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A Cytogenetic Study on Some Perennial Trigonella (Fenugreek) in Iran

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Abstract

Trigonella (Fenugreek) is a plant in the family of Papilionaceae. Its origin is Iran and west Asia and then, it has been taken to other parts of the world. At present, it is grown in most European, Asian and African countries. The research cytogenetically studied some perennial trigonella (fenugreek). It was assessed as a case study in Iran. After germination of seeds, the root apical meristem was used for karyotypic studies. Then, it was stabilized, hydrolyzed and stained. At the next stage, the microscopic sample was provided and the morphology of chromosomes was studied. The results showed that all assessed genotypes were diploid with 2n=2x=16 and the chromosomal basis of x=8. At the next stage, genomes including length of long and short arms of chromosome, the ratio of long arm to short arm and the ratio of short arm to long arm were analyzed. Then, the results were studied through Levan's (et al.) table, the types of chromosomes were identified and Karyotypic symmetry of species was assessed according to the Stebbins' method. According to the Stebbins' bilateral table, genotypes of 460 (T.elliptica, Zanjan, Iran), 770 (T.elliptica, Avaj-Qazvin, Iran) and 5310 (T.elliptica, Shurjestan-Fars, Iran) were classified as group 1A (IA), the genotype of 162 (T.elliptica, Hossein Abad-Fars, Iran) was classified as group 2A, and finally, the genotype of 76 (T.elliptica, Gazal Park-Tehran, Iran) was classified as group1B. Genotypes plot showed similar results regarding the parameters of A1 and A2 and Stebbins' table symmetry classes. At the end, the proximity and remoteness of studied species was chromosomally identified and the results were presented as dendrogram.

Keywords: Trigonella, cytogenetic, chromosome, Stebbins' table, dendrogram.

Introduction

According to the research literature, some similar studies have already been done, but they do not exactly include the title of this paper. Lakshmi et al. studied the *strains of T. Foenum graecum (which were morphologically similar) and a strain T. corniculata specie.* Karyotypic evidences show that the evolutionary paths of these two species of chromosomes are from long chromosomes to short ones and from symmetric karyotypes to asymmetric ones. The chromosome numbers which Martin et al. reported regarding a species of trigonella were 2n=14, 2n=16, 2n=30, and 2n=46. Also, they observed the B and satellite chromosomes. Aykut et al. have stated the chromosome numbers of 2n=14, 2n=16, and 2n=18 regarding some species of trigonella[1]. In 1842, the chromosomes were firstly observed by Nageli. Two years later, the *chromosomes behavior during mitosis was described by this scientist. Today, Nageli's observations* are accepted as first mitotic description. The detailed mitoses were reported in 1882 by Fleming. Chromosomes were defined as the most logical agents or determinants of transmission by cytogenetic observations. The fact that the chromosomes are transmitted to two new daughter cells caused the researchers to conclude that the chromosomes are composed of materials which convey the genetic information. Primary chromosomal studies showed that there are big differences in chromosome numbers of plants species. Therefore, determining the chromosome numbers of plants was an important issue at that time. The chromosome number of rice was determined by Kuwada in 1910 and also, the chromosome numbers of most famous cultivated and wild plants were determined at that time. The chemical pretreatment methods, especially the squash method, led to great progresses regarding the chromosome numbers of plants[2].

The genus of trigonella is in the family of Papilionaceae and its 32 species are distributed in different parts of Iran. It is known as an important medical, agricultural and pasture plant. Also, it is used in traditional medicine very much. The seeds of 5 populations of perennial trigonella were studied through this research. The studies used the Stebbins' bilateral table to determine the *evolutionary status* and study the karyotypic symmetry of genotypes. Also, the parameters such as DRL (*difference of relative length)* of large to small chromosome, *Intra-chromosomal asymmetry index* (*A1*), *Intra-chromosomal asymmetry index* (*A2*), and the percent of TF (total form) were calculated. The chromosome types were determined by Levan method. Finally, the proximity and remoteness of studied species was chromosomally identified and the results were presented as dendrogram (table-1)[2].

Table-1
Karyotypic details of different *Trigonella* genotypes

Genotype	2n	X*	A1	A2	SC	DRL	TF	VRC	KF
76	2n =16	8	0.32	0.20	1B	5.78	37.86	6.77	14m+2sm
162	2n =16	8	0.35	0.22	2A	6.70	43.03	5.29	14m+2sm
460	2n =16	8	0.25	0.19	1A	6.79	39.24	6.87	16m
770	2n =16	8	0.39	0.16	1A	5.46	38.74	7.49	8m+8sm
5310	2n =16	8	0.39	0.16	1A	7.93	40.16	6.70	12m+4sm

* X=mean chromatin length (µm); A1= Intra asymmetry chromosomal index; A2= Inter asymmetry chromosomal index; SC= Symmetry Classes; DRL= Difference of range relative length; TF= total form percentage (%); VRC= Value of relative chromatin; KF=karyotype formulae.

