EGYPTIAN JOURNAL OF BOTANY (EJBO)

# The internal transcribed spacer (ITS) as a DNA barcode in identifying some plants from family Amaranthaceae

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ARTICLE HISTORY

Submitted: April 2, 2025

Accepted: July 26, 2025

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DOI: 10.21608/ejbo.2025.372489.3252

EDITED BY: Wafaa Amer

The molecular barcoding was used to evaluate and confirm the identification of some Amaranthaceae plants in Egypt. The ITS region was selected due to its ability to identify and classify taxa at the generic and species levels, universality, and ease of amplification and sequencing. Amaranthaceae family; the three different subfamilies Amaranthoideae, Salsoloideae, and Salicornioideae in the scope of our study belong to the family Amaranthaceae. The sequence lengths of the ITS1, 5.8S, and ITS2 regions of eight species were 462 bp, 457 bp 482 bp, 498 bp, 448 bp, 427 bp, 451 bp, and 461 bp with *Aerva lanata* (PP412070.1), *Aerva javanica* (PP412069.1*), Haloxylon salicornicum* (PP410311.1), *Haloxylon scoparium* (PP410317.1), *Salicornia herbacea* (PP412073.1), *Salicornia europaea* (PP412072.1), *Sarcocornia fruticose* (PP412071.1), and S*arcocornia perennis* (PP410320.1), respectively. Each sequence was aligned individually at BLAST to confirm each species and determine its relation to other sequences. Multiple sequence alignment (MSA) involved 35 nucleotide sequences of nuclear (ITS region) was done using MEGA X software by Maximum Likelihood method with the highest log likelihood (-902.82) and phylogenetic relationship. The results of eight nucleotide sequences have been uploaded to the GenBank database NCBI with accession number**.**

**Keywords:** Amaranthaceae family, DNA barcode, Nuclear ITS region

## INTRODUCTION

Amaranthaceae s.l. is a widespread and cosmopolitan family consisting of over 170 genera and 2000 species (Xu et al., 2024). mostly annual or perennial herbs and subshrubs just a few are vine, trees, or shrubs (Blunden et al., 1999). Many species are economically important plants, that provide non-conventional protein sources due to their excellent nutritional value, significantly contributing to human nutrition, also have multipurpose applications and represent an important potential source of biologically active substances exhibiting various activities such as antioxidant, anticancer, antibacterial, antifungal, and others (Todorović et al., 2022). In addition, some are used in the food industry. Species belonging to this family are primarily found worldwide, especially in the tropic and subtropical regions also adapted to saline environments and inhabit coastal and continental habitats, arid areas, and deserts.

The family Chenopodiaceae is regarded and defined as a member of the Amaranthaceae s.l. alliance phylogenetically (Kadereit et al., 2003; Ogundipe & Chase, 2009) which classified the Amaranthaceae s.l. into seven subfamilies: Betoideae,Camphorosmoideae,Chenopodioideae, Corispermoideae, Salicornioideae, Salsoloideae, and Suaedoideae. Recent detailed studies based on molecular phylogeny have greatly improved our understanding of this group. The most recent revisions of Chenopodiaceae by Fuentes-Bazan et al. (2012) have been approved for generic delimitations and species classifications. The majority of the species in this family are herbaceous, annual, biennial, or perennial, and some of them are succulent structures. They have alternating or opposite leaves. They are

widespread in Africa, Asia, Europe, and North and South America (Gelin et al., 2003).

