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Karyotypic Study and Chromosome Evolution in Some Iranian Local Onion Populations

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Abstract
A karyotypic study was performed on 12 Iranian local onion (*Allium cepa* L.) populations. A number of mitotic cells at metaphase stage for each population were prepared. Chromosomes of suitable mitotic cells were counted and various parameters, including long arm (L), short arm (S), total length of chromosome (TL), relative length of chromosome (RL), arm ratio (AR), r-value, total chromosome volume (TCV) and centromeric index (CI), were measured. All populations were diploid with 2n=2x=16 and they were differentiated by their karyotype formula and parameters. Chromosome length varied in the populations and the highest quantity of chromatinic material was found in BehdashtShahrud onion. The onion populations under study occupied classes 1A and 2A of Stebbins' karyotype classification, indicating the presence of a primitive symmetrical karyotype in these populations. The mean chromosome length ranged from 8.54 to 11.97 µm. Haploid genome length was in the range of 67.79 to 96.65 µm and mean centromeric index (CI) of complements varied from 41.1 to 43.7%.The chromosome types were detected as mostly metacentrics "m" and some submetacentrics "sm", showing the karyotype formula of 8m (two populations), 7m+1sm (eight populations) and 6m+2sm (two populations). The cluster analysis using chromosomal parameters and based on Ward's minimum variance algorithm assigned the populations into two groups.

Keywords: *Allium cepa*, Centromeric index, Chromosome type, Karyotype, Onion

Introduction

The genus *Allium* includes agronomically useful species. The taxonomic position of onion and related genera has long been a matter of controversy. In early classifications of angiosperms, *Allium* species were placed in the Liliaceae but later, they were more often included in the Amaryllidaceae on the basis of their florescence structure. *Alliumcepa* is one of the oldest cultivated vegetables, recorded for over 4000 years (Fritsch and Friesen 2002, Phillip and Jenderek 2003). It is a biennial plant growing vegetatively one year and produces its seeds in the second year after prior exposing to a period of cold temperatures (Kovatch 2003). The *Allium* genus has great economic significance because it includes several important vegetable crops and ornamental species. All plant parts of onions except the seeds may be consumed by human, and wild species are exploited by local inhabitants.

Onion is propagated by seeds, bulbs or sets (small bulbs).

Currently, there are different opinions about the number of species in this genus (Xingjin *et al.* 2000). Estimations indicate about 750 species in the genus *Allium* (Stearn 1992) and 650 more synonymous species names exist (Gregory *et al.* 1998). The species of section *cepa* are diploid (2n=2x=16) although the occasional occurrence of individual tetraploid bulbs has been reported contrary to what is found in other *Allium* groups. The chromosomes are either metacentric or submetacentric which differs somewhat in their length; only the satellite chromosome pair is subtelocentric (subacrocentric) and the satellites being attached to the short arms. Most species of the section *cepa*have very small dot like satellite, as in other subgroups of this genus (Fritsch and Friesen 2002).

In spite of the cytological similarities between the species of section *cepa*, there are strong crossing barriers between them preventing interspecific gene flow even where sympatric distribution of two species occurs. Although in modern breeding programs, many classical cultivar groups have been crossed, the boundaries between the different taxa are becoming blurred, making it difficult to place materials within the scheme (Fritsch and Friesen 2002). To expand the genetic variation of onion, onion has been crossed with other *Allium* species, *e.g.A. sativum* (Yamashita*et al.* 2002). Since onion is regarded as an important crop worldwide, for decades there have been well-established onion breeding and seed production programs in the world, developing short intermediate and long-day cultivars. Today, almost 90% of the cultivars used come from local breeding programs (Martinez *et al.* 2000, Paknia *et al.* 2007). Chromosome identification is essential for biotechnological studies including genome analysis, somatic hybridization and ploidy manipulation (Yamamoto and Tominaga 2004). Karyotype features allow individual species to be distinguished. Thus, chromosome variation, although not always large, has accompanied evolutionary divergence of the taxa studied, a general phenomenon observed in both the plant and animal kingdoms (Acosta *et al*. 2005). Knowledge of karyotype relationships is an important prerequisite for effective plant genetic and breeding studies (Martinez-Gomez *et al.* 2003) and also provides valuable information related to the mechanism of genome evolution (Wilkinson 1994). Widely different karyotypes have been found among plants with the same chromosome number, and the relationships between karyotypes are not easily inferred from the morphology of the chromosomes (Brighton 1976). Distinguishing chromosomes based on the length may be charged with significant error due to variation in chromosome contraction within the chromosome complement in either one or different cells (Bajer 1959).

