Journal of Applied Biological Sciences
E-ISSN: 2146-0108 15(2): 206-213, 2021
Research Article

GENETIC DIVERSITY AND MOLECULAR CHARACTERIZATION OF NATURAL *PANCRATIUM MARITIMUM* L. POPULATIONS VIA SRAP MARKERS IN TURKEY

Hasan Pinar[1]*, Mustafa Unlu[2], M. Murat Hocagil[2]
Mehmet Yaman[1], Merve Arefe Yigit[1]

[1]*Erciyes University, Department of Horticulture, Kayseri, Turkey*
[2]*Alata Horticultural Research Institute, Mersin, Turkey*

**Corresponding Author:*
E-mail: hpinarka@yahoo.com

(Received 07th January 2021; accepted 15th February 2021)

ABSTRACT. Turkey with diverse ecological conditions is among unique countries allowing culture of several species nationwide. Among these species, *Pancratium maritimum* belonging to *Amaryllidaceae* family grows in sandy environments. Plant flowering period is between August – October. *Pancratium maritimum* species are common in coastal zones of Greece, France and Spain and common in coastal zones of Samsun, Giresun, Trabzon, Antalya, Mersin and Adana provinces of Turkey. This study was conducted to investigate the genetic diversity in 58 different *Pancratium maritimum* genotypes collected from coastal zones of Mersin province with the use of SRAP marker technique. Fourteen SRAP combinations were used and 81 scorable bands were obtained with these markers. Average polymorphism ratio was identified as 49.92%. According to UPGMA method, *Pancratium maritimum* genotypes were divided in two main groups and genetic similarity values varied between 0.88 – 1.00. Present findings revealed that SRAP marker system could be used to identify genetic relationships among *Pancratium maritimum* genotypes.

Keywords: *Pancratium maritimum, SRAP, polymorphism, marker, sea daffodil.*

INTRODUCTION

Turkey is a unique country in terms of plant diversity and genetic resources. Diverse ecological conditions allowed Turkey to have such great genetic diversity. Several species are either naturally grow or economically cultured nationwide [1, 2, 3].

Amaryllidaceae family is composed of about 1100 monocotyledonous species [4]. Just because of glamorous white flowers, they have been cultured as ornamental plants in Europe and America for centuries [5,6]. These species are widespread in the Mediterranean, Atlantic and Black Sea costs [7]. Sea daffodil is common in coastal zones of Greece, France and Spain. In Turkey, it naturally grows in coastal zones of Kırklareli, Istanbul, Bolu, Bartın, Sinop, Samsun, Giresun, Trabzon, Antalya, Mersin and Adana provinces [8,9]. Besides being an attractive ornamental plant, sea daffodil is also prominent with alkaloids, thus it is also used as a medicinal plant [6;10]. Berkov et al. conducted a study on leaves and roots of *Pancratium maritimum* with the use of GC/MS and reported that of 16 alkaloids defined in that study, 5 were identified for the first time in this plant [11]. Since this plant is common along the coastal zones, tourism exerts a

serious threat to this species [9]. Serious reductions have been encountered in the population of this plant in Italy, Greece and Spain.

P. maritimum L. is considered as a foreign-pollinating plant [9, 12] and pollination largely depends on and varies with different organisms within the pollination zones. Species of the same genus may differ in phenotypic characteristics, leaf blade width and length, root diameter and density. Some of these traits may be stable, but some may exhibit epigenetic variations influenced by environmental factors. Thus, the potential transfer of such variations for generations through subsequent plants should be investigated [13]. Genetic characterization plays a significant role in protection strategies of the species [14]. Analyses of individuals of different populations with the molecular markers revealed high polymorphism at genomic level. Various marker systems are employed in the identification of genetic diversity. SRAP (Sequence-related amplified polymorphism) marker system is the prominent one among these systems. SRAP was used for marker formation and mapping of grass species [15], walnut [16], apricot [17] and quince [3]. In addition to these studies, different marker systems such as RAPD [29], SSR [30], ISSR [27] were used to determine genetic diversity in *Pancratium* species.

This study was conducted to investigate the genetic relationships of 58 sea daffodil (*Pancratium maritimum* L.) genotypes collected from coastal zones of Mersin province with the aid of the SRAP molecular marker system.