In eukaryotic cells, ribosomal RNA organizes into two subunits: the large ribosomal subunit (LSU) and the small ribosomal subunit (SSU) (Richard et al., 2008). The internal transcribed spacer (ITS) is one of the most frequently used polymorphic regions, consisting of non-coding RNA sequences in the primary RNA transcript. Ribosomal RNA genes (rDNA) are part of repeat units arranged in tandem arrays. Nuclear rDNA includes ITS1, ITS2, and the 5.8S subunit, collectively known as the ITS region (Ferreira-Cerca et al., 2005; Giudicelli et al., 2015, 2017). The ITS region of rDNA repeats is highly conserved and commonly amplified using universal PCR primers to study phylogenetic genomic relationships of plants at lower taxonomic levels (Baldwin et al., 1993; Baldwin et al., 1995; Yao et al., 2009). It has recommended a barcode technology based on nucleotide sequences, including fragments of two plastid coding regions, ribulose 1,5-biphosphate carboxylase (rbcL), and maturase (matK), along with one non-coding region, trnH-psbA (Osman,2024). The internal transcribed spacer (ITS) was recently, recommended as a new plant barcoding region for species identification (CBOL, 2009). The ITS1 and ITS2 loci, as single individuals of polymorphisms among repeated units, are widely used as universal primers for various organisms and for phylogenetic reconstruction, enabling comparisons of maternally inherited chloroplasts for evolutionary studies (Poczai & Hyvnen, 2010).

The nuclear ITS region and the plastid trnH-psbA intergenic spacer could serve as universal plant barcodes. One of the most critical steps for accurate taxonomic identification in DNA meta-barcoding is to have an accurate DNA reference sequence dataset for the marker of choice (Quaresma et al., 2024). To compare mini-barcoding and meta-barcoding, a technique rapidly evolved and can fill the gaps left by other methods using smaller DNA segments for universal polymerase chain reaction primers to amplify multiple DNA barcodes and identify many species in the field of herbal molecular identification (Gao et al., 2019). The nuclear internal transcribed spacer (ITS) regions have high accuracy and efficiency for plant species identification and DNA barcoding (Raiyemo & Tranel, 2023).

The present study aims to identify and use DNA barcoding data for phylogenetic analysis and interrelationship of eight wild-type species from the family Amaranthaceae. In addition, the main objective of this study is to utilize the sequences of ITS region as useful molecular markers to determine the genetic relationships within three different subfamilies *Amaranthoideae*, *Salsoloideae*, and *Salicornioideae* belonging to the family Amaranthaceae in Egypt. Furthermore, to study functional annotation and homology modeling of sequences of ITS regions among these species using Basic Local Alignment Search Tool (BLAST).

## MATERIALS AND METHODS

### Plant sampling

Green leaves of eight taxa belonging to the family Amaranthaceae collected in 2021 from their natural populations in Elba Mountain, Halaib, Red Sea Governorate, EL-Negaila and Ras Elhekma, the North Coast, Marsa Matruh Governorate, Egypt were used in the present study (Table 1). The collected materials were developed and identified based on morphological identification (Tackholm & Boulos, 1974; Boulos, 1999). Healthy, fresh, and succulent plant parts for each species were gathered from different locations as bulk transferred into liquid nitrogen, kept frozen and stored at - 80°C until DNA extraction.

### Nucleic acid extraction and nested PCR detection

Healthy dried leaf tissues from eight species were used. DNA extraction was carried out using SIGMA® Plant High Molecular DNA extraction KIT®. DNA quality was tested using agarose gel electrophoresis, visualized by pre-added RedSafe® (5uL/100mL) under UV light. In this study, one pair of primers (ITS-F and ITS-R) were selected from the literature and the BOLD primer dataset platform (http://www.boldsystems.org/index.php/Public_Primer_PrimerSearch). The entire noncoding nuclear internal transcribed spacer (ITS) region, including ITS-F (5'-ATGCGATACTTGGTGTGAAT-3') and ITS-R (5'-GACGCTTCTCCAGACTACAAT-3') primers, was amplified to yield a PCR fragment approximately 500 ± 50 bp in length, following the protocol described by Dong et al. (2013). Each 50µL PCR reaction contained 1× Flexi buffer, 50 ng of DNA template, 2.5mM $MgCl_2$, 10µM dNTPs, 0.4µM of each primer, and 1 U of Promega® Green GoTaq® DNA polymerase. Amplification was carried out using a standard PCR profile with an annealing temperature of 55°C.

