MOLECULAR INSIGHT INTO WADI HAGUL RARE DIVERSITY:*Echinops spinosus* AND *fagonia molis*, PLANT SPECIES

NAYIRA H. ABBAS[1], MAGDA M. I . ELARABY[1], HALA F. S. AHMAD[1] AND ESRAA A. ELSHERBENY[2*]

[1]Botany Department, Faculty of Science, Ain Shams University, Egypt
[2]Genetics Resources Department, Desert Research Center, Cairo, Egypt
*Corresponding Author: **Esraa A. Elsherbeny**

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Genus Echinops belongs to the family Asteraceae (*Compositae*), comprises about 120 species distributed through the Mediterranean region to central Asia and Tropical Africa (Kadereit and Jeffrey, 2007). In Egypt, *E. spinosus* L. is among five species representing this genus. It is common throughout the Sahara of Sinai and the Red Sea coast (Boulos, 2009).

In the Mediterranean and Saharo-Sindian regions, subtropical regions of North and South America and South Africa, the genus Fagonia comprises 40 species. Early evidence of (El Hadidy, 1966) described 18 species of Fagonia and constructed an artificial key for their identification (El Hadidy, 1966; Täckholm, 1974). Recent biosystematics research mostly depends on molecular methods, but karyology and morphology still necessary information of any organism (Peruzzi and Altınordu, 2014). The karyotype approach involved the size, number, and appearance of chromosomes of eukaryotic cell. Also, includes chromosomal measurements such characteristics, along with morphological and molecular data, which can be exploited to understand patterns and mechanisms of evolution and speciation in plants (Roma-Marzio *et al*., 2015). Karyotype evolution is a notable feature in the diversification of many plant species; until now there is a strong argument about the role of chromosomal alternations in the diversification process (Roalson *et al.*, 2007). Over the last decades, many karyological investigations had been performed and provided essential information for evolutionary rate and plant systematic (Stace, 2000). Variation in total and relative chromosomal size, morphology and stainable properties can be obtained from karyological analysis (Sharma and Sen, 2002). Indeed, the chromosome morphology characters have been proved to be a useful method to differentiate genomes at the generic and sub generic levels in

plants, animals and humans (Albers *et al.*, 2007).

DNA barcode is a molecular phylogeny method that utilizes short and standardized DNA sequences in a familiar gene to identify plants and other organism species (Xiwen *et al.,* 2015). Different varieties of phylogenetic markers have been applied, but the widely used is DNA barcoding markers due to their facility identifying problematic taxa (El-Sakaty *et al.*, 2014; El-Atroush *et al*., 2015). Recently, any credible plant barcode should be multi-locus with a coding region of *rbc*L because of its responsibility toward producing enzyme RuBisCo and evolving noncoding region (Kress and Erickson, 2007). The plastid gene *rbc*L gene consists of a 599 bp region at the 5′ end of the gene. It' is straightforward recovery and supplies a good backbone to the barcode dataset (Chase*et et al.,* 2005; Newmaster *et al.*, 2006).

The current investigation was aimed to identify and conserve *Echinops spinosus* and *Fagonia molli* and also to determine the chromosome numbers karyotype and ideograms for species.

DNA barcoding, as a tool primarily for species identification, can be used in two specific ways to address biodiversity conservation: 1) as a means of more accurate and eventually more rapid biodiversity monitoring both before and after conservation actions, and 2) by providing data that will assist in estimations of phylogenetic diversity for setting conservation priorities

MATERIAL AND METHODS

Plant materials

The plants were collected from the upstream site of Wadi Hagul within the two different seasons April (spring) at daily temperature 20ºC ± 2 and night temperature 10ºC ± 2 and October (autumn) at daily temperature 30ºC ± 2 and night temperature 19ºC ± 2.

Wadi Hagul is located in the northern portion of the Eastern Desert of Egypt within the Cairo-Suez district; it occupies approximately 350 km^2 representing about 0.16% of the Egyptian Eastern Desert (Abd El Aal, 2017). It is bounded by latitudes 29°48'28" - 29°57'43" N. and longitudes 32°09'32" - 32°17'27" E. Its main channel extends for about 40 km with a width of 6-10 km and it runs to debouch into the Suez Gulf.

