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Taxonomic considerations among and within some Egyptian taxa of *Capparis* and related genera (Capparaceae) as revealed by RAPD fingerprinting

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Abstract
Taxonomic considerations among and within some Egyptian taxa of *Capparis* and related genera (Capparaceae) as revealed by RAPD fingerprinting.- This investigation was carried out to assess the taxonomic relationships among eight taxa of the Egyptian members of Capparaceae based on random amplified polymorphic DNA markers, and to compare the results with those obtained from morphological studies. A total of 46 bands were scored for three RAPD primers corresponding to an average of 15.3 bands per primer. The three primers (A03, A07 and A09) revealed eight polymorphic RAPD markers among the studied taxa ranging in size from 200 bp to 1000 bp. Jaccard's coefficient of similarity varied from 0.28 to 0.84, indicative of high level of genetic variation among the genotypes studied. UPGMA cluster analysis indicated three distinct clusters, one comprised *Cleome amblyocarpa* and *Gynandropsis gynandra*, while another included two clusters at 0.74 phenon line; one for *Capparis decidua*, and the other for *Capparis sinaica* and all varieties of *Capparis spinosa.* The four varieties of *Capparis spinosa* were segregated at 0.84 phenon line. However, one of these varieties was more closely related to *Capparis sinaica* than to the other three varieties of *C. spinosa*. The RAPD analysis reported here confirms previous studies based on morphological markers.

Key words: *Capparis; Cleome;* cluster analysis; Egypt; genetic relationships; *Gynandropsis*; RAPD-PCR; taxonomy; UPGMA.

Resumen
Consideraciones taxonómicas sobre algunos taxones egipcios de *Capparis* y géneros relacionados (Capparaceae) a partir de RAPDs.- El objetivo de este trabajo es investigar las relaciones taxonómicas entre ocho taxones pertenecientes a las Capparaceae en base a marcadores de tipo RAPD, y comparar los resultados con los obtenidos previamente en estudios morfológicos. Se han contabilizado un total de 46 bandas para tres pares de cebadores, con una media de 15,3 bandas por cebador. Los tres pares de cebadores (A03, A07 y A09) revelan ocho marcadores polimórficos entre los taxones estudiados, de entre 200 y 1000 pares de bases. El coeficiente de similaridad de Jaccard varía entre 0,28 y 0,84, indicativo de un alto nivel de variación genética entre los genotipos estudiados. El análisis UPGMA muestra tres grupos distintos, el primero comprende *Cleome amblyocarpa* y *Gynandropsis gynandra*, mientras que el segundo incluye dos grupos a la altura del valor 0,74 del dendrograma: uno se corresponde con *Capparis decidua*, y el otro comprende *Capparis sinaica* y todas las variedades de *Capparis spinosa.* Las cuatro variedades de *C. spinosa* se segregan a la altura del valor 0,84 del dendrograma. Sin embargo, una de estas variedades está más relacionada con *C. sinaica* que con las otras variedades de *C. spinosa*. El análisis de RAPD confirma los resultados de estudios anteriores basados en caracteres morfológicos.

Palabras clave: *Capparis; Cleome;* análisis *cluster*; Egipto; *Gynandropsis*; RAPD-PCR; relaciones genéticas; taxonomía; UPGMA.

INTRODUCTION

The family Capparaceae Juss. is a fairly large family (45 genera and 675 species), mainly subtropical, being most conspicuous in tropical seasonally dry habitats and with diversity in floral structure (Mabberley, 1997). The family is sometimes divided into eigth subfamilies and four tribes (Pax & Hoffman, 1936), or into two subfamilies: Capparoideae and Cleomoideae (Jafri, 1974). Actually, the two major subfamilies are quite distinct and have been elevated to family status by some authors (Airy Shaw, 1965; Hutchinson, 1967). Daniel & Sabnis (1977) determined flavonoids and phenolic acids in seven members of the Capparaceae and in five members of the Cleomaceae. The results supported the proposal (made on anatomical grounds) that Cleomaceae could be recognized as a separate family. Recently, this proposal was also supported by Kamel *et al.* (2009). The type genus of each of the subfamilies is by far the largest and houses the majority of the species: *Capparis* L. has about 250 species and *Cleome* L. has 200 species. This imbalance suggests that plants with extreme morphological traits may have been segregated into smaller genera, making the larger genera paraphyletic.