Hybrids obtained from the pollination of *Allium cepa*by other species were easily identified due to the presence of acrocentric chromosomes characteristic for the paternal genotypes (Keller *et al.* 1996). The onion breeding is slow process primarily due to the biennial nature of this outcrossing species (Alan *et al.* 2003). Alteration in chromosome

number and structure raises question about the origin, extent and evolutionary relationships of chromosome variants. Cytological variation also needs to be taken into account when conservation strategies are planned for the restoration of depleted populations, or the establishment of new ones, as the mixing of cytotypes can lead to hybrids (Murray and Young 2001). Cytotaxonomic studies provide taxonomic insight, at different hierarchical levels, not only through the determination of chromosome numbers, but also through the elaboration and comparison of karyotypes, in addition to the analysis of interphase nuclei and of chromatinic condensation standards in prophase. Cytotaxonomic studies can also contribute to discussions on evolutionary trends through chromosome changes.

The aims of this study were i) to quantify the cytological variation among 12 Iranian local onion populations, using karyotype analysis and ii) to establish karyologic relationship among the populations.

Materials and Methods

Seeds of 12 Iranian local onion populations (Table 1) obtained from Seed and Plant Improvement Institute (SPII), Karaj, Iran, were germinated on damp filter paper in petri dishes at room temperature. For the analysis of somatic chromosomes, 1-1.5 cm long fresh root tips were collected from rapidly growing germinating seeds. Different protocols for pretreatment were tested and the best result was obtained from 8 mM of 8-hydroxyquinoline in darkness at room temperature for 2.5 h. Sample roots were subsequently washed three times with distilled water (each 5 min) at room temperature. They were then fixed in Carnoy's fixative (glacial acetic acid: ethanol; 3:1) for overnight at 4°C. After thorough washing with distilled water, accesses transferred to 70% (v/v) aqueous ethanol and stored in a refrigerator until use. Hydrolysis was carried out with 1 M HCl for 8-12 min at 60°C. Thereafter, root tips were stained in 2% (w/v) aceto-carmine for 7-8 h at 20°C according to Ostergren and Haneen (1962). The stained root tips were squashed in a drop of 45% (v/v) acetic acid. The slides were frozen in liquid nitrogen to permit coverslip removal and permanently mounted.

At least five well-spread metaphase plates from different individuals were analyzed per population. The best metaphasic plates were photographed, using a camera attached to the BX50 Olympus microscope and then scanned at 200-resolution and loaded in Photoshop 8.0. Chromosome morphology was described using nomenclatures proposed by Levan *et al.* (1964). For numerical characterization, arm ratio (AR) of each pair, relative length of chromosome (RL), ratio between the longest and the shortest chromosome pair (r-value), form percentage (%F) and total form percentage (%TF) were calculated.. AR, RL, r-value, %F and %TF were calculated as follows:

$$AR = \frac{L}{S};\quad RL = \frac{TL}{\sum TL};\quad r-value = \frac{S}{L};$$

$$\%F = \frac{S}{TL}\times 100;\ \%\mathrm{TF} = \frac{\sum \mathrm{S}}{\sum \mathrm{TL}}\times 100$$

Table 1. Names and photoperiod requirement of 12 Iranian local onion populations

Population No.	Native names	Photoperiod requirement
1	White Ghom	Long day
2	Red Azar Shahr	Long day
3	White Kashan	Long day
4	Dorche Isfahan	Long day
5	Gholy Geseh Zanjan	Long day
6	Behdasht Shahrud	Long day
7	Ramhormoz	Medium day
8	Red Ray	Long day
9	White Khomein	Long day
10	Red Neyshabur	Long day
11	White Neyshabur	Long day
12	White Sary	Median day

where *S* and *L* are short and long arms of chromosome , respectively, *TF* is total length of chromosome and %*TF* is total form percentage of chromosome.

The karyotype symmetry classes of Stebbins (1971) were further quantitatively differentiated into finer karyotype evolutionary gradations, the parameter of chromosome-dispersion (Lavania and Srivastava 1999). The values of Dispersion Index (DI) for a given karyotype were estimated from the following equations:

$$CG = \frac{S\bar{x}}{TL\bar{x}} \times 100$$

Where *CG* is the centromeric gradient, $S\bar{x}$ is the length of median short arm and $TL\bar{x}$ is the total length of median chromosome.

$$CV = \frac{SD}{\bar{X}} \times 100$$

Where *SD* is standard deviation, $\bar{X}$ is mean chromosome length and *CV* is coefficient of variation for chromosome length.

