

## Karyological Studies On *Vicia sativa* L. subsp. *incisa* (Bieb.) Arc. var. *incisa*

Çiler MERİÇ, Feruzan DANE

Trakya University, Faculty of Science and Letters, Department of Biology, Edirne-TURKEY

Received: 04.05.1998

Accepted: 16.10.1998

**Abstract:** *Vicia sativa* L. subsp. *incisa* (Bieb.) Arc. var. *incisa* was investigated karyologically (chromosome number, karyotype and mitotic division).

The chromosome number of *Vicia sativa* subsp. *incisa* var. *incisa* was counted for the first time and it was found to be  $2n=14$ . In the karyotype, 1 submedian (SM) and 6 subterminal (ST) chromosome pairs were found, in one of which a SAT-chromosome was detected. A satellite was on the short arm of the 3rd chromosome pair. In the cells of the root tips, the course of mitosis was regular.

The presence of subsp. *incisa* var. *incisa* in the flora of Turkey was also confirmed with the specimens (A1(E) Edirne: EDTU 6851, EDTU 6852, EDTU 6853, EDTU 7287, ISTE 74462; A2(E) İstanbul: EDTU 7288).

**Key Words:** *Vicia sativa* subsp. *incisa* var. *incisa*, karyotype, mitotic division.

### *Vicia sativa* L. subsp. *incisa* (Bieb.) Arc. var. *incisa* Üzerinde Karyolojik Çalışmalar

**Özet:** *Vicia sativa* subsp. *incisa* (Bieb.) Arc. var. *incisa* karyolojik olarak (kromozom sayısı, karyotip ve mitoz bölünme) incelendi. *V. sativa* subsp. *incisa* var. *incisa*'nın kromozom sayısı ve morfolojisi ilk defa bu çalışmada saptandı ve  $2n=14$  olarak bulundu. Karyotip analizinde 1 çift submedian (SM) ve 6 çift subterminal (ST) kromozom belirlendi. Subterminal kromozomlardan 3 nolu çiftin SAT-kromozom olduğu ve satellitlerin kromozomların kısa koluna bağlı bulunduğu gözlemlendi. Kök ucu hücrelerinde mitoz bölünmenin düzenli olduğu saptandı.

Ayrıca subsp. *incisa* var. *incisa*'nın Türkiye Florasındaki varlığı A1(E) Edirne'den (EDTU 6851, EDTU 6852, EDTU 6853, EDTU 7287, ISTE 74462) ve A2(E) İstanbul'dan (EDTU 7288) toplanan örneklerle kanıtlandı.

**Anahtar Sözcükler:** *Vicia sativa* subsp. *incisa* var. *incisa*, karyotip, mitoz bölünme.

### Introduction

The genus *Vicia* L. (*Leguminosae*, *Vicieae*) comprises about 166 species, chiefly located in Europe, Asia and North America, extending to the temperate regions of South America and tropical Africa (1). Most of them are diploids with a basic number  $x=5, 6$  or  $7$  (2, 3); only six species are polyploids (4). The chromosome numbers of half of the 166 species are known (1, 2). Cytological investigations have mainly concerned the determination of chromosome numbers and the examination of chromosome morphology (2, 5, 6).

Cytological studies have tended to focus on the two most important economic species; *V. faba* L. and *V. sativa* (1, 2, 4). *V. sativa* shows considerable variation in almost

every trait, but particularly in leaflet morphology and in basic chromosome number (2, 7, 8). It includes 6 subspecies (1):

subsp. <i>sativa</i>	$2n=12$	(2, 6, 7)
subsp. <i>nigra</i> (L.) Ehrh.	$2n=12, 14$	(7, 8)
subsp. <i>incisa</i> var. <i>cordata</i> (Wulfen ex Hoppe) Arc.	$2n=10$	(9, 10)
subsp. <i>incisa</i> var. <i>incisa</i> (Bieb.) Arc.	-	-
subsp. <i>macrocarpa</i> (Moris) Arc.	$2n=12$	(9)
subsp. <i>amphicarpa</i> (L.) Batt.	$2n=14$	(8)
subsp. <i>devia</i> J.G. de Costa	-	-

