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Karyotype Analysis of *Pisum sativum* L.

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ABSTRACT

Karyotypic analysis of *Pisum sativum* L. var. meteor was carried out to determine the chromosomal classification. The chromosome number of *Pisum sativum* L. var. meteor was counted for the first time and it was found to be 2n=14. In the karyotype, two metacentric (m) and five submetacentric(sm) chromosome pairs were found. Two of the latter carry satellites of different sizes on their long arms. The total chromosomal length of the diploid (2n) was 224 µm; whereas, the length of haploid set was 112 µm. The average chromosomal length was 16 µm. The maximum and minimum chromosomal length was 19.99 µm and 11.11 µm, respectively. The karyotypic formula was K (n=7) =2 m+5 sm.

Key Words: *Pisum sativum;* Karyotype; Chromosomes; Idiogram

INTRODUCTION

The garden pea (*Pisum sativum* L.) is one of the important legumes. Taxonomically, pea is within the Vicieae family of the legumes, and is thus closely related to *Vicia* and *Lathyrus* (Ellis & Poyser, 2002). Pea is one of the oldest subjects of scientific genetics (Knight, 1799; Mendel, 1866). Nevertheless, there are still uncertainties regarding the unambiguous identification of its seven chromosome pairs and the assignment of genetic linkage groups to individual chromosome types (Fuchs *et al.*, 1998). Surprisingly, it is only relatively recently a coherent picture has emerged of overall structure of the genome, both in terms of karyotype and organization of the genetic linkage groups (Ellis & Poyser, 2002). The karyotypic studies have been extensively used in characterizing and distinguishing chromosomes of different species. The cytological characters of the *Pisum sativum* have been investigated by a number of cytologists. Bhattacharya and Bhattacharya (2003) found variation in chromosome number in root tips of *Pisum sativum* cultivars and its wild types. The C-banding with subsequent computer analysis of the chromosomes of all cultivars of *Pisum sativum* showed similar results (Samatadze *et al*., 2002).

Karyoptype data provided by Mukherjee and Sharma (1987) revealed the importance of structural alterations in the evolution of different strains of *Pisum sativum.* According to Errico *et al.* (1996) *Pisum sativum* contained two pairs of satellited chromosomes. The pea has a wide spectrum of cultivated varieties exhibiting marked seed morphological differences from one another. The various interspecific and intraspecific morphological differences were thought to be caused by genetic differences by the earlier geneticists. But now even intraspecific variation has been considered to be occurred due to structural changes in chromosomes, which can easily be detectable under microscope (Narkhede *et al.*, 1987). Neumann *et al.* (2002) used three pea lines with reconstructed karyotypes for analysis and subsequent purification of individual chromosome types using flow cytometry and sorting. These lines possessed chromosomal translocations allowing discrimination of three to four chromosome type based on the different size of translocation chromosome.

Although there are current technical and financial obstacles to study pea genetics and genomics, this plant has many interesting mutants available for study. These are rich resource for biology and some of these, especially those involved in the regulation of compound leaf form (Hofer & Ellis, 1998) will be difficult to identify and study in other organisms. An important tool for the exploitation of pea genetics in the near future will be comparative genetics (Ellis & Poyser, 2002). The small genome bearing legumes *Lotus japonicus* and *Medicago truncatula* are taxonomically related to pea (Doyle *et al*., 1997) and can provide a route for circumventing the problems of scale in the genome. The present study, in addition to reconfirm the chromosome number, was aimed at presenting the chromosomal morphology and the karyotype of *Pisum sativum* L. var. meteor This work may form a foundation for further insights in using various banding techniques.

MATERIALS AND METHODS

The seeds of *Pisum sativum* L. var. meteor were obtained from Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan. Seeds were germinated in dark at 28°C on moist filter paper in Petri dishes. Three day old actively growing root tips 4-5 mm in length were excised from the germinating seeds. Root tips for karyotypic studies were treated with 0.05% aqueous colchicine for 5 h at 25°C and were fixed in a mixture of ethanol: glacial acetic acid (3:1) for 24 h in refrigerator (Inceer *et al.*, 1999). The root tips were hydrolyzed with 1 N HCl for 10 min at 60°C in an oven. They were stained with Feulgen reagent for 2 h in

Table I. Chromosome types, chromosome length and arm ratio of *Pisum sativum* L. var. meteor