DI = Proportionate measure of *CG* with respect to *CV*

Total volume of chromosome (TVC) was estimated, using the following formula:

$$TVC = \pi r^2 \times TL$$

Where π is the ratio of a circle's circumference (=3.14), "r" and TL are the average chromosome radius and total chromosome length, respectively.

To estimate karyotype asymmetry, two numerical parameters were used according to Romero-Zarco (1986) method as follows:

$$A1 = \frac{\sum_{1}^{n} \frac{S\bar{x}}{L\bar{x}}}{n}$$

Where $S\bar{x}$ and $L\bar{x}$ are the mean length of the short and long arms of each pair of homologs, respectively, n is the number of homologs and $A1$ is intrachromosomal index.

$$A2 = \frac{S}{\bar{X}}$$

Where S and $\overline{X}$ are standard deviation and mean chromosome length, respectively and $A2$ is interchromosomal index.

The resultant data were first examined for normality test and then analyzed according to a completely randomized design with five replications of metaphase cells. Tukey's test was carried out for population mean comparisons (Coulaud *et al.* 1999, Guillermo Seijo and Fernandez 2003; Mahdavi and Karimzadeh 2010, Karimzadeh *et al.* 2011). Cluster analysis was performed using Ward's minimum variance method and squared Euclidean distance coefficient. Principal component analysis (PCA) was carried out to differentiate the studied populations based on karyotype parameters (Jolliffe 1986).