The chromosome numbers and morphology neither

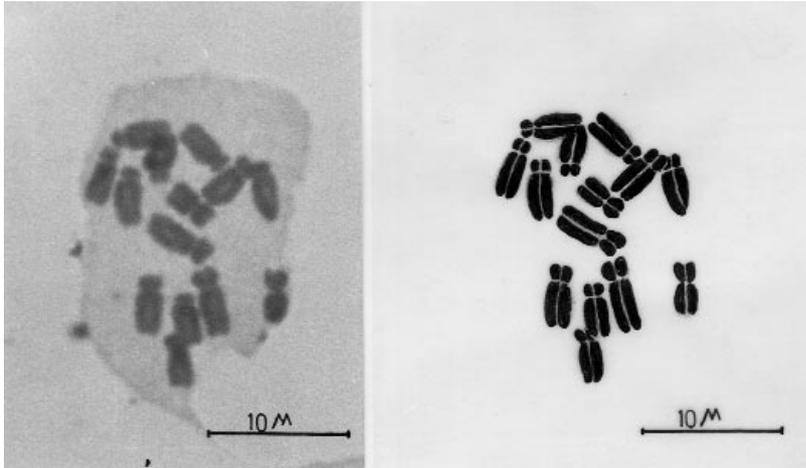


Figure 1. Microphotograph of somatic metaphase chromosomes of the *V. sativa* subsp. *incisa* var. *incisa* ( $2n=2x=14$ ).

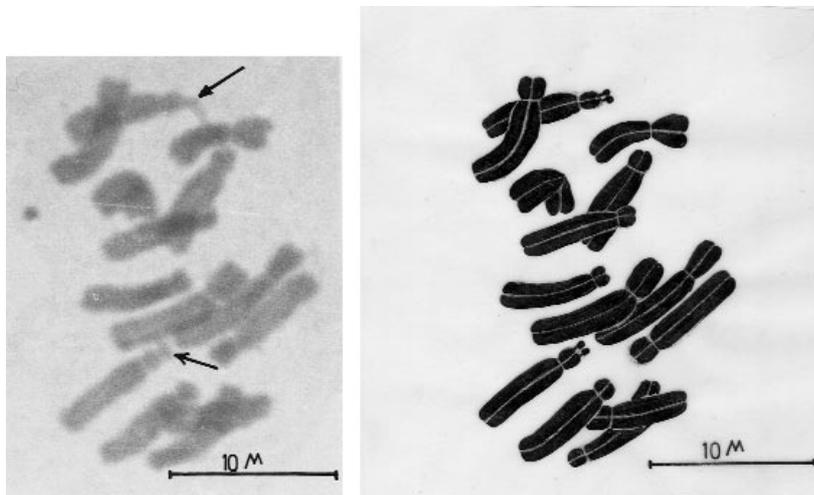


Figure 2. The arrows indicate the position of satellites on prometaphase chromosomes with lower spiralization.

for subsp. *devia* nor for subsp. *incisa* var. *incisa* are known (1, 7, 8).

In this study, we described the chromosome number and morphology in the cells of root tips of *V. sativa* subsp. *incisa* var. *incisa*. In addition, its mitotic divisions were examined.

### Material and Method

*Vicia sativa* subsp. *incisa* var. *incisa* plants were collected from the natural population in the centre Edirne in European Turkey. Voucher specimens were placed in the Herbarium of Trakya University (EDTU).

The chromosome preparations were made by using a standard root-tip squash technique (11). Seeds were germinated in darkness at 25°C on moist filter paper in petri dishes. Actively growing root tips 1 cm. in length were excised from the germinating seeds. Root tips for

karyotype analyses were pretreated with 0.5% colchicine for 2 hours at 25°C, then fixed in Battaglia (5 ethyl alcohol, 1 acetic acid, 1 formalin, 1 chloroform) for 5 min. The root tips were hydrolyzed with 1 N HCl for 16 min. at 60°C in an oven. They were stained with Feulgen reagent for 2 hours in darkness at 25°C. Dissected meristems were squashed and counterstained with acetic orcein. Slides were made permanent by processing through a series of acetic-ethyl alcohol, ethyl alcohol-xylol and xylol and covered with Entellan. The slides were examined under an Olympus Photomicroscope and photographs were taken with the same microscope. The karyograms were drawn from the metaphase plates. Karyotype analyses were carried out according to the method described by Naranjo et al. (12).

