}	276.66^{cde}	34.00^{efghi}	24.05^{bc}	18.60^{ef}	98.86^{de}	0.20^{defg}
L11	11.33^{abcd}	64.73^{bcdef}	580.28^{a}	36.26^{efghi}	28.00^{b}	21.95^{bcdef}	157.21^{a}	0.30^{cdef}
L12	9.66^{bcde}	69.90^{b}	334.16^{bcde}	43.88^{cdefgh}	25.00^{b}	18.55^{ef}	101.11^{cde}	0.21^{defg}
L13	12.36^{abc}	69.06^{bc}	323.05^{bcde}	47.61^{bcdef}	34.23^{a}	20.43^{cdef}	136.11^{abcd}	0.45^{bc}
L14	10.71^{abcde}	51.05^{h}	236.42^{de}	32.06^{efghi}	26.45^{b}	20.53^{bcdef}	80.28^{e}	0.20^{defg}
L15	13.38^{ab}	56.21^{efgh}	337.21^{bcde}	36.91^{defghi}	24.01^{bc}	24.26^{abcdef}	126.93^{abcde}	0.20^{defg}
L16	12.00^{abc}	60.16^{cdefgh}	334.85^{bcde}	39.71^{defghi}	27.21^{b}	21.43^{bcdef}	108.90^{abcde}	0.21^{defg}
L17	11.23^{abcd}	59.95^{cdefgh}	361.36^{bcde}	38.73^{defghi}	24.66^{bc}	23.01^{abcdef}	127.78^{abcde}	0.21^{defg}
L18	11.28^{abcd}	61.30^{bcdefg}	420.61^{bc}	72.66^{a}	24.83^{b}	29.71^{a}	121.66^{abcde}	0.33^{cde}
L19	12.93^{abc}	65.55^{bcde}	324.18^{bcde}	67.45^{ab}	29.11^{b}	22.21^{bcdef}	114.43^{abcde}	0.11^{g}
L20	10.26^{abcde}	66.28^{bcd}	400.28^{bcd}	47.48^{bcdef}	27.38^{b}	17.43^{f}	150.43^{ab}	0.21^{defg}
L21	12.46^{abc}	63.38^{bcdef}	328.06^{bcde}	48.45^{bcdef}	25.45^{b}	22.66^{abcdef}	137.78^{abcd}	0.26^{defg}
L22	11.45^{abc}	59.83^{cdefgh}	414.05^{bc}	38.66^{defghi}	27.23^{b}	23.60^{abcdef}	145.06^{abcd}	0.15^{fg}
L23	13.18^{abc}	58.45^{defgh}	328.18^{bcde}	31.43^{efghi}	28.21^{b}	25.23^{abcde}	113.05^{abcde}	0.20^{defg}
L24	12.88^{abc}	79.71^{a}	468.31^{ab}	64.66^{abc}	28.45^{b}	26.26^{abcd}	146.68^{abcd}	0.21^{defg}
L25	10.68^{abcde}	68.93^{bc}	274.71^{cde}	60.10^{abcd}	27.81^{b}	23.70^{abcdef}	133.88^{abcd}	0.20^{defg}
S. 2019 mean	12.50	62.71	410.23	44.49	26.55	22.70	117.12	0.29
S. 2020 mean	9.70	56.82	278.86	37.08	23.25	23.33	132.84	0.27
Total mean	11.11	59.77	344.54	40.79	25.14	22.70	124.98	0.28

(See figure on next page.)

Fig. 2 Morphological, economical, and biochemical traits of 25 basil landraces evaluated over two seasons in 2019 and 2020. **a–h** Morphological, economical, and biochemical traits of 25 basil landraces were graphed based on the average of 6 plant replicates from 2019 and 2020 seasons. The traits recorded were **a** plant height, **b** number of branches per plant, **c** plant fresh weight, **d** flowered cyme length, **e** number of flowers per cyme, **f** plant dry weight, **g** root length, and **h** root dry weight. **i–p** The traits of 25 basil landraces were graphed based on the average of 3 plant replicates from each season. The traits recorded were **i** plant height, **j** number of branches per plant, **k** plant fresh weight, **l** flowered cyme length, **m** number of flowers per cyme, **n** plant dry weight, **o** root length, and **p** root dry weight

that exhibit this trait, growers can increase the medicinal and culinary value of their crop.

