Molecular cytogenetic analysis in seven species of *Lachenalia* (Liliaceae) with the chromosome numbers of $2n=18, 22, 23, 26$ and 28 by DAPI staining and FISH using 5S rDNA and 18S rDNA probes

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ABSTRACT. Molecular cytogenetic analysis in seven species of *Lachenalia* (Liliaceae) with $2n=18, 22, 23, 26$ and 28 was studied using DAPI staining and fluorescence *in situ* hybridization (FISH). *Lachenalia latimerae* ($2n=18$), *L. juncifolia* ($2n=22$) and *L. zeyheri* ($2n=23$) would be considered as diploids which had basic chromosome numbers of $x=9, 11$ and 11 , respectively. They showed similarities in the characters of DAPI bands and FISH signals of 5S rDNA and 18S rDNA sites. *Lachenalia aloides* var. *vanzyliae* ($2n=28$) and *L. orchioides* ($2n=28$) showed tetraploidy ($X=7$). *Lachenalia paucifolia* ($2n=26$) and *L. longituba* ($2n=28$), which belonged to the genus *Polyxena* by different system, considered as diploids which might have in respect to their basic chromosome numbers of $x=13$ and 14 . *Lachenalia paucifolia* and *L. longituba* showed different characters of DAPI bands and FISH signals of 5S rDNA and 18S rDNA and showed different characters from the other species of *Lachenalia* studied here.

KEYWORDS: DAPI, 18S rDNA, FISH, 5S rDNA, *Lachenalia*, Molecular cytology

Lachenalia Jacq. ex Murray is one of the perennial bulbous genera, which belongs to the Liliaceae or sometimes to the Hyacinthaceae, and is endemic to the western part of South Africa and Namibia.

The chromosome numbers of 65 species and nine varieties of *Lachenalia* have been reported as $2n=10, 12, 14, 15, 16, 17, 18, 21, 22, 23, 24, 26, 27, 28, 29, 30, 32, 40, 42, 44, 49$ and 56 (e.g., Gouws 1965; Ornduff and Watters 1978; Nordenstam 1982; Johnson and Brandham 1997; Hamatani *et al.* 1998, 2004, 2007; Spies *et al.* 2009) and thus, $2n=14$ and $2n=16$ were abundant phenomena (Johnson and Brandham 1997).

Molecular systematic analyses based on DNA sequences (e.g., Spies 2004; Hamatani *et al.* 2008). suggested as follows: The taxa which had same basic chromosome numbers showed close relationships on the phylogenetic tree based on DNA sequences; the taxa with basic chromosome number of $x=7$ ($2n=14, 15, 28$ or 42) and the taxa with $x=8$ ($2n=16$ or 17) showed similarities to each other by DNA sequences.

Hamatani *et al.* (2009) performed molecular cytogenetic fields using 4'6-diamidino-2-phenylindole (DAPI) staining and fluorescence *in situ* hybridization (FISH) with 5S ribosomal DNA (rDNA) and 18S rDNA probes for the taxa which had chromosome numbers of $2n=14$ or 16 . They demonstrated that the taxa with $2n=16$ showed quite similar characters, and the taxa with $2n=14$ showed several characters, showed the traces of speciation from plural ancestral species and showed including natural hybrids.

The present molecular cytogenetics with DAPI staining

and FISH were completed for the taxa of *Lachenalia* which had chromosome number not of $2n=14$ or 16 but of $2n=18, 22, 23, 26$ and 28 , for the detail confirming speciation in the *Lachenalia*.

MATERIALS and METHODS

Plant materials. Plants of seven species of *Lachenalia* used in this study were tabulated in Table 1. They were cultivated in the Hiroshima Botanical Garden.

Pre-treatment-Chromosome preparation. Root-tips of the plants listed in Table 1 were harvested. The methods of pre-treatment, fixation, hydrolysis and squash of root-tips followed Hamatani *et al.* (2009).

Label with rDNA probes and method of FISH. The fragments of 5S rDNA and 18S rDNA were obtained from the total genomic DNA of *Cucumis sativus* 'Borszczagowski' using polymerase chain reaction (PCR). They were amplified according to Sogin (1990) and Hizume (1993), respectively. For FISH experiments, each rDNA probes were labeled by Hamatani *et al.* (2009). FISH method also followed Hamatani *et al.* (2009). The prepared samples were observed by fluorescence microscope FXA (Nikon) with UV-1A, B-2A, and G-2A filters. The obtained images were compounded and analyzed by using Photoshop Elements 2.0 (Adobe).

