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Karyotype and Seed Protein Analysis of *Muscari neglectum* (Liliaceae/Hyacinthaceae) Populations in North-East of Iran

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Abstract: Five specimen of 9 populations and 17 populations of *Muscari neglectum* were analysed for karyotypic characters and seed proteins analysis respectively using multivariate statistical methods. Karyotypes were asymmetrical and placed in 2A and 2B classes of Stebbins karyotype classification. The level of ploidy were tetraploid, autopentaploid, hexaploid. Some of the popoulations showed decreasing and increasing aneuploidy. Seed protein analysis showed 32 bands which some of them were specific for a population. Cluster analysis of populations on the basis of karyotype and seed protein data carried out and showed close relation of the populations.

Key words: *Muscari neglectum*, asymmetrical karyotype, seed protein electrophoresis, Liliaceae, Hyacinthaceae

INTRODUCTION

Muscari nglectum Guss. is a species of bulbous plants traditionally belonging to Liliaceae, subfamily Liliodeae, tribe Scilleae (Engler, 1887) but now usually placed in Hyacinthaceae, subfamily Hyacinthioideae, tribe Hyacintheae (Speta, 1998). This species is distributed in the Mediterranean region, Europe, Iran, Iraq, Caucasus and Afghanistan (Parsa, 1950; Davis and Stuart, 1984; Townsend and Guest, 1985; Rechinger, 1990). The populations of *Muscari neglectum* have similar morphology with variation in karyotypic characters. There are some reports of karyotype features of this species from other countries and Iran (Stuart, 1966; Federov, 1969; Love, 1973, 1974, 1976; Karlén, 1984; Dalgic, 1991; Jafari *et al*., 2008). Moreover, to our knowledge, seed protein electrophoresis have not previously been used in the biosystematics study of *Muscari neglectum*. The aim of this study was, to investigate the variation in karyotypic characters and seed protein analysis and to distinguish relationship between them. The present research carried out for first time in Iran.

MATERIALS AND METHODS

Bulbs of five specimen of nine populations for karyotypical study and 17 populations for seed protein study were collected from different localities in North-East of Iran in April until May 2005, 2006 (Table 1). Voucher specimen were deposited in herbarium of Islamic Azad University of Mashhad (IAUM). For karyotypic analysis, a pretreatment at room temperature for 3 h was usually applied before fixation of the root tips of populations in 0.002 M 8-Hydroxyquinoline. After fixation in a cold mixture of ethanol and acetic acid (3:1), the following procedure involved the maceration in 1 HCl at 60 for 5-8 min, washing in water, cutting off the meristems and squashing them in a drop of 45% acetic acid (Krahulcova, 2003). Chromosomes were described according to Levans terminology (Levan *et al*., 1964). Karyotypes were compared using total form of karyotype and calculated the ratio of the longest to the shortest chromosome (Verma, 1980). Symmetry Karyotypes were determined using Stebbins two way system (Stebbins, 1971).

Table 1: The locality of studied populations of *Muscari neglectum*

Locality	Altitude (m)	No. of populations	Karyology study	Electrophoresis study
Bazangan	863	2	x	x
Kashmar	1560	1	x	x
Benhang	1440	5	x	x
Ghandeshtan	1470	1	x	x
Manzar	1427	1		-
Arefi village (Torogh)	1370	2	x	x
Moghan	1352	1		x
Sad-Kardeh	1330	1		x
Kardeh village	1300	2		x
Ghoujghi	1400	1	x	x
Dargaz	1764	1	x	x
Kurdineh	644	1		x

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For analysis of seed protein, 300 mg of each sample (30-40 seeds) was homogenized to obtain a fine powder. Protein were extracted in pre-cooled mortar and pestle over ice with a 100 mM Tris phosphate buffer (pH 6.8), 0.5 mM EDTA (pH 8) and Mercaptoethanol. The resulting mixture was centrifuged at 12000 g for 20 min. The crude extracts were boiled for 5 min in 0.5 M Tris-HCl (pH 6.8), 10% Mercaptoethanol and 3% glycerol (Sanches-Yelamo *et al*., 1995). Protein electrophoresis by SDS-PAGE used 20 mg of protein in each lane. Vertical slab gels 1 mm thick were electrophoresied at a constant current of 30 mA for 4 h. Coomasie Brilliant Blue R-250 was used for overnight gel staining follow by trichloroacetic acid as fixative. Cluster analysis carried out on the basis of seed protein data. Also, to estimate population similarity and indicate by protein electrophoresis patterns, Jaccards index were determined (Digby and Kempton, 1994). Each protein band was considered as a qualitative character and coded as 1 (presence) versus 0 (absence). (Carreras *et al*., 1997). The resulting data matrix was used for cluster analysis using Ward method in order to identify the most variation protein bands among the populations studied. Also pearson coefficient and cluster analysis for karyotype data calculated. Statistical analysis used MINITAB ver. 11. Soft ware C.