Overall, when considering the fresh weight and dry weight yields as well as oil distillation, the top five highest productivity landraces were selected from the 25 basil Egyptian landraces. Among these landraces, L5, L11, and L24 were found to have the best productivity ranks (Table 5). By leveraging this information, growers and breeders can take steps toward improving the productivity and economic value of their basil crop.

3.3 Pairwise correlation of basil morphological traits

In order to examine the relationships between all studied traits of basil plants, we conducted a comprehensive Pearson pairwise correlation analysis. By exploring the correlation between various traits, we gain a better understanding of their interdependencies and their impact on basil production. Notably, we found a significant positive correlation between plant height with each of number of flowers (correlation coefficient: 0.659) and cyme length (0.647); plant fresh weight and dry weight

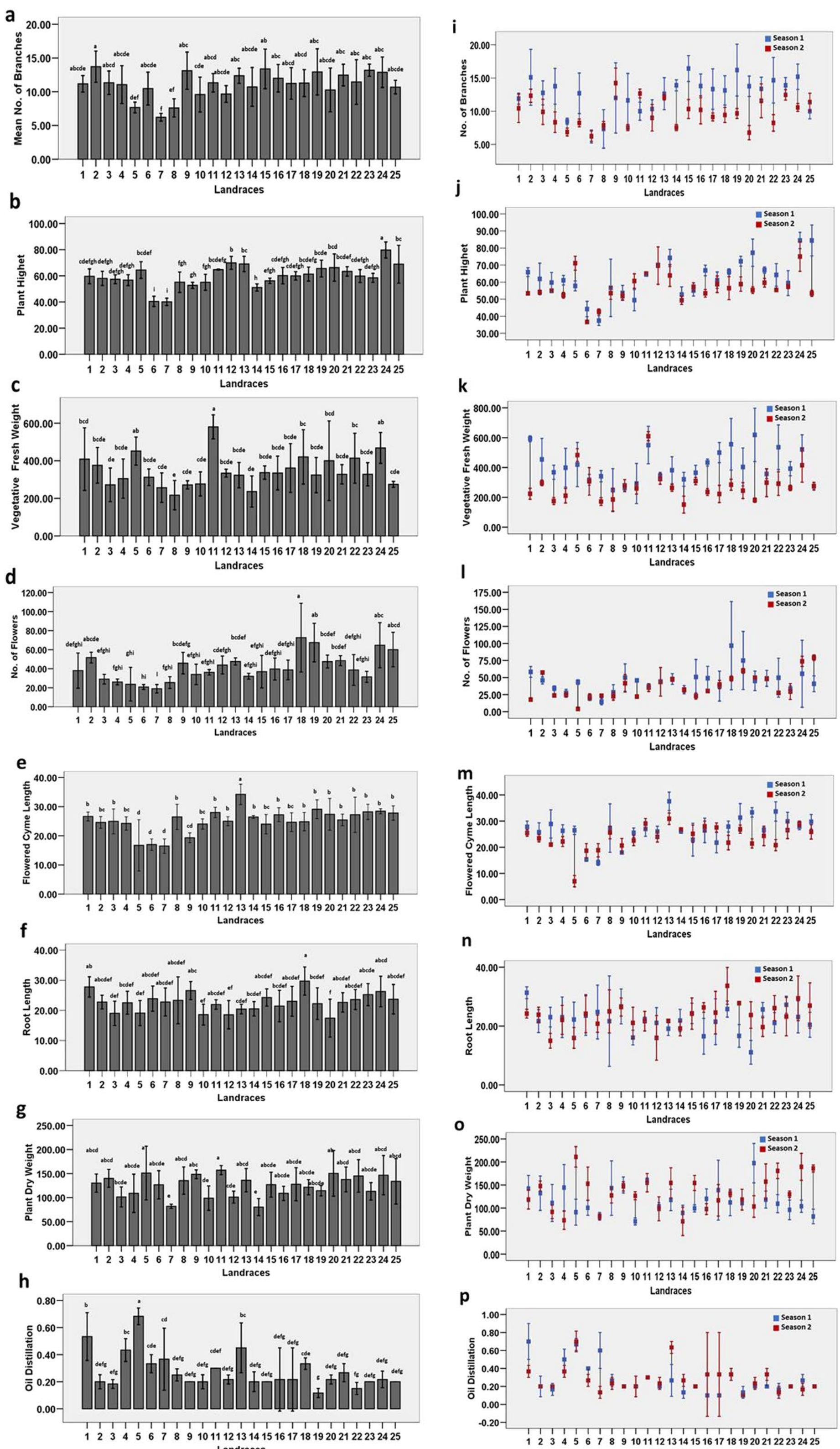

Fig. 2 (See legend on previous page.)

Table 5 The five top-performing Egyptian basil landraces ranked by their economic features

Top ranked landraces	Plant fresh weight	Plant dry weight	Oil distillation
1	L11	L11	L5
2	L24	L5	L1
3	L5	L20	L13
4	L18	L9	L4
5	L22	L24	L7

(0.614); number of branches and flowers (0.535) (Table 6). The identification of a strong correlation between these traits highlights the importance of these traits in determining the reproductive potential of basil plants. The improvement of plant fresh and dry weight would be considered in the plant breeding program.

3.4 Relationship of basil landraces based on their morphological characteristics

In order to investigate the relationships among basil landraces based on the morphological level, we conducted a clustering analysis based on their morphological and production traits. A dendrogram was generated to depict the correlation patterns among the landraces, considering the overall traits (Fig. 3). Remarkably, there were six clusters demonstrated the landraces that have strong correlations, specifically belonging to Cluster 1 (L17, L22, L15, L2, and L20), Cluster 2 (L4, L23, L3, and L10), Cluster 3 (L1, L7, and L5), Cluster 4 (L16, L24, L12, and L14), Cluster 5 (L13 and L21), and Cluster 6 (L8 and L9). These generated dendrogram revealed a visual representation of the correlation structure, facilitating the identification of landrace groups with shared characteristics.

