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Karyosystematic Study on Some Almond and Peach Species Grown in Iran

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Abstract: Almond belongs to the genus *Prunus* from the family of *Rosaceae* with about 20 species in the forms of tree, spinous and spinousless shrub. One of the most important economically species is sweet almond. To determine the relationship between three wild and domestic species of almond (*Amygdalus communis*, *A. trichamygdalus* woronow, *A. lycioides* spach) and peach (*Prunus persica* L), the karyosystematic studies were carried out using karyological methods. The root meristem cells were used in this study. For chromosome morphological studies, ten suitable metaphase plates were chosen in each sample and photographed. Also, the standard karyotype was prepared separately for the species as well as population and different chromosomes parameters including the complete length of the chromosomes, the long and short arms length, the arms relative length (AR), % TF (Total Form Percentage) and the chromosomes relative length were measured. There was a significant difference among all of the identified species. It was determined that all the species were diploid and their chromosome numbers were $2n=16$. The mean chromosomes size in Iranian samples of this species was about $2.43 \pm 0.03 \mu$. Also there was a significant difference among all of the homologous chromosomes due to the measured cytological characters. Similarity and difference among the species evaluated based on cytological parameters including the chromosomes length, the long arm and short arm length, the arms relative length and the chromosomes index. Statistical analysis showed that *A. communis* had the most similarity with *A. lycioides* and the species *A. trichamygdalus* and *P. persica* had the most similarity with each other. All studied species were cytologically classified into three groups.

Key words: Almond, Peach, *Prunus*, Cytogenetic, Chromosome parameters, Taxonomy

İran`da Yetiştirilen Bazı Badem ve Şeftali Türleri Üzerinde Karyosistematik Araştırma

Özet: *Rosaceae* familyasının *Prunus* cinsine ait olan badem, ağaç, sivri ve sivri olmayan çalı şeklinde yaklaşık 20 türden oluşmaktadır. Bu türler içerisinde ekonomik öneme sahip en önemli tür tatlı bademdir. Bademin 3 yabani ve yerli türü (*Amygdalus communis*, *A. trichamygdalus* woronow, *A. lycioides* spach) ile şeftali (*Prunus persica* L) arasındaki ilişkileri belirlemek amacıyla karyosistematik çalışmalar karyolojik metotlar kullanılarak gerçekleştirilmiştir. Bu çalışmada, kök meristem hücreleri kullanılmıştır. Kromozom morfolojisi araştırması için her bir örnekten 10 metafaz sayfası seçilerek fotoğraflanmıştır. Ayrıca, popülasyonun yanı sıra, türler içinde ayrı standart karyotip hazırlanmıştır. Denemede kromozom tam uzunluğu, uzun ve kısa kol boyu, kollara bağlı uzunluk (AR), % TF (Total Form Percentage) ve kromozoma bağlı uzunluk gibi farklı kromozom parametreleri ölçülmüştür. Sonuçlar, tanımlanan türler arasında anlamlı bir farklılığı olduğunu göstermektedir. Tüm türlerin diploid ve kromozom sayılarının $2n=16$ olduğu belirlenmiştir. Bu türlerin İran örneklerinde ortalama kromozom boyu $2.43 \pm 0.03 \mu$ olmuştur. Ayrıca tüm homolog kromozomlar arasında ölçülen sitolojik özelliklerden dolayı anlamlı farklılıklar oluşmuştur. Türler arasındaki benzerlik ve farklılıklar, kromozom boyu, uzun ve kısa kol boyu, kollara bağlı uzunluk ve kromozom indeksi gibi sitolojik parametrelere göre değerlendirilmiştir. İstatistik analiz sonuçları en fazla benzerliğin *A. communis* ile *A. lycioides* türleri arasında olduğunu göstermiştir. Aynı şekilde *A. trichamygdalus* ile *P. persica* arasında da benzerliğin olduğu tespit edilmiştir. Değerlendirilen tüm türler sitolojik olarak 3 grupta sınıflandırılmıştır.