RESULTS

The somatic chromosome number and detail of karyotype of each population were presented in Table 2. The basic chromosome number of *Muscari neglectum* is x = 9 (Speta, 1998). The population of Benhang was hexaploid with one metacentric and one telocentric chromosome which fall in 2B class. This population didn't have satellite. The population of Bazangan 2 was hexaploid with long, medium and short chromosome were m, M, respectively. The karyotype of the population was symmetrical. This population fall in 2A class. The population of Torogh was hexaploid. Its karyotype was trimodal which fall in 2B class. The population of Dargaz was tetraploid which fall in 2B class. The population of Goujghi was autopentaploid. Its karyotype was trimodal and it falls in 2B class. They were without satellite. The population of Ghandeshtan was pentaploid. Its karyotype was bimodal. Long chromosome were st, sm medium and short chromosome were M and m which fall in 2B class. The population of Kashmar was hexaploid with one st which fall in 2B class. The satellite was observed. The population of Torogh 2 was autopentaloid. Long chromosome was st, sm, medium and short chromosome were M, m. It falls in 2B class and they were without satellite. The population of Benhang 1 was autopentaploid with trimodal karyotype. Long chromosome were st, sm, medium and short chromosome were m, M. It had one st. This population fall in 2B class. Only Dargaz population was tetraploid (2n = 36) and just Bazangan 2 fall in 2A class (Fig. 1A-I).

Seed protein: The results of SDS-analysis showed, total number of bands were 32. Bands 1-11, 13, 15, 25, 32 were presented in all sampled populations. In Moghan 20 bands were observed, which only band 17 existed in this population and Benhang 5. With Rf = 0.676 this band width in Benhang 5 was 2 times more than Moghan. Also, band 24 only observed in this population with Rf = 0.79. In Jaghargh observed 20 bands which 19 was specific for this population with Rf and seed protein molecular weight (spmw) 0.699 and 1092760, respectively. In Ghandeshtan and Kashmar populations observed 19 bands with spmw = 999160. In Benhang 5 existed 21 bands without specific band with spmw = 1184560. In Benhang 3 observed 22 bands without specific band with spmw = 1236860. In Dargaz observed 18 bands but band 31 with Rf = 0.909 that existed in all populations, it wasn't in this population. This population had minimum bands. Ghoujghi had 24 bands with spmw = 1401850. Band 18 with Rf = 0.687 existed only in Dargaz and Ghoujghi but the band width in Dargaz was seven times of Ghoujghi. In Bazangan 1, Torogh 1, 2, Kardeh village 1, 2 had 22, 23, 24 and 26 bands with spmw = 1279760, 1344760, 1436960, 1346960 and 1484960, respectively. The most number of bands were 25 band in Sad-Kardeh and Kardeh village 2 and the least of them was 18 in Goujghi (Fig. 2A, B, 3A, B). The population of Bazangan 1, 3, Ghandeshtan and

