Karyotypes of Amphidiploid of *Scilla scilloides* Complex in Taiwan with Special Reference to Secondary Constriction of the A Genome

Masahiro Hizume* and Fukashi Shibata

Faculty of Education, Ehime University, 3 Bunkyo-cho, Matsuyama, Ehime 790–8577, Japan

Received September 16, 2015; accepted May 30, 2016

Summary  Several bulbs of *Scilla scilloides* collected at two localities in Northern Taiwan were studied for their karyotypes. All plants had 2n=34 chromosomes and their karyotypes, including the characteristic chromosomes of A and B genomes, were considered to be AABB genome constitution. The karyotype of each plant had two, three or four secondary constricted chromosomes. In all plants, two chromosomes with clear secondary constriction having an elongated thread and satellite corresponded to the b1 chromosome of the B genome. In some plants, two or one a2 chromosomes of the A genome had secondary constriction at the proximal region in addition to the b1 chromosome. All plants formed, at maximum, four nucleoli with various sizes in a nucleus, indicating that they had four NORs in contrast with the number of secondary constrictions. Size variation of nucleoli seemed to correlate with incidence of secondary constriction. The variation of secondary constriction or NOR in AABB plants of Taiwan populations was discussed with AABB karyotypes in other localities.

Key words  Amphidiploid, Chromosome, Genome, Karyotype, NOR, Nucleolus, *Scilla scilloides*, Secondary constriction, Taiwan.

The genus *Scilla* is composed of about 90 species and most species are distributed in Europe and around the Mediterranean Sea, with two species growing separately in the Himalayas and East Asia. *Scilla scilloides* is the only species growing in a broad area of East Asia; China, Korea, and Japan (Noda 1976, Wang and Tang 1980). Morinaga (1932) had reported two genomes with different basic karyotypes in *S. scilloides* and three cytotypes in vicinity populations of Fukuoka, Japan. Cytogenetic studies in this species were extensively performed in Japan and revealed that *S. scilloides* was a complex species composed of two genomes, A genome (n=8) and B genome (n=9) possessing different karyotypes (Araki 1971, Noda 1974, Haga and Noda 1976). Diploids and/or polyploids with various combinations of two genomes were recognized in populations over different habitats (Sato 1935, Okabe 1938, Haga and Noda 1956a, b, 1976, Araki 1971, 1972, 1975, Araki et al. 1976). To reveal the geographic distribution of plants with various genome constitutions and an origin for amphidiploids having AABB genomes, many populations were analyzed for karyotype in Japan (Haga and Noda 1976, Ibara 1977), then investigated in other parts of East Asia including Korea (Araki et al. 1979, Araki 1985, Choi and Bang 1990, Bang and Choi 1991, 1993, Choi et al. 2004), China (Fu and Hong 1989, Yu and Araki 1991, Yu et al. 1992, Hizume et al. 1993, Ding et al. 1998, 2005a, b) and Taiwan (Chang and Hsu 1974, Uchino et al. 1992, Ding et al. 1998). From the deposits of cytological analyses, we were able to draw geographic distributions of diploids of A and B genomes, and we found that amphidiploid and polyploids with various genome constitutions were as follows. Diploid of the A genome is growing in a broad area from Korea to Northern and Southwestern China, and Taiwan. BB diploids were distributed in Japan and Southeastern China. AABB plants were found in Japan, the Korean Peninsula, and Northeastern China, around the overlap area of distributions of AA and BB diploids in Southeastern China, and Northern Taiwan. The other polyploids such as allopolyploids and hybrids between diploid and amphidiploid, were scattered in many localities where diploid and amphidiploid are growing together.

Karyotype of the AABB genome (common-type) which had secondary constriction on only subtelocentric b1 chromosomes of the B genome and not on a2 chromosomes of the A genome was established by hard cytogenetic analysis (Araki 1971, Haga and Noda 1976, Yu and Araki 1991) and genomic *in situ* hybridization (Hizume and Araki 1996). The AA diploid has secondary constriction on a2 chromosomes that is homologous to b1 chromosomes (Araki 1971). The AABB and ABBB plants which seemed to be hybrids between AABB and BB plants had a2 chromosome without secondary constriction derived from common-type AABB karyo-
type (Haga and Noda 1976). After in situ hybridization (ISH) using rDNA probes, the b1 chromosomes had large rDNA signals and a2 chromosomes had faint or no signal (Hizume and Araki 1994, Choi et al. 2008). The ISH result indicated that the absence of secondary constriction of the a2 chromosome in AABB plants and their hybrids was caused by loss of rDNA copies from secondary constricitions of the a2 chromosome and not by differential amphiplasity (Hizume and Araki 1994) reported in interspecific hybrids of Crepis (Navashin 1934). This was supported by ISH results of a hybrid between Korean AA and Japanese BB diploids in which both nucleolar chromosomes had large rDNA signals and two nucleoli (Hizume and Araki 1994). A similar phenomenon of loss of secondary constriction or deletion of rDNA in polyploids was reported frequently such as in Milium and Scilla autumnalis (Bennett and Thomas 1991, Vaughan et al. 1993).