Anahtar kelimeler: Badem, Şeftali, *Prunus*, Sitogenetik, Kromozom parametreleri, Taksonomi

Introduction

Almond and peach species classified under the *Amygdalus* subgenus within the *Prunus* genus and belong to the *Prunoideae* subfamily from the *Rosaceae* which distinguished from other subgenera (*Prunophora* and *Cerasus* sections) by the corrugated seed shell (Hesse, 1971). Almond has many wild species and more than 30 species recognized in four geographical areas in the southwest of central Asia from Turkey and Syria to the mountains of Indochina and Afghanistan. Some of important Iranian almond species include *A. elaeagnifolia*, *A. scoparea*, *A. horriba*, *A. eburnea*, *A. glauca*, *A. reticulata*, *A. hussknechti*, *A. urmiensis*, *A. lycioides* (Sabeti 1972). Cytogenetical studies are important methods in different plant taxonomy using study of the cell nucleus and its contents, specially by studying and counting the chromosomes as well as ploidy levels determination (Darlington and Lacour 1979). The study of the difference in the numbers of the chromosomes and the ploidy level are used as a control for crossing between the species and for the somatic hybrids from the cellular combination (Bauchan and Curly 1998). Using chromosomes morphological characters, we can find the similarities between the species and these similarities can be used in breeding programs by combining the genes of the related species (Lewis, 1980; Gostjeva 2001). Cytogenetical studies have several roles including obtaining quantitative information on the plants evolutionary history and also the similarities among the species (Sheidaie *et al.* 2001). *Prunus* species are characterized by small chromosomes which are difficult for karyotype analysis (Hesse 1971; Oginuma 1987; Saleesses and Bonherta 1993; Schuster 1996). Most of chromosome studies in this genus carried out mainly on apricot (*P. armeniaca* L.) and peach [*P. persica* (L.) Batsch] (Jelenkovic and Harrington 1972; Kliphuis and Barkoudah 1977; Medeira and Warden 1986; Warden and Medeira 1986; Saleesses and Bonherta 1993; Yamamoto *et al.* 1999; Gostjeva 2001) as well as almond [*Prunus dulcis* (Mill.) D.A. Webb] (Martinez-Gomez *et al.* 2005) using slide preparation and staining method. Schuster and Ahne (1999) studied a kayotyping analysis on *Prunus avium* L. and observed that the number of the chromosomes in this species were $2n=2x=16$. Also using method of C banding, they found four metacentric pairs and four sub-metacentric pairs arrange in this species. Martinez-Gomez *et al.* (2005) studied on chromosomes of almond (*Prunus communis*) and showed that the chromosomes numbers of this species were $2n=16$ and the chromosomes were symmetric metacentric type. Aim of this study is to determine relationship among three wild and domestic almond species as well as peach by using karyological methods.

Materials and Methods

The plant materials including four plant species were collected from different regions of Iran and ten samples were selected from each species for cytological studies. Plant species, their local names as well as sampling regions were listed in Table 1.

Table 1: Plant species, their local names and sampling regions.

Plant Species	Local Name	Sampling Region
<i>Amygdalus communis</i>	Sweet almond, Common almond, Tangras, the spinous almond	Arasbaran, Fereydonshahr, Isfahan, Urmia, Marivan, Maragheh, Salmas
<i>A. lycioides-sub horrida</i>	Makhmali almond	Ghazvin, Shahroud, Karaj, Neyshaboor, Alborz, Sabzevar, Sanandaj, Azarbayjan
<i>A. trichamygdalus</i>	Holou	Isfahan, Sabzevar, Kordestan, Maragheh
<i>Prunus persica</i>	Holou	Karaj, Ghazvin, Sanandaj, Saggez, Khoy

The root meristem cells were used in this study. For this purpose, seeds of different species were collected, cultured and after germination their radicles were used for cytological studies. These root meristem cells were treated by 0.002M of 8-hydroxy quinolin solution for 3 hours at 4°C. Then cells were placed in the solution of Lewitsky (1:1 of chromic acid %1 and formaldehyde %10) for 36-48 hours at 4°C, and then washed for 3 hours under the current water and roots were hydrolyzed by hydroxide sodium (1N) solution (Agayev, 1998, 2002). Roots were washed with diluted water for 1.5 hours and stained with aceto iron hematoxiline using Agayev (1998, 2002) method. After staining, samples were kept in 30-35°C for 16 hours and then Cytase was used to solve the cell walls for 2 hours in room temperature. Finally, after squashing, for chromosome morphological studies, ten suitable metaphase plates were chosen in each sample and photographed microscopically (Agayev, 1998). Also, the standard karyotype was

prepared separately for the species as well as population and different chromosomes parameters including the complete length of the chromosomes, the long arm length, the short arm length, the arms relative length (AR), % TF (Total Form Percentage) and the chromosomes relative length were measured.

