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Genetic Diversity Among Populations of the Medicinal Plant *Achillea fragrantissima* (Asteraceae) in Egypt

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SUBSTANTIAL variation, in morphological traits was observed among 20 populations of *A. fragrantissima* in Egypt. Such variation was reflected in the clustering of the examined populations as major groups, one representing populations in the mountainous area of South Sinai and the other populations growing at lower elevations in the middle of Sinai and the desert west of the Suez canal from Suez in the east to Cairo in the west. Five populations in the eastern part of Sinai near Nuwieba and Taba on the Gulf of Aqaba were loosely assigned to the first group. The clustering of *A. fragrantissima* populations based on ISSR markers also showed two major groups more or less similar to the groups obtained from the analysis of morphological traits. The populations growing at high elevations in South Sinai, under lower temperature and higher humidity, were characterized by high number of total and polymorphic ISSR markers compared to other populations. Unique ISSR markers were observed in the fingerprinting of seven populations including five populations growing in the high mountains of Saint Catherine area in South Sinai and two populations growing at low elevation South east of Cairo. A noteworthy observation is that unique bands are found in populations that possess traits associated with plant size and seed yield as well as better vigor. These are important criteria for selection of populations for conservation and commercial use of *A. fragrantissima*.

Keywords: *Achillea fragrantissima,* Genetic diversity, Egypt, ISSR markers, Conservation

In many countries traditional medicine is wide spread and most of the medicinal plants are harvested from the wild. Consequently up to 10 000 medicinal plant species of the estimated 50 000 medicinal species might be endangered (Akerele

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et al., 1991 and Edwards 2004). In Egypt and other arid countries, the medicinal plants are threatened due to weak regeneration under frequent environmental stresses such as drought and salinity which do not support viable populations (Batanouny, 1999). In addition, heavy overuse by overgrazing and uncontrolled collection, uncontrolled tourism, mining and quarrying and other human activities resulted in habitat destruction and fragmentation; a problem that has been exacerbated by the lack of knowledge and awareness, the paucity of research, and the diminishing number of competent plant systematists (Ayyad , 2003 and Badr *et al.*, 2014a). In Egypt, a medicinal plants conservation project that was conducted in the last decade recommended conservation of threatened medicinal plant species for sustainable development (MPCP unpublished report, 2008). However, few studies are available on the conservation genetics of these plants.

Referring to the general agreement on tariffs and trade (GATT), it is important to assess the value of medicinal plants as important biological resources. The conservation strategies should integrated into the development plans of the Egyptian economy and ensure their sustainable use (Badr *et al.,* 2014a). Saving rare, threatened, and endangered medicinal plant species requires diversity studies that should include extensive phenomic measurements and genetic finger-printing of threatened populations using molecular technologies and bioinformatics approaches for estimating the genetic diversity. Morphological measurements provide quantifying genetic variation while simultaneously assessing genotype performance under relevant growing environments (Fufa *et al.,* 2005).

DNA-based molecular markers are reliable sources of genetic diversity because they provide unique genetic information for each species independent of age, physiological conditions and environmental factors (Kalpana *et al.,* 2004). These markers have a great utility in the drug analysis and are widely used for the characterization of medicinally important plant species (Tharachand *et al.,* 2012). Of the PCR based methods, the robustness repeatability makes the Inter-Simple Sequence Repeats (ISSRs) less prone to changes in band patterns with changes in DNA-template concentration. In ISSRs, the di- and tri-nucleotide repeat types of microsatellite are specifically targeted, because these are widespread in the nuclear genome. ISSR locus heritability has demonstrated an exceedingly close approximation to classic Mendelian ratios and thus ISSR markers are considered dominant markers and consequently effectively act as bi-allelic loci (band presence *vs.* absence) (Zietkiewicz *et al.,* 1994 and Godwin *et al.,* 1997).

The distribution of *Achillea fragrantissima* (Forssk.) Sch. Bip., of the family Asteraceae (Compositae), is restricted to the semiarid regions in North Africa and the Middle East where it is often recorded as associated species in limestone soils. In Egypt, its occurrence extends from Cairo southwards in the Wadis and sandy plains of the Eastern desert eastwards to Suez, it is also encountered in Sinai and some Oasis of the Western desert (Boulos, 2002). *Achillea fragrantissima* is a fragrant, white woolly perennial herb, up to 1 m height, with a bitter taste. Leaves

small, 0.2-1 x 0.15-0.3 cm, sessile with serrate margins. Flowers yellow in discoid head. Flowering and fruiting occur during late spring and summer. Plants withstand the hot summer while keeping green leaves (Boulos, 2002).

Fresh or dry leaves and flowering shoots of *A. fragrantissima* are used for the treatment of cough and as aromatic bitter stomachic, anthelmintic and hypoglycaemic treatments as well as for treating diabetes, intestinal colic, lowering blood cholesterol level and as a carminative, dysmenorrheal and various infections (Boulos, 1983; Yaniv *et al.*, 1987; Atayat, 1993 and Batanouny, 1999) . The aerial parts contain compounds with therapeutic and pharmacologic uses and exert biological activities against microorganisms, insects, animals and viruses. Essential oil from *A. fragrantissima* exerts a bactericidic effect on several gram-positive and gram-negative bacterial strains, as well as on *Candida albicans* (Barel and Yashphe, 1989 &1991) and its extracts have antiviral activity against polio in a concentration dependent manner at complete non-toxic concentration range 10–100 μg/ml (Soltan and Zaki, 2009)

The present study aimed at investigating the genetic diversity among populations of *A. fragrantissima* in Egypt based on morphological variation and ISSR markers profiling. The results should contribute to the selection and conservation of useful germplasm of this important medicinal plant for future sustainable exploitation.