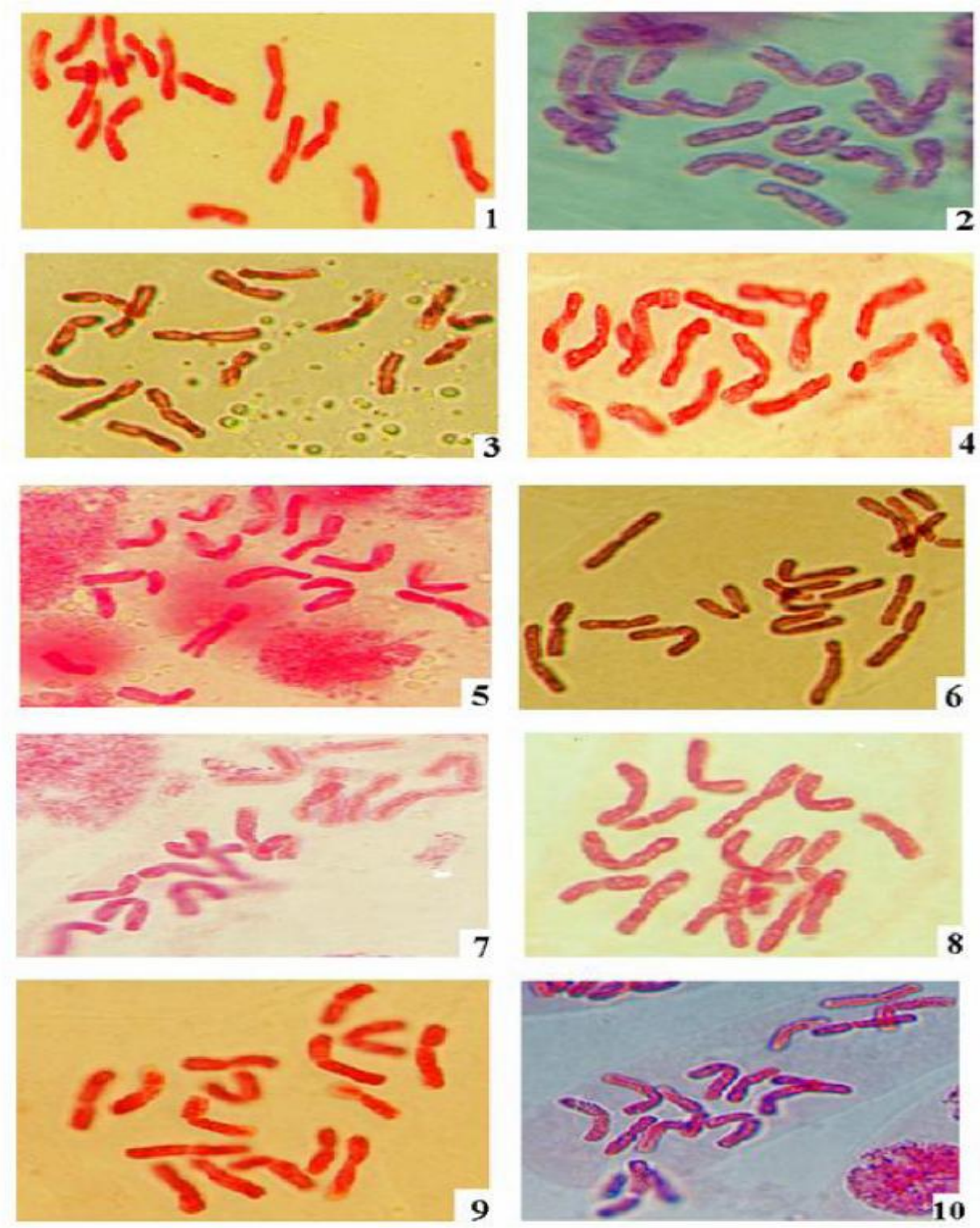
Results and Discussion

Karyotype formula and parameters for 12 Iranian local onion populations are summarized in Table 2. Figure 1 illustrates the mitotic metaphases and Figure 2 demonstrates their

Table 2. Haploid karyotypic parameters measured on the 12 Iranian local onion populations. CV%= coefficient of variation; S%= symmetry index; TF%= total form percentage; DI= dispersion index; ST= Stebbins asymmetry categories; A1= intrachromosomal index; A2= interchromosomal index; KF= karyotype formula; SAT= satellite position; Pop.= population

Pop. No.	CV%	S%	TF%	DI	ST	A1	A2	KF	SAT
1	15.886	59.09	42.46	6.794	1A	0.256	0.159	7m+1sm	2L, 6L
2	14.240	66.36	42.57	6.217	1A	0.253	0.149	7m+1sm	4L, 6lL
3	13.004	66.16	41.93	5.521	1A	0.266	0.123	7m+1sm	6L, 8L
4	12.813	67.04	40.97	5.384	1A	0.304	0.128	6m+2sm	3L, 6L
5	13.794	66.85	42.77	6.009	1A	0.245	0.138	7m+1sm	nd*
6	11.958	68.64	42.34	4.938	1A	0.256	0.120	8m	3L, 6L
7	13.346	67.80	43.36	5.885	2A	0.227	0.133	7m+1sm	2L, 6S
8	13.666	67.41	40.51	5.632	2A	0.317	0.137	6m+2sm	3L
9	14.831	61.94	42.48	6.304	1A	0.245	0.148	7m+1sm	3S, 6L
10	12.758	68.64	43.46	5.357	1A	0.239	0.127	8m	4L, 6L
11	13.904	67.97	42.20	5.794	1A	0.258	0.139	7m+1sm	6L, 7L
12	13.998	65.78	42.58	6.023	2A	0.230	0.140	7m+1sm	6L, 7L

* nd = Not detected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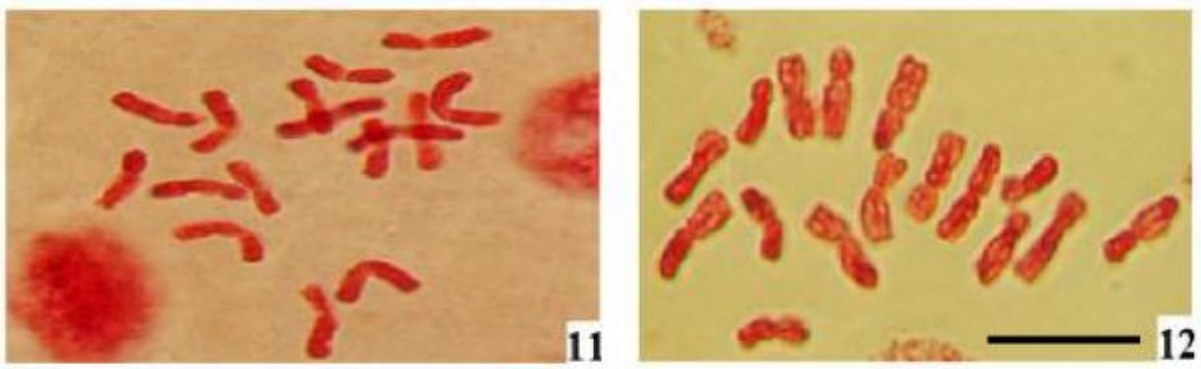

Figure 1. Somatic cells of the studied 12 onion populations (2n=16). Scale bar = 5 µm.
(for population names see Table 1)

respective idiograms. All populations were diploid with 2n=2x=16 chromosomes. The chromosome number (2n=2x=16) identified for Iranian local populations in the present study was in agreement with the number reported by Xingjin *et al.* (2000) and Fritsch and Friesen (2002). In our best knowledge, this is the first published cytological work n Iranian local population.