**Table 1.** List of the species and their subfamilies distribution

| Location | Subfamily | Plant name | Code |
|---|---|---|---|
| Gabal Elba Mountain | Amaranthoideae | *Aerva lanata* | S1 |
| | | *Aerva javanica* | S2 |
| EL-Negaila | Salsoloideae | *Haloxylon salicornicum* | S3 |
| | | *Haloxylon scoparium* | S4 |
| Ras Elhekma | Salicornioideae | *Salicornia herbacea* | S5 |
| | | *Sarcocornia europaea* | S6 |
| | | *Sarcocornia fruticosa* | S7 |
| | | *Sarcocornia perennis* | S8 |

**Sequence alignment and data analysis**

Each sequence measured in this study was BLASTed against the local database, and the percentage of identical sites was calculated and taken as the species discrimination rate of the measured sequence. All sequences were submitted to Gene Bank. Finally, the identification success rate of DNA barcoding was calculated as the product of sequencing success rate and species discrimination rate. All nucleotide sequences of eight ITS regions were searched against the NCBI database., the National Center for Biotechnology Information GenBank Database (http://www.ncbi.nlm.nih.gov). The homology searches were performed with Basic Local Alignment Search Tool of several sequences (BLASTn online program) based on their homologies with sequences published in the DDBJ/EMBL/GenBank database, which are available using the NCBI database. To investigate the phylogenetic tree and relationships among the sequences of 35 ITS regions from the GenBank NCBI database which include 8 ITS regions of the eight studied wild species, other species belonging to Amaranthaceae family, and *Arabidopsis thaliana* (LN610093.1) and *Nicotiana tabacum* (MH566981.1) from two different families were aligned. The IQR of the data is defined as the difference between the 75$^{th}$ percentile and the 25$^{th}$ percentile. The solid lines in the middle of the box represent the median, and the dotted lines represent the average.

Identification at the genus level was successful when all hits with maximal percent identity scores > 95% involved a single genus. Species identification was confirmed only when the highest percent identity included a single species and scored > 95% (de Groot et al., 2011). A Phylogenetic analysis tree was reconstructed and drawn by Maximum Likelihood (ML) method to detect positive selection under site models by MEGA X (Kumar et al., 2018) and visualized with iTOL v6 Interactive Tree of Life (Letunic & Bork, 2024).

## RESULTS AND DISCUSSION

**Morphological identification**

Morphological identification was based on morphological characteristics, the eight collected samples were: *Aerva lanata* (S1), *Aerva javanica* (S2), *Haloxylon salicornicum* (S3), *Haloxylon scoparium* (S4), *Salicornia herbacea* (S5), *Salicornia europaea* (S6), *Sarcocornia fruticose* (S7), and *Sarcocornia perennis* (S8), respectively (Figures 1-8).

**Figure 1.** Sample of *Aerva lanata*

**Figure 2.** Sample of *Aerva javanica*

**Figure 3.** Sample of *Haloxylon salicornicum*

**Figure 4.** Sample of *Haloxylon scoparium*

**Figure 5.** Sample of *Salicornia herbacea*

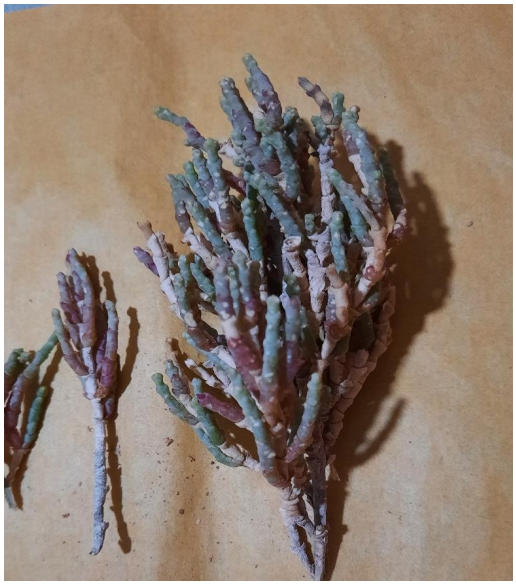

**Figure 6.** Sample of *Salicornia europaea*


**Figure 7.** Sample of *Sarcocornia fruticose*

**Figure 8.** Sample of *Sarcocornia perennis*

**Molecular identification and DNA barcoding**

The ITS gene region, comprising approximately 500±50 base pairs, was successfully isolated using the universal primers on conserved regions (ITS region), enabling the genetic identification of eight wild plant species from the Amaranthaceae family. In molecular biology, the 5.8S rRNA is used as a reference gene for miRNA detection and is a non-

