

## Karyotype Analysis of several Ecotypes of *Capsicum annuum* L. in Iran

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### Abstract

*Capsicum* (pepper) is a member of the *Solanaceae* family and this genus has a great economic importance in food, drug, spices and industry. In this study, seeds of ten ecotypes of *Capsicum* spp. were obtained from the plant gene bank of Seed and Plant Improvement Institute of Karaj, Iran. The standard karyotype was prepared for the ecotypes and the characteristics of the chromosomes including long arm, short arm, total length (TL), arm ratio and centromeric index were calculated and chromosome types were determined. The number of chromosomes in somatic cells of all genotypes was 24 ( $2n=2x=24$ ). All genotypes had a pair of satellite chromosome. The first 2 principal component analysis (PCA) justified over 99% of the total variations determined for cytological parameters. The highest total haploid length (51.65  $\mu\text{m}$ ) was detected in G7 while G8 demonstrated the least (43.46  $\mu\text{m}$ ). Cluster analysis was carried out for chromosomal parameters, classifying genotypes in three classes.

**Keywords:** ideogram, chromosomal diversity, pepper, Iran

### Introduction

*Capsicum* L. (pepper) is a member of the *Solanaceae* family and is cultivated in temperate and tropical regions (Lippert *et al.*, 1966; Eshbaugh, 1993; Pozzobon *et al.*, 2005). Fruits of this plant are used as spice, vegetable and herbal remedy. *Capsicum* originated in the new world and is under cultivation in different regions of Iran especially in north of the country.

Pepper has important roles in various aspects of economy, food and pharmaceuticals. It has the highest content of vitamin C among all plants and has important medicinal properties such as prevention of heart disease, actuation of blood ambulation and antioxidant characteristics (Salehi, 2006).

*Capsicum* has at least between 20-30 species, from which five of them have become domesticated: *Capsicum annuum*, *C. frutescens*, *C. chinense*, *C. pubescens* and *C. baccatum* (Lippert, 1966; Eshbaugh, 1993; Lanteri, 1993; Pozzobon *et al.*, 2005; De Teodoro-Pardo *et al.*, 2007).

Studies on *Capsicum* species have shown that they contain 24 chromosomes ( $2n=2x=24$ ), similar to many species of *Solanaceae* family. There are two distinct groups present in the genus; some species have 24 chromosomes ( $2n=2x=24$ ) while other species have 26 chromosomes ( $2n=2x=26$ ). The most common chromosome number in the genus is  $x=12$  (Smith and Heiser, 1951; Lippert *et al.*, 1966; Pickersgill, 1971, 1977, 1991; Limaye, 1989; Moscone, 1993; Pozzobon *et al.*, 2005).

Karyotypes in different species with 24 chromosomes are very similar with each other.

The species with 24 chromosomes have symmetrical karyotypes. They generally have one pair of acro-centrics and the rest of the chromosomes are metacentric. In contrast with, the species with 26 chromosomes display more asymmetrical complements, with more sub-metacentric (sub-telocentric) chromosomes and often one telo-centric chromosome (Lanteri, 1993; Moscone *et al.*, 2007; De Teodoro-Pardo, 2007).

Most species of *Capsicum* with 26 chromosomes have been found in South America as wild plants but all domesticated capsicums have 24 chromosomes (Pozzobon *et al.*, 2005).

Researches on *Capsicum* in Iran have been concentrated on investigating the performance of landraces pepper and foreign cultivars, genetic variation of genotypes in the country and improving cultivation techniques.

Karyotypic and cytogenetic studies on the existing genotypes by using appropriate statistical methods for analysis of data could be used for classifying different capsicum ecotypes in terms of affinity with each other. This information could be used to compile the best breeding program. The objective of this experiment was to study the chromosomal diversity and to count the number of chromosomes in the commercially important pepper genotypes in Iran.

### Material and methods

Seeds of ten ecotypes of *Capsicum annuum* were collected from the plant gene bank of Seed and Plant Improvement Institute of Karaj, Iran. The samples were studied and cytogenetic survey was performed. Root tips

were obtained by germinating seeds. Seeds were plated on moisturized filter paper in Petri-dishes and finally germinated at 25°C. When the length of roots reached to 1-2 cm, the root tips were pretreated with 8-hydroxyquinoline 0.002M for two and half hours at room temperature. Samples were fixed simultaneously in 3:1 absolute ethanol: glacial acetic acid. Then, samples were stored in 70% alcohol at 4°C. The root tips were hydrolyzed in 1N HCL for 13 minutes at 60°C. Due to staining the chromosomes, samples were placed in 0.1% Aceto Orcein for more than 48 hours at 4°C. The squashed preparations of root tips were made in 45% acetic acid. For cytological studies, images were taken with a DMC-FX55 Panasonic digital camera. The standard karyotype was prepared for the ecotypes and the parameters of the chromosomes including long length, short length, total length (TL), total arm ratio and centromer index were calculated. Chromosome types were determined according to Levan *et al* (1964).