Analysis of variance indicated significant differences among populations for most of the karyological parameters (Table 3). Means are shown in Table 4. Chromosome variation among populations of the same species has been observed in many plant groups (Maffei *et al.* 1999). Among the populations studied, the highest total chromosomes length, the longest chromosomes, shortest chromosome, centromeric index (CI) and total chromosome length were observed in BehdashtShahrud population (11.97, 6.93, 5.046, 0.442 and 96.65 µm, respectively) and the lowest values were detected in White Khomein. White Sary onion had higher total volume (34.5 μm^3) and White Ghom possessed the largest dispersion index (6.974) among the populations examined. Karyotype asymmetry was determined for all populations in which TF%, S% and Romero-Zarco (1986) indices showed higher degree of symmetry; the karyotypes of BehdashtShahrud population were the most asymmetric. Total F% analysis showed that symmetrical karyotype had median to nearly median chromosome with a moderate fluctuation in TF% values (from 40.51% in Red Ray to 43.46% in Red Neyshabur). The maximum asymmetry value is 50% where all chromosomes are metacentric (Huziwara 1962). The gradual alterations to shifting of TF% values may be due to the chromosomal abnormalities. The structural alterations in chromosome morphology, as well as the variations of secondary constricted chromosomes may be due to chromosome duplication or translocations between chromosomes with or without secondary constrictions at a very early stage of evolution (Das 1991, Das *et al.* 1998, Mohanty *et al.* 2004).

Table 3. Mean squares (MS) for karyotypic parameters of the studied Iranian native onion population**.** L= long arm; S= short arm; TL= total chromosome length; AR= arm ratio (L/S); r-value= (S/L); TCV= total chromosome volume (μm^3); CI= centromeric index, F%= form percentage of chromosome

SOV	df	MS							
		L	S	TL	AR	r-value	TCV	CI	F%
Population	11	1.578***	0.806**	4.470**	0.018**	0.003*	0.215ns	0.0004*	0.027ns
Error	48	0.435	0.271	1.319	0.006	0.001	0.162	0.0001	0.026
CV%		11.0	11.6	11.0	5.7	4.9	7.4	2.9	11.3

ns Not significant at 5% probability level,
*, **, *** Significant at 5%, 1% and 0.1% probability levels, respectively

In our study, the mean chromosome length ranged from 8.54 to 11.97 µm. Haploid genome length varied from 67.79 to 96.65 µm (Table 4). The mean centromeric index in the complement differed from 41.1% to 44.2% (Table 4). DI has been found as a useful parameter to differentiate quantitatively the closely related karyotypes belonging to the same class of symmetry (Lavania and Srivastava 1999). In this study, the higher values of DI indicated higher levels of karyotype specialization. The CV% estimated for the homology of chromosome/chromosome arms, spread over populations, is considered to serve as a general guiding parameter to detect the extent of gradual variation (Table 2). As a whole, karyotypes of the populations examined had predominance of either "m" (centromere at median region) or "sm" (sub-metacentric) chromosome types (Table 2). In other words, the most common haploid formula among Iranian local *Allium cepa* populations was 7m+1sm (eight populations), followed by 6m+2sm, (two populations) and 8m (two populations) (Table 2). Unal *et al.* (1997) with the analysis of *Allium enginii* karyotypes identified 16 somatic chromosomes (2n=2x=16) with total haploid chromosome length of 80.29 µm. The chromosome length was changed from 8.43 to 11.83 µm and the arms ratio was ranged between 1.02 and 1.46. All of the eight haploid chromosomes of *Allium enginii* were metacentrics (Unal *et al.* 1997), while in our study, the *Allium cepa* populations showed differed chromosome types.

Table 4. Means (±S.E.) of karyotypic parameters of the studied Iranian native onion populations. L= long arm; S= short arm; TL= total chromosome length; AR= arm ratio (L/S); r-value= S/L; CI= centromeric index, Pop.= population