Capparis is a polymorphic genus and is distributed in the tropical and subtropical regions of the old and new world. According to Iltis (2001), there are about 110 *Capparis* taxa in the old world. The study of the reproductive characters in *Capparis* is problematic due to the difficulty of preserving the flowers (Hedge & Lamond, 1970). In Egypt, Täckholm (1974) recognized six species of *Capparis*, whereas Boulos (1999) recognized three species and four varieties.

Cleome has nine species in Egypt. Unresolved problems are still in need of further studies concerning *Cleome gynandra* L., which has been treated in the recent past as belonging to a separate genus *Gynandropsis*, and it was so treated in Graham's manuscript, and in Täckholm (1974), Jafri (1977) and Boulos (1999). Iltis (1957) gave convincing reasons for restoring this species to *Cleome*, and his treatment was later followed by Ridley (1967), Stewart (1972) and Thulin (1993).

During this study, we realized that morphological variability has led to much confusion in distinguishing species using the diagnostic characters proposed by different authors (Zohary, 1960; Davis, 1965; Jacobs, 1965; Al-Gohary, 1982; Higton & Akeroyd, 1991; Heywood, 1993; Fici & Gianguzzi, 1997; El-Karemy, 2001). Species identification is hard or even impossible when only vegetative parts are present, which is often the case during collection. Additional information about the genotype of plants is very much needed to resolve taxonomic problems in these genera. Because the genotype is not influenced by environmental factors, evolution of closely related taxa can be investigated from an objective point of view with molecular techniques (Hillis, 1987). In addition, some molecular marker assays, e.g., the use of random amplified polymorphic DNA (RAPD), allow the detection of DNA polymorphisms by randomly amplifying multiple regions of the genome by polymerase chain reaction (PCR) using single arbitrary primers designed independently of target DNA sequence (Welsh & McClelland, 1990; Hadry *et al.*, 1992; Williams *et al.*, 1993; Karp *et al*., 1996). Therefore, it has been extensively used as a genetic marker for estimating genetic, taxonomic, and phylogenetic relationships of plants and animals (Williams *et al.*, 1990; Wachira *et al*., 1995; Kapteyn & Simon, 2002; Belaj *et al*., 2003; Deshwall *et al.*, 2005). The method does not require any prior characterization of the genome to be analyzed unlike universal PCR where sequence information is a prerequisite for designing primers. RAPD analysis requires only a small amount of genomic DNA and can produce high levels of polymorphism and may facilitate more effective diversity analysis in plants (Williams *et al.*, 1990). The technique is therefore simple, fast, and efficient, and requires little tissue for assays. RAPD has proved to be a good marker to assay and evaluate the genetic relationships among species and even among populations and individuals of the same species (Tingey *et al.*, 1993; Warburton & Bliss, 1996).

Publised studies on *Capparaceae* based on molecular markers are scarce. Application of RAPD technique has been previously performed for the conservation of isolated populations of the extensively grazed range plant *Capparis decidua* in Saudi Arabia (Abdel-Mawgood *et al.*, 2006). Inocencio *et al.* (2005) used genetic fingerprinting technique (AFLP) to determine the relationships among species of *Capparis*. Genetic distances, based on AFLP data were estimated for 45 accessions of *Capparis* species from Spain, Morocco and Syria. The results

Table 1. Materials, locations, habitats and abbreviations used in the RAPD study.

