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KARYOTYPE STUDY IN SOME *LATHYRUS* L. ACCESSIONS OF IRAN*

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Abstract – Twenty accessions of cultivated grass pea (*Lathyrus sativus* L.) and wild species of *Lathyrus* L. collected from western and southern regions of Iran were evaluated for their mitotic metaphase chromosomal characteristics. All populations comprising 16 populations of cultivated *L. sativus* and 4 wild populations of *Lathyrus* L. were diploid, 2n=2x=14 chromosomes. There were significant variations among populations in the number of metacentric, submetacentric and subtelocentric chromosomes, the longest and shortest chromosome lengths, total haploid complement, arm ratios and centromeric index. The karyotypic formula of wild *Lathyrus* spp. populations were quite varied from those in native grass pea cultivars in having either two or three pairs of subtelocentric chromosomes and accordingly containing one or two metacentric chromosomes. In addition, total chromosome length (TCL) in wild *Lathyrus* L. accessions was less than those for grass pea populations. The mean length of the largest chromosome differed significantly among populations, with populations 5 having the largest chromosome (7.2μm). The mean length of the shortest chromosome did not differ among populations, with wild populations from Ilam (populations 17, 18 and 19) having the lowest value. The arm ratios and centromeric index (CI) data revealed adverse trends, whilst wild populations of *Lathyrus* L have the highest arm ratios and the lowest CI values. There were only slight differences in the mean of arm ratios among the native grass pea populations considering a range of 1.3 to 1.9 for this morphological trait.

Keywords – Grass peas, *Lathyrus*, cultivated and wild accessions, chromosome, karyotype, idiogram

1. INTRODUCTION

Iran is considered a center of origin for many crop species. The wide range of geographical and geological conditions coupled with the climatologically diverse environments provide for this enormous diversity.

The grass pea (*Lathyrus sativus* L.) is an annual pulse crop belonging to the family Fabaceae and the tribe Vicieae. Grass pea is valued as a nutritious staple food and fodder crop primarily due to its relatively high protein content of 18-34% dry weight in grain, 17% in mature leaves and its high lysine content [1]. The *Lathyrus* genus is believed to have originated in southwest and central Asia [2]. Because of its tolerance to drought, flood and moderate salinity [3, 4], this genus may be a source of new and useful traits for the closely related genera for future improvement of commercially valuable species.

The genus *Lathyrus* L. of the family Leguminosae includes annual and perennial species, most of which are self-pollinating [5]. All *Lathyrus* species have 2n=14 chromosomes, with the basic number being n=x=7 [6]. However, Khawaja et al. [7] reported an American and British form of *Lathyrus palustris* as a perennial plant and natural autohexaploid, having 2n=42 chromosomes. The induced tetraploids (2n=28) of *Lathyrus ordoratus* and *L. pratensis* were also produced by Khawaja et al. [8] and studied for their chromosomal abnormalities. Although there is little or no variation in chromosome number of the *Lathyrus* species, there are variations in chromosome size, in arm size, and in number, size

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and location of nucleolar organizer regions (NORs) [6, 9, 10, 11]. Nandini et al. [12] have determined the 2C nuclear DNA amounts of 24 *Lathyrus* L. species using flow cytometry and observed a more than two-fold variation, ranging from 10.2 pg in *L. basalticus* to 24.2 pg in *L. latifolius*. They also found significant intraspecific variation for 2C nuclear DNA amounts in some species of *Lathyrus*.

Genetic diversity studies using different markers including karyotype morphological markers are essential for further improvement of commercially valuable grass pea cultivars. However, such studies were not carried out for grass pea in Iran. The objective of the present study was to compare chromosome morphology of twenty populations of cultivated and native *Lathyrus* spp.

2. MATERIALS AND METHODS

Twenty accessions of cultivated grass pea (*L. sativus* L.) and wild species of *Lathyrus* L. collected from western and southern regions of Iran were evaluated (Table 1). Fig. 1 presents the geographic origin of *Lathyrus* spp. accessions used in this study.