**Results and discussion**

Chromosome number in all studied genotypes was  $2n=2x=24$  (Fig. 1). The results were similar to other research results that showed domesticated *capsicum*s contain 24 chromosomes (Limaye, 1989; Pozzobon, 2005; De Teodoro-Pardo, 2007; Moscone *et al.*, 2007).

All genotypes had a pair of chromosome with satellite located at the end of short arms. Satellites in different genotypes were detected on chromosome pairs 10, 11 and 12. These chromosomes were often smaller and were identified as sub-metacentric. These results are similar to the results of other researches that have reported the existence of satellites at the end of short arm of sub-metacentric chromosomes in different species of *capsicum* (Pozzobon *et al.*, 2005; De Teodoro-Pardo, 2007). The ideogram of the species is presented in Fig. 2.

Karyotypes in all genotypes were nearly symmetrical and at least one pair of chromosomes in each genotype

were identified as sub-metacentric (Tab. 1). Other studies also showed that the karyotypes of this genus in domesticated species are symmetrical (Lanteri, 1993; Moscone *et al.*, 2007).

Minimum length of chromosomes was observed in the genotype number 4 with 3.62 μm and maximum length of chromosomes belonged to genotype number 7 with 4.30 μm. The total length of chromosomes (genome size) ranged from 43.46 to 51.65 μm. Also in this research average of total length of chromosomes was 3.87 μm (Tab. 2). These findings are similar to other results that showed the total length of chromosomes length in different species of capsicum is about 3 μ (Pozzobon, 2005; De Teodoro-Pardo, 2007).

Research in Mexico on various selections of *Capsicum annuum* has shown many changes in the total length of chromosomes. These changes indicate a very high chromosomal diversity in *capsicum*, even in different genotypes of the same species (De Teodoro-Pardo, 2007).

Principle Component Analysis (PCA) identified that the first component with 68.16% and the second component with 31.71% justified over 99% of the total variations determined for cytological parameters. In first Compo-

Tab. 1. Karyotypic profile of genotypes

Genotype	Chromosome number	Karyotype formula	Satellite location
1	2n=24	10m+2sm	12S
2	2n=24	10m+2sm	11S
3	2n=24	10m+2sm	12S
4	2n=24	10m+2sm	12S
5	2n=24	11m+1sm	10S
6	2n=24	10m+2sm	11S
7	2n=24	11m+1sm	12S
8	2n=24	11m+1sm	12S
9	2n=24	10m+2sm	11S
10	2n=24	10m+2sm	11S

