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Cytogenetical Analysis of Iranian Wild Almond Species

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Abstract

In this study, the karyo-systematic studies on the Iranian wild almond species *A .communis* L., *A. corduchoruom* Bornm., *A. trichamygdalus* woronow, *Amygdalus lycioides* Var. *horrida* Spach were done by the karyological methods. The meristem cells of the root tip were used for these studies. In each species, ten suitable metaphase plates were chosen and photographed so that the morphology of the chromosomes was completely obvious. The standard karyotype was prepared for the species separately and the parameters of the chromosomes, including the total length of the chromosomes, long arm, short arm, arm ratio, and centromer index, were calculated. There was a significant difference between all of the species that can be employed to recognize the species. All of the studied species were diploid, and the numbers of the chromosomes was 2n=16. The base number of the chromosomes in all of the species was X=8. In Iran, the average size of chromosomes in species of this genus was 2.42 micrometer. . Also, there was a significant difference between all of the homologous chromosomes according to the measured cytological characters. The similarity and the difference between the species were evaluated on the basis of the cytological specificities. The domestic species of *A. communis* L. had the most similarity with the species of *Amygdalus lycioides* Var. *horrida* Spach, and the species of *A. trichamygdalus* woronow and *A. corduchoruom* Bornm, also had the most similarity with each other. Finally, the studied species were classified into two cytologically groups.

Keywords: Almond, Breeding, Cytogenetic, Interspecific hybridization, *Prunus*.

Introduction

Almond is from the family of Rosaceae named *Prunus amygdalus* and the sub family of Prunoidae that grows in the semi-arid and steppe areas. The genus of the almond is used with the names of *Prunus* or *Amygdalus*, but due to the holes or the furrow on shell, it is distinguished from the other *Prunus* (*prunuphora* and *cerasus* sections) (Sabeti, 1972). Today, the cytogenetical science plays an important role in the study of the cell nucleus and its contents. Specifically, it influences the review and the counting of the chromosomes and the study of the different levels of ploidy of the plants in classifying the plants (Darlington and Lacour, 1979).

The study of the difference in the numbers of the chromosomes and the ploidy level are used as a control to cross between the species and for the somatic hybrids from the cellular combination (Bauchan and Curley, 1998; Sheidaie *et al*., 2001). By the morphological characters of the chromosomes, we can discover the similarity between the species, which can be used in the breeding programs by combining the genes of the species near to each other (Lewis, 1980; Gostjeva, 2001). Tavakoli *et al*., (2009) presented a short essay related to the cytological study some of the almond species and peach such as *A. communis* L., *A.lycioides* Var. *horrida* Spach, *A. trichamygdalus* Woronow and *P. persica* L.. The results showed that the number of the chromosomes, chromosomes type, the number of satellite and their location were different among species. Also the number of chromosomes for all of study species was 2n=16. Mart´ınez-Gómez *et al*. (2005) presented an essay related to the study of the chromosomes of almond (*A. communis* L.). They showed that the number of the chromosomes of this specie was 2n=16 and the chromosomes were reported as symmetrical of the metacentric type. Schuster and Ahne (1999) presented an essay on the analysis of karyotype related to the *Prunus Avium* L., reporting that the observed numbers of the chromosomes in this species equalled 2n=2x=16. By using the method of banding C, four metacentric pairs and four sub-metacentric pairs were clearly observed. *Prunus* species are characterized by small chromosomes that are difficult to karyotype (Hesse, 1971; Oginuma, 1987; Salesses and Bonherta, 1993; Schuster, 1996).

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Chromosomes are studied by slide preparation and staining have been reported mainly from apricot (*P. armeniaca* L.), peach [*P. persica* (L.) Batsch] (Jelenkovic and Harrington, 1972; Kliphuis and Barkoudah, 1977; Medeira and Warden, 1986; Warden and Medeira, 1986; Salesses and Bonherta, 1993; Yamamoto *et al.*, 1999; Gostjeva, 2001) and almond (*Prunus dulcis* (Mill.) D.A. Webb) (Martínez-Gómez *et al.*, 2005). Houshmand *et al.*, (2009) presented a short report from the karyotype study in *A. scoparia* spach. The number of chromosomes was 2n=2x=16. The karyotypic status was reported with four submetacentric chromosomes, two submetacentric chromosomes, a telocentric chromosome and a chromosome with satellite.