3.5 SCoT polymorphism and diversity pattern of Egyptian basil landraces

The genetic diversity pattern and polymorphism of the 25 Egyptian basil landraces were analyzed using eleven SCoT primers (Table 1). The SCoT marker profiles can be illustrated with the following example: SCoT3 revealing the 25 landraces (Fig. 4). A total of 226 bands were generated using SCoT3 primer. In general, the total bands per primer ranged from 226 bands (SCoT-3) to 95 (SCoT-48). Despite this variability, the amplification of all 25 landraces using SCoT primers resulted in the detection of a high level of polymorphism.

The phylogenetic tree between the landraces based on the 11 SCoT primers was generated using the Squared Euclidean Distance matrix that effectively divided all basil landraces into two distinct clades. The first clade, labelled as clade 1, was further divided into two subclades: subclade 1.1 comprising six landraces (L6, L13, L4, L2, L3, and L15), and subclade 1.2 including L7, L14, L18, L21, L17, L16, and L1 (Fig. 5). On the other hand, the second clade, labelled as clade 2, consisted of 12 landraces that were also divided into subclade 2.1 (L23, L24, L19, L5, and L12), and subclade 2.2 (L9, L10, L11, L25, L8, L20, and L22). Interestingly, L6 and L13 were the closest landraces that were separately collected from Fayoum and Port-Said, followed by L7 and L14, which also were found in the same governorates. These findings could be crucial in designing conservation strategies for these landraces, as may have evolved from a common ancestor. Additionally, L23 and L24 originated from Assiut and Sohag, respectively, in subclade 2.1 were the closest among all the landraces in clade 2. Following them, L11 and L25, which are part of subclade 2.2, were found to be closely related, along with L9 and L10; the former two landraces are assigned to Cairo.

