

CYTOLOGY OF *CROCUS VALLICOLA* AND ITS ALLIES (IRIDACEAE)

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ABSTRACT. Chromosome counts and karyotypes are given for the following taxa: *Crocus kotschyanus* subsp. *kotschyanus* $2n = 8$ and 10 , subsp. *hakkariensis* and *cappadocicus* $2n = 10$, subsp. *suwarowianus* $2n = 20$; *C. autranii* $2n = 32$; *C. gilanicus* $2n = 24$; *C. karduchorum* and *C. ochroleucus* $2n = 10$; *C. vallicola* and *C. scharojanii* $2n = 8$. A natural hybrid between the last two species is reported, and also a rare putative autotetraploid of *C. kotschyanus* subsp. *kotschyanus*. The distribution of the material examined is mapped.

INTRODUCTION

As a result of extensive studies on wild-collected material the genus *Crocus* has been shown to be of enormous cytological complexity. In addition to a wide range of chromosome numbers characterizing different species—from $2n = 6$ to $2n = 64$, Brighton *et al.* (1973)—many cases of intraspecific variation have been recorded, both in chromosome number and morphology (Brighton, 1976, 1977a, 1977b; Rafinsky & Passakas, 1976; Šopova, 1972).

This paper deals with *C. vallicola* Herb. and the species considered to be taxonomically allied to it and complements the preceding paper of Mathew (1980). This group, which occurs in Turkey and its eastern neighbours, comprises *C. vallicola* Herb., *C. scharojanii* Rupr. (including *C. lazicus* [Boiss. & Bal. ex] Boiss.), *C. kotschyanus* C. Koch subsp. *kotschyanus*, subsp. *hakkariensis* Mathew, subsp. *cappadocicus* Mathew, subsp. *suwarowianus* (C. Koch) Mathew, *C. autranii* Albov, *C. gilanicus* Mathew, *C. karduchorum* [Kotschy ex] Maw and *C. ochroleucus* Boiss. & Gaill.

C. ochroleucus has previously been investigated more fully than the other species. Counts of $2n = 10$ have been recorded by Mather (1932), Pathak (1940), Karasawa (1937, 1943) and Feinbrun (1958), but of these only the material studied by Feinbrun came from a documented wild locality—Manara, Upper Galilee, Israel. Pathak noted that there were five bivalents at first metaphase of meiosis in this species.

C. kotschyanus has been reported as having $2n = 8$ (Mather, 1932; Karasawa, 1958), *C. karduchorum* as $2n = 8$ (Karasawa, 1961) and $2n = 20$ (Mather, 1932), and *C. scharojanii* as $2n = 8$ (Zakharyera & Makushenko, 1969).

MATERIALS AND METHODS

The corms used for this survey were almost all from known localities and a large number of these collections are still in cultivation at the Royal Botanic Gardens, Kew.

Squashes of feulgen-stained root tips were made using the method previously described (Brighton *et al.*, 1973) and permanent slides are filed in the Cytogenetics Section of the Jodrell Laboratory, Kew. Voucher

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specimens have been placed in the Kew Herbarium (K). Drawings were made using a Zeiss large drawing apparatus and photographs were taken on a Zeiss photomicroscope.

RESULTS

The results of this investigation are summarised in Table 1 (p. 411) and the distribution of the collections examined cytologically is shown on the map (Fig. 7, p. 409). Each of the species of the *C. vallicola* group is dealt with in detail below; their karyotypes are illustrated in Fig. 1–6.

C. kotschyanus has the widest distribution of all the species in this group. It occurs in western Transcaucasia and NE Turkey, along the Anatolian diagonal (Davis, 1971) and into the Cilician Taurus and Amanus mountains in the southwest. It also occurs in the mountains of Hakkâri in south-eastern Turkey. Mathew (1980) has included *C. suwarowianus* C. Koch in *C. kotschyanus* as a subspecies and has described two new subspecies within *C. kotschyanus*.