Material and Methods

Twenty populations of *A. fragrantissima* were collected from sites covering most areas where the species is distributed in Egypt as common populations mostly from Sinai and Eastern desert from Suez in the east to Cairo in the west (Fig. 1). Voucher specimens representing all populations have been deposited at the Herbarium of Botany Department, Faculty of Science, Tanta University, Tanta, Egypt (TANE). Detailed morphological criteria of a number of plants of each population were scored based on careful examination of 28 traits including 18 quantitative traits and 10 qualitative traits. The average value of each quantitative trait ± standard deviation was calculated and the state of the qualitative traits was determined based on the deacription of the targeted populations with reference to Boulos (2002). For data analysis, the morphological traits were given codes ranging between 0 and 3 as given in the Supplementary (Table 1.).

DNA extraction

DNA was extracted and purified from the germinated seedlings of individual samples representing all populations using the Thermo Scientific Gene JET

Genomic DNA Purification Mini kit following the protocol of the manufacturer istructions. However, some samples of DNA were extracted from material collected from the field using the CTAB method with some modifications (Saghai-Maroof *et al.,* 1984).

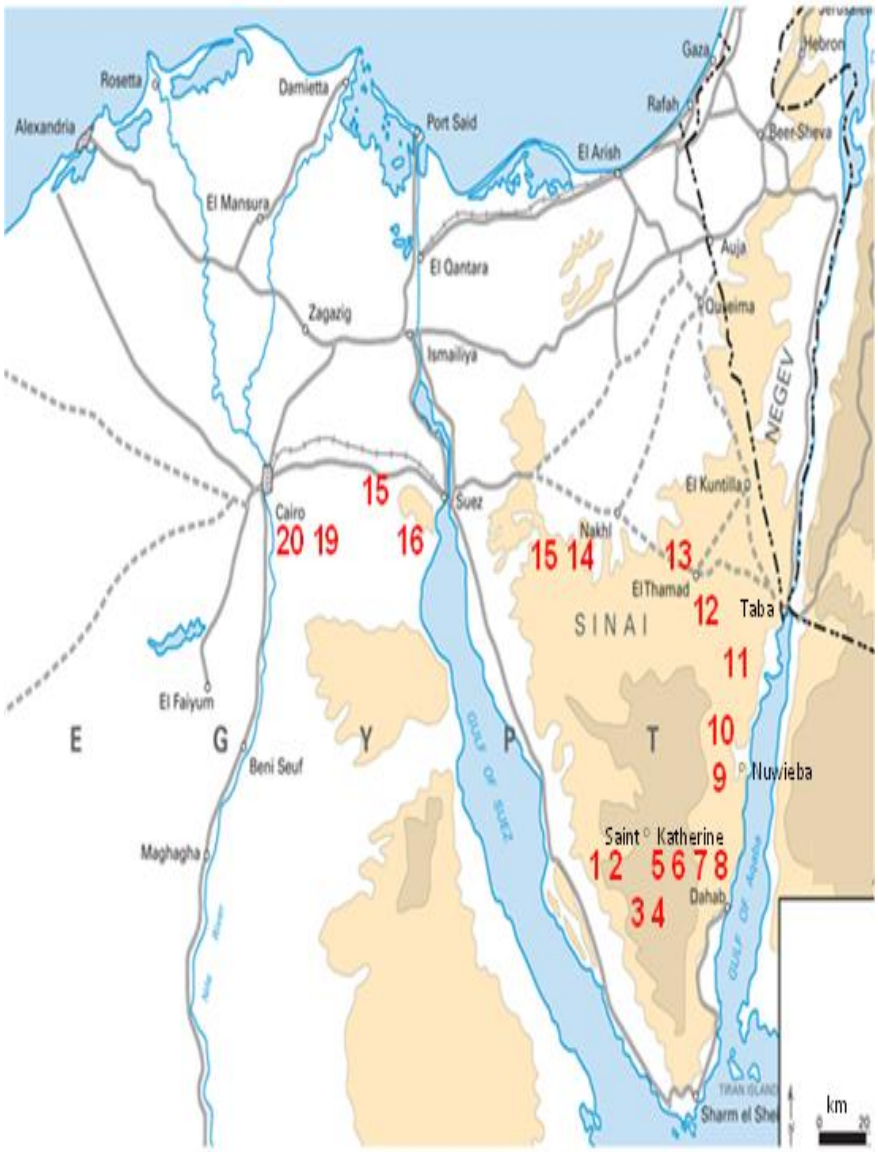

Fig. 1. Map illustrating the areas and the sites of collection of the studied populations of *A. fragrantissima* (F1 – F20) plotted as coded in Table 1.

TABLE 1. Codes, sites, GPS information and elevation of the sites from which *A. fragrantissima* populations were collected.

Code	Site	GPS location	Elevation (m)
F1	Al-Tarfa (35 Km west of Saint-Katherine)	28º 40´ 29.00" N 33º 49´ 59.00" E	1154
F2	El-Sheikh Awad, (Wadi Gharba)	28º 39´ 05.00" N 33º 39´ 06.00" E	1138
F3	Wadi El-Arbaein, Saint Katherine	28º 32´ 45.00" N 33º 57´ 14.00" E	1684
F4	Wadi El-Shak, Saint Katherine	28º 32´ 25.00" N 33º 55´ 53.00" E	1831
F5	Wadi El-Faranga (24 Km east of Saint-Katherine)	28º 45´ 55.00" N 33º 03´ 44.00" E	1281
F6	Wadi El-Shogyrate (35KM east of Saint-Katherine)	28º 46´ 16.00" N 34º 04´ 24.00" E	1240
F7	Wadi Zigzaga (50 Km east of Saint-Katherine)	28º 46´ 18.00" N 34º 07´ 37.00" E	1083
F8	Wadi Al-Nawamees (60 Km east of Saint-Katherine)	28º 49´ 13.00" N 34º 20´ 17.00" E	791
F9	Nuwieba, Wadi Wateer	29º 20´ 47.00" N 34º 32´ 05.00" E	694
F10	Wadi Grafi, Near Nuwieba.	29º 31´44.00" N 34º 38´ 09.00" E	764
F11	Wadi Grafi, 20 Km south of Taba	29º 39´ 17.00" N 34º 41´ 22.00" E	693
F12	Nakhl-El-Hasna, 47 Km before Hasna	30º 18´ 48.00" N 33º 45´ 32.00" E	339
F13	Wadi El-Gabry (45 Km after Mitla pass)	30º 01´ 33.00" N 33º 14´ 33.00" E	467
F14	Mid Sinai Mitla Pass	30º 00´ 51.00" N 32º 57´ 11.00" E	444
F15	50 Km after Ahmed Hamdi tunnel	29º 59´ 48.00" N 32º 54´ 14.00" E	419
F16	Wadi Hagul, Cairo Suez Road	29º 59´ 13.20" N 32º 05´ 49.40" E	302
F17	Bir-Gindali, south of Qattamia	29º 55´ 38.40" N 31º 48´ 21.60" E	390
F18	Wadi Abo-Syaal, Qattamia-Sukhna Road	29º 44´ 43.80" N 31º 53´ 49.20" E	293
F19	Wadi Om-Khourba, Qattamia-Sukhna Road, south east of Cairo	29º 43´ 39.60" N 31º 56´ 00.00" E	245
F20	Wadi Hof, south of Cairo	29º 52´ 43.00" N 31º 22´ 28.00" E	132