Taxa	Location and habitat	Abbreviation
Capparis spinosa L. var. *spinosa*	Sinai,Wadi Feiran, on rocks	CP SP
C. spinosa L. var. *canescens* Coss.	Ain El Sheikh Omran, near Dakhla Oasis, on canal banks	CP OV
C. spinosa L. var. *deserti* Zohary	134 km North of Siwa Oasis	CP DS
C. decidua (Forssk.) Edgew	Wadi Gemal Protectorate, 25 km from the entrance, Red Sea	CP DC
C. spinosa L. var. *inermis* Turra	Mersa Matruh, Agiba beach, west Mediterranean coast	CP OR
C. sinaica Veill.	Ras Sedr Road, 5 km from Ras Sedr, Suez Gulf, Sinai	CP SN
Cleome amblyocarpa Barratte & Murb.	Al-Arish, Abu Shenar village, N Sinai	CL AM
Gynandropsis gynandra (L.) Briq.	Ismailia, Abu Soweir, in cultivated land of mango and vegetables	GY GY

of this analysis supported the differentiation of four of the five taxa involved.

To our knowledge, there is no published information on the use of RAPD-PCR markers for the characterization of genetic relationships of the *Capparis* species that we study in this contribution. Therefore, the present study was undertaken to assess taxonomic relationships and divergence within and among the species of the genus *Capparis* from Egypt using RAPD markers, and to compare the results with those obtained from morphological studies.

A secondary aim was to determine whether *Gynandropsis* must be considered an independent genus or a synonym of *Cleome*

MATERIAL AND METHODS

A total number of eight taxa from different populations from Mersa Matruh were collected along the western Mediterranean coast, Sinai Peninsula and Ismailia region (Table 1). Ten individuals per population were included in the study. Young, healthy leaves were frozen or dried in plastic bags with silica gel until extracted in the Molecular laboratory, at Cairo University (Egypt). At least three independent leaf samples were collected for each species, in order to account for any artifactual amplification. Further descriptions of morphological traits and flowering were noted in separate data sheets for all individuals. The nomenclature follows Zohary (1966), Täckholm (1974), Thulin (1993) and Boulos (1999). A voucher of each species used in this study was deposited in Cairo University Herbarium (CAI). Initially, total genomic DNA extraction was carried out by SDS-proteinase K treatment (Brown, 1991) but this yielded poor quality DNA, as indicated by the brownish pellets. Subsequently, a modified version of the CTAB DNA extraction protocol described by Brown (1991) yielded high-quality genomic DNA.

Because the RAPD-PCR technology is sensitive to changes in experimental parameters, a total of ten primers were initially screened against ten individuals selected from every taxon. Experiments were carried out with varying concentrations of magnesium chloride (1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 mM) and DNA template (5, 10, 15, 20, and 25 ng), in order to optimize the PCR conditions. The length of the denaturation stage of the amplification was also examined. When trying to optimize annealing temperatures, we ran test reactions at 34°C, 35°C, 36°C, and 37°C. The decamer primers could be clearly amplified at 34°C. A subset of 10 primers for further analyses was based on the following criteria: (i) consistent, strong amplification products, and (ii) production of uniform, reproducible fragments between replicate PCRs. An initial screening resulted in selection of three decamer oligonucleotides from the "A" RAPD primer kit that produced clear and reproducible amplification product, and estimated fragment size (bp), *e.g.* OPA03700 stands for the 700 bp marker generated by primer 03, kit A, from Operon Technologies Inc. (Alameda, CA, USA).

Amplifications were performed in 50-µl reaction volumes containing 1unit of *Taq* polymerase BioChain, 10 mM Tris-HCl (pH 9.0), 25 mM KCl, 4 mM $MgCl_2$, 10 mM of each dNTP, 1µM each of random primer and 25 ng of template DNA. The negative control consisted of all reagents, except the DNA template in the reaction mixture. The cycling regime for the reaction was as follows:

Table 2. RAPD markers in the studied taxa. For abbreviations of taxa, see Table 1. (0=Absent, 1=Present).