C. kotschyanus subsp. *kotschyanus*

Intraspecific cytological variation is quite common in *Crocus*. In this subspecies plants with $2n=8$ and 10 were found in var. *kotschyanus*, and each had a discrete distribution (Fig. 7).

$2n=8$ cytotype. This occurs in the Cilician Taurus mountains in southern central Turkey (Fig. 7). The karyotype consists of four pairs of acrocentric chromosomes of decreasing size with satellites on the short arms of the smallest pair (Plate 4d, Fig. 1a–c). In one collection (76.384) the satellites are clearly heteromorphic (Fig. 1b), minute in one chromosome and very large in its homologue, suggesting the occurrence of tandem duplication.

Material of *C. kotschyanus* subsp. *kotschyanus* var. *leucopharynx* B. L. Burt, long known in cultivation, was examined and also found to have $2n=8$ chromosomes, which are very similar to those of var. *kotschyanus* but without satellites visible on the smallest pair (Fig. 1c). Meiosis is regular, with four bivalents at first metaphase, and from one to three chiasmata per bivalent.

$2n=10$ cytotype. All the collections of *C. kotschyanus* subsp. *kotschyanus* from Hatay have 10 small acrocentric chromosomes, similar to those of the $2n=8$ cytotype. One pair of chromosomes has very small short arms bearing satellites (Plate 4e, Fig. 1d–f). The karyotype has been designated 10a. In one case (71.169) it is difficult to distinguish structure within the short arm which seems to be entirely heterochromatic (Fig. 1f).

One collection from Doğanşehir (Baron 2116, 73.29) has $2n=20$ with larger chromosomes than other 20 cytotypes, and a karyotype (Fig. 3a) similar to a duplication of the $2n=10$ (10a) cytotype of subsp. *kotschyanus* (Fig. 1d–f). The similarity in the size and morphology of the chromosomes suggests that it might be an autotetraploid of the $2n=10a$ cytotype, although meiotic studies are needed to confirm this. This is further supported by the isolated locality of this collection (see Fig. 7) which is further south than the distribution of the other $2n=20$ subspecies, subsp. *suwarowianus*, and closer to Hatay where the $2n=10$ (10a) cytotype of subsp. *kotschyanus* occurs. It also has lilac-coloured flowers whereas subsp. *suwarowianus* is predominantly white-flowered. For these reasons it is included here under subsp. *kotschyanus* and regarded as autotetraploid.

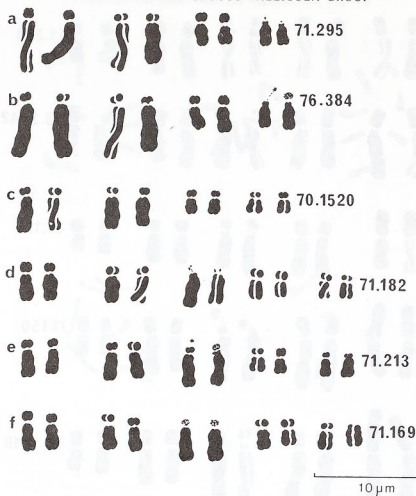


FIG. 1. Karyotypes of *C. kotschyanus* subsp. *kotschyanus*: a, b, var. *kotschyanus*, $2n=8$ cytotype; c, var. *leucopharynx*, $2n=8$; d–f, var. *kotschyanus*, $2n=10$ cytotype (10a).

C. kotschyanus subsp. *hakkariensis*

This subspecies with $2n=10$ also has an acrocentric karyotype, designated 10A, which has larger chromosomes with longer short arms than the 10a karyotype of subsp. *kotschyanus*. The two subspecies are separated geographically, the $2n=10$ (10A) subsp. *hakkariensis* plants having been collected from Hakkâri Vilayet where it is quite common, whereas the 10a subsp. *kotschyanus* plants are so far known only from Hatay. The karyotype consists of four pairs of acrocentric to sub-median chromosomes and one pair of acrocentrics with secondary constrictions in the short arms (Fig. 2a, b).