Table 1. *Lathyrus* accessions collected from different sites of Iran

Species	Accession	Collection site
Lathyrus sativus	1- Khoramabad-1	Khoramabad, Lorestan
Lathyrus sativus	2- Khoramabad-2	Khoramabad, Lorestan
Lathyrus sativus	3- Khoramabad-3	Khoramabad, Lorestan
Lathyrus sativus	4- Khoramabad-4	Khoramabad, Lorestan
Lathyrus sativus	5- Khoramabad-5	Khoramabad, Lorestan
Lathyrus sativus	6- Khoramabad-6	Khoramabad, Lorestan
Lathyrus sativus	7- Khoramabad-7	Khoramabad, Lorestan
Lathyrus sativus	8- Khorambid	Khorambid, SafaShar, Fars
Lathyrus sativus	9- Dehbid	Dehbid, SafaShar, Fars
Lathyrus sativus	10- Bavanat	JafarAbad, Bavanat, Fars
Lathyrus sativus	11- Hami	Hami-Sarchahan, Bavanat, Fars
Lathyrus sativus	12- Eghlid	Sarhad-Chahardangheh, Eghlid, Fars
Lathyrus sativus	13- Naghadeh	Naghadeh, west Azarbayjan
Lathyrus sativus	14- Borojen	Borojen, Charmahal-Bakhtiari
Lathyrus sativus	15- Semirom-1	Semirom, Isfahan
Lathyrus sativus	16- Semirom-2	Semirom, Isfahan
Lathyrus L.	17- Ilam-1 (wild)	Darrah-Shahr, Ilam
Lathyrus L.	18- Ilam-2 (wild)	Ilam, Ilam
Lathyrus L.	19- Ilam-3 (wild)	Ilam, Ilam
Lathyrus L.	20- Khoramabad-1 (wild)	Khoramabad, Lorestan

Five mitotic metaphases were selected at random from plants of each population. The root-tips of seedlings of 1 to 3 cm in length were pretreated in a saturated solution of α–bromonaphthalene for 5 hrs at 20° C and fixed in a chromic acid-formaldehyde fluid (1:1 of 1% chromic acid + 10% formaldehyde) for 24 hrs at 4° C. Then, the root tips were hydrolyzed in 1 N HCl at 60° C for 10 min, stained for 24 hrs in hematoxylin stain at 30° C, and squashed in 45% glacial acetic acid. Chromosome images were taken with a Nikon model Eclipse E600 microscope equipped with Fuji digital camera model HC-300Zi.

Fig. 1. Geographic location of *Lathyrus* spp. accessions used in this study. Wild accessions 15, 16 and 17 collected from Ilam, 20 collected from Khoramabad and the remaining grass pea populations (1-16) are from the other regions as indicated in this Fig. and Table 1

The number of chromosomes, karyotype formula, mean of chromosome length (μm), total length of the haploid complement, mean of long/short arm ratio, and mean of centromeric index (CI) were determined. CI was calculated as the percentage of the total length of a chromosome encompassed by its shorter arm, and the mean of CI for the chromosome complement was used for each accession. Chromosome nomenclature, based on the centromere location followed that proposed by Levan et al. [13], i.e. metacentric (m), submetacentric (sm) and subtelocentric (st). Total haploid complement length (TCL) was also calculated. These were compared among populations by analysis of variance and mean comparison using Fisher's least significant difference (LSD) test.

3. RESULTS AND DISCUSSION

The 16 populations of cultivated *L. sativus*, as well as 4 wild populations of *Lathyrus* L. were diploid, with 2n=14 chromosomes (see Table 2 and Figs. 2 and 3). This is in agreement with previous studies in *Lathyrus* species which indicate that all known species in the section *Notholathyrus* are diploid [6, 9, 11]. There were significant differences among populations in the number of metacentric, submetacentric and subtelocentric chromosomes, longest and shortest chromosome lengths, total haploid complement, arm ratios and centromeric index (Table 2). These results are in agreement with those of Battistin et al. [11] who reported significant variation among populations within each species and among species of four tested *Lathyrus* species. These results are also consistent with those of other researchers. Although they observed a conserved chromosome number in the species of this genus, they found variations in chromosome size and arm size within and between the *Lathyrus* species [6, 9, 11].