The objective of the present study to cytogenetically analyse the domestic and wild species of almond in Iran and to determine the relationship between them.

Materials and Methods

Plant material

The plant materials include four plant species collected from the different parts of Iran and from each species; ten specimens were chosen to study the cytology that is shown in Table 1.

Seed germination and chromosome preparation

The first step in the cytogenetic studies was to obtain the meristem cells from the new and suitable root tips. Seeds were collected from the different species after planting and germination of the seeds. Their appearing radicle was used to do the cytological studies. After providing the meristems of the root tip from the germinated seeds radicle, they were treated by the solution of 0.002 molar 8-hydroxy quinolin for three hours inside a refrigerator at 4˚C. After extracting the roots from the solution of the pre-treatment, they were placed in the solution of Levitsky (one part chromic acid %1 and one part formaldehyde %10) for 36-48 hours in 4˚C and then washed for three hours under the current water. The hydrolysis on roots was down by hydroxide sodium one normal (Agayev, 1998, 2002) – I don't know what you are trying to say here. Then, the roots were washed with the diluted water for a half an hour. After the hydrolysis stage, aceto_iron _hematoxline was used for staining the roots. They were stained by the method of Agayev (1998, 2002). Then, the specimen was preserved in 30-35 ˚C for 16 hours. The Cytase was used to solve the cells wall for two hours in room temperature. In the stage of the squash, sampels preparation to photography (Agayev, 1998) - I'm not sure what you mean. Finally, the chromosomes measurement and analysis was done using Excel and SPSS softwares.

Results

Chromosome type was used as a key to the distinction of species in terms of centromere location, chromosome length and the number of satellites. The cytological analysis showed that the four species of almond (*Amygdalus spp*) were diploid with 2n=16 chromosome (Fig.1), which correlated to previous reports (Kliphuis and Barkoudah, 1977; Singh *et al.*, 1984; Soodan *et al.*, 1988; Martínez-Gómez *et al.*, 2005; Tavakoli *et al*., 2009). In study of *A.communis* L., from a total of 8 chromosomes (pairs), one chromosome was big. Satellites were available on one chromosome pair and they were located at the end of short arm of chromosome of number two (Fig.1). Also, number four and seven of chromosomes and the other chromosomes were sub-metacentric and metacentric, respectively (Table 1). However, the chromosome complement of this species was reported as symmetric with a predominance of metacentric chromosomes (Martínez-Gómez *et al.*, 2005). The range of chromosome size was between 1.51 to 2.68 micrometer. The average of total length of chromosomes was 1.92 micrometer, and the average of arms ratio in this species estimated 1.39 micrometer (Table 1). In study of *Amygdalus lycioides* Var. *horrida* Spach from a total of 8 chromosomes, one chromosome and two chromosomes were observed big and small, respectively. Satellites were available on one chromosome and they were located at the end of short arm of chromosome number two (Fig. 1). Also, the number of four chromosome was sub metacentric and the other chromosomes were metacentric (Table 1). Range of chromosomes size was 1.82 - 3.03 micrometers. The average total length of the chromosomes was 2.26 micrometer, and the average of arms ratio in this species estimated 1.33 micrometer (Table 1). In study of *A. trichamygdalus* woronow from the total of 8 chromosomes, one of big chromosome and one of small chromosome were observed. Satellites were available on two chromosomes, and they were located at the end of short arm of chromosome of number two and four (Fig. 1). Also, eight homolog chromosomes were sub metacentric, and the other chromosomes were metacentric (Table 1). The range of the chromosome size was 1.89 - 4.70 micrometer. The average of total length chromosomes was 2.92 micrometer, and the average of arms ratio in this species estimated 1.40 micrometer (Table 1). In study of *A. corduchoruom* Bornm from a total of 8 chromosomes, one of big chromosome and one of small chromosome were observed. Satellites were available on two chromosome and they were located at the end of short arm of chromosome of number two and three (Fig. 1). Also, six chromosomes were sub-metacentric and the other chromosomes were metacentric (Table 1). The range of chromosomes size was 1.86 - 3.73 micrometers. The average of total length chromosomes was 2.58 micrometers and the average of arms ratio in this species estimated 1.37 micrometer (Table 1).