Table 2: Details of karyotype of *Muscari neglectum*

Pop.	TL	S	L	T (L/S)	TV (μm³)	DRL (%)	S (%)	TF (%)	2n	Class	KF
Benhang 5	414.7	135.7	279.0	118.8	78	2.30	28.6	32.7	53	2B	m12+sm11+st2+(M)+(t)
Bazangan 2	292.5	124.8	167.7	70.5	49	1.34	53.8	42.7	53	2A	M6+m17+sm3
Torogh 1	334.1	131.8	202.2	84.5	76	1.41	50.0	32.7	53	2B	M6+m15+sm5
Kashmar	389.8	153.6	236.2	88.1	70	2.30	30.0	39.4	53	2B	M6+m10+sm9+(st)
Ghoujghi	309.8	122.2	187.5	74.3	69	1.80	40.0	39.5	48	2B	M3+m13+sm7
Ghandeshtan	301.4	115.2	189.4	86.0	37	1.90	35.7	38.2	48	2B	M2+m11+sm9+st1
Torogh 2	214.4	85.8	128.6	79.8	21	2.48	27.3	40.0	48	2B	Sm5+M5+m12+st1
Bazangan 1	402.6	158.4	244.2	73.8	100	2.50	30.4	39.3	45	2B	M5+m12+sm5+(st)
Dargaz	238.1	99.2	138.9	50.0	35	3.50	31.6	41.7	36	2B	M7+m7+sm3