ISSR primers and ISSR finger-printing

Twenty ISSR primers (Operon Nippon EGT CO. LTD.) have been secured for DNA fingerprinting. The name, sequence, annealing temperature and GC ratio of the selected 20 ISSR primers are given in Table 2. Isolated genomic DNA was used as template in the amplification reactions using Thermo scientific Maxima Hot start PCR Master Mix (2X). A total of 25 µl reaction mix was prepared (12.5 µl Maxima Hot Start PCR Master Mix (2X), 0.5 µl Primer, 0.5 µl Template DNA and 11.5 nuclease-free water-R0581). Amplification conditions were optimized using a gradient Biometra Uno thermal cycler, Germany. After several experiments for optimizing the best conditions, a program for polymerase chain reaction (PCR) was standardized with following settings, initial denaturation at 95°C for 4 min, followed by 35 cycles of 30 sec. at 95°C for denaturation, 30 sec. for annealing according to each primer annealing temperature, and 1min at 72°C for extension and a final extension of 5 min at 72°C and stored at 4°C till removal of PCR tubes within 12 hr.

Separation of ISSR amplification products

The amplification products were separated by mixing 20 µl of the PCR-products of each primer and 2 µl of loading buffer and loading the mix into the agarose wells. Electrophoresis was made in 1.7 % agarose gel prepared in 0.5 X TAE buffer at 70 V for 3 hr. The ISSR fingerprinting was visualized using a Gel Works 1D advanced gel documentation system (UVP, UK) and photographed under UV light with Camera. The size of each band was estimated using 100 bp DNA ladder (Fermentas) as a standard marker. In the meantime, the clear unambiguous and reproducible ISSR bands were considered for scoring. For data analysis, each ISSR band was considered a single locus and scored as 1 for its presence and 0 for its absence.

Data analysis

The genetic diversity among the 20 populations of *A. fragrantissima* was estimated based on variation in both morphological traits and molecular finger-printting separately and in combination. The morplological and molecular data were analyzed using two software programs; the Community Analysis Package (CAP) version 4.0 (Seaby and Handerson, 2007) and the NTSYS-pc package version 2.02 (Rohlf 2005). The Euclidean dissimilarity coefficient and distance measures were calculated according to Legendre and Legendre (1983) using the CAP software. The CAP software was also used to construct genetic distance trees to illustrate the distance among the examined populations based on Ward (1963). For tree construction, the agglomerative cluster analysis method in the NTSYS-pc software was also used to construct trees elucidating the relationships among the examined populations using the Neighbor Joining method (Saitou and Nei, 1987) and the UPGMA method (Sokal and Michener, 1958).

Results

Morphological variation among populations of A. fragrantissima

Substantial variations, in the morphological quantitative traits have been observed among the 20 populations and are detailed in Supplementary Table 2

(Available on request). The measured morphological traits indicated variation between seven populations of *A. fragrantissima* (F1 to F7) growing in the Saint Catherine area in South Sinai at eleveations ranginging between 1083 m and 1831 m above sea level, four populations (F8 to F11) growing east of Saint Catherine and extending to Taba and Nuwieba on the Gulf of Aqaba at elevations ranging between 791 m asl and 693 m above sea level (Table 1 and Fig. 1) and nine populations (F12 through F20) collected from localities in more dry and flat areas in the middle of Sinai and the northern part of the eastern desert of Egypt from Suez to Cairo at elevation ranging from 467 m above sea level at Wadi El-Gabry in the middle of Sinai) to 132 m asl at Wadi Hof, south of Cairo (Fig. 2 and Table 1). The populations growing in South Sinai area are growing at high elevations and moderate temperatures, and have in general larger plant size compared to populations at lower elevations in a more arid sites (Table 2).

