Cytological Investigations on Eight Carex Species in Korea (Cyperaceae)

Kyong-Sook Chung1*, Takuji Hoshino2, Tomomi Masaki2 and Hyoung-Tak Im3

1Department of Medicinal Plant Science, Jungwon University, Goesan-gun, Chungbuk 28024, Korea
2Department of Biosphere-Geosphere Science, Faculty of Biosphere-Geosphere Science, Okayama University of Science, Okayama 700-0005, Japan
3Department of Division of Biological Science, Chonnam National University, Gwangju 61186, Korea

Received April 18, 2017; accepted May 30, 2017

Summary Carex L. is one of the most species-rich genera in the flora of Korea with about 157 taxa. Somatic or meiotic chromosome numbers of eight Carex taxa from the Korean Peninsula are reported, including first counts for C. macrandrolepis H. Lév. (n=37II) and C. splendentissima U. Kang & J. M. Chung (2n=12). Furthermore, there are first chromosomal investigations from Korea populations: C. bostrychostigma Maxim. (n=22II), C. capricornis Meinsh. ex Maxim. (n=35II), and C. breviculmis R. Br. (n=33II). None of the chromosomes has distinct primary constrictions. Carex sect. Mitratae exhibits high variation in chromosome numbers with aneuploidy (chromosome number increases with genomic duplication) and/or agmatoploidy (chromosome number increases without genomic duplication) whereas C. sect. Siderostictae shows polyploidy. Chromosome dynamics have played an important role in Carex species diversity in the Korean Peninsula.

Key words Carex, Chromosome number, Cyperaceae, Flora of Korea, Agmatoploidy, Chromosome dynamics.

Genus Carex L. (Cyperaceae) is one of the largest angiosperm genera in the temperate zone with more than 2000 species worldwide (Reznicek 1990, The Global Carex Group 2015). The genus exhibits great variance in chromosome numbers from 2n=12 to n=132 with continuous haploid numbers from n=6 to n=48 (Tanaka 1949, Davies 1956, Roalson 2008, Hipp et al. 2009). The chromosome number variance has been postulated to be due to the non-localized centromeres (holocentric chromosomes, no primary constrictions visible) that facilitate increases (agmatoploidy) and/or decreases (symploidy) in chromosome number by fission and fusion, respectively (Hoshino 1981, Nishikawa et al. 1984, Luceho and Guerra 1996, Chung et al. 2011, Hiph et al. 2013). Polyploidy and aneuploidy have been hypothesized to play an important role on species diversity in the genus (Hipp 2007, Rothrock et al. 2009, Hiph et al. 2010, Yano et al. 2010, 2014, Chung et al. 2012, Escudero et al. 2012). In particular, variation levels in chromosome numbers are significant in the species that have undergone recent and rapid speciation (Hipp et al. 2009, Chung et al. 2011).

About 157 taxa of Carex have been recorded from Korea, which makes the genus the most species-rich genus out of 1044 genera in the flora of Korea (Oh 2007, Kim et al. 2008). Although chromosome number variation is a well-known characteristic in Carex speciation, only a few chromosome studies have been conducted on Korean Carex populations. Kim (2006) and Lee and Kim (2008) reported the somatic chromosome numbers of C. blepharicarpa var. stenocarpa Ohwi (2n=20), C. siderosticta Hance (2n=12), and C. okamotoi Ohwi (2n=12). Recently, somatic chromosome numbers of Carex sect. Siderostictae were counted from multiple populations in Korea (Chung et al. 2013), and meiotic chromosome investigations were reported for several species (Chung et al. 2016). The studies demonstrated that Carex chromosomes were very small, about 1µm long in size; the chromosomes might have evolved from large chromosomes (about 2–4µm long) with small numbers (2n=12) to smaller chromosomes (less than 1µm long) with high numbers, and chromosome number dynamics (e.g., aneuploidy, agmatoploidy, symploidy, and polyploidy) have contributed to species diversity (Chung et al. 2013, Chung et al. 2016). Cytological information of Korean Carex species will be helpful to understand species diversity in the flora of Korea, as well as chromosome evolution in Carex as a whole.

In this paper, we report somatic or meiotic chromosome numbers of eight Carex species in four sections from Korean populations and discuss their significance in taxonomic and cytological aspects.