MATERIALS AND METHODS

Sampling

Fifty-eight sea daffodil (*Pancratium maritimum* L.) genotypes collected from coastal zones of Mersin province constituted the plant material of the present study. Genotypes that spread along the coasts of Erdemli (Alata) and Akdeniz (Kazanlı) districts were used in the study (Figure 1).

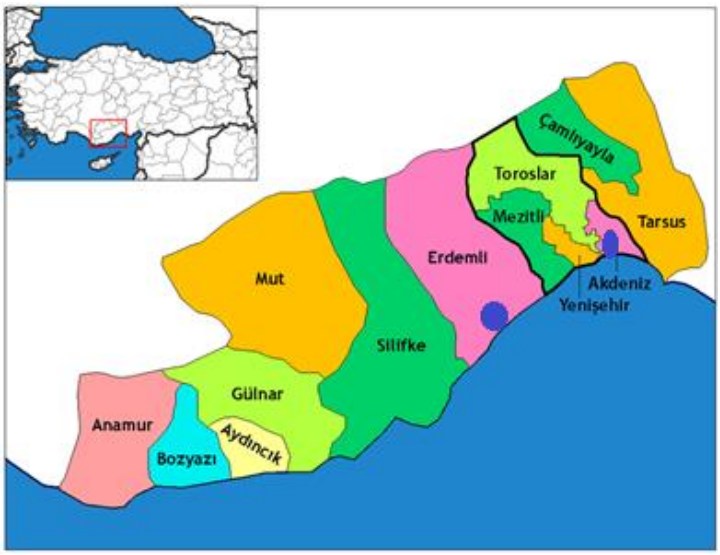


Fig. 1. *Mersin province map*

PCR Analyses

The leaf tissues were pulverized in a muller under liquid nitrogen, then genomic DNA was extracted with CTAB method according to [26]. DNA concentrations were determined with a spectrophotometer (BioTek Instruments, Inc., Winooski, VT, United States). The DNA solutions in the samples was brought to a working concentration of 10 ng/lL, and stored at- 20 C.

DNA samples were mixed with PCR components, and pre-screening tests were conducted with the use of 216 SRAP primer combinations (12 forward and 18 reverse primers) [28]. Based on the results of these tests, 14 SRAP marker combinations yielding the greatest number of polymorphic bands were used (Table 1). PCR components were prepared for each genotype as to have a total volume of 15 µl including 2 µl DNA (20 ng), 1.5 µl 10xPCR Buffer, 0.2 µl Taq DNA polymerase (5u/µL), 1 µl dNTP (2.5 mM), 1.5 µl $MgCl_2$ (25 mM), 2 µl 10 mM SRAP marker and 6.8 µl H_2O. PCR conditions were as follows: initial denaturation at 94 ^oC for 3 minutes, 35 cycles at 94 ^oC for 1 minute, 38 ^oC for 45 seconds, 72 ^oC for 2 minutes and a final extension at 72 ^oC for 10 minutes [16, 18]). PCR products were electrophoresed in 1X TAE buffer and 2% agarose gel and ethidium bromide-stained samples were imaged in gel imaging unit (Kodak Gel Logic 200 Imaging System).

Data Analysis

DNA band profiles were recorded either as 1 with a band and as 0 without a band. Data were subjected to statistical analyses with NTSYSpc 2.11 software [19] and monomorphic and polymorphic fragments were analyzed by using Dice similarity matrix [22]. The genetic similarity matrix and ultra-metric distance matrix produced from UPGMA-based dendrogram with COPH module nested in the same software was compared using Mantel's matrix correspondence test [20]. UPGMA (Unweighted Pair Group Method with Arithmetic Average) was used to create the dendrogram. For each marker used, the total number of bands, the number of polymorphic bands and polymorphism ratios were determined. The polymorphism ratio was calculated as (Number of polymorphic bands X 100 / Total number of bands). The GenAlEx ver. 6.5 software was run to determine allele frequency (p and q), number of effective alleles (Ne), Shannon's information index (I), expected (He), and unbiased expected heterozygosity (uHe) [21].

RESULTS AND DISCUSSION

Genetic relationships among sea daffodil genotypes collected from coastal zones of Mersin province were assessed through 14 different SRAP primer combinations. A total of 81 scorable bands were obtained from these SRAP primers. Of these bands, 41 were identified as polymorphic. The average number of bands per marker was calculated as 5.28, the average number of polymorphic bands was calculated as 2.92 and polymorphism ratio was calculated as 46.92%. Primer base lengths varied between 100 – 900 bp (Table 1).