Table 2. Chromosome number (2n), karyotype formula, the longest chromosome length (LCL), the shortest chromosome length (SCL), total haploid complement length (TCL), long arm/short arm ratios (L/S) and centromeric index (CI) of 20 *Lathyrus* L. Iranian accessions

			Chromosome length (µm)				
Accession	**2n**	**Karyotype**	**LCL**	**SCL**	**TCL**	**L/S**	**CI**
1- Khoramabad-1	14	8m + 6sm	$6.2^{a-d} \pm 0.6$	$5.0^{a} \pm 0.4$	$40.0^{a} \pm 2.2$	1.8 ± 0.5	36.9
2- Khoramabad-2	14	8m+4sm + 2st	$6.5^{abc} \pm 0.9$	$4.7^{a} \pm 0.8$	$39.5^{ab} \pm 0.9$	1.4 ± 0.2	40.3
3- Khoramabad-3	14	8m + 6sm	$5.7^{a-e} \pm 1.0$	$4.6^{a} \pm 0.5$	$36.9^{ab} \pm 1.6$	1.5 ± 0.4	39.0
4- Khoramabad-4	14	8m +4sm + 2st	$6.8^{ab} \pm 0.3$	$4.9^{a} \pm 0.8$	$38.4^{abc} \pm 1.2$	1.6 ± 0.08	35.4
5- Khoramabad-5	14	8m+6sm	$7.2^{a} \pm 0.9$	$4.3^{a} \pm 0.5$	$41.1^{a} \pm 2.0$	1.5 ± 0.2	42. 5
6- Khoramabad-6	14	6m + 8sm	$5.9^{a-e} \pm 0.6$	$4.0^{a} \pm 0.3$	$37.8^{abc} \pm 1.5$	1.6 ± 0.7	39.9
7- Khoramabad-7	14	8m + 6sm	$6.8^{a} \pm 0.4$	$4.8^{a} \pm 0.6$	$41.0^{a} \pm 1.8$	1.4 ± 0.6	43.7
8- Khorambid	14	6m+8sm	$5.9^{a-e} \pm 0.5$	$4.3^{a} \pm 0.7$	$39.3^{a} \pm 1.3$	1.4 ± 0.08	39.8
9- Dehbid	14	8m +4sm +2st	$5.3^{b-f} \pm 0.8$	$4.1^{a} \pm 0.6$	$33.9^{a-d} \pm 1.3$	1.6 ± 0.3	40.2
10- Bavanat	14	8m+6sm	$5.5^{b-e} \pm 0.2$	$4.4^{a} \pm 0.3$	$36.9^{abc} \pm 1.9$	1.5 ± 0.3	43.9
11- Hami	14	10m+4sm	$4.9^{b-f} \pm 0.4$	$4.3^{a} \pm 0.6$	$32.8^{c-d} \pm 1.4$	1.3 ± 0.06	46.1
12- Eghlid	14	4m+6sm+2st	$4.5^{b-f} \pm 0.4$	$4.0^{a} \pm 0.2$	$30.7^{c-d} \pm 1.0$	1.8 ± 0.1	37.1
13- Naghadeh	14	10m + 4sm	$4.9^{b-f} \pm 0.7$	$4.1^{a} \pm 0.9$	$32.3^{cd} \pm 1.9$	1.4 ± 0.2	42.7
14- Borojen	14	8m+4sm+2st	$6.0^{a-d} \pm 0.3$	$4.7^{a} \pm 0.2$	$34.2^{de} \pm 3.1$	1.7 ± 0.09	38.0
15- Semirom-1	14	6m+6sm+2st	$6.6^{a-d} \pm 0.8$	$3.8^{a} \pm 0.1$	$39.0^{ab} \pm 2.7$	1.5 ± 0.07	41.9
16- Semirom-2	14	6m+4sm+4st	$5.1^{b-f} \pm 0.6$	$3.7^{a} \pm 0.3$	$32.5^{de} \pm 2.1$	1.9 ± 0.1	35.6
17- Ilam-1 (wild)	14	2m+6sm+6st	$4.2^{b-f} \pm 0.2$	$3.9^{a} \pm 0.4$	$28.8^{e} \pm 1.0$	2.2 ± 0.2	30.6
18- Ilam-2 (wild)	14	4m+6sm+4st	$4.1^{b-f} \pm 0.4$	$3.9^{a} \pm 0.5$	$28.9^{e} \pm 1.7$	2.0 ± 0.5	28.2
19- Ilam-3 (wild)	14	4m+4sm+6st	$4.6^{ab} \pm 0.5$	$4.1^{a} \pm 0.6$	$30.0^{de} \pm 1.2$	2.1 ± 0.3	31.0
20-Khoramabad-1 (wild)	14	2m+8sm+4st	$5.6^{b-e} \pm 0.7$	$4.2^{a} \pm 0.1$	$35.2^{cd} \pm 0.9$	1.7 ± 0.2	39.2