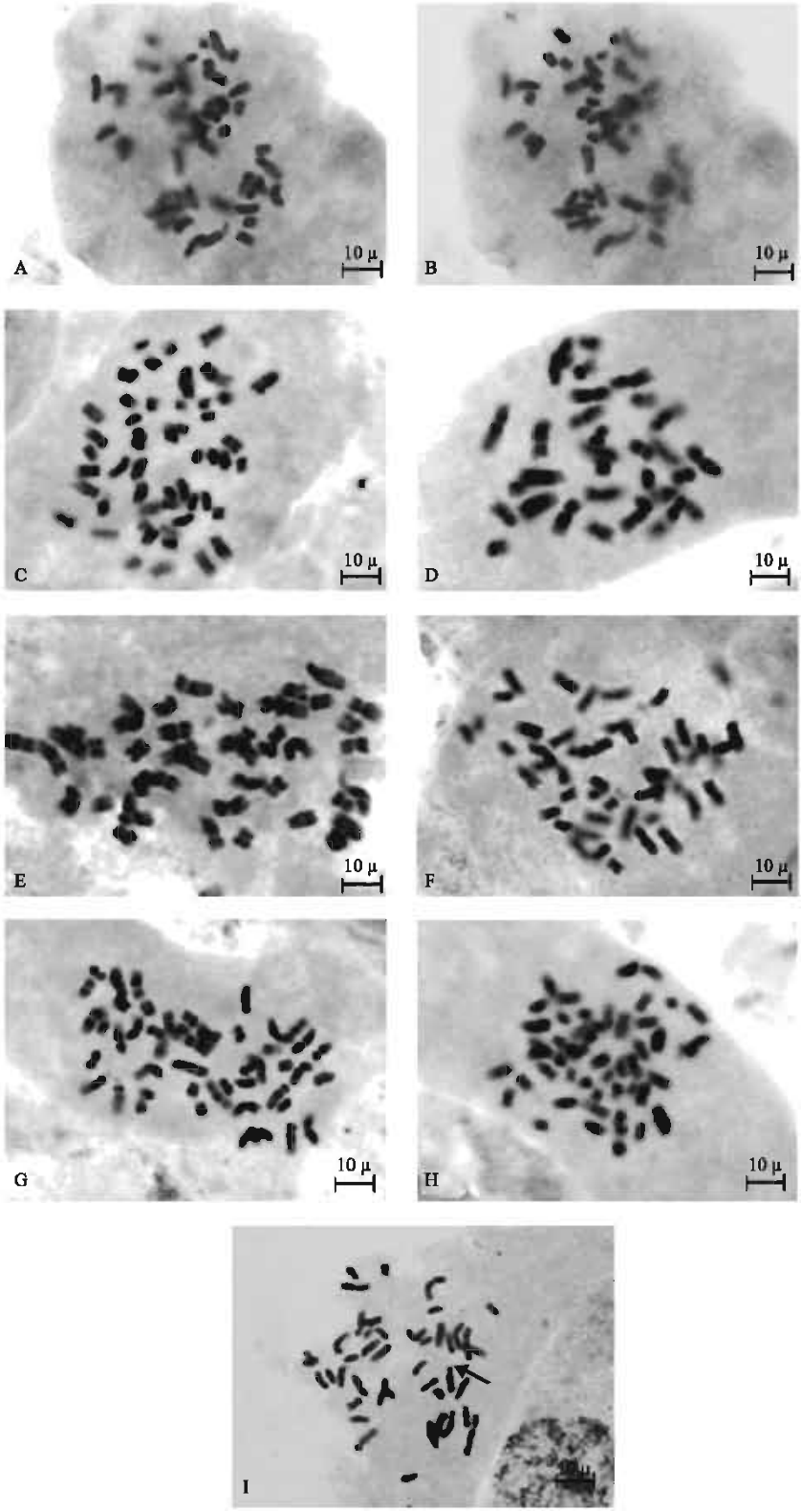

Fig. 1: Somatic chromosomes in (A, B) Benhang 5, (C) Torogh 1, (D) Dargaz, (E) Ghojghi, (F) Ghandeshtan, (G) Kashmar, (H) Torogh 2 and (I) Bazangan 1

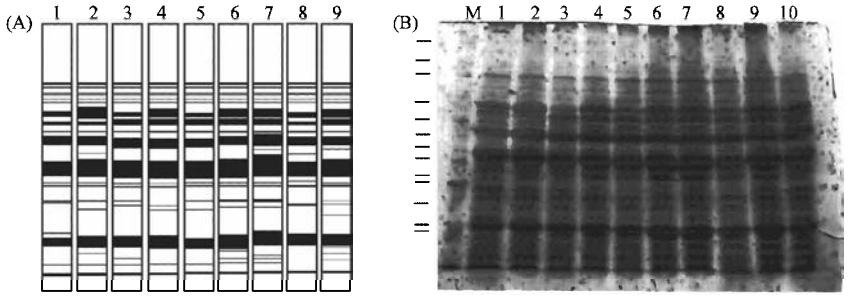

Fig. 2: Diagram of seed protein bands in (1) Moghan, (2) Benhang 4, (3) Jaghargh, (4) Ghandeshtan, (5) Kashmar, (6) Benhang 5, (7) Benhang 3, (8) Dargaz, (9) Ghoujghi and (10) Moghan (repeated)

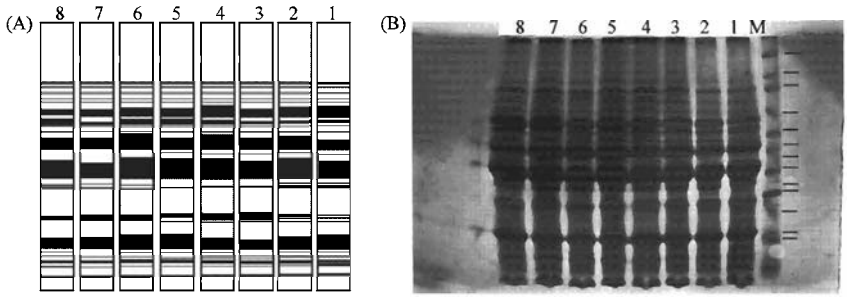

Fig. 3: Seed protein bands in (1) Bazngan 1, (2) Bazangan 2, (3) Bazangan 3, (4) Torogh 1, (5) Torogh 2, (6) Kardeh village 1, (7) Kardeh village 2 and (8) Kardeh

Hierarchical cluster analysis of observations

Euclidean distance, ward linkage amalgamation steps

Step	No. of clusters	Similarity level	Distance level	Clusters joined		New cluster	No. of Obs. in new cluster
1	8	91.07	6.205	4	6	4	2
2	7	90.23	6.790	7	8	7	2
3	6	80.70	13.407	5	7	5	3
4	5	74.63	17.619	2	3	2	2
5	4	72.87	18.843	4	5	4	5
6	3	45.76	37.674	2	4	2	7
7	2	37.06	43.721	2	9	2	8
8	1	7.13	64.508	1	2	1	9

Final partition

No. of clusters	1			
	No. of observations	Within cluster sum of squares	Average distance from centroid	Maximum distance from centroid
Cluster 1	9	3533.022	16.393	39.365