Methodology

Firstly, the seeds were provided from gene bank of research institute of forests and rangelands. The seeds were germinated after being put inside the petri dish at a temperature of 22^0C. When the length of roots reached 1-1.5 cm, they were cut and the stages of pretreatment (one percent of saturated solution of alpha bromo naphthalene), stabilization (Levinsky solution including chromium trioxide and formaldehyde 40% in equal ratios), hydroxylation (one normal sodium hydroxide) and staining (hematoxylin 4% and one gram of ammonium sulfate III) were respectively performed. Then, the slides were created using the squash method and the chromosomal images were provided. Chromosomal studies included the following stages[3]:

Image analysis: the images enlarged by Olympus microscope, the slides were provided by digital color video camera and then, the karyotypes were created by Micro measure software for each genotype. Calculation of cytogenetic parameters: each slide was assessed, three cells were selected (at least), and the cytogenetic parameters were calculated as: chromosome TL (total length), the percent of relative length of each chromosome (%RL), the length of long arm (LA), the percent of relative length of LA, the length of short arm (SA), the percent of relative length of SA, arm ratios (L/S) and centromere index (CI) which defines the ratio of length of SA to TL[4].

Results and Discussion

Regarding the basis chromosome numbers, there was no different basis chromosome among the species (x=8). All assessed genotypes were diploid with 2n=2x=16. The studied species included metacentric and sub-metacentric chromosomes regarding the chromosomal types. This showed that there is karyotypic symmetry among them[5]. The studies used the Stebbins' bilateral table to determine the evolutionary status and study the karyotypic symmetry of genotypes. Also, the parameters such as DRL *(*difference of relative length) *of large* to small chromosome*,* Intra-chromosomal asymmetry index (***A1***), Intra-chromosomal asymmetry index (***A2***), and the percent of TF were calculated[6]. Then, according to the Stebbins' bilateral table, the studied genotypes were symmetrically classified. According to this table, genotypes of5310 (T.elliptica, Shurjestan-Fars, Iran) and 770 (T.elliptica, Avaj-Qazvin, Iran) included the highest value regarding the intra-chromosomal asymmetry index. Also, genotypes of 460 (T.elliptica, Zanjan, Iran), 770 (T.elliptica, Avaj-Qazvin, Iran) and 5310 (T.elliptica, Shurjestan-Fars, Iran) were classified as group 1A (IA), the genotype of 162 (T.elliptica, Hossein Abad-Fars, Iran) was classified as group 2A, and finally, the genotype of 76 (T.elliptica, Gazal Park-Tehran, Iran) was classified as group1B. Genotypes plot showed similar results regarding the parameters of A1 and A2 and Stebbins' table symmetry classes[7].

The changes of A2 and DRL parameters were assessed in studied genotypes. The results showed that there was a positive and direct relation between them. Also, the changes of A1 and TF% parameters showed that there was a negative and reverse relation between them[8] (figure-1 and 2 and table-2).

The variance analysis showed that there were significant differences among the genotypes due to some chromosomal features at 5% and 1% levels[9]. Therefore, there were large variations among the studied germplasms regarding the assessed features[10]. After confirming the differences among the genotypes by variance analysis, they were compared and classified through Duncan's multiple range tests[11].

Conclusion

Cluster analysis based on the Ward method was used to group the genotypes and species. According to the dendrogram, the genotypes of 5310 and 770 belonged to the same family, included 2n=2x=16 chromosomes and they were classified as a cluster (class 2); and the genotype of 162 was classified in other cluster[12]. Also the results showed that there was the minimum distance between the genotypes of 5310 and 770; and the maximum genetic distances belonged to the genotype of 162[13].