The results are shown as the mean ± SE when appropriate.
Lowercase letters indicate significant differences at the 5% level (Fisher's LSD).

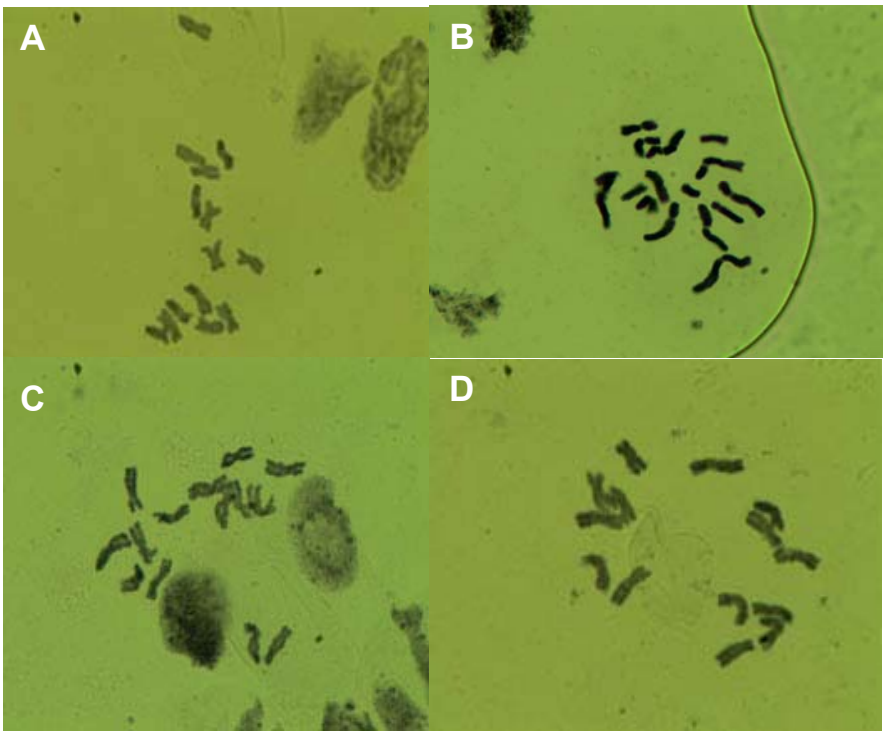

Fig. 2. Mitotic metaphase plates of five native grass pea (*Lathyrus sativus)* populations (A: Hami 1, B: Khoramabad5, C: Khorambid, D: Bavanat, E: Borojen and a wild *Lathyrus* sp. population (F: Khoramabad1-wild)