Table 6 Pairwise Pearson correlation analysis between the traits used to morphologically evaluate basil landraces

Traits	No. of branches	Plant height	Plant fresh weight	No. of flowers	Cyme length	Root length	Plant dry weight	Oil distillation
No. of branches	1							
Plant height	0.335	1						
Plant fresh weight	0.205	0.502*	1					
No. of flowers	0.535**	0.659**	0.275	1				
Cyme length	0.482*	0.647**	0.151	0.500*	1			
Root length	0.331	− 0.075	0.151	0.337	− 0.034	1		
Plant dry weight	0.27	0.453*	0.614**	0.29	0.135	0.208	1	
Oil distillation	− 0.439	− 0.043	0.259	− 0.346	− 0.299	0.015	0.141	1

** Correlation is significant at the 0.01 level 2-tailed

* Correlation is significant at the 0.05 level 2-tailed

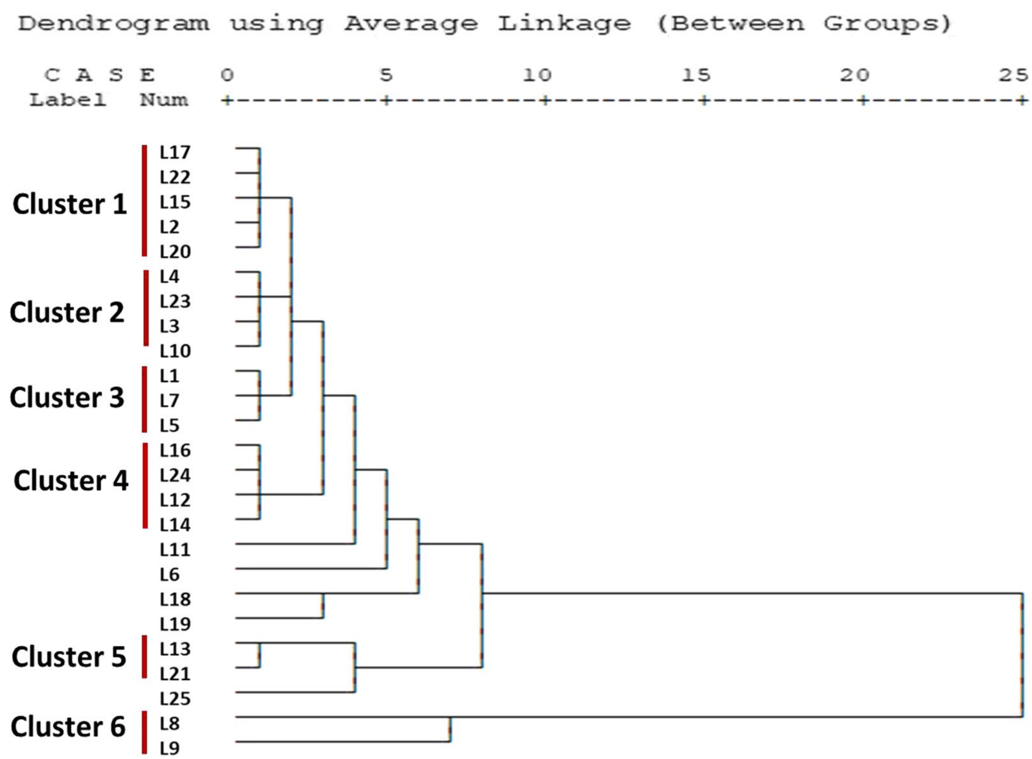

Fig. 3 Generated dendrogram for depicting the correlation patterns among the landraces based on their morphological characteristics. Six clusters revealed the landraces that have strong correlations: Cluster 1 (involved landraces: L17, L22, L15, L2, and L20); Cluster 2 (L4, L23, L3, and L10); Cluster 3 (L1, L7, and L5); Cluster 4 (L16, L24, L12, and L14); Cluster 5 (L13 and L21); Cluster 6 (L8 and L9)