C. kotschyanus subsp. *cappadocicus*

The third subspecies with $2n=10$ was collected from the area around Tunceli, on the Munzur Dağ range and in the south of Kayseri Vilayet (Fig. 7). Its karyotype, designated 10M, consists of one pair of metacentric

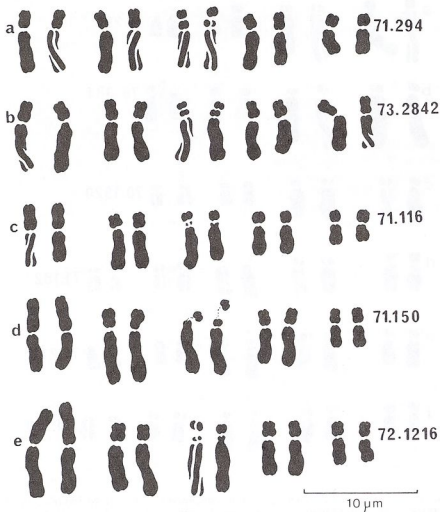


FIG. 2. Karyotypes of: a, b, *C. kotschyanus* subsp. *hakkariensis*, $2n=10$ (10A); c-e, *C. kotschyanus* subsp. *cappadocicus*, $2n=10$ (10M).

chromosomes, three pairs of acrocentrics, one with secondary constrictions in the short arms, and a pair of small metacentric or sub-median chromosomes (Plate 4f, Fig. 2c-e).

C. kotschyanus subsp. *suwarowianus*

C. kotschyanus subsp. *suwarowianus* is widely distributed, occurring in western Transcaucasia and north-eastern Turkey southeastwards to Sivas Vilayet in central Turkey, unlike the other subspecies which are more restricted in distribution. It has $2n=20$ and a complement of small chromosomes. The karyotype consists of three pairs of acrocentrics and seven pairs of small acrocentric to metacentric chromosomes (Plate 4g, Fig. 3b-d). Satellites were observed on the short arms of the third acrocentric pair and, in one case (72.1585), on the smallest pair of chromosomes.

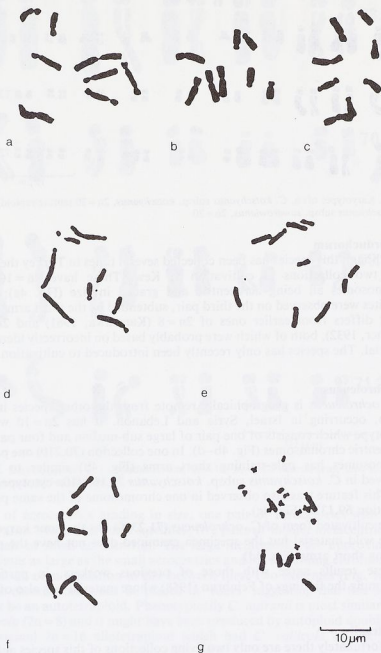


PLATE 4. Chromosomes of *Crocus*: a, *C. vallicola*, $2n=8$; b, *C. scharojanii*, $2n=8$; c, *C. scharojanii* $\times$ *C. vallicola*, $2n=8$; d, *C. kotschyanus* subsp. *kotschyanus*, $2n=8$; e, *C. kotschyanus* subsp. *kotschyanus*, $2n=10$ (10a); f, subsp. *cappadocicus*, $2n=10$ (10M); g, subsp. *suwarowianus*, $2n=20$. All from root tips.

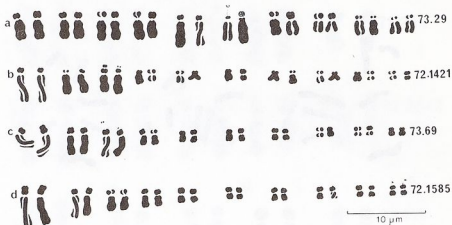


FIG. 3. Karyotypes of: a, *C. kotschyanus* subsp. *kotschyanus*, $2n=20$ (autotetraploid); b–d, *C. kotschyanus* subsp. *suwarowianus*, $2n=20$.