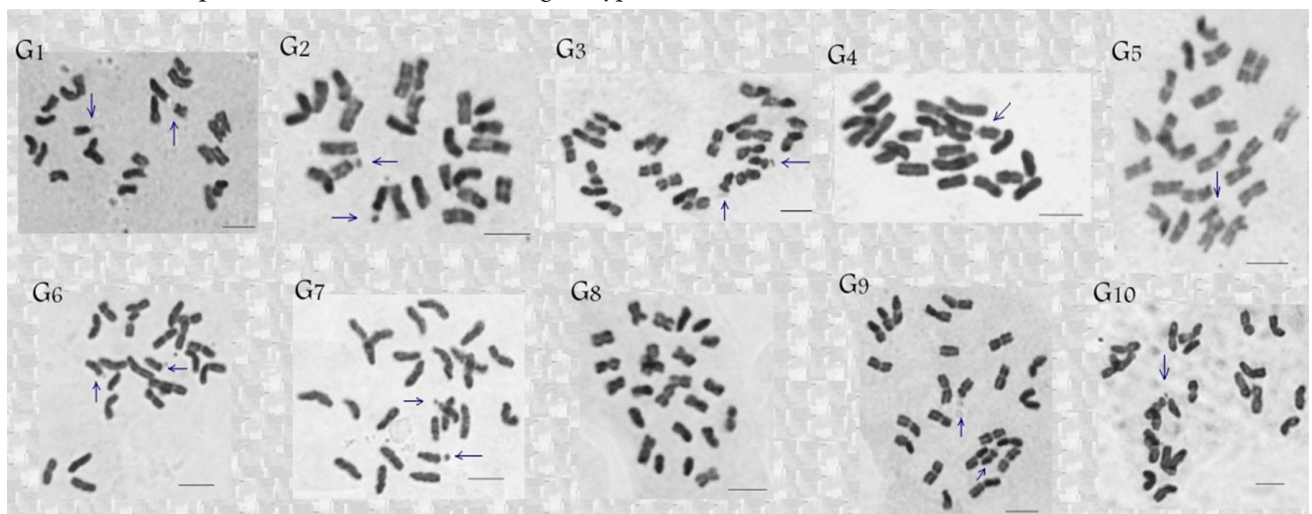


Fig. 1. Mitotic metaphase chromosome in 10 studied genotypes of *capsicum annuum* L. Arrow points to satellited chromosomes. Scale bar = 5 μm

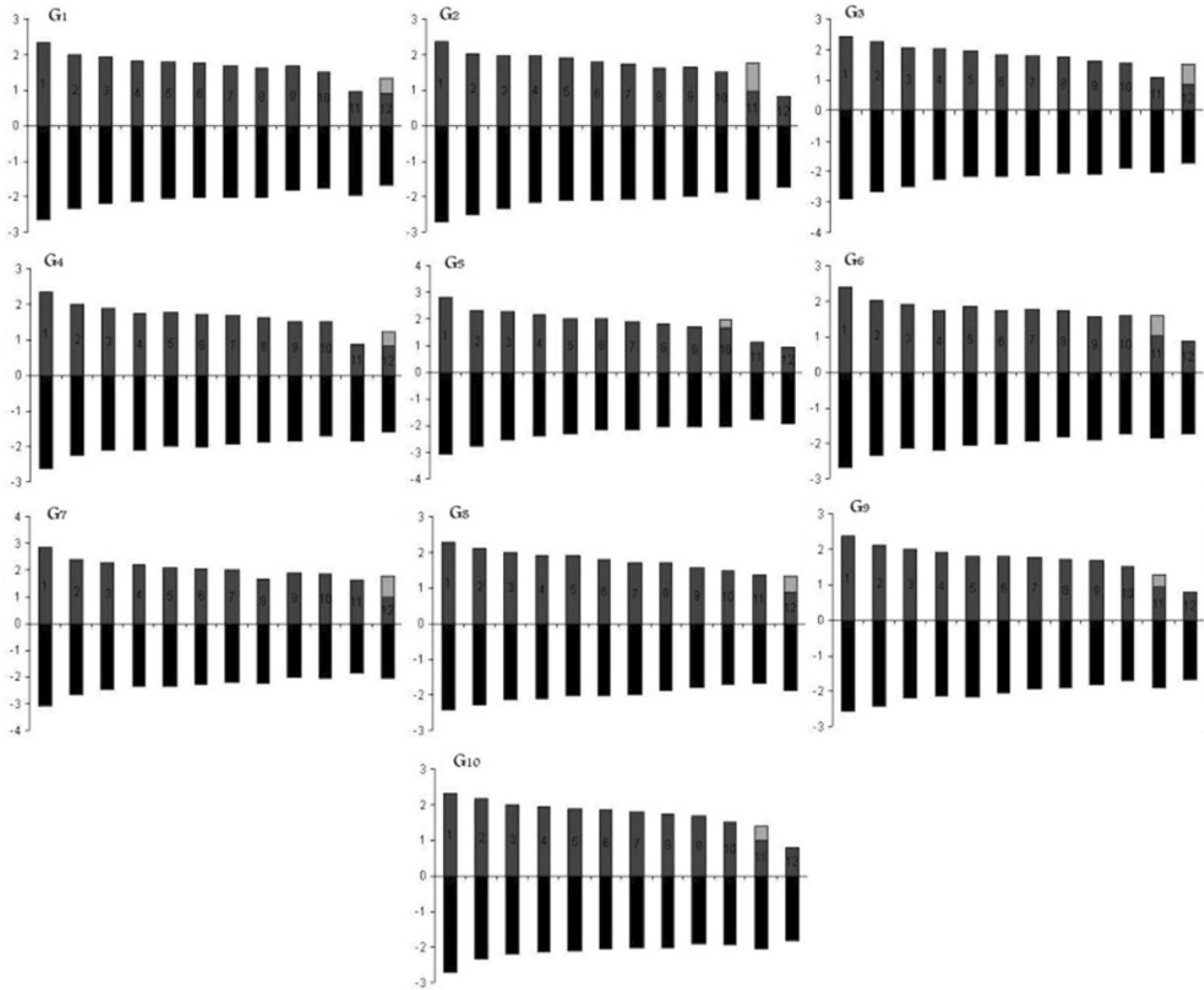


Fig. 2. Ideograms of 10 studied genotypes of *Capsicum annum* L.