Chromosome Pair	Centromeric position	L (µm)	S (µm)	C (µm)± SEM	SAT (µm)	I (µm)	L / S (µm)	R (µm)
1	Submedian region	12.82	7.17	19.99 ± 0.07	-	35.87	1.79	17.95
2	Submedian region	12.55	5.93	18.48 ± 0.23	-	26.68	2.11	16.59
3	Submedian region	10.82	6.18	17.00 ± 0.12	-	36.35	1.75	15.27
4	Submedian region	10.34	5.88	16.22 ± 0.06	2.59	36.25	1.76	14.56
5	Median region	7.35	7.18	14.53 ± 0.05	-	49.42	1.02	13.05
6	Median region	7.30	7.08	14.38 ± 0.08	-	49.24	1.03	12.91
7	Submedian region	7.01	4.10	11.11 ± 0.11	3.12	36.90	1.71	10.56

L = Long arm length S= Short arm length C= Total Chromosome length
SAT=Satellite I= Centermere index L/S= Arm ratio
SEM= Standard error of mean R= Relative length Mean chromosomal length = 15. 96 µm
H = Length of Haploid chromosome complement = 111.71 µm

dark at 25°C. Squashed root meristems were then counterstained with 2% acetic orcein. Slides were sealed with Fine Fix and examined under Kyowa Medilux-12 Photomicroscope using an oil immersion objective (100 X). Photographs were taken with the same microscope. The idiogram was prepared with measurements taken on enlarged micrographs of five well spread metaphase plates. The classification of chromosomes, the length of long and short arm, arm ratio, centromeric index and relative chromosomal length were measured/ calculated (Table I). The classification of chromosomes into Metacentric (M), metacentric (m), submetacentric (sm) and telocentric (T) was based on the analysis of metaphase chromosomes (Levan *et al*., 1964)

RESULTS

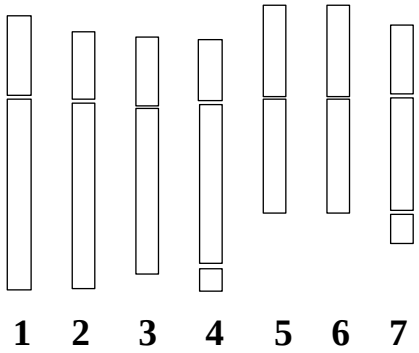
The chromosome numbers of species were determined to be 2n=14 (Fig. 1). Data on individual chromosome of this variety regarding length of long arm, length of short arm, total length of chromosome, arm ratio, centromeric index and relative chromosome length along with chromosomal classification based on ratio and centromeric index as proposed by Levan *et al.* (1964) is given in the Table I .The total length of the haploid set is 111.71 µm and the total length of the diploid chromosomes was measured 223.42 µm. The average chromosomal length was 15.96 µm. As a result of classification five chromosomes pair of this variety were found to be submetacentric (sm) and two metacentric (m). Chromosomes pair 1-4 and seven have centromeres at submedian region while chromosome pairs 5 and 6 have centromeres in the median region (Fig. 2). Two of the chromosome pairs 4 and 7 bear satellites on their long arms having sizes 2.59 and 3.12 µm, respectively.

DISCUSSION

The karyotypic analysis of *Pisum sativum L.* var. meteor exhibited the diploid chromosome number 2n=14. The karyotype of the variety consists of two metacentric and five submetacentric chromosomes. The ideogram (Fig. 2) confirms the karyotypic formula K (n=7) =2m+5sm. These findings agree with reports of chromosome number for *Pisum sativum* L. (Mukherjee & Sharma, 1991; Neumann *et al*., 1998; Samatadze *et al.,* 2002). Bhattacharya and Bhattacharya (2003) studied the mitotic chromosome number in *Pisum sativum* cultivars and wild types. The normal chromosome number (2n=14) was observed for all the cultivars, except for local *Pisum sativum* wild type, which showed significant variation in chromosome number. Chromosome pairs 4 and 7 carry satellites on their long arms having 2.59 and 3.12 µm size, respectively. These results are in agreement with earlier studies (Folkeson, 1990; Ellis & Poyser, 2002; Stamadze *et al*., 2002; Fuchs *et al*., 1998).

Fig. 1. Mitotic metaphase chromosomes in *Pisum sativum* L. var. meteor

Fig. 2. Ideogram of *Pisum sativum* L. var. meteor



Pisum sativum L. var B-22 showed instability of somatic chromosome number which can be observed in the meristematic regions of root and shoot tips. This variation in somatic chromosomes may range from 2n=14-5 (Maitra & Ghosh, 1991). This variability of chromosome number was also observed during present studies in some cells where the chromosomes are synchronized in metaphase.

The chromosome number and karyology of *Pisum sativum L.* var meteor is being reported for the first time. According to the observations the variety has 2n=14 and the chromosomes are metacentric and submetacentric.

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