coding RNA component of the large subunit of the eukaryotic ribosome. The 5.8S ribosomal RNA sequences analyzed were approximately 460 base pairs in length, with a mean slightly below this value, indicating a consistent central tendency. The interquartile range (IQR) of sequence lengths was between 450 bp and 470 bp, reflecting a narrow range of variation. However, there is a significant outlier at the upper end, measuring around 500 bp. These observations suggest that while the GC content of ITS sequences is relatively stable and lacks major outliers, the sequence lengths are also consistent but with a notable extreme value at the upper end. The box in the plot represents the IQR, defined as the difference between the 75th and 25th percentiles. The solid lines in the middle of the box represent the median, and the dotted lines represent the average (Figure 9 and Table 2)**.**

**Phylogenetic analysis**

DNA barcoding analysis was carried out to identify and classify important plants from the family Amaranthaceae for their conservation, using (ITS) region for phylogenetic comparative analysis. Blast results for ITS gene for eight samples included sample codes, accession numbers, organism names, and sequence lengths; all sequences were submitted to GenBank (Table 3).

The analysis focused on multiple sequence alignments (MSA) of the ITS region and 35 sequences. Two species from different families included *Arabidopsis thaliana* (LN610093.1) and *Nicotiana tabacum* (MH566981.1) as out-groups from the NCBI database during BLASTN searches, indicating the consistency and reliability of the inferred phylogenetic relationships. With E-value = zero, high identity and similarity. The ITS sequences recorded an average length of 460±75 bp, with a minimum and a maximum length of 427 to 498 bp, respectively. The total GC % ratio was 64.10%, with the highest GC % ratio found in species S3 (64.11), as opposed to species S1 (54.64). Based on BLAST Similarity search, the top hits for each plant sample sequence were retrieved independently. Based on the morphological inspection and limited by the BLAST nucleotide database filtered by > 91% pair wise identity (PI), the species (S1) matched *Aerva javanica* (MH547518.1; PI = 100.00%), and the species (S2) matched *Aerva javanica* (OQ458804.1; PI = 99.50%). Both sequences *Aerva lanata* (PP412070.1) and *Aerva javanica* (PP412069.1) were clustered together in a single clade (group I), indicating their close genetic.

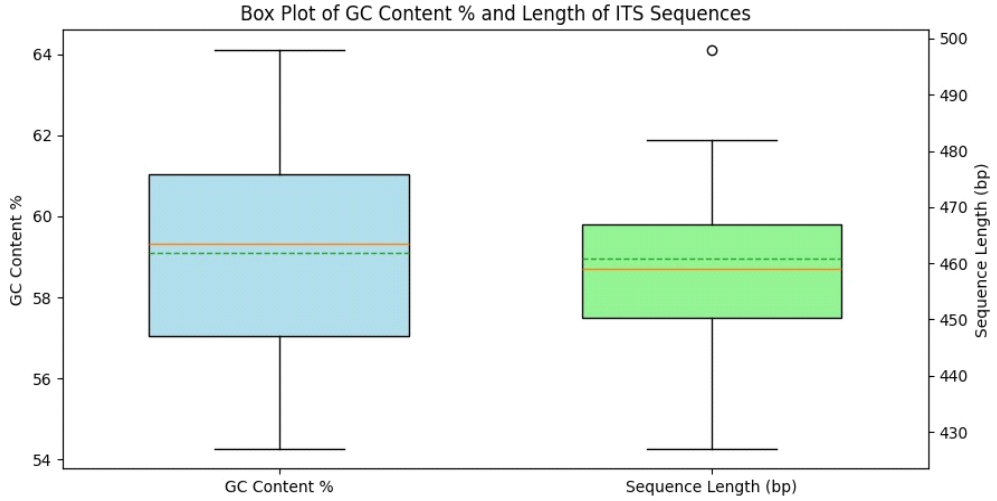


**Figure 9.** Distribution of GC content and sequence lengths of the ITS haplotypes from all species

**Table 2.** Distribution of GC content and sequence lengths of the ITS haplotypes from all species

| Length | GC Content | |
|---|---|---|
| 8.0 | 8.0 | Count |
| 460.75 | 59.11 | Mean |
| 21.55 | 3.5 | std |
| 427.0 | 54.27 | min |
| 498.0 | 64.11 | max |

