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Chromosome Numbers and Karyotypes of Four Species *Rubus* L. from North of Iran

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ABSTRACT

Karyotype study was performed across 7 populations of 4 *Rubus* species growing in Iran. The results showed that the chromosome numbers of *R. sanctus* (2n=2x=14) diploid, *R. caesius,* and *R. discolor* were (2n=4x=28) tetraploids, *R. persicus* (2n=8x=56) octoploid. The chromosome numbers of *R. discolor*, and *R. persicus* were reported here for the first time. Karyotypic formula also varied between the studied species, 5m+sm+t (Joybar population) & 6m+t (Babolsar population) in *R. sanctus,* 5m+2t in *R. caesius & R. discolor*, 4m+3t in *R. persicus*. In addition, the four studied species showed intrachromosomal (A1; range = 0.38-0.58), and inter-chromosomal (A2; range = 0.10-0.14) asymmetry and they were segregated into 3A (*R. sanctus*), 3B (*R. caesius*), 2C (*R. discolor*) and 3C (*R. persicus*) karyotypic symmetry classes. Total form percentage and the asymmetry indices, suggesting changes in the chromosome structure of *Rubus* species, occurred during their diversification. The chromosome number ranged from 2x to 8x in the studied species; it also confirmed that polyploidy within this genus cytogenetic phenomenon caused this variability in this genus.

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Introduction

Raspberries (*Rubus* L.) belonging to *Rosaceae* family, subfamily *Rosoideae* includes 750 species (Robertson, 1974; Lu, 1983; Gu *et al*., 1993; Thompson, 1995). This genus is distributed on all continents, with the exception of Antarctica (Thompson, 1995, Focke, 1910, 1911, 1914; Hummer, 1996). The number of Raspberries species occurring in Iran varies according to different authors. Boissier (1867) reported seven *Rubus* species from Iran while Rechinger (1969) reported 7 species & 5 hybrids; Khatamsaz (1992) reported 8 species and 5 hybrids.

All the *Rubus* species of Iran are shrub, bushes, branch with prickles in the inflorescence with long branches, white- pink panicle inflorescence, and complex carpels, with exception *R. saxaitilis* L, which is a perennial herb with many small prickles (Khatamsaz, 1992). It is economically and ecologically important, edible fruit specifically for the West, Northwest, and Center of the country. The extract of this genus is used as home-remedy which is useful for sore throat, diabetes, Hemorrhoids, and digestive disorders (Ipek *et al*., 2011). In spite of numerous valuable properties of this genus, more studies and research are required to better understand its chromosome features and genetic variation in the field of plant taxonomy (Palmer *et al*., 2003). Meanwhile, cytogenetic studies are useful in determining the relationship between species (Mirzaie-Nodoushan and Naderkhani, 2000; Sheidai *et al*., 2001). Regarding morphological studies presented in the past, recent research has focused on biosystematics affairs and many new characteristics are studied such as chromosome structure, protein, enzyme, and *etc*. These new features are applied for classic taxonomy ambiguities classification (Mirzaie-Nodoushan *et al*.,

2003). Karyotypes studies are applied for diversity of species, gen formation, and chromosome trends. Variations in chromosomes affect the gen structure and extend to phenotypes species size of chromosome. Chromosome structures can cause different phenotypes (Sheidai and Aynamdar, 1991). Further, karyotypes research is important across species population in different habitats, because every population of any species could adapt to different habitats and locality. Karyotypes study could reveal the different role of adaption (Ramak-Masomi and Khosravi, 1989). According to (Bammi, 1965), somatic chromosomes of *Rubus* are small and unique structures. Many recent studies have worked on this genus based on chromosome number. Nybom (1980) and Jennings (1988) reported 2x-14x. Thompson (1995) studied 113 species and 9 sub-genus x=7 and reported different polyploidies 2x-12x. Other studies on European *Rubus* (298 species) reported (2n=21) triploids, (2n=28) tetraploids and (2n=35) pantaploids (Iwatsubo*et al*., 1995). (Thompson, 1997) reported x=7 and verified polyploidy 2x=14 and two unknown 13x, 18x. Naruhashi*et al*. (2002) reported one species from *Chamaebatus*, 16 subgenus *Malachobatus*, and 20 species from subgenus of *Idaeobatus* in Taiwan; *Chamaebatus* (Hexaploids), *Malachobatus* different polyploids (tetraploid, hexaploid, octoploid) and *Idaeobatus* (diploids). Also, Naruhashi*et al*. (2002) studied chromosome numbers of 10 *Rubus* species in Southwest China and reported that all studied species were diploids (2n=2x=14), and different levels of polyploidies were also observed. Further, Karyotype formula was presented metacentric and sub-metacentric while no acrocentric and telocentric were seen.