C. karduchorum

Although this species has been collected several times in Turkey there are only two collections in cultivation at Kew. These have $2n=10$, the chromosomes all being acrocentric and graded in size (Fig. 4a); large satellites were observed on the third pair, subtended by the short arms. This count differs from earlier ones of $2n=8$ (Karasawa, 1961) and $2n=20$ (Mather, 1932), both of which were probably based on incorrectly identified material. The species has only recently been introduced to cultivation.

C. ochroleucus

C. ochroleucus is geographically remote from the other species in this group, occurring in Israel, Syria and Lebanon. It has $2n=10$ with a karyotype which consists of one pair of large sub-median and four pairs of acrocentric chromosomes (Fig. 4b–d). In one collection (70.210) one pair of chromosomes has pale-staining short arms (Fig. 4b) similar to those observed in *C. kotschyanus* subsp. *kotschyanus* 71.169 (10a cytotype, Fig. 1f). This feature was also observed in one chromosome of the same pair in collection 69.1324 (Fig. 4c).

The cultivated form of *C. ochroleucus* (71.2300) has the same karyotype as the wild material but the specimen examined does not have the paler-staining short arms (Fig. 4d).

These results agree with those of previous workers, in particular confirming the findings of Feinbrun (1958) whose material was also of wild origin.

C. autranii

Unfortunately there are only two living collections of this species at Kew, both from the Caucasus area of USSR. *C. autranii* is unique cytologically in being the only species of *Crocus* so far found to have $2n=32$. Moreover, the chromosomes are unusually large in a genus where, as a general rule, size tends to diminish with an increase in number. The karyotype consists of two pairs of large metacentric chromosomes, four pairs of sub-medians, three



FIG. 4. Karyotypes of: a, *C. karduchorum*, $2n=10$; b, c, *C. ochroleucus*, $2n=10$; d, *C. ochroleucus*, cultivated form, $2n=10$.

pairs of acrocentrics grading in size, one pair of acrocentrics with small short arms which bear small satellites, five pairs of smaller metacentrics and one pair of small acrocentrics. The large metacentrics are approximately five times as large as the small acrocentrics and the remaining chromosomes exhibit an even gradation in size between these two extremes (Fig. 5a).

The chromosomes can be placed in 'fours' suggesting that this species might be an autotetraploid. Phenotypically *C. autranii* is most similar to *C. vallicola* ($2n=8$) and it might have been produced by autopolyploid doubling of an original $2n=16$ allotetraploid which had *C. vallicola* as one of its parents.

C. gilanicus

Two collections of this species, from north-western Iran, were studied and found to be unusual cytologically. *C. gilanicus* has $2n=24$ and a karyotype which comprises one pair of large metacentric chromosomes, two

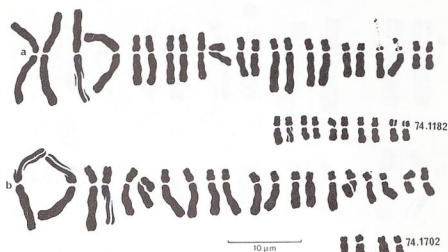


FIG. 5. Karyotypes of: a, *C. autranii*, $2n=32$; b, *C. gilanicus*, $2n=24$.

pairs of sub-medians, six pairs of acrocentrics grading in size, one pair of small metacentrics, and two pairs of small acrocentrics (Fig. 5b).

As noted above, the largest chromosomes in *Crocus* tend to be found in species with low chromosome numbers, but the large metacentrics of *C. autranii* and *C. gilanicus* are approximately the same size as those of *C. vallicola* with $2n=8$ (Fig. 6a–b). *C. autranii* and *C. gilanicus* have similar karyotypes apart from their difference in number. The species are also fairly close geographically.

C. vallicola

C. vallicola ($2n=8$) grows mainly in the Zigana and Soğanli passes of north-eastern Turkey (see Fig. 7). The karyotype of this species is distinctive, consisting of one pair of large metacentric chromosomes, three pairs of acrocentrics, one pair of which have secondary constrictions in the long arms (Plate 4a, Fig. 6a–b). All the collections have this pattern although in some specimens one of the secondary constrictions is less obvious than the other.