Wadi Hagul lies within an arid desert climate with deficient rainfall, high temperature, and high evaporation rate. The geologic and vegetation structures of the Wadi Hagul led to the recognition of three main sections: upstream, middle stream and downstream sections (Zahran and Willis, 2009). These sections represent the natural xeric habitat that xerophytic plants mainly inhabit. The dominant species are *Fagonia mollis, Echinops spinosus* and other xerophytic plants.

Karyotype analysis

Seeds of the two studied plants (*Echinops spinosus* and *Fagonia mollis*) were collected from the upstream site in Wadi Hagul. The lateral roots, 1.5 - 2.0 cm length, were excised into glass vials containing 2 ml of 0.05% colchicine for three hours at room temperature. Fixation was done using ethanol-acetic acid (3: 1) fixative. Then, root samples were washed thoroughly with water and macerated with the enzymatic mixture (4% cellulase, 1 % pectenase, 75 mM KCl and 7.5 mM EDTA) on the glass slides in the moisture chamber at 37^{o}C for 40 min. The enzymatic mixture was then washed with distilled water and each root tip was chopped into fine pieces, flamed by forceps and squashed and stained by the acetoorcein solution. These stained samples were used to automatically scan the chromosome image of the examined material (Fukui and Kakeda, 1994). The samples were examined using the Image Processing Analysis System (KS - Chromo). Karyotype analysis was carried out using karyostar demo. & micro measure computer program in the c-metaphase stage and analyzed using the video test karyotype software (Reeves, 2001).

DNA barcode analysis

Fresh plant leaf samples from *F. mollis* and *E. spinosus* 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 Red Safe® (5ul /100 ml) under UV light. The primer pairs for gene rbcLaF (5` -ATG TCA CCA CAA ACA GAG ACT AAA GC-3`) and rbcLaR (5`- GTA AAA TCA AGT CCA CCR CG- 3`) were used to amplify *rbc*L region (White *et al*., 1990). PCRs of 50 ul reaction mixture (1x Flexi buffer, 50ng DNA template, 2.5 mM $MgCl_2$, 10uM dNTPs, 0.4 uM of each primer, and1U Promega© Green Go Taq™ enzyme) were performed, standard PCR profile with 50ºC annealing temperature was used to amplify *rbc*L gene.

RESULTS AND DISCUSSIONS

The mitotic chromosome numbers of the studied plants, *Echinops spinosus* and *Fagonia mollis* are shown in Table (1), while the metaphase plates for these taxa are presented in Fig. (1). The karyotype analyses are represented in certain chromosomal features of both studied plants: chromosomal number, area of chromosomes, chromosomal length, arm ratio, the position of centromeres, and centromeric index.

The diploid chromosome number of *E. spinosus* is 2n=18, and the area of the chromosomes ranged between 4.66 µ to 0.61 µ, while the chromosomal length ranged between 5.63 µ to 1.53 µ while its long and short arm is varied between 3.04µ – 0.83µ and 2.60 µ - 0.71 µ, respectively. Consequently, the arm ratio of *E. spinosus* ranged between 1.17 - 1.11. Additionally, the centromeric index of *E. spinosus* is observed between ‘47.49 to 46.03’ and the position of centromeres is metacentric for all chromosomes (Fig. 1).

The diploid chromosome number of *F.mollis* is 2n=18 and its chromosomal area are fallen between 4.30 μ to 2.0 μ, while the chromosomal length ranged between 4.56μ to 2.07μ , while its long and short arm were ranged between 2.47 μ - 1.11 μ and 2.09 μ - 0.96 μ, respectively. Therefore, the arm ratio of *F. mollis* ranged between 1.41 - 1.10. Moreover, the centromeric index of *F. mollis* is observed between 47.63 - 45.52 and the position of centromeres is metacentric for all chromosomes (Fig.1).

The karyotype is the phenotypic feature of the chromosome complement seen at mitotic metaphase to give chromosomal information in evolution, plant systematic and ranges of possibilities to understand taxa affinities (Levin, 2002). In the present study of *E. spinosus* verified its chromosomal number as 2n=18 (Table 1), while Ismael *et al.*, (2009) have been analyzed the chromosomal number of different *Echinops* species and record *E. gmelini* chromosomal number being 2n=26 and its related *E. acantholepis* (2n=14) showed strongly divergent karyological patterns of difficult interpretation, along with *E. przewalskyi* counts its chromosomal number being 2n=32. Our results indicated that the *F. mollis* karyotype analysis was 2n=18 (Table 1), however, evidence of Kučera *et al.*, (2016) reported that the chromosomal number of India *F. cretica* was 2n=22, while Baquar (1970) described that the chromosomal number of this species outside of India are different being 2n = 18, 20 and 22. Other report recorded by Zaidi (2003), showed that the 2n of *F. schweinfurthii* being 20 and 22.