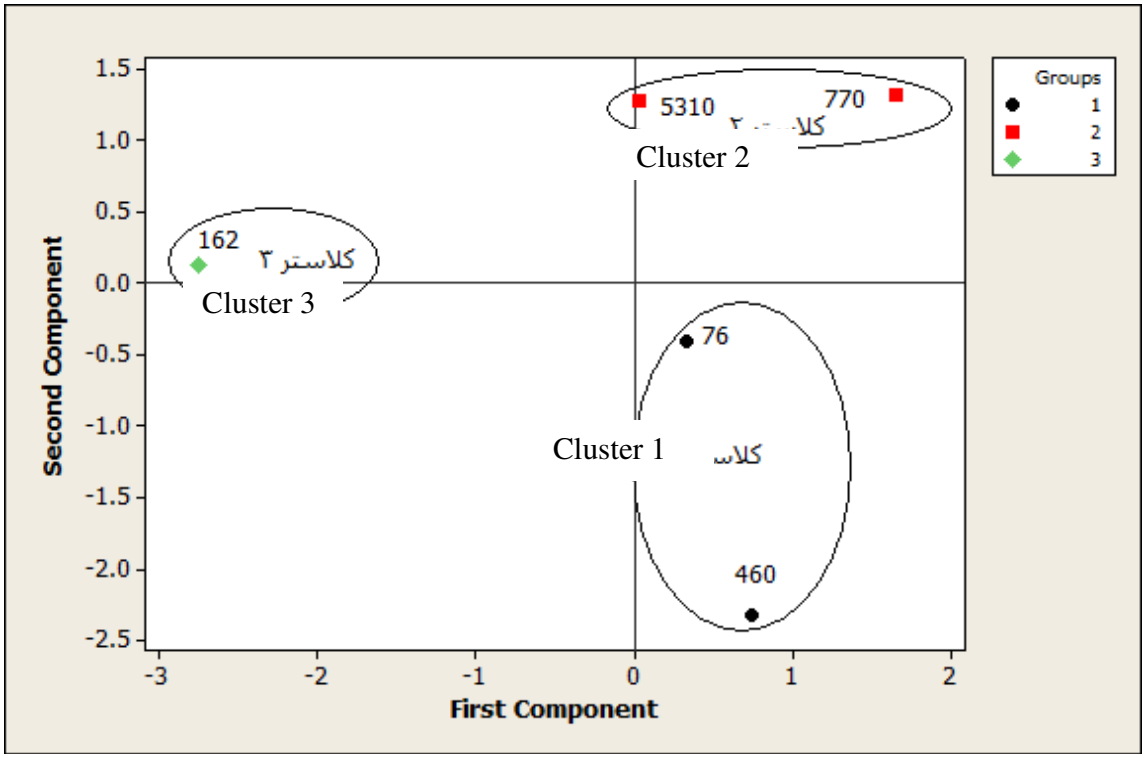

Figure-1

Dispersion diagram genotypes according to parameter A1, A2 ; companion with kinds symmetry suggestion by Stebbins: ● – genotypes belonging to 1A class, ▲ – genotypes belonging to 2A class ■genotypes belonging to 2B class.

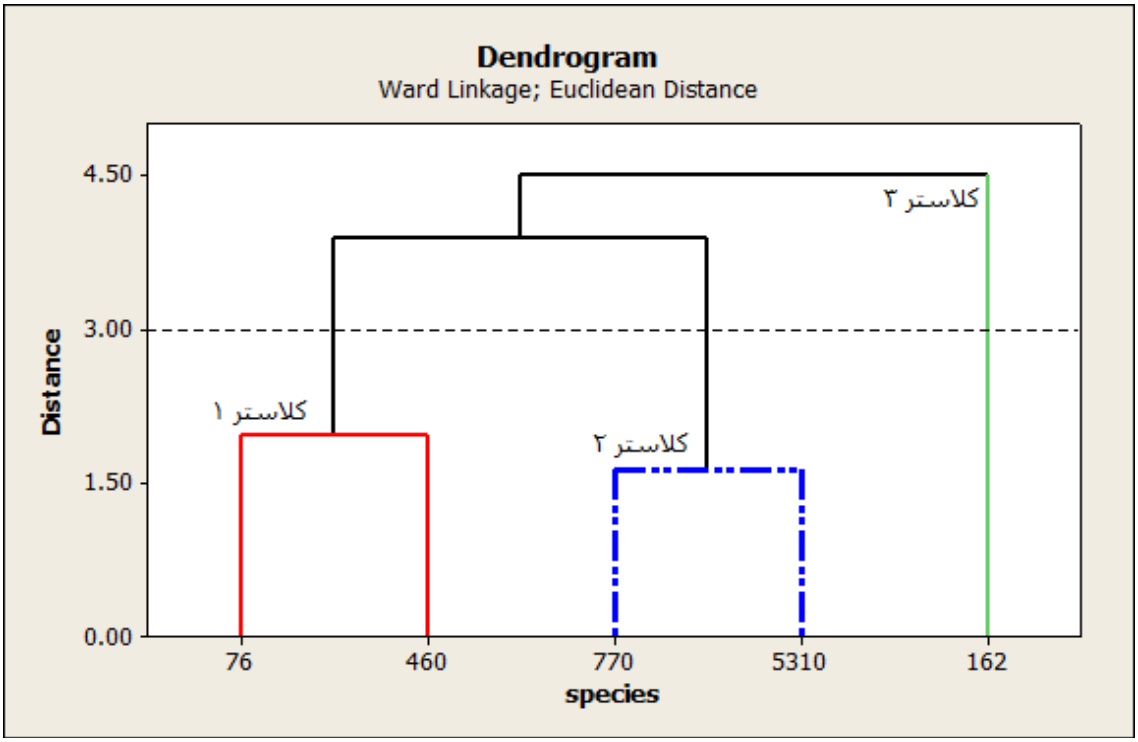

Figure-2

Dendrogram gained of cluster analysis via five species of *Trigonella*

Table-2
Specific values of variance percentage and coefficients of specific vectors in analyzing main components

Second component	First component	Name of traits
0.06	0.60	TL
-0.23	0.57	SA
-0.66	0.04	CI
0.25	0.56	LA
0.67	-0.03	AR
2.25	2.74	Specific values
0.45	0.55	Variance percentage
1.00	0.55	Accumulative variance percentage

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