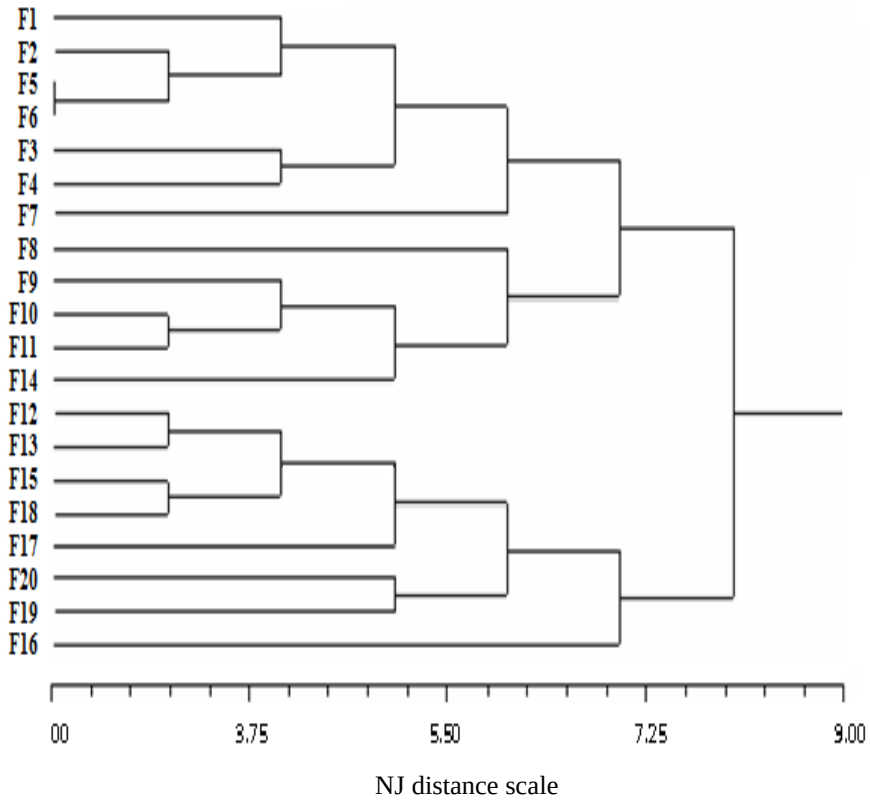

Fig. 2. NJ tree showing the distance among the populations of *A. fragrantissima* (F1-F20), based on the analysis of morphological traits using the NTSYS-pc software (For populations site details see Table 1, Fig. 1) .

TABLE 2. The name, sequence, annealing temperature and GC ratio and of the selected 20 ISSR primers used for fingerprinting *A. fragrantissima*.

Serial	Primer name	Primer sequence	Annealing temperature (ºC)	GC ratio
01	17898A	$(CA)_6AC$	42ºC	50.0%
02	17898 B	$(CA)_6GT$	42ºC	50.0%
03	17899 A	$(CA)_6AG$	44ºC	57.1%
04	17899 B	$(CA)_6GG$	44ºC	57.1%
05	HB-8	$(GA)_6GG$	44ºC	57.1%
06	HB-9	$(GT)_6GG$	44ºC	57.1%
07	HB-10	$(GA)_6CC$	44ºC	57.1%
08	HB-11	$(GT)_6CC$	38ºC	72.7%
09	HB-12	$(CAC)_3GC$	38ºC	72.7%
10	HB-13	$(GAG)_3GC$	38ºC	72.7%
11	HB-14	$(CTC)_3GC$	38ºC	72.7%
12	HB15	$(GTG)_3GC$	29.9ºC	72.1%
13	807	$(AG)_8T$	34.3ºC	47.2%
14	809	$(AG)_8G$	35.7ºC	52.9%
15	814	$(CT)_8TG$	35.7ºC	50.0%
16	825	$(AC)_8T$	35.1ºC	47.2%
17	834	$(AG)_8YT$	35.1ºC	47.2%
18	841	$(GA)_8YC$	38.8ºC	52.9%
19	UBC-820	$(GT)_8C$	41.0ºC	52.9%
20	UBC-827	$(AC)_8G$	41.0ºC	52.9%

Genetic diversity based on morphological variation

The genetic distance among populations was similar in all genetic distance trees; a neighbor-joining distance tree constructed using NTSYS-pc is shown in Fig. 3. In this tree, the examined populations are divided into two main groups at a total distance of 8 distance; one comprising populations F1, through F11 representing populations that were collected from Saint Catherine mountains in south Sinai and the populations from east Sinai as well as population F14 from the Mitla pass in the middle of Sinai. The site of the latter population is a location where hills surround a narrow Wadi and the plants were flourishing on the Wadi sides that seem to have been disturbed by road construction few years ago. The second group comprises populations F12 through F20 which is more or less corresponds to populations collected from locations in middle Sinai, and the desert west of the Suez Canal from Suez in the east to Cairo in the west.

In the first group, the seven populations F1, F2, F3, F4, F5, F6 and F7, growing in Saint Catherine area of South Sinai, were separated as one cluster from other five populations (F8, F9, F10, F11 and F14) that were recognized as a second cluster at a distance of 7.0. In the former cluster, the population F7 was distinguished from the populations F1 to F6 at a distance of 6.0; at a distance of 5.0; F3 and were differentiated from F1, F2, F5 and F6. In the second cluster, F8 was clearly

distinguished the population F14 as well as populations F9, F10, F11. In the second group, population F16 from Wadi Hagul west of Suez was clearly separated, at a distance of 7.0, from the other seven populations that were then divided into two culsters at a distance of 6.0; one comprised populations F19 and F20, and the other comprised populations F12, F13, F15, F18 and F17 (Table 1 and Fig. 2).