C. scharojanii

The two collections of the Turkish *C. scharojanii* studied come from the Soğanli and Zigana passes where this species grows with *C. vallicola* ($2n=8$) and *C. kotschyanus* subsp. *suwarowianus* ($2n=20$). *C. scharojanii* has $2n=8$ chromosomes and a karyotype which comprises one pair of metacentric chromosomes, one pair of sub-metacentrics and two pairs of acrocentrics. The same karyotype was observed in both collections (Plate 4b, Fig. 6c–d). *C. scharojanii* from the Caucasus has been combined by Mathew (1980) with *C. lazicus*, the former name having priority. The one living collection at Kew of wild material from the Caucasus has a karyotype which comprises one pair of metacentric chromosomes and three pairs of acrocentrics, one pair of which has very small satellites on the short arms (Fig. 6e). This karyotype differs from the Turkish collections, but with the

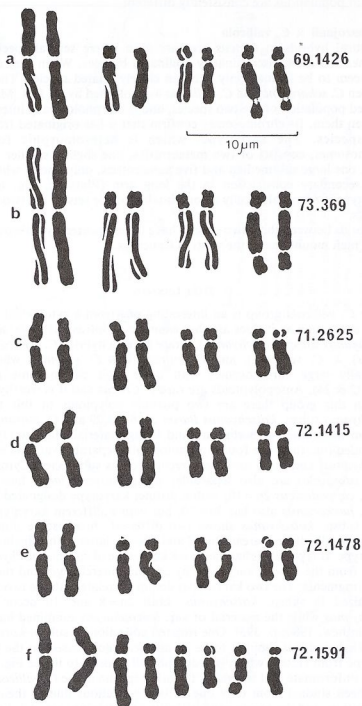


FIG. 6. Karyotypes of: a, b, *C. vallicola*, $2n=8$; c, d, *C. scharojanii*, $2n=8$; e, *C. scharojanii* (Caucasian), $2n=8$; f, *C. scharojanii* × *C. vallicola*, $2n=8$.

limited sampling available it is not possible to say whether the Russian and Turkish populations are consistently different.

C. scharojanii* × *C. vallicola

Natural hybrids in *Crocus* are rare even where several species grow together and are indiscriminately pollinated by bees. When they do occur they seem to be formed only between closely related species. The hybrid between *C. scharojanii* and *C. vallicola* was collected by Dr J. R. Marr from a mixed population of the two species, and is morphologically intermediate between them. Its chromosomes confirm that it has originated from these two species. The karyotype, which is heteromorphic for most chromosomes, consists of two metacentrics, one slightly smaller than the other, one large sub-median and five acrocentrics, only one of which has a clear secondary constriction in the long arm (Plate 4c, Fig. 6f). This karyotype is morphologically a combination of the sets of the two putative parents.

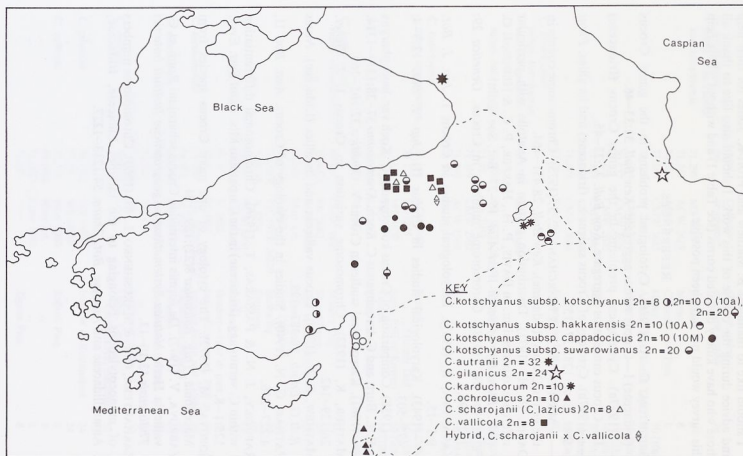
Hybrids between these two species have been reported by Yabrova (1947) in the high mountains of the western Caucasus.