DNA barcode

In recent years, attention has been paid to the benefits of DNA barcoding for deciphering the phylogenetic relationships between closely related taxa. However, at lower taxonomic levels of medicinal plants, the problem is that no specific DNA barcoding is sufficient to resolve inter-and intraspecific relationships. We, therefore, turned our attention to the universal *rbc*L coding plastid genes. Therefore, the universal *rbc*L DNA barcode approach has been utilized to identify the plant species by using a short sequence of a universal standard part of a genome instead of using the whole genome. (Ali *et al.*, 2015). In the present study, both plants′ DNA barcode analysis revealed that *E. spinosus* tree (Fig.2) was divided into two main clads. The *rbc*L phylogenetic analysis showed that *E. spinosus* belonged to the family Asteraceae and supported *Echinops species*. On the other hand, *F. mollis* tree (Fig. 3) was divided into two clads, belonging to the family Zygophyllaceae and supported to *F. latistipulata* (bootstrap support of 93%). These results approved that *rbc*L region was effective in the identification of both studied plants. Recent evidence of Elsherbeny (2016) provided preliminary as-

sessment data to assist for more comprehensive DNA barcoding applications in wild medicinal plants. It was found that *rbc*L was helpful for barcoding some medicinal plant species in the family Labiatae. *rbc*L region should be involved as a standard for comparison to other barcoding due to the advantage that this gene is easily amplified and sequenced in most plants and it is regarded as a benchmark locus in phylogenetic investigations by providing a reliable placement of a species into plant family and/or genus (Hassel *et. al.*, 2013).

Karyotype analysis and DNA barcode of Egyptian flora have been utilized to study the plant evolution and systematic of some plant groups as well to form a genetic database of natural plants which is endangered and susceptible to become rare (Badr and Sharawy, 2007). In this consideration, karyotype analysis and DNA barcoding have been performed for *Echinops spinosus* and *Fagonia mollis*. To the best of our knowledge, there is no literature have been reported for the chromosomal karyotype and DNA barcode of the two studied plants in Egypt, hence it will be essential to discuss and report this work.

Chromosomes in the Egyptian flora karyotype analysis of plant have been applied to address some plant group′s systematic, so it was essential to study some plants′ karyotype in Wadi Hagul as participation in documenting the chromosomal number and shape of these plants. Karyotyping was performed for *Echinops spinosus* and *Fagonia mollis* plants growing in Wadi Hagul. To our knowledge, no literature was available, dealing with a chromosomal karyotype of the two studied plants, so it was necessary to do; however, no polyploid chromosome are recorded in these plants. So, this work aims to provide a base for nature conservation and other applied programs for a genetic study.

On the other hand, a few molecular phylogenies at taxonomic levels have been assessed through RAPD, SSR, ISSR, AFLP and IRAP markers applied for germplasm identification and genetic diversity. The increasing demands for direct genome analysis based on DNA barcodes are currently one of the powerful techniques existing to employ in specific fields particularly in systematic and taxonomy. In the current study, the studied plants have been used to determine rbcL sequence divergence can reasonably guide gene choice in phylogenetic across abroad scale in Egyptian plants. DNA barcoding not only helps in the identification of species but can also define species boundaries, flagging of new species and species delimitation.

SUMMARY

Genetic studies have been performed to distinguish two plants inhabiting a natural valley renowned as Wadi Hagul of Egypt throwing karyotype and DNA barcode analysis. The two plants were morphologically identified as *Echinops spinosus* and *Fagonia mollis*. As a result, the karyotype approach exhibited a significant variant among the certain

chromosomal features involving chromosomal number, area of chromosomes, chromosomal length, arm ratio, the position of centromeres, and centromeric index. The chromosomal number of both plants was 2n=18, while the DNA barcode identified their species as *E. spinosus* of the family *Asteraceae* and *F. mollis* of the family *Zygophyllaceae* based on *rbc*L phylogenetic analysis.