Primer code	Nucleotide sequence (5'–3')	Marker (bp)	CP SP	CP OV	CP DS	CP DC	CP OR	CP SN	CL AM	GY GY
A03	AGT CAG CCA C	1000	0	0	0	1	0	1	1	1
		750	1	1	1	1	1	1	0	1
		200	1	1	1	1	1	1	0	0
A07	GAA ACG GGT G	1000	1	1	1	1	1	1	1	0
		750	1	1	1	1	1	1	1	1
		500	1	1	1	1	0	0	0	0
A09	GGG TAA CGC C	1000	0	0	0	0	0	0	1	1
		600	1	1	1	0	1	1	0	0
		250	0	0	0	0	0	0	1	1

pre-denaturation (94°C, 4 min) followed by 35 cycles consisting of denaturation (94°C, 1 min), annealing (34°C, 1 min) and extension (72°C, 2 min) with a final extension (72°C, 10 min). PCR was carried out with a gene Amp 2400 PCR System (Perkin Elmer DNA Cycler). Replication of the RAPD reaction for every combination of template DNA and primer was carried out to ensure reproducibility. Only reproducible RAPD markers were included in the analysis. RAPD fragments together with a size marker 10000 bp DNA ladder (Pharmacia Biotech) were separated electrophoretically on 2% agarose gels in 1X TBE buffer for approximately 4 hours, stained with ethidium bromide, photographed on a UV transilluminator (Sambrook *et al.*, 1989), and the banding patterns were compared.

RAPD bands were discerned from the agarose gel. Any fragment thought to be artifact, based on the controls or those too difficult to score with certainly, were not included in the data set and only distinct reproducible, well–resolved fragments were scored (1) for presence and (0) for absence of bands and assembled into a data matrix (Table 2). The genetic similarity between different pairs of individuals was estimated according to Jaccard's coefficient. A dendrogram following the unweighted pair group arthimetic average method UPGMA algorithm in the Multi Variate Statistic Package (MVSP) for windows version 3.13 (Kovach, 1999) was generated with the Jaccard's coefficient based on the entire marker generated (Rohlf, 1992).

RESULTS AND DISCUSSION

A necessary precondition for any RAPD analysis is the establishment of PCR conditions that ensure reliable and reproducible results (Ramser *et al.*, 1996). Various parameters likely to affect PCR amplification were optimized. However, only data from optimum amplification conditions in our experiments are presented here.

To investigate the intra-varietal polymorphism among the studied taxa, the different RAPD profiles within the different species and varieties were compared. Figures 1 and 2 represented RAPD profiles obtained from primers A03 and A07, respectively. A total of 46 bands were scored for the three RAPD primers corresponding to an average of 15.3 bands per primer. The three primers revealed eight polymorphic RAPD markers among the studied taxa, ranging in size from 200 bp to 1,000 bp, and one monomorphic marker at A07 of 750 bp (Table 2). Within *Capparis*, some fragments were shared by two species, and that was clearly observed for *Capparis sinaica* and *C. decidua* (A03, 1000 bp). Some bands that were present in different genera were recorded: a band at A03 (750 bp) was present in all studied taxa except in *Cleome amblyocarpa;* a band at A03 (200 bp) was present in all species of *Capparis*; and a band at A07 (1000 bp) was present in the genus *Capparis* and in *Cleome amblyocarpa.* Species-specific RAPD markers suitable for discriminating the studied taxa of *Capparis* and their allied genera were also detected.