**Table 3.** Blast results for the ITS gene for eight samples including sample code and the accession number

| Length | GC% | Plant name - | Acc. No | Code |
|---|---|---|---|---|
| 462 bp | 54.64 | *Ouret lanata* | PP412070.1 | **S1** |
| 457 bp | 54.14 | *Aerva javanica* | PP412069.1 | **S2** |
| 482bp | 64.10 | *Haloxylon salicornicum* | PP410311.1 | **S3** |
| 498bp | 62.85 | *Hammada scoparia* | PP410317.1 | **S4** |
| 448 bp | 57.81 | *Salicornia europaea (Salicornia herbacea)* | PP412073.1 | **S5** |
| 427bp | 60.42 | *Salicornia europaea* | PP412072.1 | **S6** |
| 451bp | 59.95 | *Salicornia fruticosa* | PP412071.1 | **S7** |
| 461bp | 58.56 | *Salicornia perennis* | PP410320.1 | **S8** |

Relatedness and belonging to the subfamily Amaranthoideae and specifically to genus *Aerva*. Species (S3) matched *Haloxylon salicornicum* (KX262585.1; PI = 100.00%), species (S4) matched *Hammada articulata* (EF453440.1; PI = 99.20%), Both sequences of *Haloxylon salicornicum* (PP410311.1) and *Haloxylon scoparium* (PP410317.1) were clustered together in a single clade (group II), indicating their close genetic relatedness and belong to subfamily Salsoloideae, and specifically to genus *Haloxylon*. Species (S5) matched *Salicornia fruticosa* (ON685418.1; PI= 91.54%), species (S6) *Salicornia europaea* (KX282378.1.1; PI = 92.10%), species (S7) matched *Salicornia fruticosa* (ON685421.1; PI=100.00%), species (S8) matched *Sarcocornia perennis* (KC555118.1; PI =100.00%) clustered together in a single clade (group III), indicating their close genetic relatedness and belong to subfamily Salicornioideae (Figure 3), as shown in in Table 3 and Figure 10.

These results indicated that molecular studies based on ITS region sequences are very important and provide a high accurate identification for confirmation of the phenotypic characterization of eight species belonging to the family Amaranthaceae in Egypt. The ITS region barcode sequencing has been effectively used for molecular identification of several wild plant species. Our results align with those of Al-Sobeai et al. (2015), Hammer et al. (2019) and Ghahramanlu et al. (2023). A phylogenetic analysis shows that the annual genus *Salicornia* is a sister group to the perennial genera *Sarcocornia, Arthrocnemum*, and *Halocnemum* (Papini et al., 2004). A phylogenetic tree showed that three samples from *H. salicornium* in Saudi Arabia clustered all haplotypes of *H. salicornium* together, with strong bootstrap support (97% and 99%) (Al-Sobeai et al., 2015). Al-Sobeai (2017) investigated by sequencing the amplified ITS region for molecular identification, genetic distance, and genetic relationships among *Haloxylon salicornicum*. The results showed that the high level of genetic similarities of *H. salicornicum* and its closely related taxa might be due to the controlled gene flow and small size of samples. The results showed 100% identification at the family level, 77% identification at the genus level, and 70% species resolution. The ITS2 marker exhibited an outstanding species-level identification efficiency of 100% when evaluated using both BLASTN and neighbor-joining (NJ) tree methods.