Results indicated that, *F. mollis* evolutionary tree was rooted into two clads, belonging to family *Zygophyllaceae* and supported to *Fagonia latistipulata* (bootstrap support of 93%). Indeed, analysis of *rbc*L sequence will *permit* to *evaluate* the taxonomy and systematic of different plants recovered with a good performance in *clarifying* genetic diversity within and between populations in the studied plants. In particular, *Echinops spinosus* and *Fagonia mollis* showed a minor divergent from the common species, therefore an urgent survey for these genotypes and their conservation is required.

REFERENCES

Abdelaal M. (2017). Current statue of the floristic composition in Wadi Hagul, Northwest Suez Gulf, Egypt. Rend. Fis. Acc. Lincei , 28: 81-92.

Albers F., J. Van der Walt J. and Geraniace (2007). The families and genera of vascular plants. Springer Berlin Heid.157-167.

Ali Z., Abulfaraj A., Idris A., Ali S., Tashkandi M. and Mahfouz M. (2015). CRISPR/Cas9-mediated viral interference in plants. Genome Biol., 16:238.

Badr A. and Sharawy S. (2007). Karyotype analysis and systematic relationships in the *Egyptian Astragalus* L. (Fabaceae). Int. J. Bot.,, 3: 147-159.

Baquar S. (1970). Natural tetraploidy in the genus *Fagonia* L. Curr. Sci., 36:172.

Boulos L. (2009). Flora of Egypt ChecklistRevised Annotated Edition, Al Hadara Publishing, Cairo, Egypt, pp. 221.

Chase M. W., Salamin N., Wilkinson M., and Dunwell J. M. (2005). Land plants and DNA barcodes: short-term and long-term goals. Philos. Trans. R. Soc. Lond. B Biol. Sci., 360: 1889-1895.

El Atroush H., Magdi M.. and Werner O. (2015). DNA Barcoding of two endangered medicinal plants from Abou Galoom protectorate. Life Sci. J., 12: 101-109.

EL Hadidy M. N. (1966). The genus *Fagonia* L. in Egypt. Candollea, 21: 13 - 54.

El-Sakaty S. I. A., Magdi M., El-Atroush H., Mohamed M. M., Abou-Salama

U. (2014). DNA barcoding of three *Bryaceae –Musci*. Egypt. J. Bot., special issue for the fourth international conference of Botany and Microbiology Sciences, 53-63

Elsherbeny Esraa A. (2016). DNA barcoding of some medicinal plants, family Labiatae. Egypt. J. Exp. Biol. (Bot.)., 12: 175-180.

Fukui K. and Kakeda K. (1994). Dynamic changes in the morphology of barley chromosomes during the mitotic metaphase stage. Jap. J. Gen., 69:545-554.

Hassel K., Segreto R., and Ekrem T. (2013). Restricted variation in plant barcoding markers limits identification in closely related bryophyte species. Mol. Ecol. Res., 13: 1047-1057.

Ismael S., Georgy A., Oriane H. and Teresa G. (2009). Molecular systematics of *Echinops* L. (*Asteraceae*, *Cynareae*): A phylogeny based on ITS and trnL-trnF sequences with emphasis on sectional delimitation. Taxon., 59:698-708.

Kadereit J. W. and Jeffrey C. (2007). Flowering plants. Eudicots. In: "The Families and Genera of Vascular Plants. (Kubitzki K. Ed.)". Springer - Verlag, Berlin, Heidelberg, pp. 61-128.

Kress W. and Erickson D.. (2007). A two-locus global DNA barcode for land plants: the coding *rbcL* gene complements the non-coding trnHpsbA spacer region. PLoS One., 2: e508.

Kucera J., Martonfiova L., Stubnova E. and Slovak M. (2016). Picris. IAPT/IOPB chromosome data 21. Taxon., 65 : 675.

Levin D. (2002). The role of chromosomal changes in plant evolution. Oxford Univ. Press.

Newmaster S., Fazekas A. and Ragupathy S. (2006). DNA barcoding in land plants: evaluation of *rbc*L in a multigene tiered approach. Canad. J. of Bot., 84: 335-441.

Peruzzi L. and Altınordu F. (2014). A proposal for a multivariate quantitative approach to infer karyological relationships among taxa. Comp. Cytogen., 8: 337-349.

Reeves A. (2001). Micro Measure: a new computer program for the collection and analysis of cytogenetic data. Genome., 44:439-443.

Roalson E., Mccubbin A. and Whitkus G. (2007). Chromosome evolution in *Cyperales*. Ran. San. Ana. Bot. Gard., 23: 62-71.