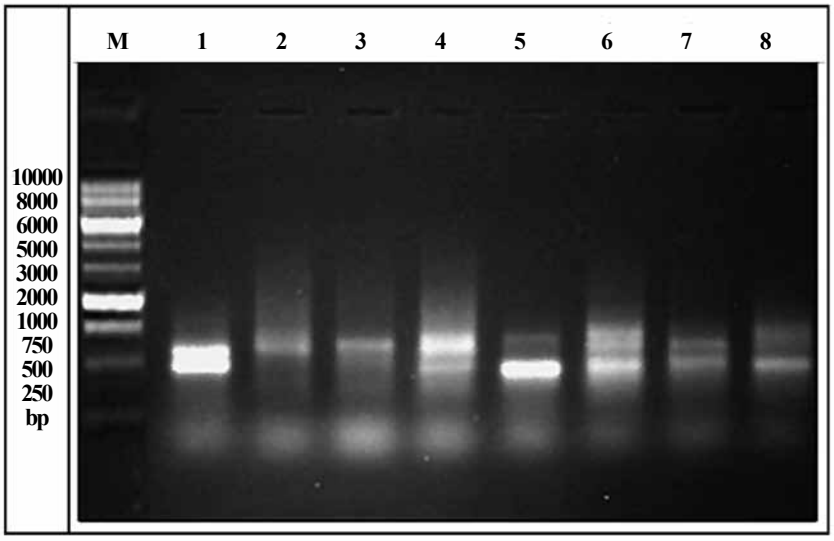

Figure 1. RAPD amplification profiles of *Capparis, Cleome,* and *Gynandropsis* species using primer A03: (lanes 1-8) from left to right. **M** = Marker, **1.** *Capparis spinosa* var. *spinosa,* **2.** *C. spinosa* var. *canescens,* **3.** *C. spinosa* var. *deserti,* **4**. *C. decidua,* **5.** *C. spinosa* var. *inermis,* **6.** *C. sinaica,* **7.** *Cleome amblyocarpa,* **8.** *Gynandropsis gynandra.*

The polymorphism in presence/absence of RAPD fragments was used to construct a dendogram (Fig. 3) based on Jaccard's similarity coefficient. In the present analysis of eight taxa, two major clusters were identified. The first included *Cleome amblyocarpa* and *Gynandropsis gynandra*, while the latter included two clusters; one for *Capparis decidua*, while the other for *C. sinaica* and all varieties of *C. spinosa.*

Cluster analysis revealed also that *C. spinosa* var. *inermis* was closer to *C. sinaica* than to the remaining three varieties of *C. spinosa*. These results supported the earlier taxonomic studies and numerical analyses (Abd El-Ghani *et al.*, 2007; Kamel *et al.*, 2009, 2010). Thus, *C. spinosa* var. *inermis* could be treated as an independent species, *C. orientalis*, as suggested by some authors (Täckholm, 1974; Al-Gohary, 1982; Inocencio *et al.*,

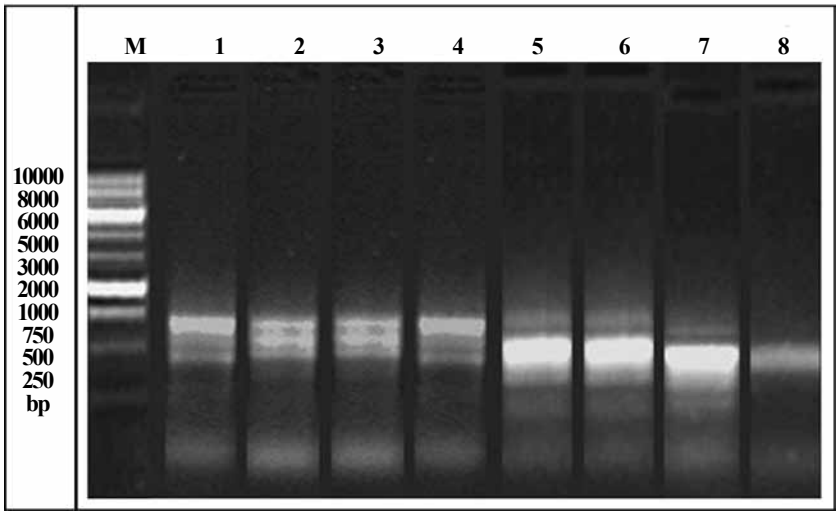

Figure 2. RAPD amplification profiles of *Capparis, Cleome,* and *Gynandropsis* species using primer A07: (lanes 1-8) from left to right: **M** = Marker, **1.** *Capparis spinosa* var. *spinosa,* **2.** *C. spinosa* var. *canescens,* **3.** *C. spinosa* var. *deserti,* **4**. *C. decidua,* **5.** *C. spinosa* var. *inermis,* **6.** *C. sinaica,* **7.** *Cleome amblyocarpa,* **8.** *Gynandropsis gynandra.*