4 Discussion

The exploration of the morphological and genetic diversity of basil landraces holds immense significance for the advancement of the progress of taxonomic research and the application of agricultural practices. The implications of this diversity are numerous, including conservation, breeding, and maintaining the country's competitive edge in the global market [18]. The preservation of genetic resources is crucial for ensuring the long-term sustainability of agricultural systems. Therefore, it is imperative that we take steps to conserve the genetic diversity of basil landraces through appropriate storage and management [23]. In addition, the genetic and morphological diversity of basil landraces provides an opportunity to identify novel traits that can be used in breeding programs [14]. These traits can help to improve the productivity, quality, and disease resistance of basil varieties, and ultimately benefit farmers and consumers alike. By carefully selecting and breeding landraces with desirable traits, we can develop new varieties that are better suited to the needs of modern agriculture and the changing environmental conditions.

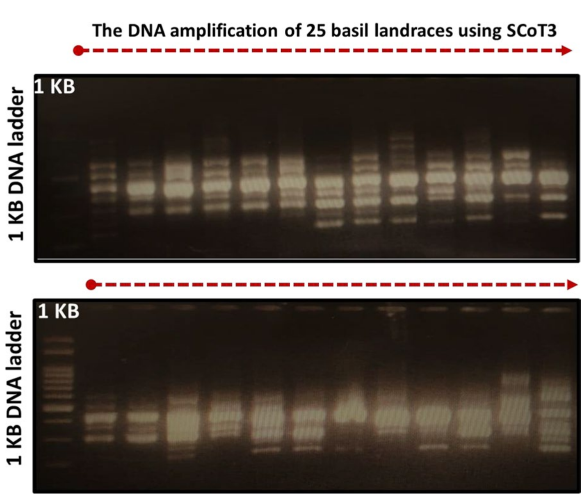

Fig. 4 SCoT marker profiles for 25 basil landraces using SCoT3. The first lane of each panel displays a 1KB ladder, and the following 25 lanes (from lane 2 to lane 26), each corresponding to a distinct basil landrace

A comprehensive exploration was conducted to examine the range of morphological and genetic diversity present in basil landraces in Egypt. We analyzed 25 different landraces of *Ocimum* sp. from 12 geographical regions across Egypt. The landraces collected for the study encompassed a diverse range of species, although the identification of some of them at the species level