Roma-Marzio F., Astuti G. and Peruzzi L. (2015). Taxonomy, typification and

karyology of *Crepislacera* (Asteraceae). Phyto. 208:45-54.

Sharma A., and Sen S. (2002). Chromosome botany. Enfield (NH): Science Publishers.

Stace C. (2000). Cytology and cytogenetics as a fundamental resource for the 20th and 21st centuries. Taxon., 49: 451-477.

Täckholm V. (1974). Student Flora of Egypt, 2nd edition. University Publishing, pp. 888.

White T. J., Bruns T., Lee S., Taylor J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetic. In: "PCR Protocols: a guide to methods and applications. (Innis M.A., Gelfand D.H., Sninsky J.J., White T.J., eds)". Academic Press, New York, USA, pp. 315-322.

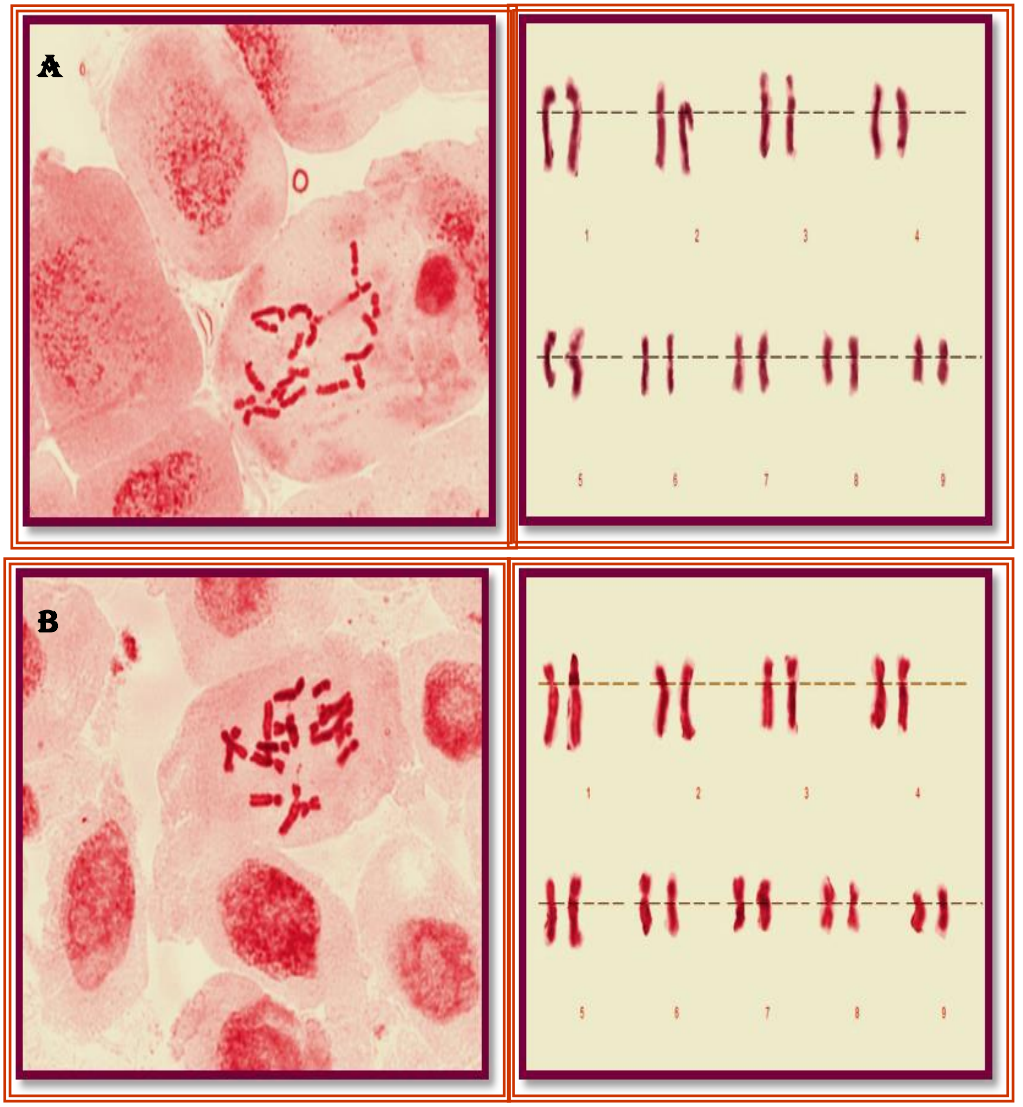
Xiwen L., Yang Y., Robert J., Maurizio R., Yitao W and Shilin C. (2015). Plant DNA barcoding: from gene to genome. Biol. Rev., 90: 157-166.