Xiao Rong *et al*. (2008) studied 12 taxa of *Rubus* genus and confirmed as 2n=2x=14 diploid and reported some features such as the structure of metacentric position, satellite chromosome, and the ratio of long/short chromosome (Wang, 2009). According to recent studies, the chromosome numbers of taxa are reliable characteristics for taxonomy studies. In this regard, different populations of *Rubus* are treated in this current study. Due to lack of information on the chromosomal status of genus *Rubus* in Iran, this study was conducted to investigate the chromosomal number and chromosomal detail of four species.

Materials and Methods

Plant materials

Karyotype studies were performed across 7 populations of *Rubus* species growing in Iran. The species were studied from *R. caesius* L. (two population); *R. sanctus* Schreber (two population); *R. discolor* Weihe & Nees (one populations); and *R. persicus* BOISS. (two populations). Details of the studied material and voucher specimens are included in table 1.

Table 1. Collection data of studied specimen or population properties.

Species	Location	ASM	Voucher code
*R.caesius*L.	Iran, Mazandaran Prov., Nour Forest Park	215	803014-GKUH
R. caesius L.	Iran, Guilan Prov., Hashtpar region	6	803023-GKUH
*R. discolor*Weihe and Nees.	Iran, Guilan Prov., Astara to Ardabil, Heyran region	290	803031-GKUH
R. persicus Boiss.1	Iran, Golestan Prov., Loveh forest	-	-
R. persicus Boiss.2	Iran, Guilan Prov., Saravan forest	90	-
R.sanctus Schreber.1	Iran, Mazandaran Prov., Nyarak- village, Joybar	40	803225-GKUH
R.sanctus Schreber.2	Iran, Mazandaran Prov., Babolsar, Mirrood	-6	-

Stratification

Germination of *Rubus* is not an easy task, and it needs some stratification due to a hard seed coat and biological barrier (Nybom, 1980; Taylor, 2005). Germination work was based on Wada and Reed, (2011) protocol. First, the seeds of *Rubus* were pretreated by sulfuric acid for 3 hours, then soaked with combination gibberellins acid 2/03 mg/L and nitrate potassium 34 mg/L. Then, the seeds were transferred into a germinator (photo-period 16 and 8 hours' dark at a temperature adjusted at 20˚C). The seed germination time ranged from 4 to 7 days when the root tips were treated.

Cytological studies

To observe the chromosome root tips, they were pretreated in 8 hydroxyquinoline solution (0/002 m) for 1 h at room temperature and

subsequently held at 5˚C for 15 h. The root tips were then fixed in a mixture of glacial acetic acid absolute ethyl alcohol (1:3) for 1 h, soaked in 1N HCl for a few hours, macerated in 1N HCl at 60˚C for 5 min, and then immersed in tap water. Meristematic cells were stained in 2% acid carmine, where the usual squashing method was applied for examining the somatic chromosome in the root tip cells. The studied species were photographed by Nickon microscope and digital Canon camera. Photomicrography was used to measure each chromosome pair S (short arm), L (long arm), TL (total chromosome length) (r=L/S) arm ration, and (r=S/L). The chromosomes were identified according to Levan *et al*. (1964); karyotype symmetry was determined according to Stebbins (1971). Further, other karyotype parameters such as total form percentage (TF %; Huziwara, 1962), coefficient of variation (CV) of the chromosome size as well as A1 and A2 indices of Romero-Zarco (1986) were determined.