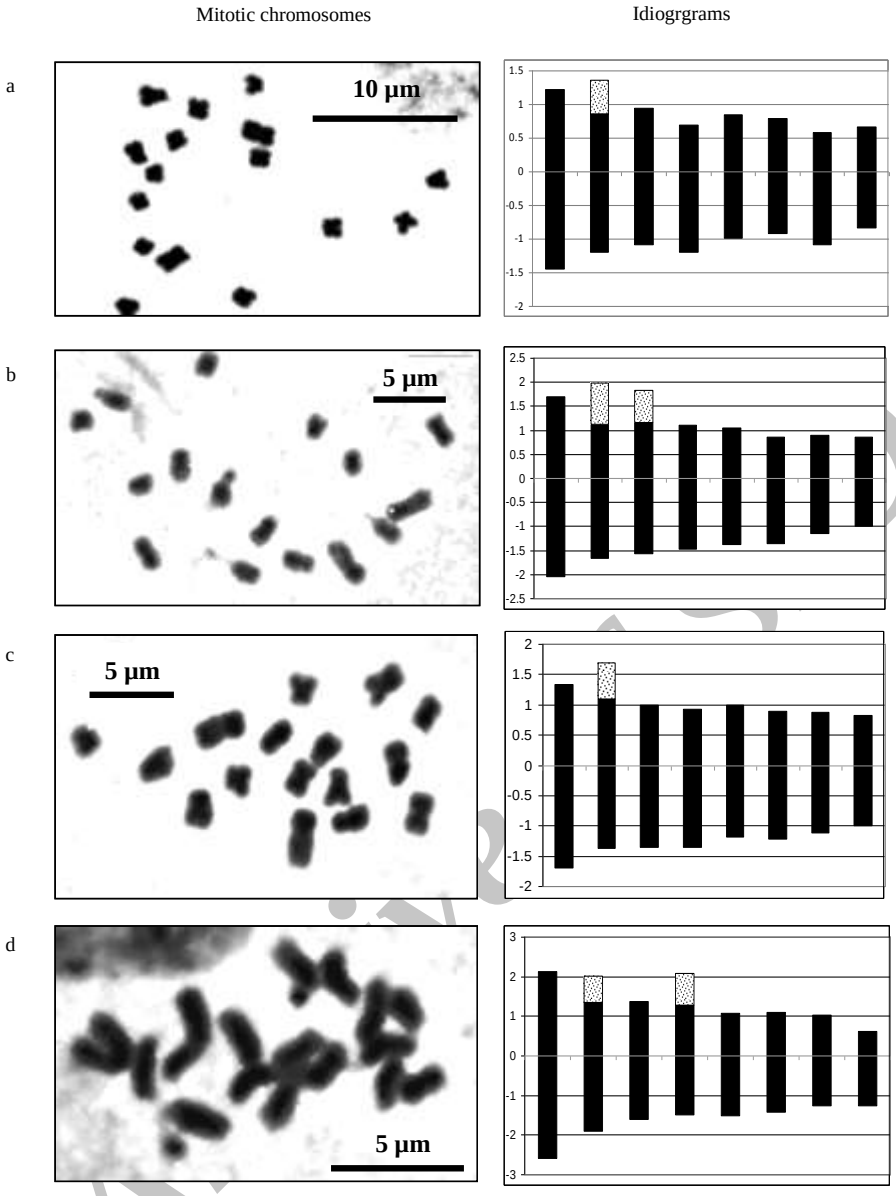

Fig. 1. Metaphasic plaques showing the mitotic chromosoms and idiograms of the four wild almond species studied: a) *Amygdalus communis* L., b) *A. corduchoruom* Bornm, C) *A. trichamygdalus* Woronow, and d) *A. lycioides* Var. horrida Spach.