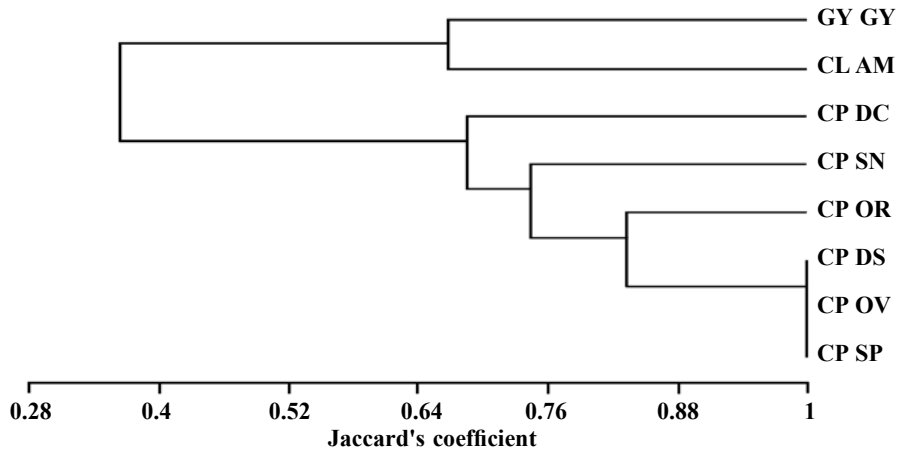

Figure 3. UPGMA dendrogram showing the relationships between the 8 studied taxa using Jaccard's similarity coefficient. GY GY = *Gynandropsis gynandra*, CL AM = *Cleome amblyocarpa*, CP DC = *Capparis decidua*, CP SN = *C. sinaica*, CP OR = *C. spinosa* var. *inermis*, CP DS = *C. spinosa* var. *deserti*, CP OV = *C. spinosa* var. *canescens*, CP SP = *C. spinosa* var. *spinosa*.

2005) in contradiction with Boulos (1999). Thus, a DNA based diagnostic assay like RAPD is able to identify genotypes directly and can therefore be of help to mitigate complications arising from earlier morphological studies. Additional future studies should focus on the taxonomic and genetic relationships among Capparoideae and Cleomoideae, not only in Egypt but in a wider geographic scale.

RAPD data does not contradict the recommendation of considering *Gynandropsis gynandra* within *Cleome* as *Cleome gynandra* (Kamel *et al*., 2010), because it is grouped with *Cleome amblyocarpa*. However, further studies including other species of *Cleome* should be undertaken to confirm these results.

Similar studies are important in detailing the level of variation and relationships within and between the species in order to plan future domestication trails and to manage properly the wild species collections which are kept in gen banks. In conclusion, we recommend that RAPD analyses are used as additional tool in the study of members of the Capparaceae.

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REFERENCES

Abd El-Ghani, M. M., Kamel, W., & El-Bous, M. 2007. The leaf architecture and its taxonomic significance in Capparaceae from Egypt. *Acta Biol. Szeged.* 51: 125-136.

Abdel-Mawgood, A. L., Assaeed, A. M. & Al-Abdallatif, T. I. 2006. Application of RAPD technique for the conservation of an isolated population of *Capparis decidua*. *Alexandria J. Agric. Res.* 51: 171-177.