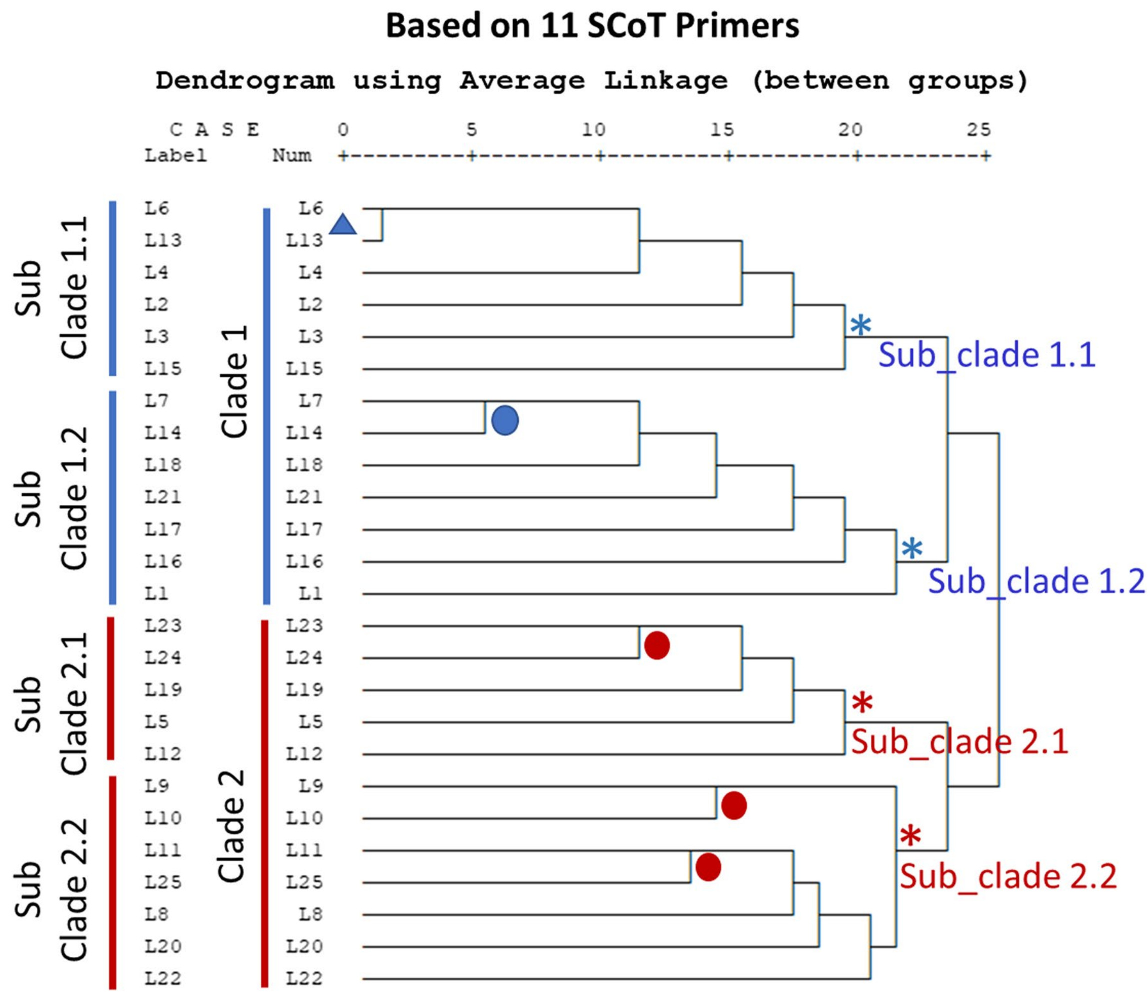

Fig. 5 Phylogenetic clustering dendrogram of 25 basil landraces across Egyptian governorate. The analysis conducted using squared elucidation distance clustering revealed two distinct clades among all basil landraces. Clade 1 included subclade 1.1 (L6, L13, L4, L2, L3, and L15) and subclade 1.2 (L7, L14, L18, L21, L17, L16, and L1). Clade 2 divided into subclade 2.1 (L23, L24, L19, L5, and L12) and subclade 2.2 (L9, L10, L11, L25, L8, L20, and L22)

proved to be challenging. Despite this, nine of those were assigned to four distinct species. These species include *O. tenuiflorum*, represented by landraces L1, L4, and L16; *O. basilicum*, represented by landraces L10, L21, and L23; *O. sanctum*, represented by landraces L14 and L17; and *O. gratissimum*, represented by landrace L19. Here, we focused on the morphological characteristics of the collected basil plants. The landraces revealed considerable variability in their morphology, indicating that there are likely to be numerous subtypes within the *Ocimum*, which could potentially have distinct physiological properties. This information is essential for proper identification and classification of species of *Ocimum* and for understanding their diversity. The observed variation in morphological parameters such as the number of branches, plant height, number of flowers, cyme length, and root length indicated that different landraces have distinct growth patterns and may be suitable for specific usages, such as culinary or medicinal applications [14].

Among the 25 landraces evaluated, we identified the top five highest productivity landraces, providing useful information for breeders. Landraces L5, L11, and L24 emerged as the most productive among the studied basil Egyptian landraces, as indicated by their superior ranks (Table 5), considering these important factors such as fresh weight, dry weight yields, and oil distillation. This finding suggests that these specific landraces have the potential to significantly enhance basil crop productivity. By focusing on these high-performing landraces to be included in the breeding programs to improve the basil plant, growers can optimize their cultivation techniques and maximize their yields. This information can be also used to identify the best-performing varieties and develop breeding programs that aim to improve their performance even further [14]. In addition, the identification of the best-performing landraces can also have environmental benefits. By focusing on the most productive varieties, breeders can reduce the amount of land required for cultivation, reducing the environmental impact of farming activities [24]. Therefore, further research in this area is warranted to develop novel basil varieties and improve the productivity of existing ones.