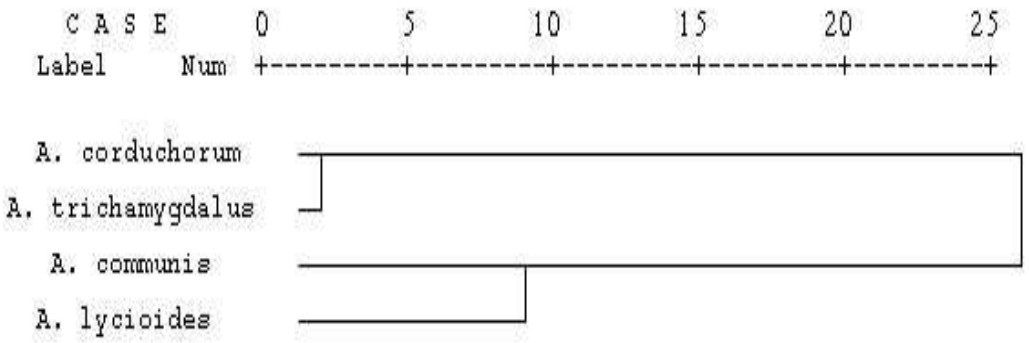

Fig. 2. Dendrogram showing the phylogenetic relationships of the studied wild species (*Amygdalus communis*, *A. corduchoruom* Bornm, *A. trichamygdalus* and *A. lycioides*) based on Ward method.

Table 1. Origin, pedigree and main agronomic characteristics of the 4 almond cultivars assayed

Specie	Local name	Somatic Number	Karyotipic Description[1]	Lengh Range	Morphometric parameters of the karyotypes [2]						
					Haploid complement	Long Arm (L)	Short Arm (S)	Ratio (L/S)	CI	L%	S%
A. communis	Sweet almond	2n=16	10m+2m(sc)+4sm	1.51-2.68	15.36	1.92	1.09	1.39	42.82	56.77	43.2
A. lycioides	Spinous almond	2n=16	12m+2m(sc)+2sm	1.82-3.03	18.08	2.26	1.28	1.33	43.69	56.64	43.36
A. trichamygdalus	Maknali almond	2n=16	10m+4m(sc)+2sm	1.89-4.7	23.36	2.92	1.62	1.4	42.12	55.48	44.52
A. corduchoruom	Ravandozi almond	2n=16	10m+4m(sc)+2sm	1.86-3.73	20.64	2.58	1.45	1.37	42.60	54.06	20.64

[1] m (metaphasic), sm (submetaphasic), sc (secondary constriction

[2] L/S= Largest/shortest chromosome;CI= Centromeric Index; L%= long arm percent; S%= short arm percent

Discussion

The analysis compared the total length of the chromosomes, long arm, short arm, and arm ratio and centromere index of the studied species. Table 1 show that the species of *A. trichamygdalu*s woronow and *A. corduchoruom* Bornm were in a subset, and the total length of chromosome and the centromere index were also arranged near each other. Cytotaxonomical research can be a key to proximity determining among species. As for the important role in linked almond root stock with domestic species, the similarity between the two species mentioned indicates that a similar gene is shared. Thus, A. orientalis Duh can be a base suitable for connection with any domestic specie (*A. communis*).

A. lycioides and *A. communis* L. are not in one group. However, traits such as the total length of chromosomes, the long arm, the short arm and the centromere index were near each other, which showed the chromosomal similarity between these two species. By comparing the total length of the chromosome, the long arm length ,the short arm length, it was found that the two species had the shorter chromosome size according to the above traits relative to the species of the another group. On the other hand, *A.communis* L. is domestic specie. It is likely that *Amygdalus lycioides* Var. *horrida* Spach is an ancestor of *A.communis* L. since in both species, the chromosome pair of number 2 is placed as a satellite. Also, in each of the two species, one sub- metacentric chromosome pair was shown on the chromosome pair number four (Table 1).

The method of cluster analysis was used to study species in relation to the total length of chromosome, the long arm, the short arm, the arms ratio and the centromere index. The cluster analysis from four species is shown in the Fig. 2.

Dendrogram from the cluster analysis was obtained using Ward's method. This method detected that the studied species were separated in two different groups. *A.communis* and *A. lycioides* Var. *horrida* Spach were placed in the first group. The species of *A.corduchoruom* Bornm and *A. trichamygdalus* woronow were placed in the second group.

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