RESULTS

Chromosome numbers and numbers of FISH signals in

Table 1. Chromosome numbers and numbers of FISH signals in seven species of *Lachenalia* studied

Species	Chromosome number (2n)	Number of FISH signals	
		5S rDNA	18S rDNA
<i>L. aloides</i> (L. f.) Engl. var. <i>vanzyliae</i> W. F. Barker	28	4	4
<i>L. juncifolia</i> Bak.	22	2	2
<i>L. latimerae</i> W. F. Barker	18	2	4
<i>L. longituba</i> (A. M. van der Merwe) J. C. Manning & Goldblatt (= <i>Polyxena longituba</i>)	28	2	2
<i>L. orchioides</i> (L.) Ait. var. <i>orchioides</i>	28	4	6
<i>L. paucifolia</i> (W. F. Barker) J. C. Manning & Goldblatt (= <i>Polyxena paucifolia</i>)	26	2	2
<i>L. zeyheri</i> Bak.	23	2	2

seven species of *Lachenalia* studied are listed in Table 1. Photographs of somatic chromosomes stained by DAPI and FISH of 5S rDNA and 18S rDNA probes of each species are shown in Fig. 1.

Lachenalia aloides var. *vanzyliae*, *L. longituba* and *L. orchioides* had commonly the chromosome number of $2n=28$ (Table 1). *Lachenalia aloides* var. *vanzyliae* showed 5S rDNA signals on four chromosomes, and 18S rDNA on four chromosomes (Fig. 1). *Lachenalia longituba* showed 5S rDNA signals on two chromosomes and 18S rDNA on two chromosomes. *Lachenalia orchioides* showed 5S rDNA signals on four chromosomes and 18S rDNA on six chromosomes. DAPI-positive bands were shown on all chromosomes excepting four chromosomes had FISH signals of 5S rDNA sites in *L. aloides* var. *vanzyliae* and *L. orchioides*, while DAPI-positive band was not shown in *L. longituba*. *Lachenalia juncifolia* had the chromosome number of $2n=22$. This species showed 5S rDNA signals on two chromosomes and 18S rDNA on two chromosomes. However, DAPI-positive band was not shown. *Lachenalia latimerae* had the chromosome number of $2n=18$. This species showed 5S rDNA signals on two chromosomes and 18S rDNA signals on four chromosomes. DAPI-positive band was not shown. *Lachenalia paucifolia* had the chromosome number of $2n=26$. This species showed 5S rDNA signals on two chromosomes and 18S rDNA on two chromosomes. Clear DAPI-positive bands were shown on all chromosomes. *Lachenalia zeyheri* had the chromosome number of $2n=23$ which showed 5S rDNA signals on two chromosomes and 18S rDNA on two chromosomes. However, DAPI-positive band was not shown.

DISCUSSION

Figure 2 shows the idiograms of the *Lachenalia* species studied and indicated the positions of clear DAPI-positive bands and FISH signals of 5S rDNA and 18S rDNA sites.

Lachenalia latimerae ($2n=18$) showed two and four signals of 5S rDNA and 18S rDNA sites, and *L. juncifolia* ($2n=22$), *L. zeyheri* ($2n=23$), *L. paucifolia* ($2n=26$) and *L. longituba* ($2n=28$) showed commonly two signals each of

5S rDNA and 18S rDNA sites, and thus, these facts vouched that these were diploids. *Lachenalia aloides* var. *vanzyliae* ($2n=28$) showed four signals each of 5S rDNA and 18S rDNA sites, and thus, it was suggested that *L. aloides* var. *vanzyliae* was tetraploid. *L. orchioides* ($2n=28$) showed four signals of 5S rDNA and and six signals of 18S rDNA sites. *Lachenalia aloides* var. *vanzyliae* and *L. orchioides* showed similarities in the characters of clear DAPI-positive bands and the 5S rDNA signals; the two taxa showed clear DAPI positive bands on their all chromosomes except for the largest four chromosomes. The four largest chromosomes 5S rDNA signals on their distal positions which did not show clear DAPI positive bands. The two species showed different characters in 18S rDNA with each other: *L. aloides* var. *vanzyliae* showed four signals on the short arms of medium-sized chromosomes while *L. orchioides* showed two signals on intercalary positions of the short arms of the medium-sized chromosomes and another four signals on distal positions of the short arms of another medium-sized chromosomes. Therefore, *L. orchioides* was suggested to be amphidiploid, or the six 18S rDNA signals of this species might be as a trace of increasing of 18S rDNA sites. Then, it was regarded that *L. latimerae* had the basic chromosome number of $x=9$, *L. juncifolia* and *L. zeyheri* had $x=11$, *L. paucifolia* had $x=13$, *L. longituba* had $x=14$, and *L. aloides* var. *vanzyliae* and *L. orchioides* had $x=7$.