Results and Discussions

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 four species of almond (*Amygdalus spp*) were diploid with $2n=16$ chromosome (Table 2 and Fig. 1), as reported in previous studies (Kliphuis and Barkoudah, 1977; Singh *et al.*, 1984; Soodan *et al.*, 1988). In species *A. communis*, one chromosome pair was bigger than others (totally 8 chromosome pairs). Satellites were available on one chromosome pair and located at the end of short arm in chromosome pair number two (Fig. 2). Also, the homolog chromosomes number four and seven as well as the other chromosomes were sub-metacentric and metacentric, respectively (Table 2), although chromosome complement of this species was reported as symmetric with a predominance of metacentric chromosomes (Martinez-Gomez *et al.*, 2005). The range of chromosome size was $1.51\text{-}2.68\mu$. The average of haploid chromosomes total length and the average of arms ratio in this species were 1.92μ and 1.39μ , respectively (Table 3). In the species *A. lycioides-sub horrida*, it was found that one chromosome pair was smaller and two chromosome pairs were bigger than others (totally 8 chromosome pairs). Satellites are available on one chromosome pair and located at the end of short arm in chromosome pair number two (Fig. 2). Also the homolog chromosome number four was sub-metacentric while other chromosomes were metacentric (Table 2). The range of chromosome size was $1.82\text{-}3.03\mu$. The average of haploid chromosomes total length and the average of arms ratio in this species were 2.26μ and 1.33μ , respectively (Table 3). In the species *A. trichamygdalus*, one chromosome pair was bigger and one chromosome pair was smaller than others (totally 8 chromosome pairs). Satellites are available on two chromosome pairs and located at the end of short arm in chromosome pairs number two and four (Fig. 2). Also eight homolog chromosomes were sub metacentric and the others were metacentric (Table 2). The range of chromosome size was $1.89\text{-}4.70\mu$. The average of haploid chromosomes total length and the average of arms ratio in this species were 2.92μ and 1.40μ , respectively (Table 3). In the species *Prunus persica*, one chromosome pair was bigger and one chromosome pair was smaller than others (totally 8 chromosome pairs). Satellites were available on one chromosome pair and located at the end of short arm in chromosome pair number two (Fig. 2). The homolog chromosome number six was sub metacentric while the others were metacentric (Table 2). The range of chromosome size was $1.83\text{-}3.58\mu$. The average of haploid chromosomes total length and the average of arms ratio in this species were 2.49μ and 1.44μ , respectively (Table 3).

Table 2. Chromosome numbers and Karyotype description of almond species

Species	Somatic Number	Karyotype Description	Length Range (μ)
<i>A. communis</i>	$2n=2x=16$	$10m+2m(sc)+4sm$	1.51-2.68
<i>A. lycioides-sub horrida</i>	$2n=2x=16$	$12m+2m(sc)+2sm$	1.82-3.03
<i>A. trichamygdalus</i>	$2n=2x=16$	$10m+4m(sc)+2sm$	1.89-4.7
<i>Prunus persica</i>	$2n=2x=16$	$10m+2m(sc)+2sm$	1.83 -3.58

Table 3. Morphometric parameters of almond species karyotypes

Species	haploid complement	Long Arm (μ)	Short Arm (μ)	Arm Ratio (L/S)	CI	L%	S%
<i>A. communis</i>	15.36	1.92	1.09	1.39	42.82	56.77	43.2
<i>A. lycioides-sub horrida</i>	18.08	2.26	1.28	1.33	43.69	56.64	43.36
<i>A. trichamygdalus</i>	23.36	2.92	1.62	1.4	42.12	55.48	44.52
<i>Prunus persica</i>	19.92	2.49	1.43	1.44	42.28	57.43	42.57