### Results

*V. sativa* L. subsp. *incisa* has two varieties; var. *incisa*

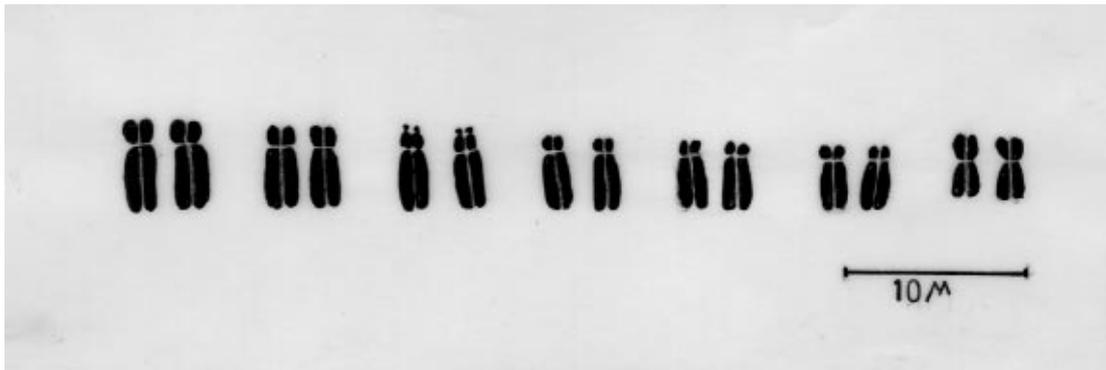


Figure 3. Karyograms of subsp. *incisa* var. *incisa*.

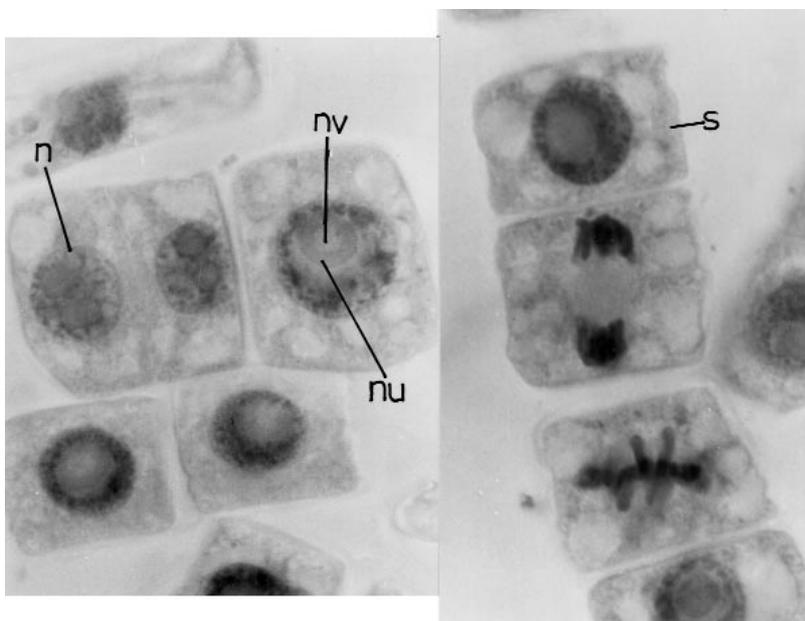


Figure 4. Some stages of regular mitotic division of subsp. *incisa* var. *incisa* (n, nucleus; nu, nucleolus; nv, vacuole of nucleolus; s, cytoplasm) 1000x.

and var. *cordata* (7). While var. *cordata* has been collected from Anatolia, var. *incisa* has not been collected anywhere in Turkey except for in 1898 and 1906. Moreover, its chromosome number was not known (7, 8). According to Tutin (8), it occurs only in Krym, Bulgaria, N.E. Greece and it is not recorded in Turkey. According to Davis (7), it was collected in 1898 by Aznavour from A2(E) İstanbul: Zekeriyaköy and in 1906 A2(A) İstanbul: Çiftlik. But there are no recorded specimens in the Herbaria of Turkey.