Pop. No.	L	S	TL	AR	r-value	CI
1	5.661±0.209^{ab}	4.272±0.173^{ab}	9.933±0.380^{ab}	1.354±0.017^{ab}	0.760±0.006^{ab}	0.429±0.002^{ab}
2	5.675±0.165^{ab}	4.402±0.171^{ab}	10.077±0.330^{ab}	1.313±0.021^{ab}	0.788±0.011^{ab}	0.437±0.004^{a}
3	5.854±0.432^{ab}	4.385±0.383^{ab}	10.239±0.809^{ab}	1.391±0.036^{ab}	0.758±0.017^{ab}	0.426±0.006^{ab}
4	6.266±0.316^{ab}	4.561±0.264^{ab}	10.827±0.577^{ab}	1.450±0.032^{ab}	0.743±0.012^{ab}	0.420±0.004^{ab}
5	6.244±0.269^{ab}	4.777±0.168^{ab}	11.022±0.408^{ab}	1.323±0.045^{ab}	0.781±0.022^{ab}	0.435±0.007^{ab}
6	6.927±0.238^{a}	5.046±0.252^{a}	11.972±0.464^{a}	1.421±0.050^{ab}	0.744±0.021^{ab}	0.421±0.008^{ab}
7	6.172±0.145^{ab}	4.925±0.149^{ab}	11.098±0.262^{ab}	1.285±0.035a^{b}	0.803±0.019^{a}	0.442±0.006^{a}
8	6.415±0.228^{ab}	4.475±0.087^{ab}	10.891±0.274^{ab}	1.500±0.058^{a}	0.714±0.021^{b}	0.411±0.008^{b}
9	4.885±0.211^{b}	3.663±0.140^{b}	8.548±0.340^{ab}	1.384±0.028^{ab}	0.760±0.015^{ab}	0.427±0.005^{ab}
10	6.550±0.527^{ab}	4.876±0.333^{b}	11.426±0.854^{ab}	1.367±0.025^{ab}	0.760±0.017^{ab}	0.428±0.005^{ab}
11	5.671±0.356^{ab}	4.141±0.258^{ab}	9.812±0.612^{ab}	1.124±0.010^{ab}	0.742±0.009^{ab}	0.421±0.002^{ab}
12	5.372±0.188^{ab}	4.0740.237^{ab}	9.446±0.423^{ab}	1.367±0.040^{ab}	0.770±0.017^{ab}	0.430±0.006^{ab}

Means within each column followed by different symbol letters are significantly different at 5% probability level

Stebbins (1971) found that increased karyotype asymmetry is associated with increased morphological specialization. In general, the karyotypes of onion populations in our study were mostly symmetrical and fell in Stebbins 1A category of symmetry, except for Ramhormoz, Red Ray and White Sary populations which fell in 2A category (Table 2). Romero-Zarco (1986) indices permitted the detection of slight differences among populations. These indices (A1 and A2) showed small ranges of between-population variations (Table 1). The karyotype asymmetry of the studied populations was very similar, in particular the values of the A1 intrachromo-somal index parameter, the highest value (0.317) was found in the Red Ray population. The diversity was found in the presence, or in the absence, of chromosomes with satellites on either short or long arm. Satellites were detected in one or two chromosome pairs in the karyotype of onion populations tested. Most of populations possessed a satellite on 6L, except in GholyGesehZanjan in which the satellite was not detected (Table 2, Figure 2).

Genetic relationship among populations was assessed by cluster analysis. Grouping based on Ward's minimum variance algorithm and squared Euclidean distance coefficient assigned the populations into two clusters (Figure 3). Cluster I consisted of nine populations (Dorche Isfahan, holyGesehZanjan,

Ramhormoz, White Ghom, Red AzarShahr, White Khomein, White Neyshabur, Red Ray, RedNeyshabur) and Cluster II contained three populations (White Kashan, BehdashtShahrud, White Sary). This clustering,based on cytogenetic data, showed close relationship between three populations (White Kashan, White Sary, BehdashtShahrud), supporting morphological characteristics of the genus (Paknia *et al*. 2007). These populations appeared to be very similar, locating closely to each other in a single cluster, while other cluster showed to be distant.

The principal component analysis (PCA) based on karyotypic parameters showed that the first two principal components account for the 84.7% of the total variations and they were projected in a two-dimensional graphic (Figure 4). The first component (46.7%) emphasized the position of the centromer, while the second component (38%) accentuated variation in complement length. The arrangement of populations based on PCA was fully agreed with the result of cluster analysis. Differences in karyotype formula and asymmetry indices found among onion populations suggest that structural changes may have contributed to the diversification of the studied populations.

In conclusion, the main object of the present report was to select onion populations with the most homology in chromosomal variations for the purpose of crossing in plant breeding programs. Crosses of Dorche Isfahan, White Ghom, White Khomein, Red Ray and White Kashan populations by GholyGesehZanjan or Ramhormoz, Red AzarShahr, White Neyshabur, RedNeyshabur and White Saryare, therefore suggested for obtaining the higher genetic variation. This type of analysis can help breeders choosing diverse parents for heterosis breeding programs aimed at varietal improvement.