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

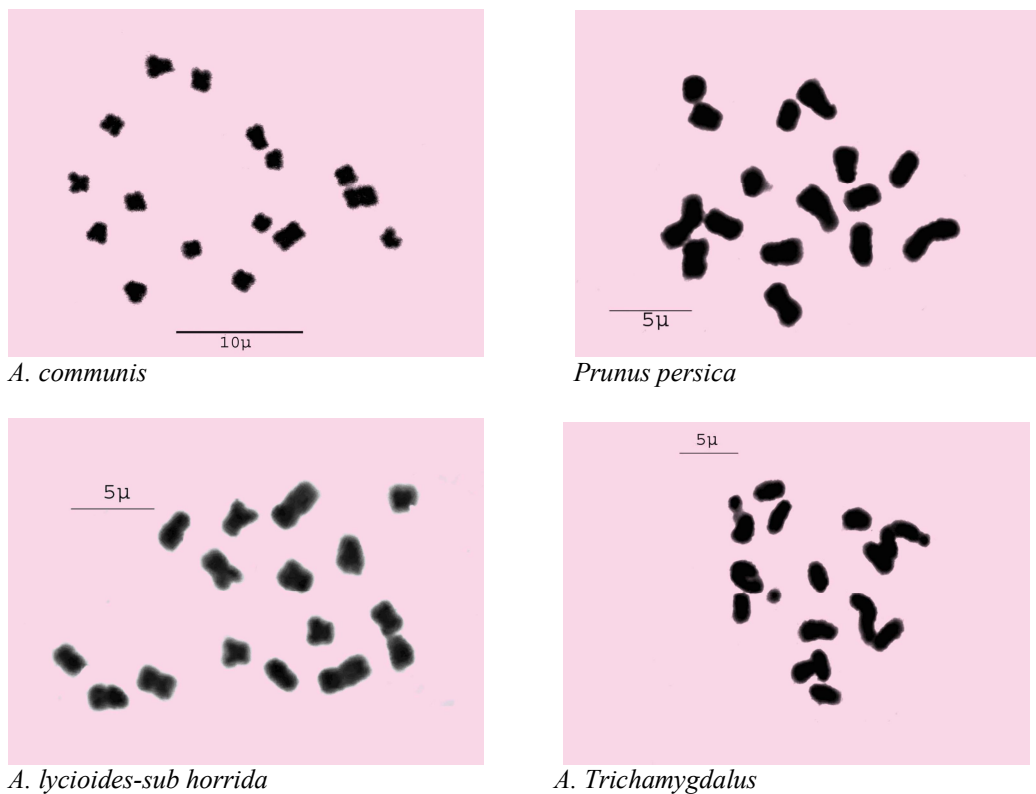


Figure 1. Mitotic metaphase of *A. communis*, *A. trichamygdalus*, *A. lycioides-sub horrida* and *Prunus persica*.

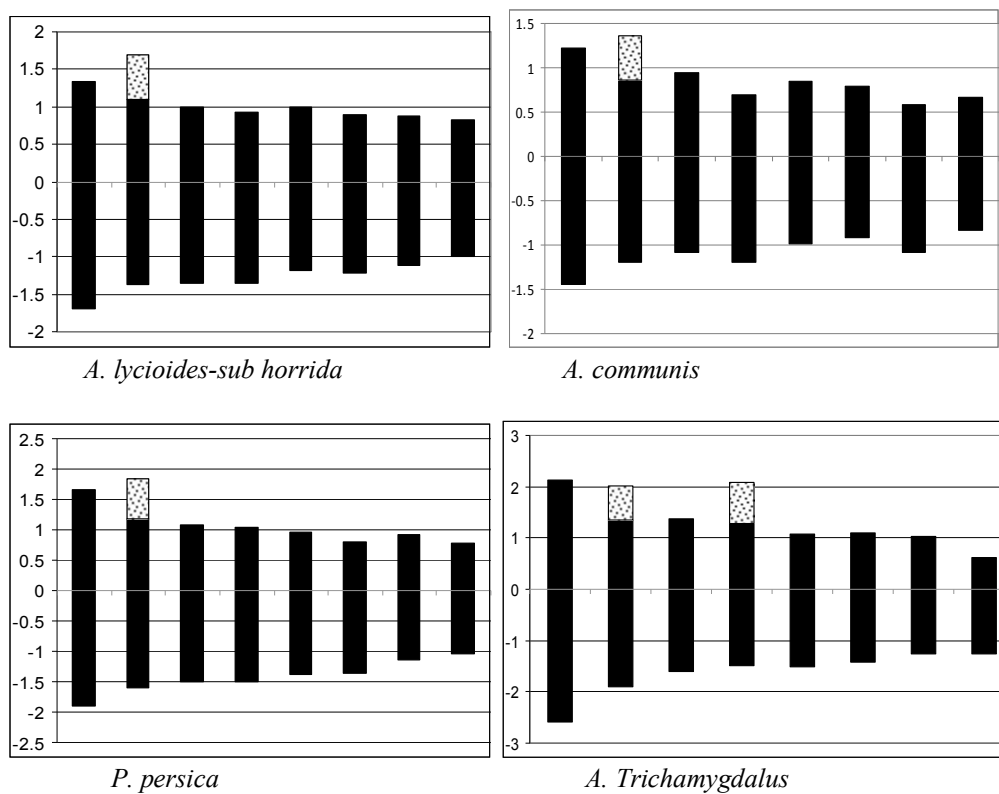


Figure 2. Mitotic metaphase idiogram of *A. communis*, *A. trichamygdalus*, *A. lycioides-sub horrida* and *Prunus persica*