Zahran M. A. and Willis A. J. (2009). The Vegetation of Egypt. 2nd ed. Springer, Netherland, pp. 437.

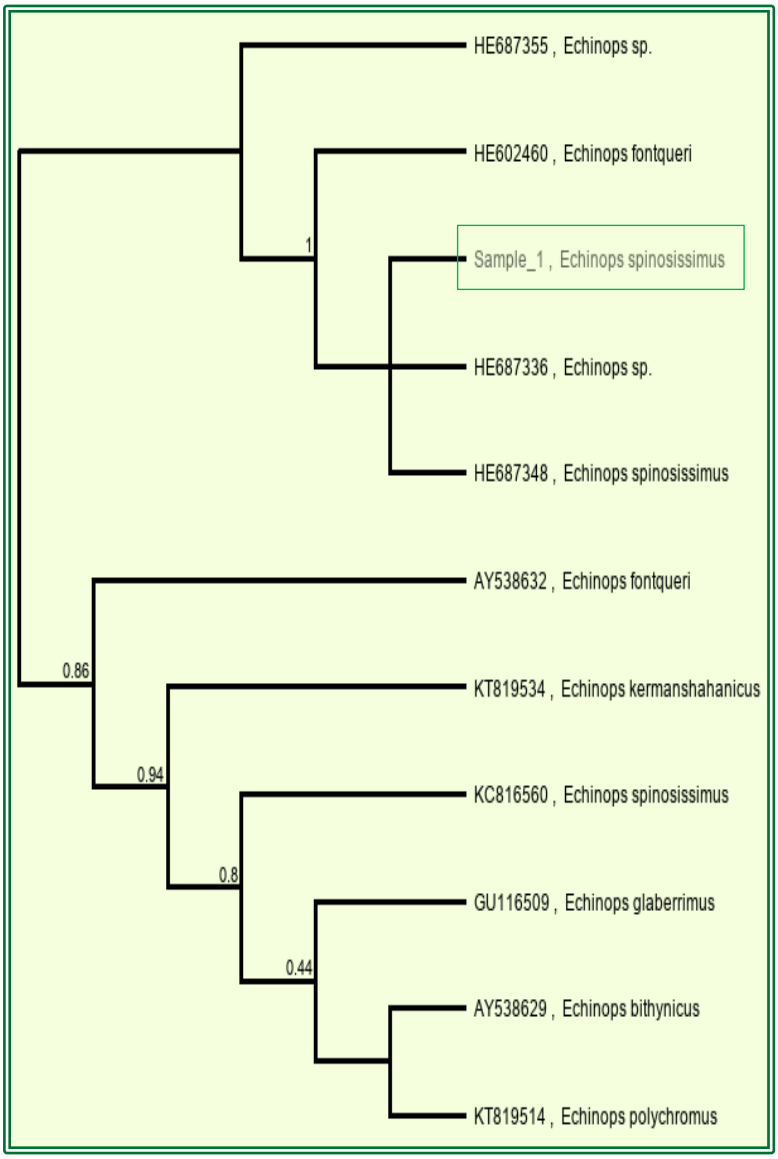
Zaidi S. (2003). Effects of electromagnetic fields (created by high tension lines) on the indigenous floral biodiversity in the vicinity of Karachi - I: Studies on PMC meiosis, meiotic products and pollen fertility. Pakistan J. Bot., 35: 743-755.

Table (1): Chromosome parameters of *Echinops spinosus* and *Fagonia mollis* are Chr. No.: chromosome pair number, CA: chromosome area, CL: chromosome length (µm), LA: long arm, SA: short arm, AR: arm ratio, CI: Centromeric index, PC: position of centromere and M: Metacentric chromosome.