DISCUSSION

The *C. vallicola* group is an interesting one from a cytological point of view, containing as it does another example of intraspecific variation (*C. kotschyanus* subsp. *kotschyanus*), a rare natural hybrid (*C. scharojanii* (*C. lazicus*) × *C. vallicola*), and *C. autranii* and *C. gilanicus* which have unusually large chromosomes with quite high chromosome numbers ($2n=32$ & 24). Autopolyploids are rare in *Crocus* and it is worthy of note that in this group there are two possible polyploids of this type (*C. kotschyanus* subsp. *kotschyanus* Baron 2116, 73.29 and *C. autranii*).

From his studies of herbarium and living material Mathew (1980) has concluded that there are four morphologically separable variants within *C. kotschyanus*, and these have been recognized as subspecies. Cytologically these subspecies are also separable: subsp. *suwarowianus* has $2n=20$; subsp. *cappadocicus* $2n=10$, with a distinct karyotype designated as 10M; subsp. *hakkariensis* also has $2n=10$, but with a different karyotype, 10A; while subsp. *kotschyanus* shows two different chromosome numbers in geographically distinct areas, $2n=8$ and 10 —the latter with a distinctive 10a karyotype. It is possible that the $2n=8$ karyotype of subsp. *kotschyanus* has arisen from the $2n=10$ karyotype by unequal interchanges and the loss of small fragments. The two karyotypes do not correlate with the two varieties recognized in subsp. *kotschyanus*: both $2n=8$ and 10 occur in var. *kotschyanus* while the material of var. *leucopharynx* examined had $2n=8$ (see Mathew, 1980, p. 393). One isolated collection of subsp. *kotschyanus* from Doğanşehir is thought to be an autotetraploid based on the $2n=10a$ cytotype from Hatay, which is geographically closest to it (see Fig. 7).

It is unfortunate that several of the other species in the *C. vallicola* group have been studied from only one or two collections due to their limited distributions and the consequent difficulty of obtaining material. In a genus such as *Crocus*, where so much variation occurs, further studies are needed to give a more accurate view of the cytology of these species.

FIG. 7. Cytological distribution of *C. vallicola* and its allies.