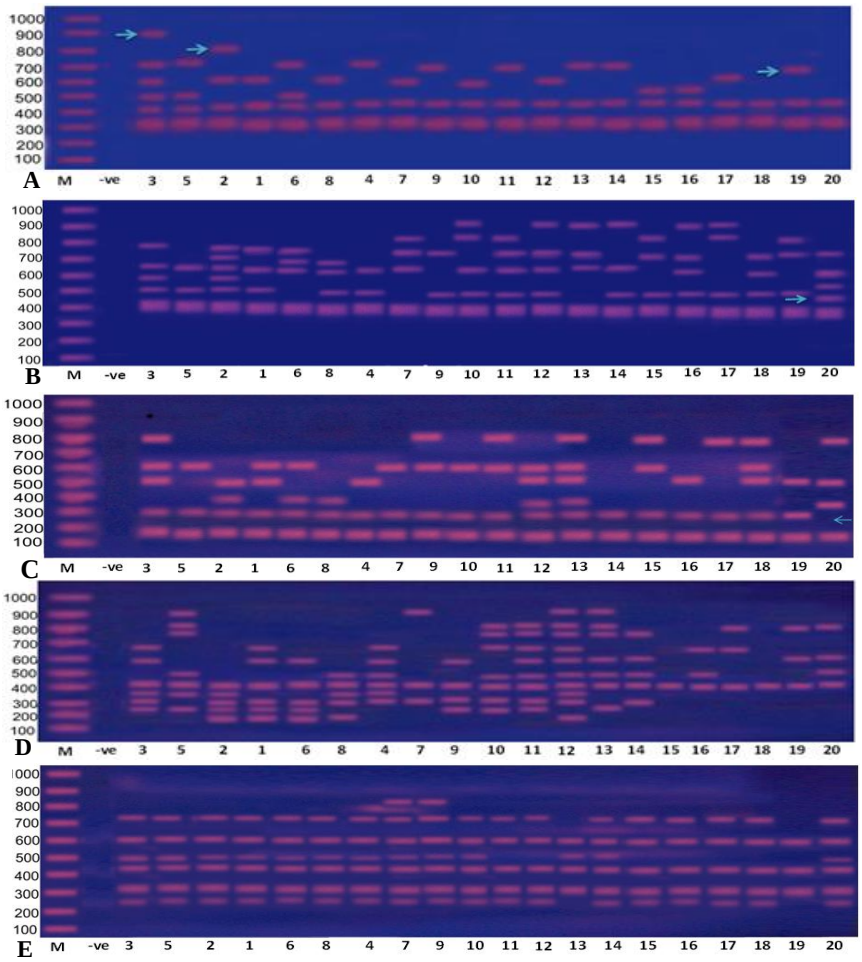

Fig. 3. Examples of ISSR finger printing produced by five primers in the 20 *A. fragrantissima* populations. A = Pr-17898B showing 3 unique bands in F3, F2 and F19, B = Pr-HB-09 showing one unique band in F20, C = Pr-8.9 showing absence of a band in F20, D = Primer HB-08 showing the largest number of bands and the highest number of polymorphic bands, E =Pr- HB-13 showing the highest number of monomorphic bands; M =100 bp marker, -ve = negative control and F1 to F20 are the codes of populations as given in Table 1 and as shown in Fig. 1, Arrows indicate unique bands.

ISSR fingerprinting in Achillea fragrantissima populations

The ISSR fingerprinting of the 20 *A. fragrantissima* populations as revealed by 20 ISSR primers was scored in supplementary Table 2 and associated photos (Available on request). Examples representing fingerprinting profiles by five primers are shown in Fig. 3. Based on the score of banding profiles for the 20 *A. fragrantissima* populations, the total number of bands as well as the number of monomorphic, polymorphic and unique bands and the percentage of polymorphism were calculated and given in Table 3. The number of bands and the percentage of polymorphism are generally higher in the populations growing in the mountainous area of South Sinai and low in populations growing at lower elevations west of the Gulf of Suez to Cairo. Ten unique bands were scored in seven populations including five populations growing in the high mountains of Saint Catherine area in South Sinai (F1 to F5) and two populations growing in south east of Cairo (F19 & F20). Three bands were scored in F3 and two in F20 and only one band in each of the other five populations (Table 3). A band that was revealed in the profiles of 19 populations by primer 809 was absent in the fingerprinting of population F20.

TABLE 3. Number of total, monomorphic, polymorphic and unique bands and the percentage of polymorphism in 20 populations of *A. fragrantissima*.

Pop. Code	Total number of bands	Monomorphic bands	Polymorphic bands	Unique bands	% of polymorphism
F1	99	24	72	1	72.8%
F2	86	24	61	1	72.1%
F3	91	24	66	3	75.6%
F4	90	24	65	1	76.3%
F5	86	24	62	1	72.1%
F6	84	24	60	-	71.4%
F7	78	24	54	-	69.2%
F8	78	24	54	-	69.2%
F9	74	24	50	-	67.6%
F10	77	24	53	-	68.8%
F11	78	24	54	-	69.2%
F12	82	24	58	-	70.7%
F13	76	24	52	-	68.4%
F14	77	24	53	-	68.8%
F15	70	24	46	-	65.7%
F16	68	24	44	-	64.7%
F17	65	24	41	-	63.1%
F18	66	24	42	-	63.6%
F19	66	24	39	1	63.6%
F20	72	24	45	+2, -1	66.7%

Genetic diversity among *A. fragrantissima* based on ISSR markers

The genetic diversity among the examined populations of *A. fragrantissima* based on ISSR markers, as expressed by a NJ tree constructed using the NTSYS software, is illustrated in Fig. 4. In this tree, the examined populations are divided as two main groups at a total distance of 8; one comprising populations F1, through F11, which were collected from south and eastern Sinai and the other comprising populations F12 through F20 which more or less corresponds to populations collected from locations in middle of Sinai, and the desert from Suez to Cairo. The topology of this tree shows that population F7 which was also differentiated from populations F1 to F6 which represent populations growing at the mountainous area of Saint Catherinein South Sinai. Populations F9 & F8 and populations F11 & F10 are clearly separated in this tree as two small clusters in group 1. These four population were collected from sites in eastern part of Sinai extending from 60 km east of Saint Catherine to Nuwieba and Taba on the Gulf of Aqaba. The second group comprised populations F12 through F20 which is more or less correspond to populations collected from locations in middle Sinai, and the desert west of the suez canal from Suez in the east to Cairo in the west. In this group, F16 is clearly differentiated from the other populations, which are divided in two clusters; one composed of the populations F12, F13 and F14 and the other composed of populations F15, F19 and F17 well as populations F18 and F20.