While determining some recent samples collected by Ç. Meriç from European Turkey (centre of Edirne in 1993 and 1994), some interesting specimens of *Vicia* L. came to light. Collection was repeated by Ç. Meriç and F. Dane from the same locality in 1997. These specimens have been identified using basic Floras (7, 8) as *V. sativa* subsp. *incisa* var. *incisa* which has not been recorded with

specimens in the Flora of Turkey.

A short description of subsp. *incisa*, individual karyotype characteristics and the course of mitosis in the cells of the root tips are given below. Voucher specimens are deposited in EDTU and ISTE. The karyotype slides are kept in EDTU.

***V. sativa* L. subsp. *incisa* (Bieb.) Arc var. *incisa***

(Syn. *V. incisa* Bieb., *V. pinpinelloides* Seb. & Mauri, *V. sativa* L. var. *incisa* (Bieb.) Boiss.)

**Morphology**

Annual, 60-80 cm., climbing. Leaflets 4-5 pairs, lower leaflets incised, upper leaflets broadly obovate, calyx-teeth about as long as the tube; Flowers 1-2 axillary,

corolla 20-23 mm; standard violet, the wing usually darker. Legume 40 x 5-6 mm., glabrous, seeds 5-7 mm. Flowering time April-May, habitat under moist wood.

Conservations status : Lower Risk Least Concern (13).

Distribution in Turkey : European Turkey, A1(E)  
Edirne; A2(E) İstanbul.

General distrubution : Crimea, Bulgaria, N.E. Greece  
and European Turkey.

Examined species: A1(E) Edirne: Trakya University, Faculty of Medicine, next to Güllapoğlu Stream, under moist wood, Çiler Meriç, 22.05.1993, EDTU 6851, 16.04.1994, EDTU 6852 and 10.5.1997, EDTU 6853, ISTE 74462, det. F. Dane, conf. K. Alpınar, the same locality, F. Dane, 1.5.1997, EDTU 6854, det. F. Dane, conf. K. Alpınar. Söğütlük, under moist wood, Feruzan Dane, 15.5.1998 EDTU 7287; A2(E) İstanbul: Belgrade forest, under moist wood, Çiler Meriç, 15.5.1998, EDTU 7288.

### Karyology

The present study includes counting of the chromosomes, the chromosome morphology and mitotic division in root tips of *V. sativa* subsp. *incisa* var. *incisa* for the first time.

Subsp. *incisa* var. *incisa* was found to be diploid. The chromosome number was determined to be  $2n=14$  (Fig. 1). The karyotype of subsp. *incisa* var. *incisa* consisted of 6 pairs of subterminal (ST), one of which, pair 3, had a satellite on the short arm (SAT) and 1 pair submedian (SM) (Fig. 2-3). In the cells of the root tips mitotic division was normally observed (Fig. 4). At the end of the telophase, 1-2 nucleoli were seen. The nucleoli were large and had a big vacuole (Fig. 4).

### Discussion

The chromosome number of *V. sativa* subsp. *incisa* var. *incisa* was reported for the first time in this study and it was determined to be  $2n=14$ . But it was determined to be  $2n=10$  for *V. sativa* subsp. *incisa* var. *cordata* by Raina & Rees (9) and Kamari et al. (10). Chromosome numbers are known to be  $2n=12$  for *V. sativa* subsp.

*sativa* (2, 6, 7),  $2n=12, 14$  for *V. sativa* subsp. *nigra* (7, 8),  $2n=12$  for *V. sativa* subsp. *macrocarpa* (9) and  $2n=14$  for *V. sativa* subsp. *amphicarpa* (8).

In *V. sativa* subsp. *incisa* var. *incisa* 6 pairs of chromosomes were subterminal (ST) and 1 pair submedian (SM). It has been found that *V. sativa* subsp. *incisa* var. *cordata* had 5 pairs of subterminal (9). According to Maxted (2) *V. sativa* subsp. *sativa* is the only taxon in the *Vicia sativa* subspecies to have a metacentric chromosome.