Acknowledgements

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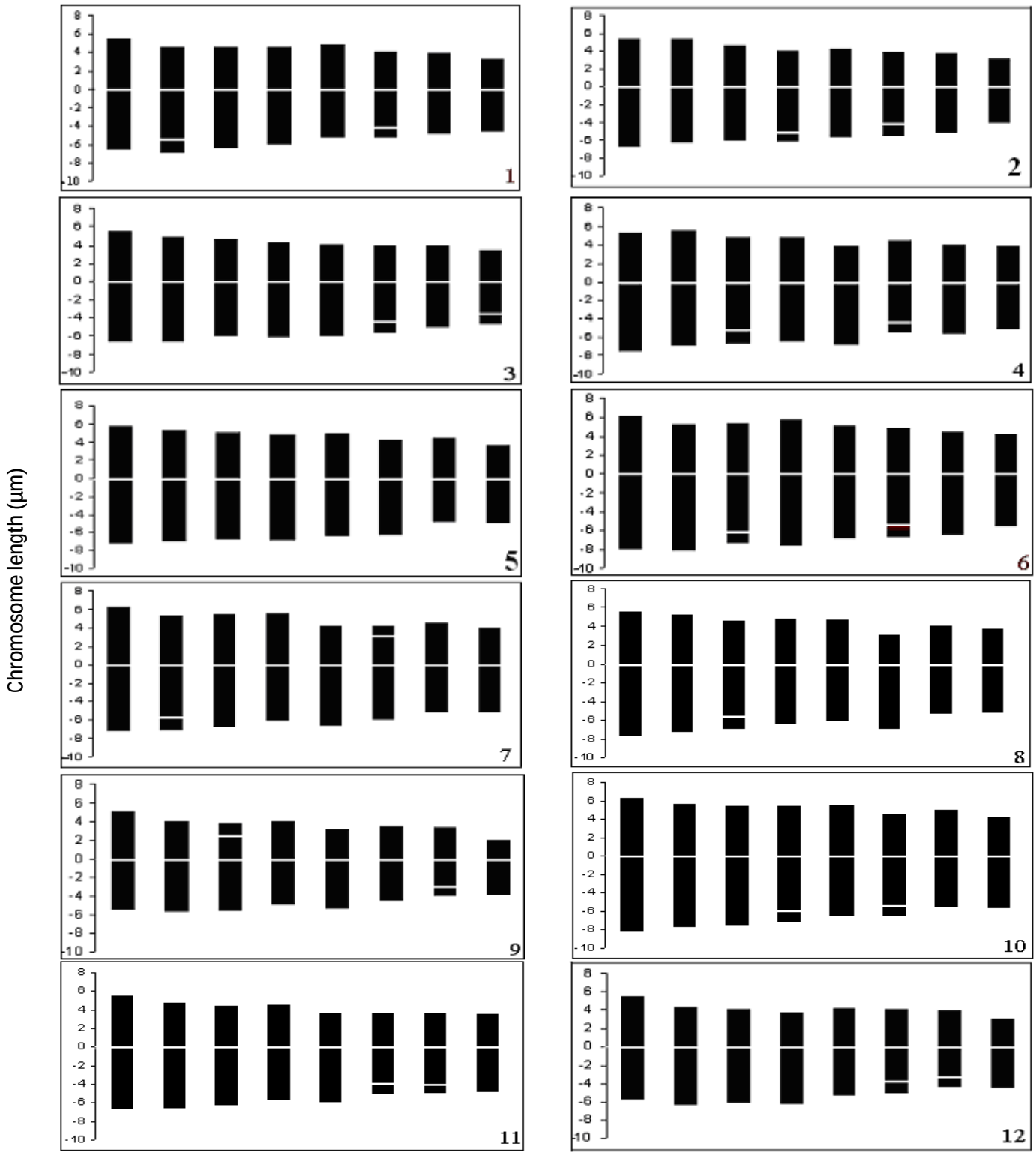

Figure 2. Idiograms of somatic cells of 12 onion populations under study (for population names see Table 1)