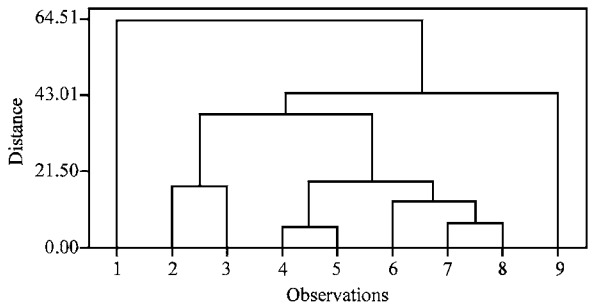

Fig. 4: Dendrogram of *Muscari neglectum* populations on the basis of karyology study. (1) Benhang 5, (2) Bazangan 2, (3) Torogh 1, (4) Kashmar, (5) Ghoujghi, (6) Ghandeshtan, (7) Torogh 2, (8) Bazangan 1 and (9) Dargaz

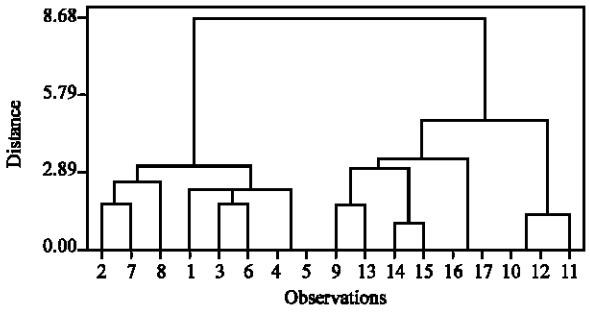

Fig. 5: Dendrogram of *Muscari neglectum* populations on the basis of seed protein bands. (1) Moghan, (2) Benhang 4, (3) Jaghargh, (4) Ghandeshtan, (5) Kashmar, (6) Benhang 5, (7) Benhang 3, (8) Dargaz, (9) Ghojghi, (10) Bazangan 1, (11) Bazangan 2, (12) Bazangan 3, (13) Torogh 1, (14) Torogh 2, (15) Kardeh village 1, (16) Kardeh village 2 and (17) Kardeh

Kashmar, Sad-Kardeh and Kardeh village had most similarity as Jaccards coefficient = 100%. Cluster analysis of karyotype and seed protein data were presented on the basis of karyotype details and absence or presence of bands respectively in Fig. 4 and 5.

DISCUSSION

The results of karyological study showed decreasing aneuploidy in Benhang 5, Bazangan 2, Torogh 1, Kashmar with 2x = 2n-1 = 53 but Goujghi, Ghandeshtan and Torogh 2 had increasing aneuploidy with 2x = 2n+3 = 48. The rest of them were autopentaploid 2x = 2n = 45 and tetraploid 2x = 2n = 36. But in earlier studies, the chromosome number were reported from Greece, Spain, Afghanistan, Iran, Cyprus with 2n = 54, 36, 18, 44, 45, 63, 70, 72 (Saito and Matsuzava, 1969; Karlén, 1984). For statistical analysis, Pearson coefficient calculated on the basis of DRL. The result of this analysis showed the population of Kashmar and Benhang 5 (hexaploid) had the most of Pearson coefficient (r = 0.994) and the population of Kashmar and Torogh 1 had the least of Pearson coefficient (r = 0.838). The maximum Pearson coefficient observed in Goujghi and Ghandeshtan population (pentaploid) with r = 0.998 and the minimum Pearson coefficient observed in Torogh 2 and Ghoujghi with r = 0.946. The population of Kashmar was close to Benhang 5. They grow at the same weather but Torogh and Kashmar have different weather and their locality was far from each other. Kashmar and Benhang weather is hot but in Torogh, it was colder and temperate.

Cluster analysis of karyotype data showed: Ghandeshtan and Kashmar populations, Torogh 2 and Bazangan 1 had similarity 91.07 and 90.23%, respectively. Benhang 5 and Bazangan 2 had maximum distance (Fig. 4).

Cluster analysis of protein data (Fig. 5) showed: Ghandeshtan and Kashmar populations, Bazanan 1, 3, Kardeh village 2 and Sad-Kardeh had similarity 100%. These results confirmed, Jaccard similarity coefficient. In the populations which had most similarity on the basis Jaccard coefficient, growing in places with same weather. The populations of this cluster growing in close locality with the same weather. The minimum similarity observed between Moghan and Ghoujghi populations because they grow in distant locality with separate weather.

In cluster analysis of protein data and karyotype data almost showed the same results. In populations, with increasing of distance, similarity decreased among them. Also different ploidy level didn't have correspondence to populations relationship.

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