Table 1. *List of SRAP primers, number of total (TFN) and polymorphic fragments (PFN), polymorphism ratio (PR)*

Primers	Base Length	TFN	PFN	PR (%)
Em1-Me1	200-530	5	2	40.00
EM3-Me4	240-650	4	0	0.00
Em3-Me6	180-800	2	0	0.00
Em4-Me11	220-880	5	2	40.00
Em7-Me1	190-900	5	5	100
Em7-Me4	120-880	9	7	77.77
Em9-Me5	520-780	2	1	50.00
Em10-Me2	110-770	8	7	87.50
Em10-Me3	150-600	9	4	44.44
Em12-Me6	100-750	9	8	88.88
Em12-Me7	180-330	3	1	33.33
Em13-Me2	150-500	4	0	0.00
Em13-Me4	330-700	5	1	20.00
Em13-Me9	250-510	4	3	75.00
Mean	**100-900**	**5.28**	**2.92**	**46.92**

In previous studies conducted with SRAP primers, base lengths were reported as between 400-1700 bp [3] and between 100-1500 bp [18]. Differences with the present findings were attributed to differences in genetic materials used in different studies.

Grassi et al. investigated the genetic diversity of sea daffodil genotypes collected from 10 different locations of coastal zones of Italy with the use of AFLP molecular marker system [13]. Researchers were able to obtain 956 bands of which 34 were polymorphic and the average number of polymorphic bands was reported as 3.56. Present findings were similar to those earlier ones. The lowest polymorphism ratio (0%) was observed in Em13-Me2, Em3-Me4 and Em3-Me6 primers and the greatest ratio (100%) was obtained from Em7-Me1 primers. Uzun et al. reported average polymorphism ratio of SRAP marker system as 90.0% and such a finding was different from the present one [3]. Smaller polymorphism ratios of this study may be attributed to the single location of the studied genetic materials.

Number of effective alleles (Ne) ranged from 1.00 (Em3-Me4 and Em3-Me6) to 1.613 Em4-Me6) (average 1.319), Shannon's information index (I) values from 0.000 (Em3-Me4 and Em3-Me6) to 0.541 (Em7-Me1) (average 0.279), expected heterozygosity (He) values from 0.000 (Em3-Me4 and Em3-Me6) to 0.358 (Em7-Me1) and unbiased expected heterozygosity (uHe) values from 0.000 (Em3-Me4 and Em3-Me6) to 0.361 (Em7-Me1) (average 0,190) (Table 2). For all these parameters, Em7-Me1 primer combination had the highest values whereas Em3-Me4 and Em3-Me6had the lowest values. Cophenetic correlation between ultra-metric similarity tree and similarity matrix was found to be relatively high ($r = 0.76$, $P<0.01$). Values of between 0.7 – 0.9 indicate a well-correlation between similarity indices and dendrogram [23]. The present value revealed that there was a high correlation between the similarity indices and the dendrogram, in other words, the present dendrogram well represented the similarity indices.

Table 2. *SRAP primers, estimated allele frequency (p and q), number of effective alleles (Ne), Shannon's information index (I), expected (He) and unbiased expected heterozygosity (uHe)*

Primers	**p**	**q**	**Ne**	**I**	**He**	**uHe**
Em1-Me1	0.760	0.240	1.554	0.404	0.288	0.291
Em3-Me4	1.000	0.000	1.000	0.000	0.000	0.000
Em3-Me6	1.000	0.000	1.000	0.000	0.000	0.000
Em4-Me11	0.901	0.099	1.237	0.223	0.149	0.150
Em7-Me1	0.758	0.242	1.576	0.541	0.358	0.361
Em7-Me4	0.475	0.525	1.471	0.401	0.270	0.273
Em9-Me5	0.660	0.340	1.484	0.453	0.298	0.301
Em10-Me2	0.736	0.264	1.343	0.277	0.192	0.194
Em10-Me3	0.894	0.106	1.245	0.214	0.143	0.145
Em12-Me6	0.629	0.371	1.613	0.495	0.342	0.346
Em12-Me7	0.954	0.046	1.104	0.133	0.079	0.080
Em13-Me2	0.884	0.116	1.276	0.271	0.178	0.179
Em13-Me4	0.941	0.059	1.110	0.077	0.055	0.056
Em13-Me9	0.704	0.296	1.454	0.423	0.282	0.285
Mean	**0.806**	**0.194**	**1.319**	**0.279**	**0.188**	**0.190**