Materials and methods

The chromosome numbers of eight species (11 individuals) in Carex from Korean populations were analyzed. Young stamens and/or roots were collected from natural populations and/or from Korea National
Arboretum (Province Gyeonggi-do, Korea) to determine chromosome numbers. For meiotic chromosome observation, immature spikes were fixed in a mixture of methanol, chloroform, and propionic acid (6:3:2) (Rothrock and Reznicek 1996). In addition, mitotic root tips were fixed in D.W. at 4°C for 24h (Chung et al. 2013). Fixed anthers and roots were squashed in 2% aceticorcein and observed at 1000×magnification and photographed (Nikon Eclipse 50i). At least three meristematic cells per sample were analyzed to determine meiotic or mitotic chromosome numbers. All voucher specimens are deposited at the Korea National Arboretum Herbarium (KH) (Table 1).

Results and discussion

The meiotic or mitotic chromosome numbers observed are tabulated with previous records (Table 1). All chromosomes are less than 1µm long except for taxa in Carex Sect. Siderostictae (C. siderosticta and C. splendentissima) (Fig. 1). In addition, primary constrictions are not found in any chromosomes.

Table 1. Species, localities, voucher specimens, and chromosome numbers of Carex collected from Korea (FRK. First report from Korea, FR. First report).

<table>
<thead>
<tr>
<th>Taxon (locality, voucher specimen)</th>
<th>Chromosome numbers counted (2n)</th>
<th>Previous counts (2n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sect. Debiles Carex bostrychostigma Maxim. Mt. Irwolsan, Yeongyang-gun, Gyeongsangbuk-do, South Korea (Chung et al. 1546, 24 April 2016, KH)</td>
<td>FRK44 (n=22II)</td>
<td>46 (Hoshino 1981)</td>
</tr>
<tr>
<td>Sect. Digitatae C. macrandrolepis H. Lév. Yongsu-ri, Hangyeong-myeon, Jeju-si, Jejuteukbyeoljachi-do, South Korea (Chung et al. 403, 19 April 2013, KH)</td>
<td>FR74 (n=37II)</td>
<td>None</td>
</tr>
<tr>
<td>Sect. Mitratae C. breviculmis R. Br. Uisin-myeon, Jindo-gun, Jeollanam-do, South Korea (Chung et al. 1401, 26 June 2016, KH)</td>
<td>FRK 66 (n=33II)</td>
<td>64 (Lange and Murray 2002)</td>
</tr>
<tr>
<td>C. sabynensis Less. ex Kunth Mt. Irwolsan, Yeongyang-gun, Gyeongsangbuk-do, South Korea (Chung et al. 1539, 23 April 2016, KH)</td>
<td>76 (n=38II)</td>
<td>40, 41, 64 (Tanaka 1948)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>42 (Chung et al. 2016)</td>
</tr>
<tr>
<td>C. tristachya Thunb. Uisin-myeon, Jindo-gun, Jeollanam-do, South Korea (Chung et al. 1403, 26 June 2016, KH)</td>
<td>42 (n=21II)</td>
<td>40, 41, 64 (Tanaka 1948)</td>
</tr>
<tr>
<td>Uisin-myeon, Jindo-gun, Jeollanam-do, South Korea (Chung et al. 1405, 26 June 2016, KH)</td>
<td>42 (n=21II)</td>
<td>42 (Chung et al. 2016)</td>
</tr>
<tr>
<td>Sect. Pseudocyperae C. capricornis Meinsh. ex Maxim. Korea National Arboretum, Socheureup, Pocheon-si, Gyeonggi-do, South Korea (Chung et al. 1607, 9 May 2016, KH)</td>
<td>FRK70 (n=35II)</td>
<td>70 (Tanaka 1948)</td>
</tr>
<tr>
<td>Korea National Arboretum, Socheureup, Pocheon-si, Gyeonggi-do, South Korea (Chung et al. 1608, 9 May 2016, KH)</td>
<td>FRK70 (n=35 II)</td>
<td></td>
</tr>
<tr>
<td>Sect. Siderostictae C. siderosticta Hance Songdeok-ri, Jangyeon-myeon, Goesan-gun, Chungecheongbuk-do, South Korea (Chung et al. 1511, 6 April 2016, KH)</td>
<td>12 (n=6II)</td>
<td>12, 24 (Hoshino and Tanaka 1977, Hoshino 1981)</td>
</tr>
<tr>
<td>C. splendentissima U. Kang &amp; J. M. Chung Jeongseon-gun, Gangwon-do, South Korea (Chung s.n. KNA, 1 April 2017, KH)</td>
<td>FR12</td>
<td>12 (Chung et al. 2013)</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td></td>
</tr>
</tbody>
</table>