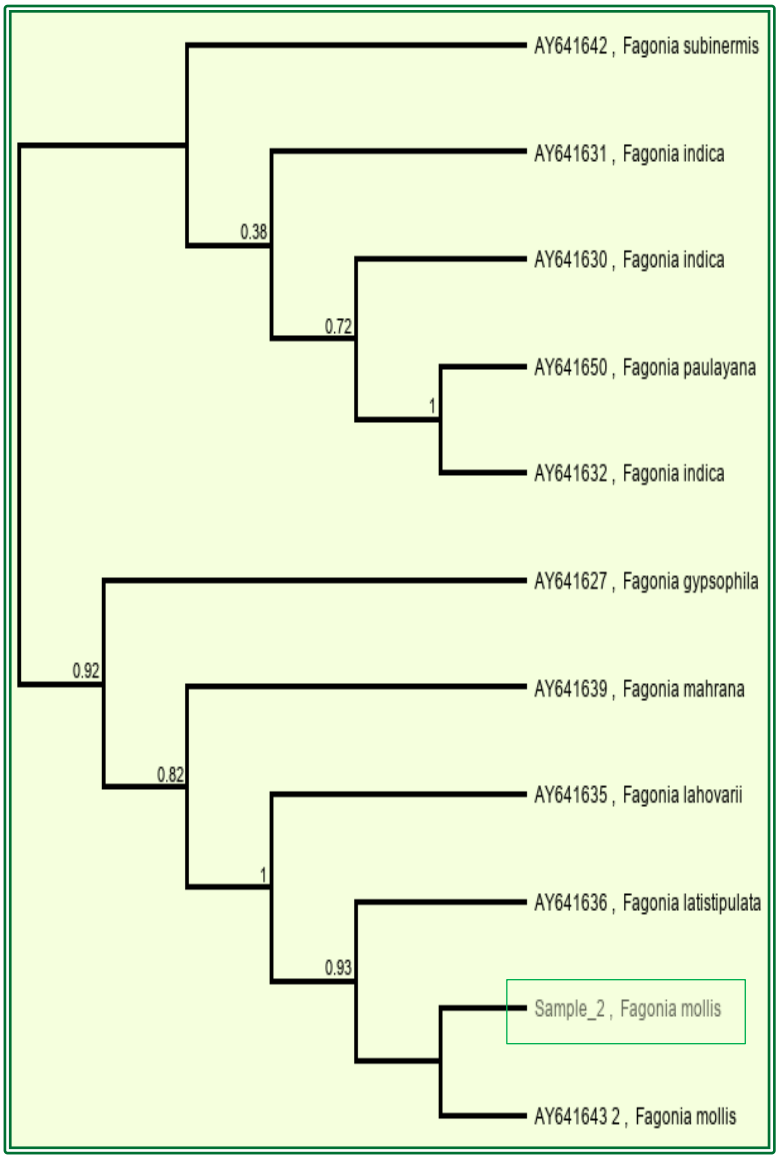
Species	***Echinops spinosus***						
Chr. No.	CA	CL	LA	SA	AR	CI	PC
1	4.66	5.63	3.04	2.60	1.17	46.10	M
2	4.53	5.43	2.89	2.55	1.13	46.85	M
3	4.46	5.36	2.89	2.47	1.17	46.03	M
4	4.39	5.14	2.71	2.42	1.12	47.17	M
5	2.88	3.57	1.89	1.68	1.12	47.07	M
6	2.78	3.49	1.86	1.63	1.14	46.70	M
7	3.19	3.53	1.89	1.65	1.15	46.60	M
8	2.62	3.30	1.73	1.57	1.11	47.49	M
9	0.61	1.53	0.83	0.71	1.17	46.11	M
species	***Fagonia mollis***						
Chr. No.	CA	CL	LA	SA	AR	CI	PC
1	4.30	4.56	2.47	2.09	1.18	45.83	M
2	4.11	4.31	2.26	2.05	1.10	47.63	M
3	3.83	4.11	2.41	1.70	1.41	41.44	M
4	3.51	3.67	1.98	1.69	1.18	45.95	M
5	3.20	3.47	1.89	1.58	1.20	45.52	M
6	3.00	3.18	1.68	1.51	1.11	47.35	M
7	2.49	3.05	1.62	1.44	1.12	47.10	M
8	2.19	2.31	1.23	1.08	1.14	46.75	M
9	2.00	2.07	1.11	0.96	1.16	46.22	M



(Fig. 1): Karyotype characteristics and metaphase chromosome of *E. spinosus* (A) and *F. mollis* (B). (9 pairs each).



(Fig. 2): Dendrogram of *E. spinosus* based on *rbc*L gene.



(Fig. 3): Dendrogram of *F. mollis* based on *rbc*L gene.