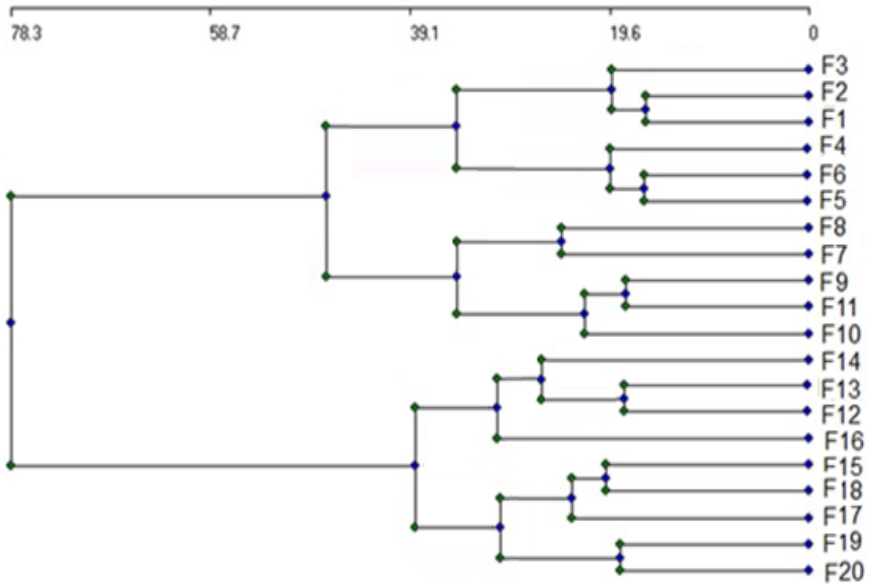

Fig.4. NJ tree showing the distance among the populations of *A. fragrantissima* (F1-F20), based on the analysis of ISSR fingerprinting using the NTSYS-pc software (For populations site details see Table 1, Fig. 1).

Genetic diversity based on morphological variation and ISSR markers

The genetic diversity among the examined populations of *A. fragrantissima* based on morphological variation and polymorphism in ISSR markers is expressed by a Ward tree constructed using the CAP software (Fig. 5). In this tree, the examined populations are divided into two main groups at a total distance of 78.3; one comprising populations F1, through F11 including the populations that were collected from south and east Sinai and the other comprising populations F12 through F20 which more or less corresponds to populations collected from locations in middle Sinai, and the west of the Suez Canal from Suez in the east to Cairo in the west. The topology of this tree resembles that of the tree based on analysis of ISSR markers but F 7 was clustered with F8 & F9 was clustered with F11 & F10 in the group 1. In additions, F14 from the Mitla pass in middle Sinia was grouped here in a cluster of F12, F13 and F16 is at close distance with populations 14 & F12 and F13. Both trees differ from the tree based on morphological variation where F14 was grouped with populations F10, F11 and F9. However, the three agree in differentiating populations of south and east Sinai from populations growing in the middle of Sinai and west of the Suez Canal from Suez to cairo.

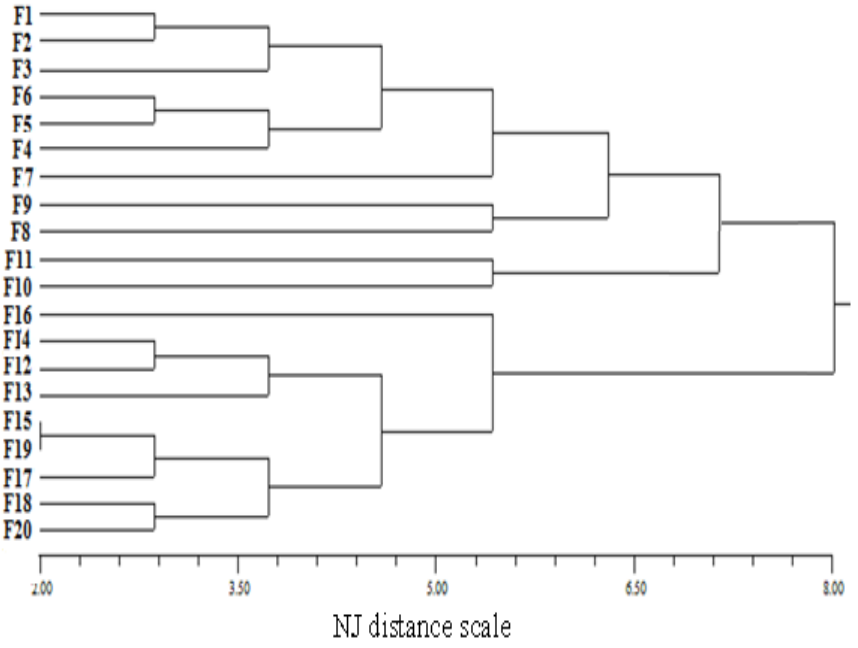

Fig. 5. A CAP Ward tree showing the relationships among the populations of *A. fragrantissima* (F1-F20) based on the analysis of variation in morphological traits and ISSR markers using the CAP software.

Discussion

The measurements of morphological traits for populations of *A. fragrantissima* in the Saint Catherine area of South Sinai showed that these populations are more similar to each other compared to populations growing in other parts of the study area. In the South Sinai area, plant height is more than its height in the populations in the middle of Sinai and to the west of Suez except for populations F19 and F20. In that mountainous area, the temperature is much lower, the humidity is higher in the non-mountainous areas in middle Sinai and west of Suez. Gurevitch (1992) reported that environmental factors, such as temperature and altitude can affect morphological characters, such as the size and compactness of *Achillea* leaves.

The populations of *A. fragrantissima* in the mountains of South Sinai at elevations ranging between 1154 m asl and 1831 m asl were clearly distinguished from the populations F12 - F20 which grow at lower elevations in middle Sinai and west of the Suez Canal from Suez to Cairo. The close genetic distance among populations growing in that area is in agreement with variation in karyotype features among populations of *A. fragrantissima* in Egypt as they have shorter chromosomes (Sayed Ahmed *et al.,* 2012) . In addition, populations F7, F8 and F16 that were distinguished based on morphological variation the two populations F10 and F11 were also differentiated based on ISSR markers are populations growing in sites in the eastern part of Sinai extending from 60 km east of Saint Catherine and extending east to Nuwieba and north to Taba on the Gulf of Aqaba. The results generally indicate closer genetic affinities among geographically closer populations.