Tab. 2. Averages of measured chromosomal traits in the studied genotypes

Genotype	Total length (μm)	Long arm (μm)	Short arm (μm)	Arm ratio	Chromosomes total length (genome size)(μm)	Centromer index
1	3.7284	2.0572	1.6713	1.3019	44.74136	0.4426
2	3.8596	2.1577	1.7019	1.353	46.31557	0.4347
3	3.9868	2.2087	1.7781	1.3128	47.84115	0.44
4	3.6224	2.0001	1.6223	1.3084	43.46864	0.4406
5	4.1907	2.2877	1.903	1.2696	50.28883	0.4484
6	3.7312	2.0371	1.6941	1.265	44.77409	0.4486
7	4.3048	2.3041	2.0007	1.2008	51.65723	0.4604
8	3.7264	1.9963	1.73	1.206	44.71647	0.46
9	3.7474	2.0467	1.7007	1.2855	44.96846	0.447
10	3.8391	2.1101	1.729	1.3025	46.06917	0.4437
Total	3.87368	2.12057	1.75311	1.28055	-	0.4466

gent total length of chromosome and short arm have the most differences and second component showed variation in centromer index and arm ratio. The value of the expression changes showed that these two components can be used for more studies of existing varieties and genotypes. Thus, both the first and the second components were used

for charting the distribution of genotypes and result is presented in Fig. 3.

Cluster analysis with UPGMA method was used on all chromosomal traits for categorizing the studied genotypes. UPGMA dendrogram based on similarity Karyotypic is presented in Fig. 4.

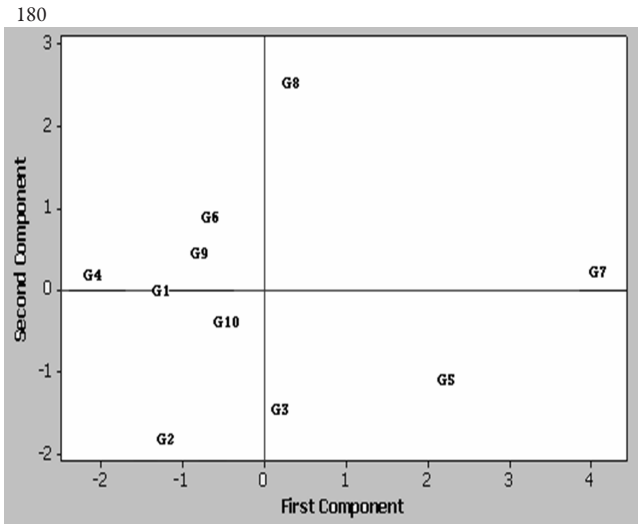


Fig. 3. Plot of the first two components from principle component analysis (PCA) for classification of genotypes

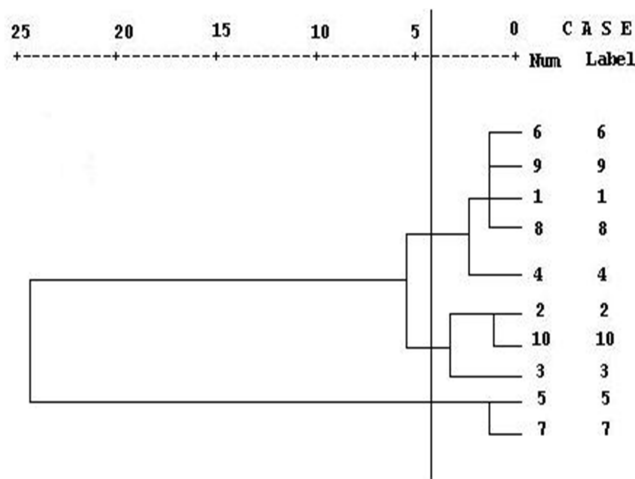


Fig. 4. Dendrogram resulting from UPGMA cluster analysis on the entire chromosomal trait

The first group included genotypes 6, 9, 1, 8, 4 and genotypes 2, 3, 10 were in second group. Genotypes 5 and 7 were classified in category III.

### Conclusions

The present investigation showed that the investigated genotypes of *Capsicum annuum*, all had 24 chromosomes and there was a significant chromosomal diversity between the genotypes which could be used to develop breeding programs for this plant.

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