Carex sect. Debiles (J. Carey) Ohwi

Carex sect. Debiles comprises about 10 taxa occurring in Asia, and five species are endemic to China (Dai et al. 2010). Out of 10 taxa, only C. bostrychostigma chromosome has been reported with 2n=46 chromosome numbers (Hoshino 1981).

C. bostrychostigma Maxim. (n=22II, Fig. 1A): From meiotic pollen mother cell observation, 2n=44 chromosome numbers of the species are confirmed, which is the first chromosome number count from Korean populations and differs from the previous count (2n=46, Hoshino 1981). The species inhabits roadsides and forest margins in Korea, Japan, and N.E. China (Hoshino et al. 2011). The species exhibits a broad geographic distribution in the section. Additional chromosome observations from Japan, Korea, and China populations will determine a chromosome number variation range in the species. Furthermore, to understand high endemism in the section, phylogenetic and geographic studies are required.
Carex sect. Digitatae (Fries) Christ

The section comprises about 30 species in Europe, Asia, and North America with high diversity (about 20 species) in East Asia (Egorova 1999, Hoshino et al. 2011). However, several species have been treated in sect. Clandestinae G. Don, Rhomboidales Kükenthal, or sect. Digitatae (Fries) Christ (Hendrichs et al. 2004, Dai et al. 2010). Among Asian Digitatae species, variation in chromosome numbers has been reported: C. lanceolata Boot (2n=70 and 72; Hoshino 1981, Hoshino and Ikeda 2003) and C. lasiolepis Franch (2n=16 and 36; Hoshino 1981, Hoshino et al. 2011).

C. macrandrolepis H. Lév. (n=37II, Fig. 1E): For the first time, the chromosome number of C. macrandrolepis is determined. The species occurs only in Taiwan, Japan, and Korea (Hoshino et al. 2011, Katsuyama 2015).

Carex Sect. Mitratae Kükenthal

About 60–80 species occur in Asia, Australia, Europe, and New Zealand with high diversity in Asia, and with great numbers of endemic species, such as 16 Chinese endemics and 53 Japanese endemics (Standley 2002, Dai et al. 2010, Hoshino et al. 2011). Coupled with high species diversity in Asia, morphological variations within and/or among species have resulted in complex taxonomic histories of taxa. The chromosome numbers of 13 species in the section have been reported (IPCN accessed on 18 March 2017). Many species exhibit variation in chromosome numbers within species, and a univalent is observed in several species such as C. caryophyllea Latourr. and C. sociata Boott (Luceño 1993, Ohkawa et al. 2000, Chung et al. 2016).

C. breviculmis R. Br. (n=33II, Fig. 1D): We observed a chromosome number of 2n=66 in meiotic pollen mother cells. However, Lange and Murray (2002) reported 2n=ca. 64 of the species from New Zealand, and chromosome numbers of C. leucochloa Bunge, a taxonomic synonym of the species, were documented as 2n=64, 68, 70, 72, 74 (Hoshino 1981, Ohkawa and Yokota 1998, Dai et al. 2010, Hoshino et al. 2011). In Korea and China, several infraspecific taxa of the species have been recognized due to morphological variation (Dai et al. 2010, Yang et al. 2014). In various Carex species, chromosome number variation coupled with morphological variance has been found (Hipp et al. 2007, Chung et al. 2011). To resolve complex infraspecific classification, additional cytological investigations with samples covering morphological character variations are...
recommended.