Results and Discussion

Details of the karyotype analyses in *Rubus* L. species are presented in the table. 2 as well as in figure 1. The chromosome number was 2n = 2x = 14 for the Joybar and Babolsar population of *R. sanctus*; 2n = 4x = 28 for the Nur and Hashtpar populations of *R. caesius*; for the Heyran pass of *R. discolor*; 2n =8x = 56 for the Loveh and Saravan population of *R. persicus*. Among the populations of *R. persicus*, the value for total chromosome length (TCL) was highest (81.66 µm) in the Loveh population, which is octoploid while it was lowest (18.52 µm) in the Joybar population of *R. sanctus*, which is diploid. The length of the longest chromosome ranged from 1.99 µm, in the Heyran pass population, to 3.69, in the Joybar population (Table 2). The total form percentage (TF%) varied from 30.50 in *R. persicus* (in the Saravan population) to 44 in *R. caesius* (Nur population) (Table 2); a higher value of TF% indicates the presence of relatively more symmetrical karyotype. The *Rubus* species were grouped as 3A, 3B, 2C and 3C classes of Stebbins karyotype symmetry. It seems that the *Rubus* species studied have asymmetrical karyotypes. Joybar and Babolsar populations of *R. sanctus* revealed the maximum symmetric karyotype among the species studied as it stands in 3A class of Stebbins' classification. On the other hand, Loveh and Saravan populations of *R. persicus* stand in 3C class showing relatively more asymmetrical karyotype, possibly due to the occurrence of chromosomal structural changes. All of the species placed indicated a high value of A1 index (0.38-0.58) of Romero-Zarco and therefore have asymmetrical karyotype. All these results suggest the role of both quantitative and qualitative changes in the genome during the *Rubus* species diversification.

Chromosome number of *Rubus* was reported x=7 (Thompson, 1995, 1997) and ranged from 2x to 14x across different species. The current research also confirmed that the polyploidy within this genus cytogenetic phenomenon has caused this variability in this genus. Further, polyploidy and hybridization mechanism occurred across this genus. Chromosome number 2n=4x=28 confirmed other reports on *R. caesius* (Heslop-Harrison, 1952; Thompson, 1995). Thompson (1995) studied 201 chromosome numbers from variable genera and 27 numbers of *Rubus* were reported from European and West Asia. All previous studies demonstrated 2x, 3x, 4x, 5x, 6x in *Rubus* genus, but 2n=4x=28, 2n=8x=56 were first found by the current experiments. Karyotype was treated in two populations of *R. sanctus*. Polyploidies in two populations (Mirrood and Joybar) as 2n=2x=14 confirmed a previous study of Thompson (1995, 1997).

According to Stebbins (1971), a cytogenetic study was applied as evidence of understanding the relationships of species, phylogenetic speciation trends of species, and plant sciences. All species have adapted themselves to two variable habitats where species adaption may cause speciation and variety of chromosomes. Finally, structure and variability of chromosomes can be used as knowledge of phylogenetic of plants' trends in the world (Ramak-Masomi and Khosravi, 1989).