In the Ryukyu Archipelago, Okinawa Island is the largest among the islands scattered between Kyushu, Japan and Taiwan. In Okinawa, AABB plants had four large rDNA signals or secondary constrictions on both a2 and b1 chromosomes and made four nucleoli in a nucleus (Hizume and Araki 1994). The karyotype of Okinawa AABB (Okinawa-type) was different from that of AABB (common-type) of most localities having secondary constriction only on the a2 chromosome. In three localities of Northern Taiwan, S. scilloides plants were studied cytologically and karyotypes of AA and AABB genome constitutions were recorded (Chang and Hsu 1974, Uchino et al. 1992, Ding et al. 1998). Only the genome constitutions of AABB plants were reported without mention of secondary constriction. To understand the relationship between AABB plants of Okinawa and other regions, we need more cytological information on karyotypes of AABB plants in Taiwan with particular attention to secondary constriction or NOR. This brief report includes conventional karyotype analysis of S. scilloides in two localities of Taiwan to reveal relationships of AABB plants in other populations. The nucleolar chromosomes of AABB plants in Taiwan are discussed.

Materials and methods

Plants

We collected 11 and 14 bulbs of Scilla scilloides (Lindl.) Druce at natural populations of Heping Island, Keelung City and Yeliu, Taipei County in Northern Taiwan, respectively, and planted them in pots in the experimental garden of Ehime University, Matsuyama, Japan. Under the cultivation and climate condition they came into flowering at late September to October, and it was the latest flowering among our S. scilloides collections from various localities. The scapes of the Taiwan plants were short with about 10–12 cm height like that of AABB plants collected in Okinawa. Under these conditions seeds were separated from other collections of Taiwan plants and used for cytological analysis.

Cytological observation

Root tips were collected from individuals planted in a pot and treated with 2 mM 8-hydroxyquinoline for 3 h, then fixed in acetic alcohol (v:v=1:3) and stored in a freezer. Fixed root tips were put in water to remove soil and dust. The root tips were treated with 1 M HCl at 60°C for 30 s, and the meristematic tissue was dissected on to a glass slide. The meristematic tissue was stained with 2% acetic orcein for 2–3 min at room temperature. The tissue on the slide was covered with a coverslip and was squashed. Microscopic images of chromosomes were taken with a digital camera. Each chromosome name of A and B genomes followed Araki (1971). The A genome has the longest metacentric a1 chromosome and subtelocentric a2 chromosomes, which have secondary constriction at the proximal region of the short arm in AA diploid and in AABB plants a2 chromosomes did not display secondary constriction. The B genome has the longest subtelocentric b1 chromosomes having secondary constriction at the proximal region of short arm and four pairs of short metacentric b6–9 chromosomes. For observation of nucleoli, the fixed roots were macerated in 45% acetic acid at 60°C for 5 min and squashed. The preparation was removed onto a coverslip and stained with 50% silver nitrate (w/w) at 65°C for 1–3 h (Hizume et al. 1980).

Results and discussion

All 26 plants of S. scilloides collected in two localities of Northern Taiwan had 2n=34 chromosomes and no small supernumerary chromosome observed frequently in this species (Araki 1985, Choi and Bang 1990, Yu and Araki 1991, Bang and Choi 1993, Ding et al. 1998). The characteristic chromosomes for each genome, such as the longest metacentric a1 chromosome of the A genome and b1 chromosomes with secondary constriction and four short metacentric chromosomes of the B genome, were easily identified in each chromosome complement of all plants examined (Fig. 1A–C). The chromosome composition of karyotypes indicated that all plants had AABB genomes supporting previous reports (Chang and Hsu 1974, Ding et al. 1998). Two to four secondary constrictions of b1 and a2 chromosomes appeared in each chromosome complement in both populations. All plants had secondary constrictions at the proximal region of b1 chromosomes. Another secondary constriction appeared on nucleolar a2 chromosomes in homozygous or heterozygous condition depending on individuals (Fig. 1A, B), and in some plants no secondary constriction appeared on the a2 chromosome (Fig. 1C).

Detection of faint secondary constriction on a2 chro-
Karyotypes of Scilla scilloides in Taiwan 281

In Taiwan, karyotypes of Scilla scilloides were reported previously by Chang and Hsu (1974), Uchino et al. (1992), and Ding et al. (1998). The karyotype of the AA diploid was reported in the population of Taouyen and possessed secondary constriction on a2 chromosomes (Uchino et al. 1992) similar to that of AA diploids of other localities in China and Korea (Araki 1985, Yu and Araki 1991, Hizume et al. 1993, Ding et al. 1998). Ding et al. (1998) reported on plants of AABB karyotype in a population of Suao without mentioning secondary constriction, suggesting that they would regard AABB karyotype of Taiwan as common-type AABB. The AABB karyotype (Okinawa-type) having four satellite chromosomes of both genomes and four similar-sized nucleoli in a nucleus, were also found in Taiwanese populations (Fig. 1A, D). The difference between the Okinawa and Taiwan populations is that some Taiwan plants had a2 chromosomes showing no secondary constriction in spite of having active NORs.