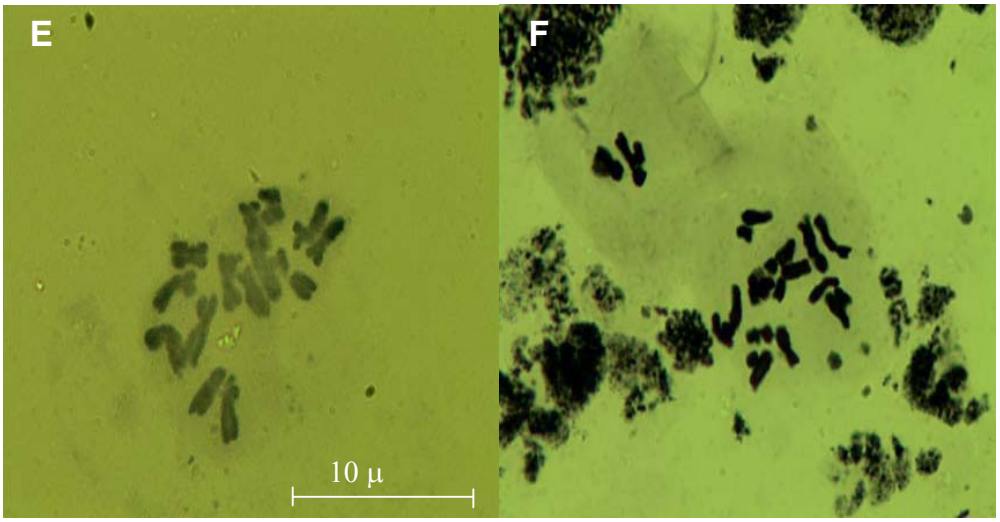

Fig. 2. (Continued)