The results of some chromosomes parameters including the whole chromosome length, the long arm and short arm length, the arms ratio (L/S) and centromeric index for studied species based on averages comparison and Duncan multiple range test (Table 4) showed that the long arm and short arm length, the whole chromosome length and the centromeric index had statistically similarity between *P. persica* and *A. trichamygdalus* species. *P. persica* and *A. trichamygdalus* species had a sub-metacentric homolog chromosome in number six and number eight chromosomes pair, respectively. *A. lycioides-sub horrida* and *A. communis* were not grouped each other, but the most parameters such as the chromosome total length, the long arm and short arm length and also the centromeric index were near each other which showed the chromosomal similarity between these two species. By comparing the total length of the chromosomes, the long arm and short arm length, it was found that these parameters were less in these two species rather than *P. persica* and *A. trichamygdalus* species. *A. communis* is a domestic species and it is supposed that *A. lycioides-sub horrida* was the ancestor of *A. communis* since in both species satellites are available on one chromosome pair and located at the end of short arm in chromosome pair number two. Also, in both species, one sub-metacentric chromosome pair was on the chromosomes pair number four.

Table 4. Comparison of almond species based on chromosome traits

Species	Total length (μ)	Long Arm (μ)	Short Arm (μ)	Arm Ratio	CI
<i>A. communis</i>	1.9182 a	1.0923 a	.8259 a	1.3259 a	42.1174 a
<i>A. lycioides-sub horrida</i>	2.2631 b	1.2768 b	.9863 b	1.3653 a	42.2798 a
<i>Prunus persica</i>	2.4859 c	1.4274 c	1.0585 c	1.3949 a	42.5968 a
<i>A. trichamygdalus</i>	2.9196 c	1.6236 c	1.2410 c	1.4402 a	43.6880 a

For better grouping of species based on chromosome parameters (the chromosome length, the long arm and short arm length, the arms ratio and centromeric index), cluster analysis was done using ward method (Fig. 3). Species could be classified in three groups. *A. communis* and *A. lycioides-sub horrida* placed separately in one cluster and two other species *P. persica* and *A. trichamygdalus* placed in a common cluster.

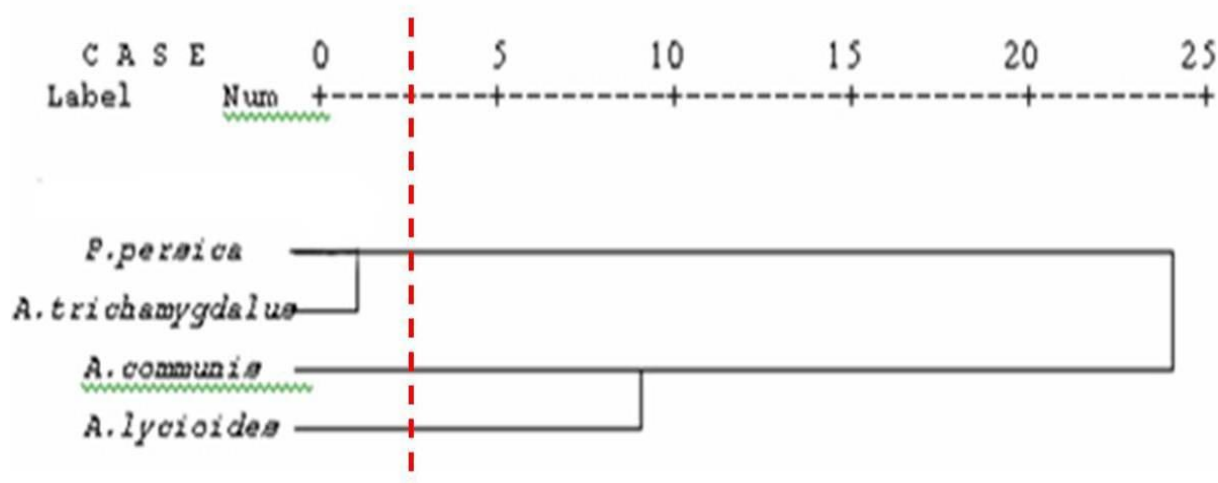


Figure 3. Cluster analysis of almond species based on ward method

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