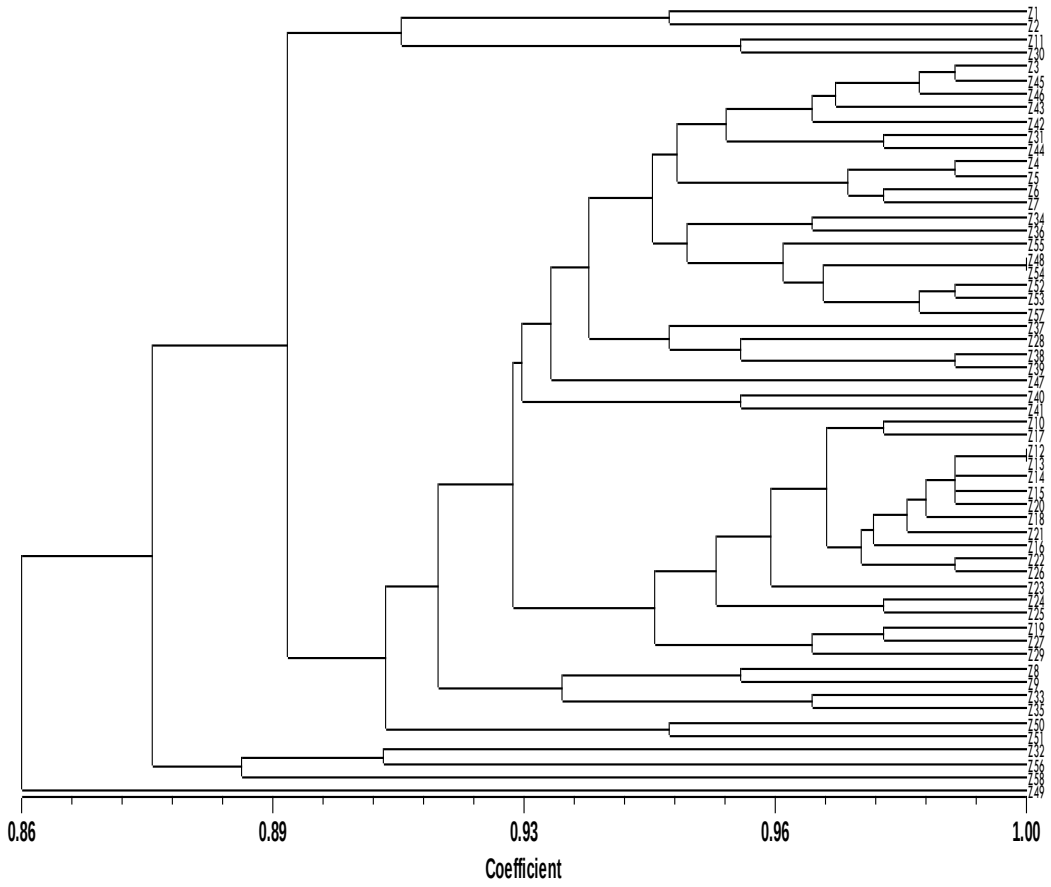


Fig. 2. *Dendrogram for 58 sea daffodil genotypes constructed with SRAP markers*

According to UPGMA dendrogram, similarity index values varied between 0.86 – 1.00. Present sea daffodil genotypes were gathered under two main groups. In the RAPD molecular marker analysis performed on different Pancratium species, it was determined that 4 main groups were formed depending on the primers used in the study and the RAPD method was sufficient to distinguish the species from each other [29]. In the current study, the first group included only genotype 49 and the other group included the rest. The genotypes 12-13 and 48-54 had a similarity index of 1.0. A narrow genetic index of similarity has been obtained between genotypes. The reason for this can be related to the collection of all materials from a single region rather than the SRAP marker system used. Similar to our current study, in the SSR marker analysis performed in the *Pancratium maritimum* species, a narrow genetic similarity within the species in terms of genetic similarity was determined [30]. Present findings are different from the findings of Sanaa and Fadhel conducted isoenzyme analyses on sea daffodil genotypes collected from different locations 10 m apart in Tunisia [24]. That study was conducted with 19 different populations and polymorphism ratios were reported as between 27.80 - 55.60%. Such differences are mostly attributed to differences in marker systems and genotypes. In RAPD and SSR analyses, similarity index in sea daffodil genotypes was reported as 0.81 [25]. Such a value was similar to the present one. On the other hand, in the SRAP analysis performed in the daffodil genotypes collected from different regions of Turkey similarity index of genotypes varied between 0.35 and 0.95 [28]. The current study is different from [25] in the literature. The reason for the difference can be explained by the fact that genotypes collected from only one region were used in the study.