Airy Shaw, H. K. 1965. Diagnoses of new families, new names, etc. for the seventh edition of Willis's "Dictionnary". *Kew Bull.* 18: 249-273.

Al-Gohary, I. H. 1982. *Morphological studies on the Cappa-ridaceae in Egypt*. M.Sc. Thesis, Faculty of Science, Ain Shams University, Cairo.

Belaj, A., Satovic, Z., Ismaili, H., Panajoti, D., Rallo, L. & Trujillo, I. 2003. RAPD genetic diversity of Albanian olive germplasm and its relationships with other Mediterranean countries. *Euphytica* 130 : 387-395.

Boulos, L. 1999. *Flora of Egypt* 1. (*Azollaceae-Oxalidaceae*). Al Hadara Publishing, Cairo.

Brown, T. A. 1991. *Essential molecular biology: A practical Approach* 1. IRL Press, Oxford.

Daniel, M. & Sabnis, S. D. 1977. Chemotaxonomic studies on Capparidaceae-Cleomaceae. *Curr. Sci.* 46: 472-474.

Davis, P. H. 1965. *Flora of Turkey* 1. Edinburgh University Press, Edinburgh.

Deshwall, R. P. S, Singh, R., Malik, K. & Randhawa, G. J. 2005. Assessment of genetic diversity and genetic relationships among 29 populations of *Azadirachta indica* A. Juss. using RAPD markers. *Genet. Res. Crop Evol.* 52: 285-292.

El-Karemy, Z. A. R. 2001. Capparaceae in the flora of Egypt. *Taeckholmia* 21: 257-267.

Fici, S. & Gianguzzi, L. 1997. Diversity and conservation of wild and cultivated *Capparis* in Sicily. *Bocconea* 7: 437-443.

Hadry, H., Bailk, M. & Schierwater, B. 1992. Application

of random amplified polymorphic DNA (RAPD). *Molec. Ecol.* 1: 55-63.

Hedge, I. C. & Lamond, J. 1970. Capparidaceae. In: Rechinger, K. H. (Ed.), *Flora Iranica* 68. Akademische Druck-u. Verlagsanstalt, Graz: 1-9.

Heywood, V. H. 1993. *Flowering plants of the world*. Oxford University Press, New York.

Higton, R. N. & Akeroyd, J. R. 1991. Variation in *Capparis spinosa* L in Europe. *Bot. J. Linn. Soc.* 106: 104-112.

Hillis, D. M. 1987. Molecular versus morphological approaches to systematics. *Annual Rev. Ecol. Syst.* 18: 23-42.

Hutchinson, J. 1967. *The genera of flowering plants.* Vol. 2. Clarendon Press, Oxford.

Iltis, H. H. 1957. Studies in the Capparidaceae III: Evolution and phylogeny of the western North America Cleomoideae. *Ann. Missouri Bot. Gard.* 44: 77-119.

Iltis, H. H. 2001. Capparaceae. In: Stevens, W. D., Ulloa-Ulloa, C., Pool, A. & Montiel, O. M. (Eds.), *Flora of Nicaragua*. *Monogr. Syst. Bot. Missouri Bot. Gard*. 85: 566-584.

Inocencio, C., Cowan, R. S., Alcaraz, F., Rivera, D. & Fay, M. F. 2005. AFLP fingerprinting in *Capparis* subgenus *Capparis* related to the commercial sources of Capers. *Genet. Resources Crop Evol.* 52: 137-144.

Jacobs, M. 1965. The genus *Capparis* (Capparaceae) from the Indus to the Pacific. *Blumea* 12: 385-541.

Jafri, S. M. H. 1974. Capparidaceae. In: Nasir, E. & Ali, S. I. (Eds.), *Flora of Pakistan* 71. National Herbarium, Islamabad: 1-42.

Jafri, S. M. H. 1977. Capparaceae. In: Ali, S. I. & Jafri, S. M. H. (Eds.), *Flora of Libya* 12. Al-Faateh University, Tripoli: 1-20.