**Table 4.** List of the top hits based on BLAST results for each of the sampled species and out-group of the family for the ITS gene

| PI% | Species | Accession no. | Sample |
|---|---|---|---|
| 100.00 | *Aerva javanica* | MH547518.1 | S1 |
| 99.50 | *Aerva javanica* | OQ458804.1 | S2 |
| 100.00 | *Haloxylon salicornicum* | KX262585.1 | S3 |
| 99.20 | *Hammada articulata* | EF453440.1 | S4 |
| 91.54 | *Salicornia fruticosa* | ON685418.1 | S5 |
| 92.10 | *Salicornia europaea* | KX282378.1 | S6 |
| 100.00 | *Salicornia fruticosa* | ON685421.1 | S7 |
| 100.00 | *Sarcocornia perennis* | KC555118.1 | S8 |
| | *Arabidopsis thaliana* | LN610093.1 | out-group |
| | *Nicotiana tabacum* | *MH566981.1* | |

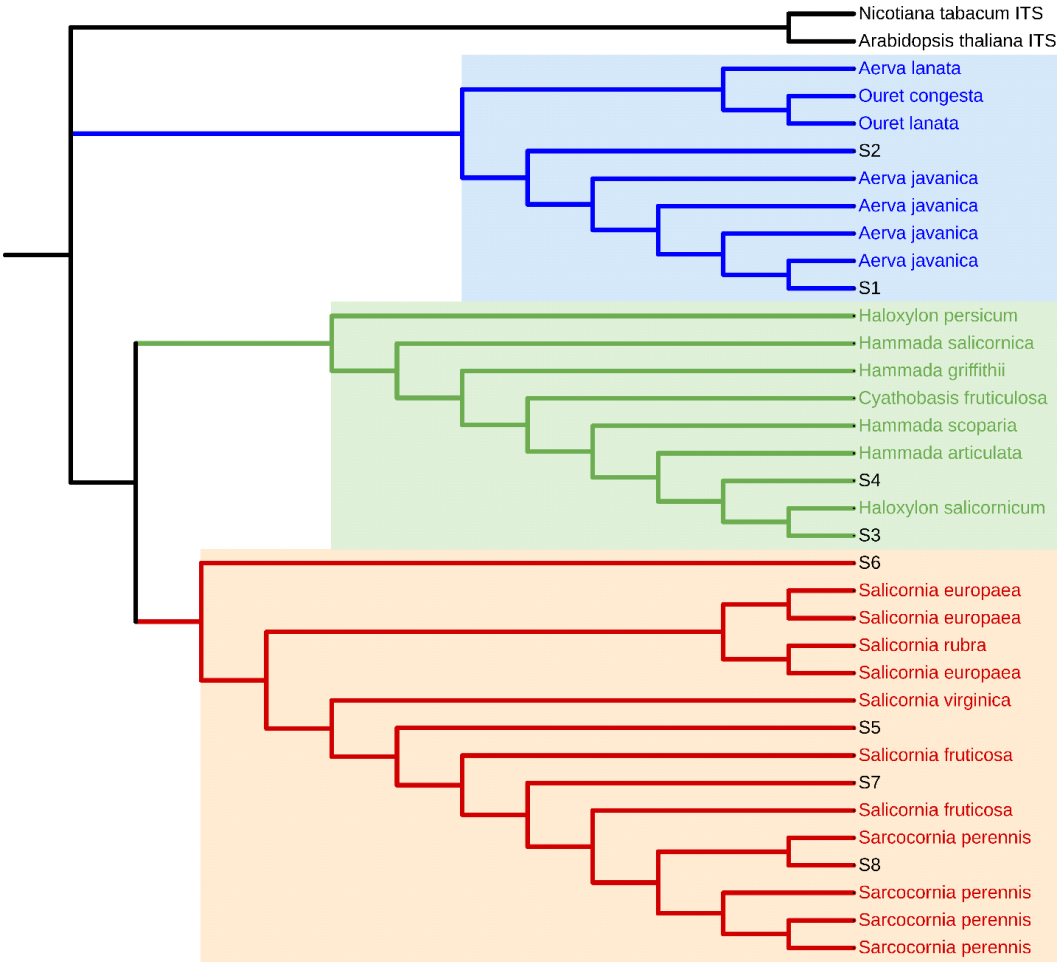


**Figure 10.** Molecular phylogenetic analysis involved 35 nucleotide sequences of the nuclear (ITS region)

## CONCLUSION

Molecular barcoding techniques, particularly those targeting the nuclear internal transcribed spacer (ITS) region, provide a reliable tool for molecular-level identification, classification, and differentiation among the three studied genera, as well as across other genera within the Amaranthaceae family.. These plants have multiple uses, and are endangered plants that need a strict conservation plan. The alignments and phylogenetic trees of 35 nucleotide sequences of the nuclear ITS region (TS1, 5.8S and ITS2) involving *Aerva lanata, Aerva javanica, Haloxylon salicornicum, Haloxylon scoparium, Salicornia herbacea, Salicornia europaea, Sarcocornia fruticose,* and *Sarcocornia perennis* showed high similarity percentages with closely related species of the *Aerva* genus, Haloxylon genus, Salicornia genus and other genera belonging to family Amaranthaceae while they showed low percentages of similarities towards relatively distantly related species and take them away from two different plant species of different families as out group of the family Amaranthaceae.

**Acknowledgments:** This study is a part of Ph.D. thesis in the Faculty of Environmental Agricultural Science, Arish University, North Sinai, Egypt. We are many thanks to all supervisors, especially deceased professor Dr. Farida Elsaid. Her groundbreaking work in genetics has profoundly influenced our research and many thanks to the Desert Research Center for supporting this study

**Conflicts of interest:** The author declares no conflict of interest.

**Authors' contributions:** The manuscript was edited and revised by all authors.

**Ethical approval:** Not applicable.

## REFERENCES

Al-Sobeai, S.M., Alamer, K.H., Amer, S.A. (2015).