CONCLUSION

It was concluded based on present findings that *P. maritimum* species were under the threat of extinction in their natural habitat [31]. Extinct genotypes result in changes in the genetic variation of the species. The present study investigated the genetic diversity in 58 *P. maritimum* genotypes collected from the coastal zone of Mersin province with the use of SRAP markers and present findings revealed that SRAP marker system could be used to identify genetic diversity in the sea daffodil genotypes. According to the results obtained, selecting genotypes that are distant from each other rather than genotypes that are genetically close to each other may be beneficial in conserving gene resources.

REFERENCES

[1] Ercisli, S. (2004): A short review of the fruit germplasm resources of Turkey. Genetic Resources and Crop Evolution 51(4): 419-435.

[2] Pınar, H., Yaman, M., Sarısu, H.C., Uzun, A., Yiğit, M.A. (2018): Bazı Kiraz Anaçlarının Genetik Akrabalık İlişkilerinin RAPD Moleküler Markır Yöntemi ile Belirlenmesi. Harran Tarım ve Gıda Bilimleri Dergisi 22(3): 326-334. (in Turkish)

[3] Uzun, A., Çil, A., Yaman, M., Coşkun, Ö.F. (2020): Genetic Diversity and Some Fruit Characteristics of Quince Genotypes Collected from Kayseri Region. Turkish Journal of Agriculture Food Science and Technology 8(2): 318-323.

[4] Georgiev, V., Ivanov, I., Pavlov, A. (2010): Obtaining and Selection of *Pancratium maritimum* L. In Vitro Cultures with Acetylcholinesterase Inhibitory Action. Biotechnology & Biotechnological Equipment 24:sup1, 149-154.

[5] Kilinc, M., Yuksel, S. (1995): *Pancratium maritimum* L. (Amaryllidaceae) Uzerinde Morfolojik, Anatomik ve Ekolojik Bir Arastırma, Doga, Turkish Journal of Botany 19, 309–320. (in Turkish)
[6] Korkmaz, E., Çelikel, F.G. (2013): Türkiye Kıyılarında Doğal Yayılış Gösteren Kum Zambağının Korunması ve Kültüre Alınması Üzerine Yapılan Araştırmalar. V. Süs Bitkileri Kongresi. 6-9 Mayıs, Yalova 855-859. (in Turkish)
[7] Sanaa, A., Boulila, A., Bejaoui, A., Boussaid, M., Fadhel, N.B. (2012): Variation of the chemical composition of floral volatiles in the endangered Tunisian *Pancratium maritimum* L. populations (Amaryllidaceae). Industrial Crops and Products 40: 312– 317.
[8] Giovino, A., Domina, G., Bazan, G., Campisi, P., Scibetta, S. (2015): Taxonomy and conservation of *Pancratium maritimum* (*Amaryllidaceae*) and relatives in the Central Mediterranean. Acta Botanica Gallica 162(4): 289-299.
[9] Demir, S., Çelikel, F.G. (2017): Türkiye'de Doğal Olarak Yetişen Kum Zambağının (Pancratium maritimum) Farklı Özellikleri ve Önemi. Türk Tarım–Gıda Bilim ve Teknoloji Dergisi 5(13): 1726-1731.
[10] Berkov, S., Evstatieva, L., Popov, S. (2003): Alkaloids in Bulgarian *Pancratium maritimum* L. Zeitschrift für Naturforschung 59: 65–69.
[11] Berkov, S., Evstatievaa, L., Popov, S. (2004): Alkaloids in Bulgarian *Pancratium maritimum* L. Zeitschrift für Naturforschung 59C: 65-69.
[12] Eisikowitch, D., Galil, L. (1971): Effect of wind on the pollination of *Pancratium maritimum* L. (Amaryllidaceae) by hawkmoths (Lepidoptera: Sphingidae). Journal of Animal Ecology 40: 673–678.
[13] Grassi, F., Cazzaniga, E., Minuto, L., Peccenini, S., Barberis, G., Basso, B. (2005): Evaluation of biodiversity and conservation strategies in *Pancratium maritimum* L. for the Northern Tyrrhenian Sea. Biodiversity and Conservation 14: 2159– 2169.
[14] İlhan, D. (2017): Bitki Biyoteknolojisinde Genetik Kaynakların Önemi. Kafkas Üniversitesi Fen Bilimleri Enstitüsü Dergisi 10(2): 134-144. (in Turkish)
[15] Gülşen, O., Sever-Mutlu, S., Mutlu, N., Tuna, M., Karagüzel, O., Shearman, R. C., Riordan, T. P. (2009): Polyploidy creates higher diversity among Cynodon accessions as assessed by molecular markers. Theoretical and Applied Genetics 118: 1309-1319.
[16] Uzun, A., Coşkun, Ö. F., Yaman, M., Pinar, H., Paris, K. (2017): Identification of Genetic Similarities among Walnut (*Juglans regia* L.) Genotypes Selected from Central Anatolia Region of Turkey with SRAP Markers. Bahçe Kültürleri Araştırma Enstitüsü 26.
[17] Pinar, H., Ercişli, S., Bircan, M., Ünlü, M., Uzun, A., Yilmaz, K. U., Yaman, M. (2017): Evaluation, genetic analysis and self- (in) compatibility characters of new promising apricot genotypes. Journal of Agricultural Science and Technology 19: 365-376.
[18] Yaman, M., Yıldız, E., Pınar, H., Uzun, A., Sümbül, A., Alkan, Ş. (2020): Determination of Genetic Diversity Using SRAP Technique in Some Walnut Genotypes Obtained by Selection. Turkish Journal of Agriculture-Food Science and Technology 8(12): 2577-2582.
[19] Rohlf, F. J. (2000): NTSYS-pc: numerical taxonomy and multivariate analysis system, version 2.1. Exeter Software: Setauket NY.
[20] Mantel, N. (1967): The detection of disease clustering and a generalized regression approach. Cancer Research 27(2 Part 1): 209-220.
[21] Peakall R., Smouse P E. (2012): GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research - an update. Bioinformatics 28: 2537-2539.
[22] Dice, L.R. (1945). Measures of the amount of ecologic association between species. Ecology 26(3): 297-302.
[23] Mohammadi, S.A., Prasanna, B.M. (2003): Analysis of genetic diversity in crop plants—salient statistical tools and considerations. Crop Science 43(4): 1235-1248.