Few studies have been conduct on *Achillea*, Rahimmalek *et al.* (2009) found that the germplasm of *A. santolina* in Iran showed low genetic diversity, despite the fact that the samples were collected from different geographical regions. However, in *A. santolina* growing in Egypt, the morphological traits showed much closer resemblance among populations compared to ISSR polymorphism but agree with ISSR data in supporting the idea of a possible gene flow in populations growing in close locations and limited gene flow among population in geographically distant locations (Badr *et al.,* 2014b). In *A. fragrantissima*, (Rawashdeh *et al.,* 2009, 2010) showed the existence of an association between morphology and molecular analysis, especially in the populations of Shoubak and Ma'an, which were recognized as separate groups. The results showed high polymorphism indicating the presence of genetic variation among *A. fragrantissima* populations. Morsy (2007) assessed the molecular variation of five populations of *A. fragrantissima* in Sinai using RAPD and isozymes markers, revealing that differences in locations were particularly reflected on DNA fingerprints. Similar results were also found in *Artemisia* species in Egypt (Badr *et al.*, 2011) and Saudi Arabia (Badr *et al.,* 2012).

Population genetic diversity in a species is affected by a number of evolutionary factors including mating system, gene flow, seed dispersal, geographic range as well as natural selection (Hamrick and Godt, 1989). Of these factors, the geographic range of a species appears to influence the levels of genetic diversity of its populations. Representative population from the geographical range of the species can help to ensure conservation of co-adapted gene complexes (Beuselinck and Steiner, 1992). In the current study, the grouping of populations growing in the mountainous area of South Sinai may be attributed to differences in environmental variables particularly temperature, humidity and soil due to the high altitude of that part of Sinai.

Meanwhile, unique ISSR markers characteristic for populations F19 and F20 south of Cairo may be regarded as molecular markers that differentiate these two populations from the populations growing west of the Suez Canal and the middle of Sinai taking into consideration the position of population F16. However, in the current study, unique ISSR markers are mainly found in populations growing in the mountainous area of Saint Catherine in South Sinai associated with traits of plant size e.g. plant height and plant crown width and seed yield e.g. number of florets in the head and weigh of 100 seeds as well as vigor estimated as the speed of seed germination. This finding is in agreement with the view of Mittal and Boora (2005) that unique markers are important criteria for selection of plant populations for conservation. Unique markers may be regarded as markers for genetic resources authentication and the establishment of property rights (Badr *et al.*, 2014a).

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References

Akerele, O., Heywood V. and Synge H. (1991) *"Conservation of Medicinal Plants"*. Cambridge University Press. Cambridge, UK.

Atayat A. (1993) *"Medicinal Plants Encyclopedia: Pharmacology-morphology and Chemist"*. Al-Arabia Association for Studies and Publication, 11th ed., Beirut, P 237 (In Arabic).

Ayyad, M.A. (2003) Case studies in the conservation of biodiversity: degradation and threats. *Journal of Arid Environments*, **54**: 165-182

Badr, A. Morsy, W., Abdelfattah, S., Shams, S. and Shehab, A. (2011) Genetic diversity in *Artemisia monosperma* and *Artemisia judaica* populations in Egypt based on morphological, karyological and molecular variations. *J. Med Plant Res.*, **6**: 66-78.

Badr, A., El-Shazly, H.H., Helail, N.S. and El-Ghanim, W. (2012) Genetic diversity of *Artemisia* populations in central and north of Saudi Arabia based on morphological variation and RAPD polymorphism. *Plant Syst Evol.,* **298**: 871-886.

Badr, A., El-Shazly H. and Sakr, M. (2014a) Role of biotechnology in conservation and sustainable use of medicinal plants in the arid regions. In: *"Recent Progress in Medicinal Plants Biotechnology and Genetic Engineering*," **Vol.** 39: Kumar, P.A. Govil, J.N. (Chief Ed.). Studium Press India and USA, Chapter 12, pp 317-333

Badr, A., Sayed Ahmed, H.I., Hamouda, M.M. and El-Khateeb, I. (2014b) Assessment of genetic diversity in Egyptian populations of *Achillea santolina* using morphological traits and ISSR markers (submitted for publication).

Barel, S., Segal, R. and Yashphe, J. (1991) The antimicrobial activity of the essential oil from *Achillea fragrantissima*. *J. Ethnopharmacol,* **33**: 187-191.

Barel, S. and Yashphe, J. (1989) Effect of the essential oil from *Achillea fragrantissima* on *Escherichia coli* cells .*Curr Microbiol.,* **19**: 337-341.

Batanouny, K.H. (1999) "*Wild Medicinal Plants in Egypt*"; An inventory to support conservation and sustainable use. With contribution by Aboutabl EA, M. Shabana and F. Soliman. Academy of Scientific Research and Technology, Egypt and IUCN, Switzerland. 208 pp + 48 colored plates.

Beuselinck, R. and Steiner, J.J. (1992) A proposed framework for identifying and utilizing plant germplasm resources. *Field Crop Res.,* **29**: 261–272.

Boulos, L. (1983) *"Medicinal Plants of North Africa"*. Reference Publications, Algonac, Michigan, USA. 52. 286 p.

Boulos, L. (2002) *"Flora of Egypt"*, vol. 3 (Verbenaceae – Compositae). Al Hadara Publishing, Cairo, Egypt. p 249, plate 60 (3), 373p.

Edwards, R. (2004) No remedy in sight for herbal ransack. *New Sci.,* **181**: 10–11.

Fufa, H., Baenziger, P.S., Beecher, B.S., Dweikat, I., Graybosch, R.A., and Eskridge K.M. (2005) Comparison of phenotypic and molecular marker-based classifications of hard red winter wheat cultivars. *Euphytica,* **145**: 133-146.

Godwin, I.D., Aiktken, E.A.B and Smith, L.W. (1997) Application of inter- simple sequence repeat (ISSR) markers to plant genetics. *Electrophoresis,* **18**: 1524-1528.

Gurevitch, J. (1992) Sources of variation in leaf shape among two populations of *Achillea lanulosa*. *Genetics,* **130**: 385-394.