Here, we conducted a comprehensive analysis of the pairwise correlations between studied morphological traits of basil landraces. By employing Pearson pairwise correlation analysis, we identified significant relationships between traits and gain insights into their interdependencies and their impact on basil production. Notably, significant positive correlations were observed between plant height and the number of flowers, plant height and cyme length, plant fresh weight and dry weight, as well as the number of branches and flowers (Table 6). These findings indicate that these traits are closely linked and have a direct influence on the reproductive potential of basil plants. For example, taller plants tend to have a greater number of flowers and longer cymes, suggesting a potential relationship between plant height and reproductive success. Similarly, a positive correlation between fresh weight and dry weight implies that heavier plants also exhibit greater dry weight, which can be indicative of higher biomass production. Understanding these correlations between various morphological traits provides valuable insights for basil production. Growers and breeders can leverage this knowledge to select and prioritize specific traits during the cultivation process or breeding programs.

In addition, the study employed a clustering analysis to explore the relationships among basil landraces based on their morphological and production traits. The generated dendrogram depicted the correlation patterns among the landraces and facilitated the identification of clusters with shared characteristics. Notably, six clusters emerged, each representing a set of landraces displaying strong correlations (Fig. 3). Cluster 1 encompasses landraces L17, L22, L15, L2, and L20, while Cluster 2 comprises L4, L23, L3, and L10. Landraces L1, L7, and L5 form Cluster 3, and Cluster 4 consists of L16, L24, L12, and L14. Cluster 5 includes L13 and L21, and L8 and L9 are grouped together in Cluster 6. This clustering analysis facilitated the identification of groups of landraces with shared characteristics, enabling targeted breeding efforts. Overall, these findings contribute to a better understanding of the morphological characteristics of basil landraces and provide valuable information for basil growers and breeders.

The use of SCoT markers has been particularly successful in distinguishing between closely related basil plant species and cultivars, making them a valuable tool in studying the genetic relationships between different basil accessions. By comparing the SCoT profiles of basil plants, we can identify genetic clusters and determine the degree of relatedness between different varieties of the *Ocimum* species [20]. This information can guide breeding programs, conservation efforts, and the development of new varieties with desired traits [14]. The genetic diversity and polymorphism of Egyptian basil landraces were investigated using eleven SCoT primers. The study demonstrated that there was a high level of genetic diversity among the 25 Egyptian basil landraces, with a wide range of polymorphism variation, indicating the existence of genetic variation within the different regions of Egypt. The phylogenetic tree based on the 11 SCoT primers showed that all 25 basil landraces were divided into two distinct clades. The identification of distinct clades and subclades can help in the design of conservation strategies that consider for the genetic diversity and relationships between different landraces. For instance, the identification of closely related landraces, such as L6 and L7 from Fayoum and L13 and L14 from Port-Said, respectively, could guide conservation strategies for these landraces, as may have evolved from similar species. In clade 2, L23 from Assiut and L24 from Sohag in subclade 2.1 were identified as the closest landraces. This was followed by L11 and L25 in subclade 2.2, along with L9 and L10, both assigned to Cairo. Overall, the phylogenetic analysis based on the SCoT primers provides valuable insights into the genetic diversity and relationships between different basil landraces [20]. This information can inform the development of conservation and breeding strategies that can help to safeguard the genetic resources of this important crop in the face of global climate change and other threats.

The comparison between the trees derived from the morphological and genetic aspects of the studied landraces revealed notable differences. However, despite these discrepancies, a few landraces showed correlation between the two approaches. Consider the different scales employed in morphological and genetic analyses, landraces L2 and L15 demonstrated a correlation in Cluster 1 of the morphological tree, while in the genetic dendrogram, they were found in subclade 1.1. This suggests a degree of agreement in the classification of these landraces, considering the different perspectives provided by morphological and genetic characteristics. Similarly, in Cluster 2 of the morphological tree, L1 and L7 appeared to be closely related, which aligns with their grouping in Subclade 1.2 of the genetic tree. These instances of correlation between the two approaches indicate a potential convergence of morphological and genetic characteristics in certain landraces, despite the overall differences observed between the trees. Further analysis and exploration of these relationships could provide insights into the underlying factors influencing the observed patterns.