[24] Sanaa, A., Fadhel, N.B. (2010): Genetic diversity in mainland and island populations of the endangered *Pancratium maritimum* L. (*Amaryllidaceae*) in Tunisia. Scientia Horticulturae 125(4): 740-747.
[25] Elibol, C., Bilgen, B.B. (2017): Genetic diversity and molecular characterization of natural Pancratium maritimum L. populations by DNA markers. Turkish Journal of Botany 41(6): 569-578.
[26] Doyle, J.J., Doyle, J.L. (1990): Isolation of plant DNA from fresh tissue. Focus 12(13): 39-40.
[27] Soliman, M.I., Rizk, R.M., El-Saied, F.M., El-Eraky, N.M. (2012): Genetic variation of some *Pancratium* L. species in Egypt related to accessions environment and geographical isolation. Journal of Biological Chemistry and Environmental Sciences 7(1): 61-80.
[28] Zeybekoglu, E., Asciogul, T.K., Ilbi, H., Ozzambak, M.E. (2019): Genetic Diversity of some Daffodil (*Narcissus* L. spp.) Genotypes in Turkey by Using SRAP Markers. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 47(4): 1293-1298.
[29] El-Hadidy, A., El-Ghani, M.A., Amer, W., Hassan, R. (2012): Morphological 56 and molecular differentiation between Egyptian species of *Pancratium* L. 57 (*Amaryllidaceae*). Acta Biologica Cracoviensia s. Botanica 54: 53-64.
[30] De Castro, O., Di Maio, A., Di Febbraro, M., Imparato, G., Innangi, M., Vela, E., Menale, B. (2016): A Multi-Faceted Approach to Analyse the Effects of Environmental Variables on Geographic Range and Genetic Structure of a Perennial *Psammophilous* Geophyte: The Case of the Sea Daffodil *Pancratium maritimum* L. in the Mediterranean Basin. PLOS ONE 11(10): e0164816.
[31] San Nayim, Y. (2020): Assessment of sand dune ecosystems with Pancratium maritimum, Bartın, Turkey. Journal of Environmental Biology 41(2): 483-490.