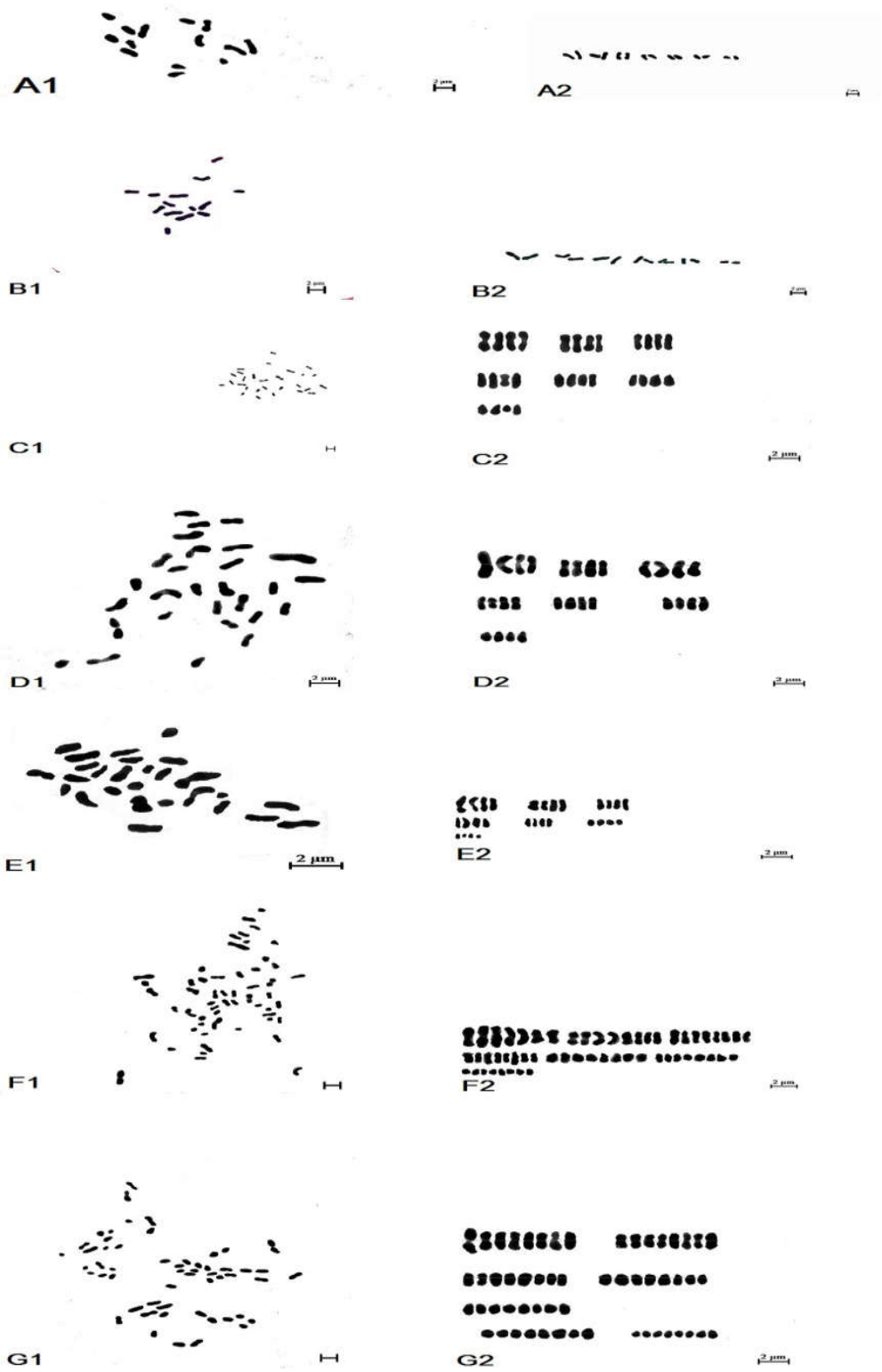

Fig. 1. Representative metaphase somatic cells and karyogrms in the studied species of *Rubus* (Rosaceae): A-B) Joybar and Babolsar populations of *R. sanctus*, showing 2n =2x=14, respectively; C -D) Nur and Hashtpar populations of *R. caesius* showing 2n =4x= 28, E) Heyran pass of *R. discolor* showing 2n=4x=28; F-G) Loveh and Saravan populations of *R. persicus*, showing 2n =8x=56 respectively. Scale bar = 2µm.

Table 2. Karyotypic details of *Rubus* species or populations*

Species	Locality	2n	Ploidy level	TCL ±Sd(µm)	L(µm)	S(µm)	L/S	X(µm)	ST	A1	A2	TF%	Karyotype formula
Rubussanctus	Joybar	14	2x	18.52±0.26	3.69	1.86	1.98	2.64	3A	0.38	0.10	36%	5m+sm+t
R. sanctus	Babolsar	14	2x	18.55±0.31	3.38	1.75	2.20	2.65	3A	0.40	0.12	38%	6m+t
R. caesius	Nur	28	4x	47.9±0.19	2.45	1.20	2.04	1.29	3B	0.46	0.14	44%	5m+2t
R. caesius	Hashtpar	28	4x	48.53±0.20	3.19	1.10	2.90	1.73	3B	0.46	0.11	34.15%	5m+2t
R. discolor	Ardabil, Heyran pass	28	4x	29.16±0.14	1.99	0.35	6.63	1.04	2C	0.48	0.13	36.31%	4m+1sm+2t
R. persicus	Loveh	56	8x	81.66±0.14	3.04	0.61	4.98	1.46	3C	0.58	0.10	30.87%	4m+3t
R. persicus	Saravan	56	8x	76.02±0.15	2.85	0.68	4.19	1.38	3C	0.54	0.11	30.50%	4m+3t

**L: the size of the longest chromosome;* S: *the size of the shortest chromosome;* L/S: *the ratio of the longest to shortest chromosome;* X: *mean chromosome length; ST: Stebbins' class; A1 & A2: symmetry indices of Romero-Zarco; TF%: total form percentage.*

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