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TABLE I

	Kew cytol. no.	Collector or source*	Locality (Turkish unless otherwise stated)	2n
<i>C. kotschyanus</i>				
subsp. <i>kotschyanus</i>	71.295	MT 4487	Pozanti	8
var. <i>kotschyanus</i>	71.343	ACW 2413	Mersin	8
"	76.384	ABM 2	Cilician Gates Pass	8
"	71.169	CMW 2685	Hatay	10 (10a)
"	78.127	W 2498	Amanus Mts, Karlik Tepe	10 (10a)
"	71.214	CMW 2719	Hatay	10 (10a)
"	70.157	CMW 2626	"	10 (10a)
"	70.161	CMW 2625	"	10 (10a)
"	71.182	CMW 2686	"	10 (10a)
"	70.208	CMW 2682	"	10 (10a)
"	71.213	CMW 2684	"	10 (10a)
"	73.29	B 2116	Doğanşehir, Malatya Vilayet	20
<i>C. kotschyanus</i>				
subsp. <i>kotschyanus</i>				
var. <i>leucopharynx</i>	70.1520	Hort. origin	—	8
<i>C. kotschyanus</i>				
subsp. <i>hakkariensis</i>	73.2842	TB, ISTE 26.119	Hakkâri Vilayet	10 (10A)
"	73.38	Rix 1953	"	10 (10A)
"	71.359	CMW 2982	"	10 (10A)
"	71.294	ACW 1569	"	10 (10A)
"	70.149	Rix 161	"	10 (10A)
<i>C. kotschyanus</i>				
subsp. <i>cappadocius</i>	71.150	ACW 2401	Tunceli	10 (10M)
"	70.173	D 31243	" , Munzur Dağ	10 (10M)
"	71.116	MT 4520	Sariz, ESE of Kayseri	10 (10M)
"	72.1420	TB 2	Tunceli	10 (10M)
"	72.1216	Rix 1610	Sivas, Zirayet Pass	10 (10M)
"	78.898	M 9042	" " "	10 (10M)
<i>C. kotschyanus</i>				
subsp. <i>suwarowianus</i>	69.1417	CMW 3838	Munzur Dağ	20
"	72.1421	Rix 2062	Sivas Vilayet	20
"	71.342	ACW 2322	Ispir to İkizdere Pass	20
"	70.160	Marr s.n.	Zigana Pass	20
"	69.1126	F 3882	Koş Dağ Pass	20
"	73.2812	TB, ISTE 26229	Sarikamiş	20
"	72.1585	A 3274	Erzurum	20
"	73.69	ACW 2300	"	20
"	75.1211	CMW 3827	Erzincan	20
"	80.4	M 9022	İkizdere—Ispir nr pass	20
"	80.8	M 9025	Ispir—Erzurum rd, 2300 m	20
<i>C. karduchorum</i>	75.288	TB, ISTE 31.134	between Hizan and Tatvan	10
"	78.899	M 9037	Gümüşdere—Hizan	10
<i>C. ochroleucus</i>	69.1144	OP 28	Lebanon	10
"	69.1324	Aw 682363	Israel: Golan Heights	10
"	70.210	Aw s.n.	" " "	10
"	71.2300	VF	Cultivated origin	10
<i>C. autranii</i>	74.1182	LA	USSR. Caucasus:	
"			Abkhazskaya, Sukhumi	32
"	77.84	T	USSR	32
<i>C. gilanicus</i>	74.1702	WS 14870	Iran: W of Rustanabad	24
"	77.10	WS 14910	" " "	24
<i>C. valliscola</i>	73.371	Marr 2066	Soğanlı Pass	8
"	69.1426	Marr s.n.	"	8
"	71.2623	Marr 1088	"	8
"	71.297	—	Zigana Pass	8

	Kew cytol. no.	Collector or source*	Locality (Turkish unless otherwise stated)	2n
<i>C. vallicola</i>	70.163	MT 4347	Zigana Pass	8
"	70.151	MT 4344	"	8
"	70.185	Marr s.n.	"	8
"	72.1588	LP 72/49	"	8
"	73.369	GW s.n.	"	8
"	73.370	Marr 1114	"	8
"	76.308A	Rix 1018	"	8
"	77.57	PT 75/137	Soganli Pass	8
"	79.691	M 9013	Zigana Pass, Stavros, 2000 m	8
"	80.101	Wa 68	Soganli Pass, 8000 m, N side summit	8
"	80.215	P s.n.	Soganli Pass, 2100 m	8
<i>C. charojanii</i>	71.2625	Marr 2073	Soganli Pass	8
"	72.1415	Cobb	Zigana Pass	8
"	80.7	M 9011	" " —Stavros	8
"	72.1478	Unknown	USSR. Caucasus: Mt Tscheget	8
<i>C. charojanii</i> × <i>vallicola</i>	72.1591	Marr s.n.	Soganli Pass	8

* Abbreviations

A = Archibald
 ABM = Allison, Ball & McNeill
 ACW = Albury, Cheese & Watson
 Aw = Awishai
 B = Baron
 CMW = Cheese, Mitchell & Watson
 D = Davis
 F = Furse
 GW = Grey-Wilson
 LA = L. Asiechvili
 LP = Leep & Pasche

M = Mathew
 MT = Mathew & Tomlinson
 OP = O. Polunin
 P = Pasche
 PT = Pasche & Taubenheim
 T = Tbilisi Botanical Garden
 TB = T. Baytop
 VF = V. Finnis
 W = Watson
 Wa = W. A. P. Marr
 WS = Wendelbo & Shirdelpur