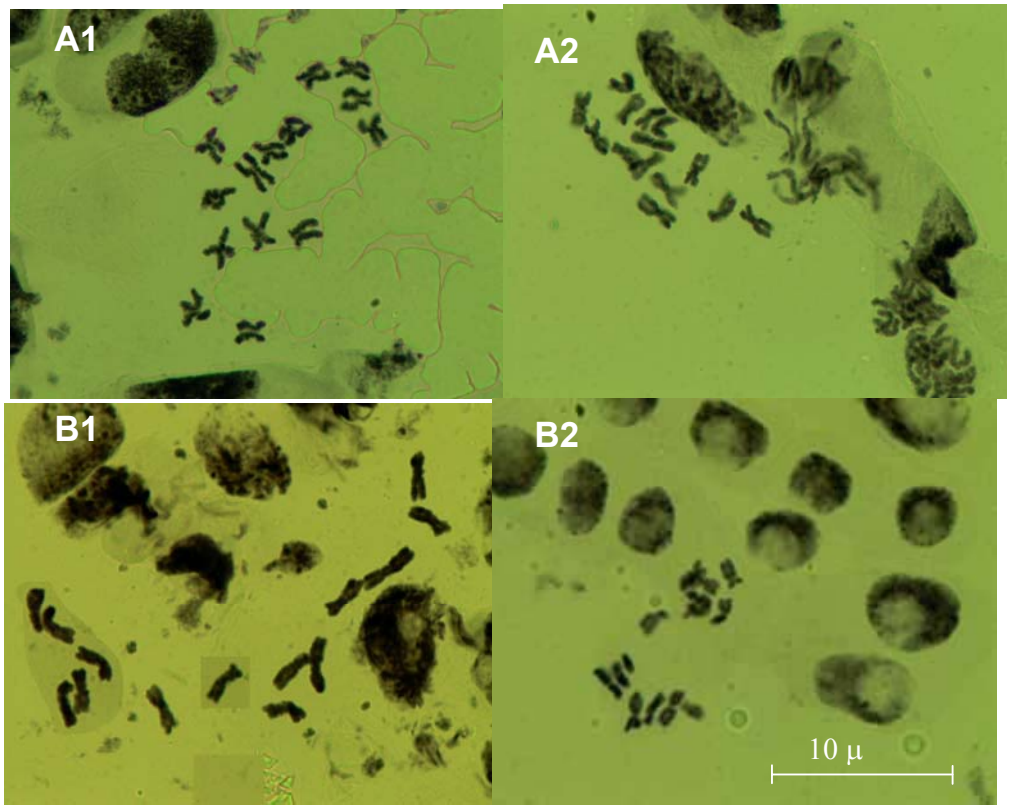

Fig. 3. Mitotic metaphase plates of two native grass pea populations (A1 and A2=Eghlid; B1 and B2= Khoramabad-4) captured on two slides (A1 and A2; B1 and B2). B1 and B2 microphotographs were taken at original 1000x and 400x magnifications, respectively

Representative examples of mitotic chromosome photomicrographs of each karyotypic formula are presented in Figs. 2 and 3. Five out of 16 grass pea populations comprising populations 1 (Khoramabad-1), 5 (Khoramabad-5), 7 (Khoramabad-7) and 10 (Bavanat) had similar karyotypes, with only metacentric and submetacentric chromosomes (8m + 6sm) (Table 2). Furthermore, 9 out of 16 grass pea populations showed similarities in their karyotypes, with metacentric and submetacentric chromosomes, which differed only in number (one or two pairs). The seven remaining populations differed for only having one

pair of subtelocentric chromosomes (Table 2). This finding suggests a more restricted degree of homology among these populations compared to the others. Karyotypic formula of wild *Lathyrus* spp. populations varied greatly from those of native grass pea cultivars in having either two or three pairs of subtelocentric chromosomes, and accordingly containing one or two metacentric chromosomes. Details of individual chromosomes (length and arms) of the 20 *Lathyrus* accessions used in this study are presented as their idiograms in Fig. 4. In addition, total chromosome length (TCL) in wild *Lathyrus* L. accessions was less than that for grass pea populations. Figure 3 indicates the similarity of two plants of the same population and the variation of two populations for chromosome morphology. Battistin et al. [9], working with the South American *Lathyrus* species, reported intra- and interspecific variations for karyotypic formula and TCL.