The karyotype of *V. sativa* subsp. *incisa* var. *incisa* was characterized by quite different chromosome types. The karyotype of this taxon consists of slightly longer chromosomes than *V. sativa* subsp. *incisa* var. *cordata* (9), *V. sativa* subsp. *sativa* (2, 5) and *V. sativa* subsp. *nigra* (5). The most obvious difference was observed in the SAT-chromosome pair morphology. *V. sativa* subsp. *sativa* (2, 5) and *V. sativa* subsp. *nigra* (5) had longer satellites which were attached to the longer arms. In *V. sativa* subsp. *incisa* var. *incisa* satellite was smaller than that in the *V. sativa* subsp. *sativa* and *V. sativa* subsp. *nigra*. Also it was attached to the shorter arms. But there were no satellites in any chromosome pairs of *V. sativa* subsp. *incisa* var. *cordata* (9, 10).

In our opinion, their identification is directly related with the degree of spiralisation. In the plates with higher degrees of spiralisation in the late metaphase, they stick to the arms and therefore are difficult to observe. However, in the prometaphase, satellites in the chromosomes with lower degree of spiralisation were clearly observed.

In the cells of the root tips, mitotic division was regularly observed. In the cytoplasm, several small vacuoles and nuclei including a nucleolus with a big nucleolar vacuole, were seen. These observations show that *V. sativa* subsp. *incisa* var. *incisa* might be used in cytological research.

### Acknowledgement

We would like to thank Prof. Dr. Kerim Alpınar (İstanbul University ISTE) who examined the specimens and confirmed their identification.

### References

1. Maxted, N.: A Phenetic Investigation of *Vicia* L. subgenus *Vicia* (Leguminosae, Viciaeae). Botanical Journal of the Linnean Society 111: 155-182 (1993).
2. Maxted, N., Callimassia, M.A., Bennett, M.D.: Cytotaxonomic Studies of Eastern Mediterranean *Vicia* Species (Leguminosae). Pl. Syst. Evol. 177: 221-234 (1991).

3. Darlington, C.D., Janaki Ammal, E.K.: Chromosome Atlas of Cultivated Plants, 156-158, George Allen & Unwin Ltd. London (1945).
4. Cremonini, R., Funari, S., Mazzuca, S.: Cytology of Vicia species: nuclear structure, karyological analysis and DNA content. Chromatin 1, 135-146 (1992).
5. Şahin, A., Babaç, M.T.: Doğu ve Güneydoğu Anadolu'da Yetişen Bazı Vicia L. Türleri Üzerinde Sitotaksonomik Araştırmalar I. Doğa Tr. J. of Botany, 14: 124-138 (1990).
6. Elçi, Ş.: Memleketimizin Önemli Fiğ Türlerinde Kromozom Sayıları Tespiti ve Kromozom Morfolojilerinin Mukayesesi, Ankara Üniversitesi Ziraat Fakültesi Yayınları, No:254, Ankara (1965).
7. Davis, P.H., Plitmann, U.: Vicia L. Flora of Turkey and the East Aegean Islands, Davis, P.H., (Ed.) Edinburgh University Press, Edinburgh, Vol. 3: 274-325 (1970).
8. Tutin, T.G.: Flora Europaea. Vol. 2: 129-136 (1968).
9. Raina, S.N., Rees, H.: DNA Variation Between and Within Chromosome Complements of Vicia species. Heredity, 51: 335-346 (1983).
10. Kamari, G., Felber, F. & Garbari, F., (Ed.): Mediterranean Chromosome Number Reports 4. Flora Mediterranea, 4: 233-301 (1994).
11. Gray, P.: Handbook of Basic Microtechnique, McGraw-Hill Book Company, New York (1964).
12. Naranjo, C.A., Poggio, L.: A New Template For Direct Morphological Classification of Chromosomes. Darwiniana 27(1-4): 39-41 (1986).
13. Ekim, T. et al.: List of rare threatened and endemic plants in Turkey. Turkish Association for Conservation of Nature and Natural Resources. Ankara, (1989).