Lachenalia latimerae, *L. juncifolia*, *L. zeyheri* and *L. longituba* did not show DAPI-positive band. *Lachenalia latimerae*, *L. juncifolia* and *L. zeyheri* showed the 5S rDNA signals near the centromeres of medium or small-sized chromosomes and showed the 18S rDNA signals near the centromeres of another medium-sized chromosomes. These points suggested that these three species were similar to each other, although they had different chromosome numbers. Hamatani *et al.* (2009) reported that *L. pusilla* which have the chromosome number of $2n=14$ showed quite different characters in DAPI bands and FISH signals from those which have common chromosome number of $2n=14$ ($x=7$) species of the genus. *Lachenalia pusilla* did not show DAPI-positive band on all chromosomes, and

Fig. 1. FISH signals of 5S rDNA and 18S rDNA sites of the somatic chromosomes at metaphase in *Lachenalia* studied. 5S rDNA probes were labeled by tetramethylrhodamine and 18S rDNA probes were labeled by biotin. Biotin-labeled signals were detected with Alexa F488. Blue areas were stained by DAPI, red signals were hybridized with 5S rDNA and yellow signals were hybridized with 18S rDNA.

A: *L. aloides* var. *vanzyliae*, B: *L. juncifolia*, C: *L. latimerae*, D: *L. longituba*, E: *L. orchioides* var. *orchioides*, F: *L. paucifolia*, G: *L. zeyheri*. Bar indicates 10 μm .

Fig. 2. The idiograms of somatic chromosomes at metaphase with DAPI positive bands, signals of 5S rDNA and 18S rDNA sites in seven species of *Lachenalia* studied.

showed the 5S rDNA signals near the centromeres of the medium-sized chromosomes and the 18S rDNA signals near the centromeres of the other medium-sized chromosomes. These phenomena suggested similarity between the four species of *L. latimerae*, *L. juncifolia* and *L. zeyheri* and *L. pusilla*.

Spies (2004) compared the sequences of *trnL-F* regions on cpDNA while Hamatani *et al.* (2008) compared internal transcribed spacer (ITS) regions. These studies based on

DNA sequences showed no genetic correlation with various different basic chromosome numbers displayed in the taxa studied. This investigation discovered that *Lachenalia latimerae* of $x=9$, and *L. juncifolia* and *L. zeyheri* of $x=11$ had similar characters in DAPI bands and FISH signals of 5S rDNA and 18S rDNA sites with each other and thus, hypothesized that the speciation of these three species and *L. pusilla* of $x=7$ might be occurred from a common ancestor. According to Spies *et al.* (2002), *L.*

unifolia ($x=11$), *L. hirta*, *L. juncifolia* and *L. pusilla* were placed in the same clade in the phylogenetic tree on the basis of the *trn* intergenetic spacer of cpDNA.

Lachenalia latimerae showed four signals of 18S rDNA. It might be regarded as one of the traces of structural change on chromosomes. Additionally, *L. longituba* which did not show DAPI-positive band showed the 5S rDNA signals on the distal position of the long arm of small-sized chromosomes and showed the 18S rDNA signals on the distal position of the long arm of another small-sized chromosomes. These characters were different from *L. latimerae*, *L. juncifolia* and *L. zeyheri*. This phenomenon was evident on the essential difference between *L. latimerae*, *L. juncifolia* and *L. zeyheri*, and *L. longituba*.

Incidentally, the results of DAPI staining for *Lachenalia aloides* var. *vanzyliae* and *L. orchioides* showed similar tendency to the results for *L. aloides* var. *aloides*, *L. viridiflora* and so on with the chromosome number of $2n=14$ and the basic chromosome number of $x=7$ (Hamatani *et al.* 2009).

Lachenalia paucifolia, *L. aloides* var. *vanzyliae* and *L. orchioides* showed clear DAPI-positive bands on their chromosomes. *Lachenalia paucifolia* showed bands on the all chromosomes, while *L. aloides* var. *vanzyliae* and *L. orchioides* showed bands on the all chromosomes except for the largest four chromosomes. *Lachenalia paucifolia* showed the 5S rDNA signals on the intercalary positions of the long arm of the medium-sized chromosomes and showed the 18S rDNA signals on the intercalary positions of the long arm of another medium-sized chromosomes. Manning *et al.* (2004) insisted to change the position of *Polyxena* into *Lachenalia* on the basis of the DNA analysis. *Lachenalia paucifolia* and *L. longituba* were changed to the present genus from *Polyxena*, however, they showed quite different characters of DAPI bands and rDNA signals. Thus, it was speculated that there might be plural strains of speciation among the species which were extended from *Polyxena*.