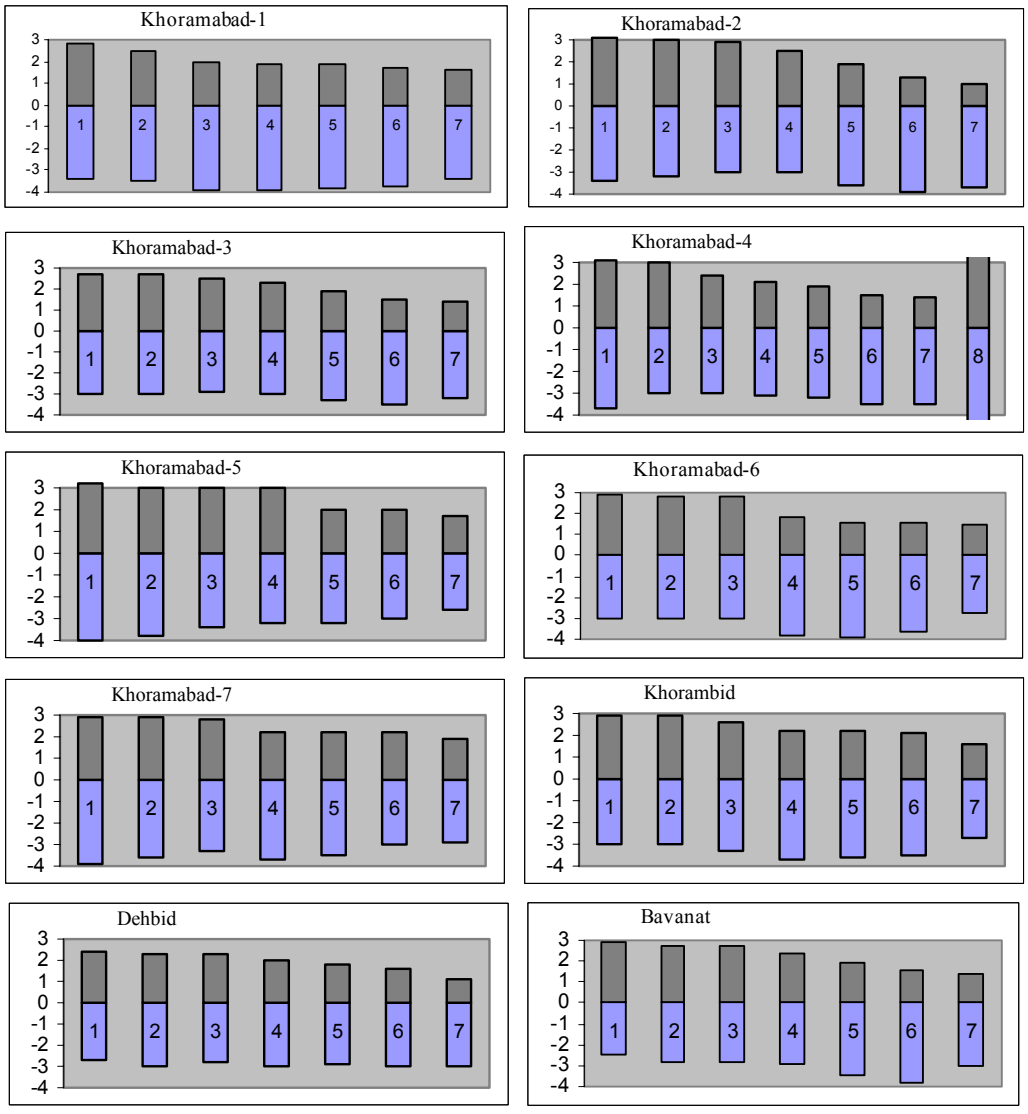

Fig. 4. Idiograms of the twenty *Lathyrus* accessions including 16 cultivated and 4 wild accessions used in this study