*C. sabynensis* Less. ex Kunth (*n*=38II, Fig. 1F): We observed meiotic chromosomes of *n*=38II from *C. sabynensis*, which is incongruent with previous reports of *n*=27II (Chung et al. 2016), 2*n*=60 (Yurtsev and Zhukova 1982) and 2*n*=40 (Krogulevich 1971). In particular, our results suggest that chromosome number variations among Korean populations conflict with the previous counts from a Korean population (Chung et al. 2016). The species is distributed in broad areas in East Asia, growing in open meadows or wet places in Japan, Korea, East Siberia, and northeast China (Hoshino et al. 2011). The broad geographic distribution associated with the morphological character variations might have resulted in the incongruent cytological characters.

*C. tristachya* Thunb. (*n*=21II, Fig. 1G): The chromosome number counts from two Korean populations are reported as *n*=21II, which agrees with previous reports from Korean populations (Chung et al. 2016). Korean populations have a consistent chromosome number of 2*n*=42, but Japanese populations exhibit variations in chromosome numbers among populations (2*n*=40, 41, 64; Tanaka 1948). The species also occurs in southeast China (Dai et al. 2010).

**Carex sect. Siderostictae Franchet ex Ohwi**

About 13 species in the section occur in Asia, and previous phylogenetic studies suggest the sections as a basal group to the rest of *Carex* species (Starr and Ford 2009, Waterway et al. 2009, Jung and Choi 2013, Chung et al. 2013). Chung et al. (2013) and Yano et al. (2014) demonstrated that chromosomes in the section are larger in size with low numbers, and polyploidization events are involved with phylogenetic lineage divergence.

*C. siderosticta* Hance (*n*=6II, Fig. 1H): Six bivalent chromosomes were observed at meiotic pollen mother cells. Previous counts from Korea are diploids with chromosome numbers of 2*n*=12 (Chung et al. 2013, Chung et al. 2016). In China, Japan, and Russia, diploids and/or tetraploids have been reported: 2*n*=12, 24 from Japan (Hoshino and Tanaka 1977, Hoshino 1981, Hoshino et al. 1993), and 2*n*=24 from China and Russia (Tang and Xiang 1989, Starodubtsev 1989, Probatova et al. 1998, Probatova 2000). Although aneuploidy is known to play an important role in *Carex* speciation, in *Carex* sect. *Siderostictae*, polyploidy seems to be a major speciation mechanism.

*C. splendentissima* U. Kang & J. M. Chung (*n*=12, Fig. 1I): Mitotic chromosomes of *C. splendentissima* are determined for the first time. The species is endemic to Korea and known to have morphological similarity to *C. ciliatomarginata* Nakai, which also has somatic chromosome numbers of 2*n*=12 (Hoshino and Tanaka 1977, Hoshino 1981, Hoshino and Shimizu 1986, Chung et al. 2013). Two species in the section, *C. splendentissima* and *C. okamotoi* Ohwi, are endemic to Korea (Kang et al. 2012, Yang et al. 2014). Both are diploids (Chung et al. 2013), but their distribution patterns differ in Korea. *C. splendentissima* has been only found in northeastern South Korea whereas *C. okamotoi* occurs mainly in southern parts of the Korean Peninsula (Kang et al. 2012, Yang et al. 2014).

The chromosome numbers for *C. macrandrolepis* (*n*=37II) and *C. splendentissima* (*n*=12) are determined for the first time in this study. Both occur only in East Asia. In particular, *C. splendentissima* has been only found in Korea with a small number of populations (Hoshino et al. 2011, Kang et al. 2012). Only one species, *C. capricornis*, exhibits variations in chromosome number comparing with previous reports (Table 1). As pointed previously, only diploid chromosome numbers are found in *C. siderosticta* from South Korea populations, but tetraploids are common in populations from China and Japan (Chung et al. 2013, Yano et al. 2014, Chung et al. 2016). Further cytological studies are needed to elucidate the chromosomal differentiation among natural populations. More cytological studies are also needed in order to explain the mechanisms of the aneuploidal diversification of four species, *C. bostrychostigma*, *C. breviculmis*, *C. sabynensis*, and *C. tristachya*, which have different chromosome numbers from...
previous reports.

Acknowledgement

This study was conducted as a part of the “Cytological study of Cyperaceae in Korea” project funded by the Korea National Arboretum.

References


Egorova, T. V. 1999. The Sedges (Carex L.) of Russia and Adjacent States (Within the Limits of the Former USSR, Missouri Botanical Garden Press, St. Louis.