Kamel, W., Abd El-Ghani, M. M. & El-Bous, M. 2009. Taxonomic study of Capparaceae from Egypt: Revisited. *African J. Pl. Sci. Biotech.* 3: 27-35.

Kamel, W., Abd El-Ghani, M. M. & El-Bous, M. 2010. Cleomaceae as a distinct family in the flora of Egypt. *African J. Pl. Sci. Biotech.* 4: 11-16.

Kapteyn, J. & Simon, J. E. 2002. The use of RAPDs for assessment of identity, diversity, and quality of *Echinacea*. In: Janick, J. & Whipkey, A. (Eds), *Trends in New Crops and New Uses*. *Amer. Soc. Hort. Sci. Press, Alexandria*: 509-513.

Karp, A., Seberg, O. & Buiatti, M. 1996. Molecular techniques in the assessment of botanical diversity. *Ann. Bot (Oxford)* 78: 143-149.

Kovach, W. L. 1999. *MVSP. Multivariate Statistical Package for IBM-PCs*, *version 3.13*. Kovach Computing Services, Pentraeth.

Mabberley, D. J. 1997. *The plant book: a portable dictionnary of the vascular plants*. Cambridge University Press, Cambridge.

Pax, F. & Hoffmann, K. 1936. Capparidaceae. In: Engler, A. & Prantl, K. (Eds.), *Die natürlichen Pflanzenfamilien* 17 (b). Engelmann, Leipzig: 146-233.

Ramser, J., Weising, K., Kahl, G., López-Peralta, C. & Wetzel R. 1996. Genomic variation and relationships in aerial yam (*Dioscorea bulbifera* L.) detected by random amplified polymorphic DNA. *Genome* 39: 17-25.

Ridley, H. N. 1967. *The flora of the Malay Peninsula*. A. Asher & Co., Amsterdam.

Rohlf, F. J. 1992. *NTSYS-PC. Numerical taxonomy and multivariate analysis system*. *Version 1.7*. Exeter Software, Applied Biostatistics Inc., New York.

Sambrook, J., Fritsch, E. F. & Maniatis, T. 1989. *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.

Stewart, R. R. 1972. An annotated catalogue of the vascular plants of West Pakistan and Kashmir. In: Nasir, E. & Ali, S. I. (Eds.), *Flora of West Pakistan.* Fakhri Press, Karachi.

Täckholm, V. 1974. *Students' Flora of Egypt*. Cairo University Press, Cairo.

Thulin, M. 1993. *Flora of Somalia* 1. Royal Botanical Gardens, Kew.

Tingey, S. V. & del Tufo, J. P. 1993. Genetic analysis with random amplified polymorphic DNA (RAPD). *Pl. Physiol.* 101: 349-352.

Wachira, F. N., Waugh, R., Powell, W. & Hackett, C. A. 1995. Detection of genetic diversity in tea (*Camellia sinensis*) using RAPD markers. *Genome* 38: 201–210.

Warburton, M. L. & Bliss, F. A. 1996. Genetic diversity in peach [*Prunus persica* (L.) Batsch] revealed by randomly amplified polymorphism DNA (RAPD) and compared to inbreeding coeffients. *J. Amer. Soc. Hort.. Sci.* 121: 1012-1019.

Welsh, J. & McClelland, M. 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucl. Acids Res.* 18: 7213-7218.

Williams, J. G. K., Hanefey, M. K., Rafalski, J. A. & Tingey, S. V. 1993. Genetic analysis using random amplified polymorphic DNA markers. *Meth. Enzymol.* 218: 704-740.

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. & Tingey, S. V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl. Acids Res.* 18: 6531-6535.

Zohary, M. 1960. The species of *Capparis* in the Mediteranean and the near eastern countries. *Bull. Res. Council Israel* 8: 49-64.

Zohary, M. 1966. *Flora Palaestina* 1. Israel Academy of Sciences and Humanities, Jerusalem.