A preliminary molecular variability within *Haloxylon salicornium* accessions growing in Saudi Arabia. *Plant Omics*, 8(6): 604-608.

Al-Sobeai, S.M. (2017). Molecular genetic relationships among *Haloxylon salicornicum* plant and its closely related taxa in Saudi Arabia. *International Journal of Biosciences,* 10(2): 81-88.

Baldwin, B.G. (1993). Molecular phylogenetics of Calcydenia (Compositae) based on ITS sequences of nuclear ribosomal DNA: Chromosomal and morphological evolution reexamined. *American Journal of Botany*, 80: 222-238.

Baldwin, B.G., Sanderson, M.J., Porter, J.M., Wojciechowski, M.F., Campbell, C.S., Donoghue, M.J. (1995). The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. *Annals of the Missouri Botanical Garden,* 82(2): 247-277.

Blunden, G., Yang, M.H., Janicsák, G., Máthé, I., Carabot-Cuervo, A. (1999). Betaine distribution in the Amaranthaceae. *Biochemical Systematics and Ecology,* 27(1): 87-92.

Boulos, L. (1999). "*Flora of Egypt: Monocotyledons (Alismataceae-Orchidaceae)*" (Vol. 4). Al Hadara Pub., Cairo, Egypt.

CBOL Plant Working Group (2009). A DNA Barcode for Land Plants. *Proceedings of the National Academy of Sciences of the United States of America*, 2009; 106: pp. 12794-12797.

De Groot, A.M. (2011). "*Language and Cognition in Bilinguals and Multilinguals: An Introduction*". Psychology Press.

Dong, B., Yi, J., Dai, L., Dai, X. (2013). Evaluation of several DNA extraction methods for obtaining total community DNA from anaerobic digestion sludge. *Procedia Environmental Sciences,* 18: 856-863.

Ferreira-Cerca S., Poll, G., Gleizes, P.E., Tschochner, H., Milkerei, T.P. (2005). Roles of Eukaryotic ribosomal protein in maturation and transport of pre 18S rRNA and ribosome function. *Molecular Cell,* 20(2): 263-275.

Fuentes-Bazan, S., Mansion, G., Borsch, T. (2012). Towards a species-level tree of the globally diverse genus Chenopodium (Chenopodiaceae). *Molecular Phylogenetics and Evolution*, 62(1): 359-374.

Gao, Z., Liu, Y., Wang, X., Wei, X., Han, J. (2019). DNA mini-barcoding: A derived barcoding method for herbal molecular identification. *Frontiers in Plant Science,* 10: 417036.

Gelin, Z., Mosyakin, S.L., Clemants, S.E. (2003). Chenopodiaceae. *Flora of China,* 5(1): 351-414.