AABB amphidiploids would be generated by hybridization between AA and BB diploids and following polyploidization. Hybridization between AA and BB diploids and the making of AB hybrid plant were realized by Araki (1971). The artificial AB hybrid between...
Korean AA and Japanese BB diploids had nucleolar a2 and b1 chromosomes carrying secondary constriction. Their secondary constrictions of both genomes had a large amount of rDNA and NOR function demonstrated by ISH with rDNA probe (Hizume and Araki 1994). The sterile intergenomic hybrids tend to become fertile in sexual reproduction by polyploidization as reported in many plants (Kihara 1930). The primitive AABB karyotype generated by polyploidization of the AB hybrid would have four natural secondary constrictions or NORs on both a2 and b1 chromosomes. On the basis of geographical distributions of plants with each genome constitution, Ding et al. (1998) suggested that the place of origin of AABB plants was plausible in central Eastern China where AA and BB diploids were growing in the same or nearby localities. It was widely accepted that AABB plants have secondary constructions only on b1 chromosomes and not in a2 chromosomes. The secondary constriction of a2 chromosomes in primitive AABB karyotypes disappeared in the early stage of cytotype differentiation into common-type karyotypes and the mechanism of this phenomenon was unsolved. On the other hand, the AABB plants with four NORs were discovered in Okinawa (Hizume and Araki 1994) and Taiwan (Fig. 1). The primitive AABB generated in China might migrate to Taiwan and Okinawa, which were sometimes connected to mainland China and Japan–Korea through land bridges made by changes in sea level. The primitive AABB plants might be relics in Taiwan and Okinawa islands after separation from mainland China. Then, in China, the primitive AABB plants had lost most of the rDNA or secondary constriction of a2 chromosomes and changed to the common-karyotype. The common-karyotype AABB plants would spread over China, Korea, and Japan but not reach Okinawa and Taiwan. This suggests that the Okinawa-type karyotype might adapt to the environmental condition of islands.

Process of variation in secondary constrictions and NOR functions among AABB plants in Taiwan populations might be explained by the following hypotheses. The first one is that the population of primitive-type AABB plants migrated from China to Taiwan and further Okinawa. In Taiwan, incoming AABB plants would lose secondary constriction or NOR function of a2 chromosomes by unknown genetic factors as occurring in China. The Taiwan population might display ongoing stages of decrease of NOR function in primitive AABB plants. This phenomenon might be replicated in artificial AABB plants made by polyploidization of the AB hybrid. Observation of the AABB karyotypes over several generations would reveal whether the secondary constriction on a2 chromosome changes. The second hypothesis is that plants of primitive karyotype migrated to Taiwan, and then in China the primitive AABB plants lost secondary constriction of a2 chromosome to common-type karyotype. The common-type AABB of China would expand again to Taiwan and then might meet primitive-type AABB coming previously in some localities of Northern Taiwan. They would be expected to perform hybridization between plants of two AABB cytotypes to generate hybrid AABB with secondary constrictions on two b1 and one a2 chromosomes like Fig. 1B. When the hybrids would make gametes by meiosis, unequal recombination might occur in the rDNA locus of a2 chromosomes to generate variation of number of rDNA repeats. Over several generations, rDNA repeats of the rDNA locus on a2 chromosomes would vary in number of rDNA repeats or NOR function among plants in Taiwan populations. The rDNA locus having a large amount of rDNA might appear as secondary constriction, while that with a small amount of rDNA did not appear as secondary constriction in spite of having NOR function. This speculation would be examined by making hybrids between the Okinawa-type and common-type AABB plants, and then watching the amount of rDNA and the expression of secondary constriction for several generations. NOR function and amount of rDNA would appear to relate to the display of secondary constriction on a2 chromosomes. It is suspected that unequal recombination occurs only in the a2 chromosome as the occurrence of loss of rDNA in a2 chromosomes, generating common-type AABB in China. The phenomenon might occur also between Okinawa Island and Kagoshima, Kyushu. The third hypothesis is that NOR function of Taiwan AABB plants was suppressed by unknown mechanism reported in a Chinese AA plant (Hizume et al. 2014). In AABB population of Taiwan suppression of NOR activity of rDNA might occur partially on a2 chromosomes by similar genetic condensation.

Application of ISH with rDNA probe to experimental hybrid AABB plants would validate these hypotheses and reveal relationships among rDNA amount, expression of secondary constriction and NOR function in amphidiploids. Future molecular phylogenetic analysis on genomic and chloroplast DNAs among populations over the distribution area, in addition to cytological and molecular cytogenetic analyses, will draw an outline of genome differentiation, the place of origin of amphidiploids, direction of parent in hybridization, and the route of migration to the present distribution in S. scilloides complex.

Acknowledgements

The authors are thankful to Dr. Yoshiko Kono and Dr. Kazuo Oginuma of University of Kochi for supplying plants collected in Taiwan.

References