Hamrick, J. and Godt, H. (1996) Conservation genetics of endemic plant species. In: "*Conservation Genetics: Case Studies From Nature*" Avise, J.C., Hamrick, J.L. (Eds). pp 281-304.Chapman and Hall. New York. USA.

Kalpana, J., Warude, P.C. and Bhushan, P. (2004) Molecular markers in herbal drug technology. *Curr. Sci.,* **87**(2): 159-165

Legendre, L. and Legendre, P. (1983) *"Numerical Ecology"*. Elsevier Scientific Publishing Company, Amsterdam, 419 pp.

Mittal, M. and Boora, K.S. (2005) Molecular tagging of gene conferring leaf blight resistance using microsatellites in sorghum [Sorghum bicolor (L.) Moench]. *Ind. J. Exp Biol.,* **43**: 462-466.

Morsy, A.A. (2007) Molecular variations of *Achillea fragranissima* (Forssk.) SCH. BIP. growing in five areas of South Sinai. *Int. J. Agric. Biol.,* **9**: 11-17

MPCP unpublished report (2008) Conservation and Sustainable Use of Medicinal Plants Project.

Rahimmalek, M., Sayed, Tabatabaei, B.E., Arzani, A. and Etemadi, B.E.(2009) Assessment of genetic diversity among and within *Achillea* species using amplified fragment length polymorphism (AFLP). *Biochem. Syst.Ecol*., **37**: 354-361.

Rawashdeh, I.M. (2010) Genetic diversity analysis of *Achillea fragrantissima* (Forskal) Sshultz Bip populations collected from different regions of Jordan using RAPD markers. *Jord. J. Biol. Sci.,* **4** (1): 21-28.

Rawashdeh, I.M., Nasri, I.H. and Amri, A. (2009) Morphological diversity among and within populations and a medicinal plant (*Achillea fragrantissima*) collected from different regions of Jordan. *Res. Crops*, **37**: 242-252.

Rohlf, F. (2005) "NTSYSpc: *Numerical Taxonomy System"* (Ver. 2.2). Exeter Publishing, Ltd., Setauket.

Saghai-Maroof, M.A., Soliman, K.M. Jorgensen, R.A. and Allard, R.W. (1984) Ribosomal DNA spacer-length polymorphism in barley. Mendelian inheritance, chromosomal location, and population dynamics. *Proc. Natl. Acad. Sci,* USA **81**: 8014-8018.

Saitou, N. and Nei, M. (1987) The neighbor-joining method: A new method for reconstruction phylogenetic trees. *Mol. Biol. Evol.,* **4**: 40-42.

Sayed Ahmed, H.I. El-Khateeb E.A. and Badr, A. (2012) Karyotype diversity in Egyptian populations of *Achillea fragrantissima* and *Achillea santolina. Egypt. J. Exp. Biol., (Bot)* **8**: 315-322.

Seaby, R. and P Henderson, (2007) *"Community Analysis Package"* 4.0. Searching for structure in community data. Lymington, UK.

Sokal, R.R. and Michener, C.D. (1958) A statistical method for evaluating systematic relationships. *Univ. Kansas Sci. Bull.,* **28**: 1409-1438.

Soltan, M.M. and Zaki, A.K. (2009) Antiviral screening of forty-two Egyptian medicinal plants. *J. Ethnopharmacol*, **126**: 102-107.

Tharachand, C., Immanuel Selvaraj C. and Mythili, M.N. (2012) Molecular markers in characterization of medicinal plants: An overview. *Research in Plant Biology*, **2**(2): 01-12, 2012.

Ward, J.H. (1963) Hierarchical Grouping to optimize an objective function. *J. Amer Statist Assoc.,* **58** (301): 236-244.

Yaniv, Z., Dafni, A, Friedman, J. and Palevitc, D. (1987) Plants used for the treatment of diabetes in Israel *J. Ethnopharmacol,* **19**: 145-151.

Zietkiewicz, E., Rafalski, A. and Labuda, D. (1994) Genome fingerprinting by simple sequence repeat (SSR)—anchored polymerase chain reaction amplification. *Genomics***,** **20**: 176–183.

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التنوع الوراثيي بين عشائر نبات القيصوم العطري الطبي في مصر

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تم تسجيل اختلافات جوهرية في صفات الشكل الظاهري بين ٢٠ عشيرة من نبات القيصوم العطري في مصر. وقد انعكس هذا التنوع بوضوح في شجرة النسب للعشائر التي تمت دراستها كمجمعاتين رئيسيتين، تمثل إحداهما العشائر التي تنمو في المناطق الجبلية جنوب سيناء و تمثل الأخرى العشائر التي تنمو في المناطق الأقل ارتفاعا في وسط سيناء والصحراء الشرقية غرب قناة السويس من مدينة السويس الي القاهرة. وقد أظهرت خمس عشائر في الجزء الشرقي من سيناء بالقرب من نويبع و طابا في خليج العقبة ارتباطا بصورة ضعيفة بالمجموعة الأولي. كذلك أظهرت شجرة النسب المبنية علي تباينات ISSR تمايز مجموعتين كبيرتين تتشابهان نسبيا مع مجموعتي الشكل الظاهري، فالعشائر التي تنمو في في المناطق المرتفعة بجنوب سيناء تحت درجات حرارة منخفضة ورطوبة تربة مرتفعة تميزت بوجود أعداد كبيرة من حزم ISSR بالمقارنة بالعشائر الأخري. وقد لوحظت حزم ISSR فريدة في البصمة الوراثية لسبعة عشائر خمس منها تنمو في منطقة الجبال المرتفعة بسانت كاترين بشمال سيناء واثنين في مناطق أكثر انخفاضا شرق القاهرة. الملاحظة الهامة الأخرى هى أن هذه الحزم الفريدة موجودة في عشائر تتميز بصفات ظاهرية مرتبطة بحجم النبات وإنتاج البذور وأيضا قوة النبات، وهذه صفات مهمة لاختيار عشائر القيصوم العطري التى يمكن اتخاذ تدابير لحمايتها وترشيد استخداماتها التجارية.