Lachenalia longituba, *L. aloides* var. *vanzyliae* and *L. orchioides* had the same chromosome numbers of $2n=28$. However, *L. longituba* might be a diploid while *L. aloides* var. *vanzyliae* and *L. orchioides* might be tetraploids. Both *L. paucifolia* and *L. longituba* which were changed their positions from *Polyxena* by Manning *et al.* (2004) showed different characters of DAPI bands and rDNA signals on their chromosomes from those of the other species of *Lachenalia* studied during the course of this investigation. These species were included molecular phylogenetically

in a large *Lachenalia* clade based on DNA sequences by Spies (2004) and Hamatani *et al.* (2008). Further studies are expected to analyze more species of *Polyxena* and *Lachenalia* which have the basic chromosome numbers beyond $x=7$ and 8.

LITERATURE CITED

- Gouws, J. B. 1965. Cytological studies in the genus *Lachenalia* Jacq. Ann. Univ. Coll. W. Cape 2:1-7.
- Johnson, M. A. T. and Brandham, P. E. 1997. New chromosome numbers in petaloid monocotyledons and in other miscellaneous angiosperms. Kew Bull. 52: 121-138.
- Hamatani, S., Hashimoto, K. and Kondo, K. 1998. A comparison of somatic chromosomes at metaphase in *Lachenalia* (Liliaceae). Chrom. Sci. 2: 21-25.
- Hamatani, S., Ishida, G., Hashimoto, K. and Kondo, K. 2004. A chromosome study in ten species of *Lachenalia* (Liliaceae). Chrom. Sci. 8: 55-61.
- Hamatani, S., Kondo, K., Kodaira, E. and Ogawa, H. 2007. Chromosome morphology of 12 species and one variety of *Lachenalia* and five species of closely related, allied genera (Liliaceae). Chrom. Bot. 2: 79-86.
- Hamatani, S., Masuda, Y., Kondo, K., Kodaira, E. and Ogawa, H. 2008. Molecular phylogenetic relationships among *Lachenalia*, *Massonia* and *Polyxena* (Liliaceae) on the bases of the internal transcribed spacer (ITS) region. Chrom. Bot. 3: 65-72.
- Hamatani, S., Tagashira, N., Ishida, G. and Kondo, K. 2009. Chromosome relationships among 13 species and one variety of *Lachenalia* (Liliaceae) with the chromosome numbers of $2n=14$ and 16 detected by FISH using 5S rDNA and 18S rDNA probes and DAPI staining. Chrom. Bot. 4: 57-63.
- Hizume, M. 1993. Chromosomal localization of 5S rRNA genes in *Vicia fava* and *Crepis capillaris*. Cytologia 58: 417-421.
- Manning, J. C., Goldblatt, P. and Fay, M. F. 2004. A revised genetic synopsis of Hyacinthaceae in sub-saharan africa, based on molecular evidence, including new combinations and the new tribe Pseudoprosperae. Edinburg Jour. Bot. 60: 533-568.
- Nordenstam, B. 1982. Chromosome numbers of southern African plants: 2. Journ. S. Afr. Bot. 48: 273-275.
- Ornduff, R. and Watters, P. J. 1978. Chromosome numbers in *Lachenalia* (Liliaceae). Journ. S. Afr. Bot. 44: 387-390.
- Sogin, M. L. 1990. Amplification of ribosomal RNA genes for molecular evolution studies. In: Innis, M. A., Gelfand, D. H., Sninsky, J. J. and White, T. J. Eds., PCR Protocols. A guide to methods and applications: 307-322. Academic Press, Inc.
- Spies, J. J., van Rooyen, P. and Kleynhans, R. 2002. The subgeneric delimitation of *Lachenalia* (Hyacinthaceae). Acta Hort. 570: 225-231.
- Spies, J. J., Spies, P., Minnaar, A., Reinecke, S. M. C., du Preez, H. and Kleynhans, R. 2009. Hyacinthaceae. In Marhold, K. (ed). IAPT / IOPB chromosome data 8. Taxon 58(4): 1281-1289.
- Spies, P. 2004. Phylogenetic relationships of the genus *Lachenalia* with other relate liliaceous taxa. Magister Sci., Univ. Free State: 1-257.