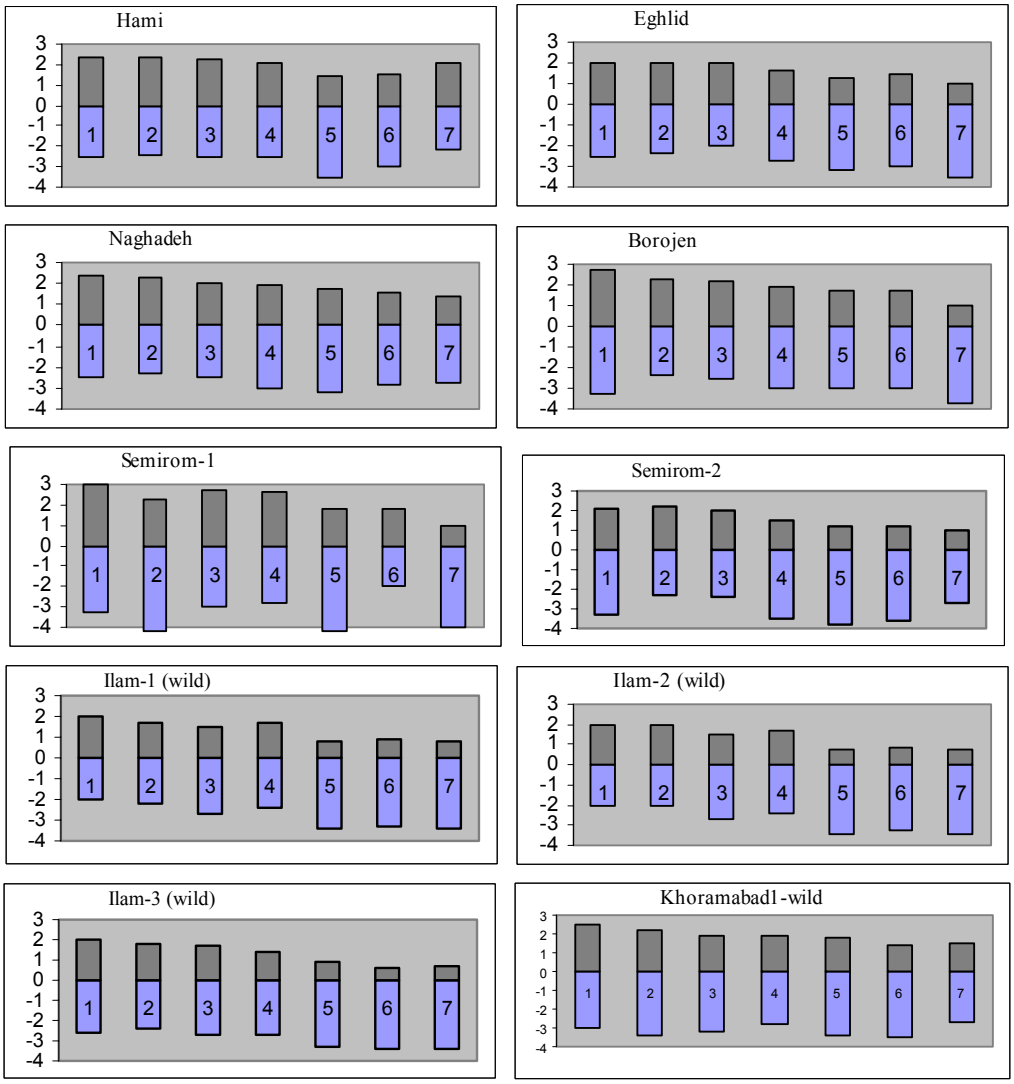

Fig. 4. (Continued)

In the present study, significant variation was observed among populations for chromosome length (Table 2). The mean length of the largest chromosome differed significantly among populations. Population 5 had the largest chromosome (7.2μm) and the highest value for TCL (41.1μm) in the studied populations (see Fig. 2 and Table 2). The mean length of the shortest chromosome did not differ among populations, with wild populations from Ilam (populations 17, 18 and 19) having the lowest value. There were significant differences among populations for the total haploid chromosome length (TCL), which reflects the size of the karyotype. The largest TCL mean was found for population 5 and 7 with 41.1 and 41μm lengths, respectively. The amplification or deletion of a chromatin segment during species diversification often result in chromosome size variation. According to Narayan [14] the within and between species variation of chromosome size in *Lathyrus* shows remarkable differences in the amount of DNA building complement size, a high percentage of which is moderately repetitive. It has been suggested that the quantitative changes in the nuclear DNA of diploid *Vicia* species [15] and the genus *Lathyrus* L. [14, 16, 17] are achieved by changes in the amounts of both repetitive and non–repetitive DNA sequences. It should be noted that differences in chromosome contraction among cells and even between chromosome size in the same cell result in intraspecific variations in chromosome size or total

complement size between plants [18]. As pointed out by Sybenga [18] the cells may be considered genotypically identical and the number of replications of the measurements can be chosen to meet the requirements of statistical analysis. It should be acknowledged that sampling of five cells per plant used in this investigation may not be sufficient. Klamt and Schifino-Wittmann [11] and Seijo and Fernandez [19], in *Lathyrus*, employed sampling of 10 cells per plant, which made a statistical analysis of the comparing size to be reliable.

The arm ratios and centromeric index (CI) data revealed adverse trends, whilst wild populations of *Lathyrus* L have the highest arm ratios and the lowest CI values. There were only slight differences in mean of arm ratios among the native grass pea populations considering a range of 1.3 to 1.9 for this morphological trait.

Based on the observations of variation in total chromosome length without major changes in the karyotype formula, Seijo and Fernandez [19] concluded that changes in the amounts of genomic DNA are proportional to the relative length of each chromosome arm and that species of *Notholathyrus* evolved in a concerted fashion. Although similar observations on *Lathyrus* species were obtained in the present study, the mentioned conclusion may not apply to these results. It should be noted that *Notholathyrus* is restricted to South America.

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