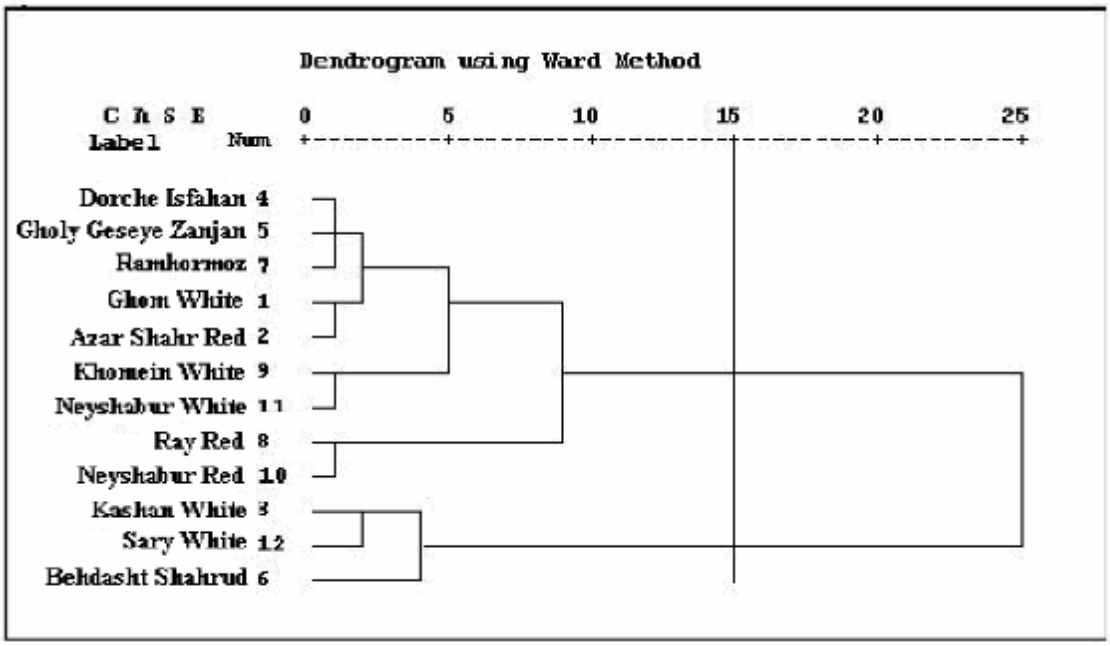

Figure 3. Dendrogram showing the phonetic relationships among the 12 Iranian native onion populations under study, constructed by the Ward's method using the matrix of karyotype distances (Cophenetic correlation coefficient= 0. 654)

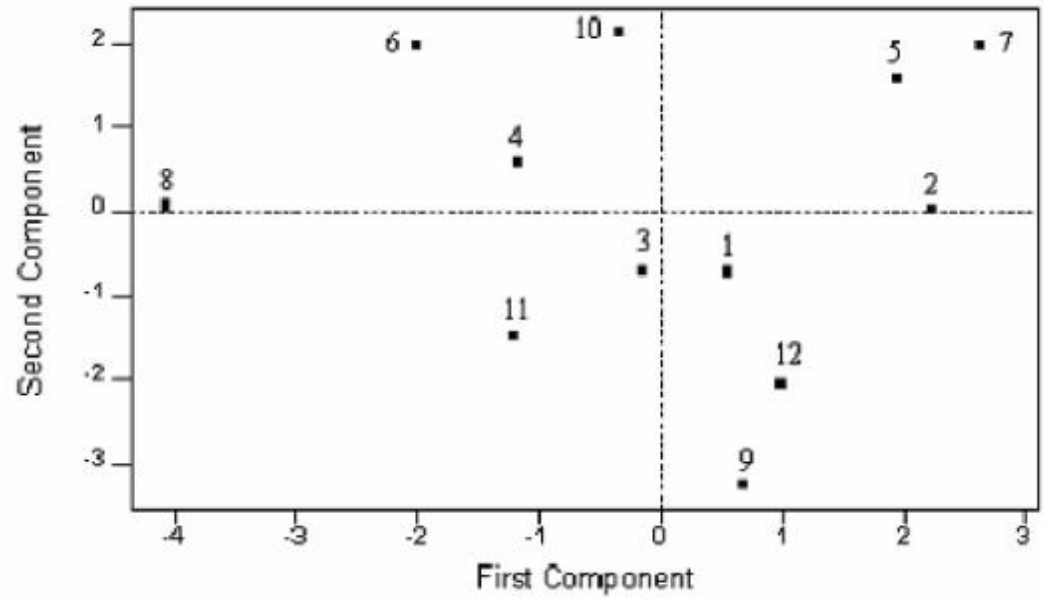

Figure 4. Diagram resulted from the principal component analysis of the studied populations. The first component was highly related with r value and the second was strongly related with the length of complements (for population names see Table 1)

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