These findings highlight the importance of using both morphological and genetic data to fully understand the diversity and relationships among landraces. While morphological characters can provide valuable information about the physical appearance and adaptability of a population, genetic markers can reveal the underlying evolutionary history and relationships among landraces. Integrating these two types of data can improve our understanding of biodiversity, conservation, and breeding programs.

5 Conclusion

In this study, we investigated the morphological and genetic diversity of 25 basil landraces collected from 12 different geographical locations in Egypt. The results showed considerable variability in the morphological traits among the collected landraces, indicating the presence of numerous subtypes within the *Ocimum* sp. The productivity and biochemical parameters were also evaluated, revealing significant differences among the landraces. This comprehensive analysis of basil landraces encompassed the exploration of morphological traits, pairwise correlations, clustering analysis, and genetic diversity using SCoT markers. The study revealed significant correlations between traits, providing valuable insights for basil production and breeding programs. The clustering analysis identified distinct groups of landraces with shared characteristics, enabling targeted breeding efforts. Furthermore, the genetic diversity analysis using SCoT markers highlighted the presence of two distinct clades and subclades, offering insights into the genetic relationships between different basil accessions. Despite differences between the morphological and genetic trees, certain landraces demonstrated correlations, suggesting a convergence of traits. These findings contribute to our understanding of the morphological and genetic characteristics of basil landraces, providing valuable information for basil growers, breeders, and conservationists. Further research and analysis in this field will contribute to the development of improved basil varieties and the preservation of the genetic resources of this important crop.

6 Perspectives

In order to further advance the characterization and identification of the basil landraces, future studies can explore the use of additional molecular markers and DNA barcoding techniques. The incorporation of multiple molecular markers and DNA barcoding techniques can offer a more reliable and accurate means of identifying and characterizing the basil landraces. This, in turn, can greatly benefit both breeding and conservation efforts.

In terms of breeding programs, identification of accessions with desirable traits for culinary or medicinal use can be a priority. This can involve further evaluation of the genetic diversity patterns and polymorphism of the landraces, as well as characterization of their phenotypic traits. This can enhance the quality and yield of the crop and help meet the increasing demand for culinary and medicinal basil products.

On the other hand, the conservation and management of basil genetic resources in Egypt is also crucial. Traditional landraces are an important part of cultural heritage and their loss can lead to irreversible damage to biodiversity. Therefore, active conservation efforts are needed to prevent loss of genetic diversity. This can involve collection, characterization, and preservation of landraces in gene banks, as well as promoting their use in sustainable agriculture practices. Furthermore, engaging local communities in conservation efforts can help raise awareness of the importance of preserving traditional landraces and ensure their long-term survival.

Abbreviations

SCoT	Start codon targeted
CTAB	Cetyltrimethylammonium bromide
SPSS	Statistical package for the social sciences

Acknowledgements

The authors express their gratitude to Dr. Nouh E. Ahmed for his assistance in conducting the SCoT gel analysis.

Author contributions

KAS, HBK, MAFAE, and YSA conceptualized the study. KAS, HBK, and MAFAE designed the methodology. YSA carried out the investigation. MAFAE and YSA implemented of the field experiment. HBK, YSA, and MAFAE performed formal analyses. KAS, MAFAE, and YSA provided resources. KAS, HBK, and MAFAE supervised the study. HBK and YSA prepared and wrote the original draft. All authors reviewed and edited the draft and read and approved the final manuscript.

Funding

This work was funded by Ain Shams Center of Genetic Engineering and Biotechnology and Agriculture research center, Cairo, Egypt.

Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Received: 7 May 2023 Accepted: 20 July 2023
Published online: 28 July 2023

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