Ghahramanlu, A., Rezaei, M., Heidari, P., Balandari, A. (2023). Species survey of Iranian barberry genotypes using ITS2 sequences and basic local alignment search tools. *Erwerbs-Obstbau,* 65(6): 2491-2499.

Giudicelli, G.C., Mäder, G., de Freitas, L.B. (2015). Efficiency of ITS sequences for DNA barcoding in Passiflora (Passifloraceae). *International Journal of Molecular Sciences,* 16(4): 7289-7303.

Giudicelli, G.C., Mäder, G., Silva-Arias, G.A., Zamberlan, P.M., Bonatto, S.L., Freitas, L.B. (2017). Secondary structure of nrDNA Internal Transcribed Spacers as a useful tool to align highly divergent species in phylogenetic studies. *Genetics and Molecular Biology*, 40(Suppl. 1): 191-199.

Hammer, T.A., Zhong, X., Colas des Francs-Small, C., Nevill, P.G., Small, I.D., Thiele, K.R. (2019). Resolving intergeneric relationships in the aervoid clade and the backbone of Ptilotus (Amaranthaceae): Evidence from whole plastid genomes and morphology. *Taxon,* 68(2): 297-314.

Kadereit, G., Borsch, T., Weising, K., Freitag, H. (2003). Phylogeny of Amaranthaceae and Chenopodiaceae and the evolution of C4 photosynthesis. *International Journal of Plant Sciences,* 164(6): 959-986.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution,* 35(6): 1547-1549.

Letunic, I., Bork, P. (2024). Interactive tree of life (iTOL) v6: Recent updates to the phylogenetic tree display and annotation tool. *Nucleic Acids Research*, 52(W1): W78-W82

Ogundipe, O.T., Chase, M. (2009). Phylogenetic analyses of Amaranthaceae based on matK DNA sequence data with emphasis on West African species. *Turkish Journal of Botany,* 33(3): 153-161.

Osman, S.A. (2024). The power of DNA barcoding for plant identification. *Egyptian Journal of Chemistry*, 67(1), 633-646.

Papini, A., Trippanera, G., Maggini, F., Filigheddu, R., Biondi, E. (2004). New insights in *Salicornia* L. and allied genera (Chenopodiaceae) inferred from nrDNA sequence data. *Plant Biosystems,* 138: 215-223.

Poczai P., Hyvönen, J. (2010). Nuclear ribosomal

spacer regions in plant phylogenetics: Problems and prospects. *Molecular Biology Reports,* 37(4): 1897-1912.

Quaresma, A., Ankenbrand, M.J., Garcia, C.A.Y., Rufino, J., Honrado, M., Amaral, J., et al. (2024). Semi-automated sequence curation for reliable reference datasets in ITS2 vascular plant DNA (meta-) barcoding. *Scientific Data,* 11(1): 129.

Raiyemo, D.A., Tranel, P.J. (2023). Comparative analysis of dioecious *Amaranthus plastomes* and phylogenomic implications within Amaranthaceae ss. *BMC Ecology and Evolution,* 23(1): 15.

Richard, G.F., Kerrest, A., Dujon, B. (2008). Comparative genomics and molecular dynamics of DNA repeats in Eukaryotes. *Microbiology and Molecular Biology Reviews,* 72(4): 686-727.

Täckholm, V. (1974). "*Students' Flora of Egypt*", 2nd ed. Cairo University, Cairo, Egypt, 888p.

Todorović, M., Zlatić, N., Bojović, B., Kanjevac, M. (2022). Biological properties of selected Amaranthaceae halophytic species: A review. *Brazilian Journal of Pharmaceutical Sciences*, 58: e21229.

Xu, H., Guo, Y., Xia, M., Yu, J., Chi, X., Han, Y., et al. (2024). An updated phylogeny and adaptive evolution within Amaranthaceae sl. inferred from multiple phylogenomic datasets. *Ecology and Evolution*, 14(7): e70013.

Yao, H., Song, J.Y., Ma, X.Y., Liu, C., Li, Y., Xu, H.X., et al. (2009). Identification of *Dendrobium* species by a candidate DNA barcode sequence: The chloroplast psbA-trnH intergenic region. *Planta Medica*, 75(06): 667-669.