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Karyotypes and Giemsa C-banding patterns of *Zebrina pendula*, *Z. purpusii* and *Setcreasea purpurea*, compared with those of *Tradescantia ohiensis*

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It has been proposed that the genera *Zebrina* and *Setcreasea* of the family Commelinaceae should be united and reunited, respectively, with the genus *Tradescantia*, mainly based on morphological studies. In the present study, karyotypes and Giemsa C-banding patterns in the root-tip cells of three *Zebrina* and two *Setcreasea* clones were analyzed, and were compared with those of a triploid *Tradescantia* clone. *Z. pendula* and *Z. purpusii* (both 2n = 24) were found to have similar karyotypes (4 M + 6 ST + 14 T; M = meta-, ST = subtelo-, T = telocentric chromosomes), while *Z. pendula* cv Quadricolor (2n = 23) had a unique karyotype (6 M + 5 ST + 11 T + 1 SA; SA = short acrocentric chromosome). The only clear difference between *Z. pendula* and *Z. purpusii* was that one and two subtelocentric chromosomes, respectively, had satellites at the short arms. Two clones of *S. purpurea* (2n = 24) had karyotypes (8 M + 8 M' + 8 SM; M' = nearly meta-, SM = submetacentric chromosomes) similar to each other. *T. ohiensis* (2n = 18) had a symmetric karyotype (9 M + 9 SM) consisting of larger chromosomes than *S. purpurea*. Many clear Giemsa C-bands were detected, in addition to centromeric bands in all chromosomes of all clones. *Z. pendula* and *Z. purpusii* commonly had single clear interstitial bands in eight telocentric chromosomes each, but they also had unique telomeric and other interstitial bands, respectively. *Z. pendula* cv Quadricolor had a unique banding pattern, i.e., satellite bands in the unique short chromosome, telomeric bands at the long arms of all metacentric chromosomes, and single interstitial bands in six telocentric chromosomes. Two clones of *S. purpurea* had telomeric bands at many chromosome arms and satellite bands in two nearly metacentric and one submetacentric chromosomes, but some differences were found between them. On the other hand, all the chromosomes of *T. ohiensis* had telomeric bands at both arms, and three submetacentric chromosomes had satellite bands. These result prove structural differentiation of chromosomes occurred among the clones, especially in *Zebrina*, and show that *S. purpurea* is relatively close to *T. ohiensis*, while *Zebrina* is obviously distant from the other two genera. Therefore, there remains a question cytologically at least for uniting *Zebrina* with *Tradescantia*.

INTRODUCTION

The family Commelinaceae is a monocotyledonous family consisting of about 600 species which are mostly distributed in tropical and subtropical regions, but partly in the temperate zone. Most of these species are natives exclusively in either the Old or the New World.

The phylogenetic relationships among the genera of this family have been examined in various studies. Most of these studies have distinguished two major tribes, Commelineae and Tradescantieae. According to Brenan (1966), Clarke had proposed in 1881 dividing the family into these two tribes plus the tribe Poliaceae, based on the morphological characteristics of capsules and stamens. Pichon (1946) later divided the family into ten groups (Commelineae, Tradescantieae and eight others) by comparing many characteristics but attaching importance to

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the structure of the androecium, free or connate petals, and the presence or absence of the aril. Brenan (1966) further classified the family into 15 groups based on 17 characters, mainly related to the structure of the inflorescence, which had not been considered well by Pichon (1946). According to Hunt (1975), however, Rohweder had supported the original two tribes in 1958 based on the structure of the inflorescence, especially those of the cincinni and bracteoles, and Martinez and Swain (1985) reached to a similar conclusion based on their chemotaxonomic study of some flavonoids.

In the tribe Tradescantieae, Hunt (1975, 1986) proposed reunion or union of five genera with *Tradescantia*, and Hunt (1980) also proposed classifying the tribe into eight sections. Martinez and Martinez (1993) analyzed flavonoids in six out of the eight sections, and divided *Tradescantia* into three (*T. fluminensis*, *T. virginiana* and *T. crassifolia*) groups, based on their own results and the geographic distributions.

Although it was proposed that *Setcreasea* and *Zebrina* should be reunited and united with *Tradescantia*, respectively, by Hunt (1975, 1986), they have various characteristics differing from those of *Tradescantia*. The present cytological study was conducted in order to examine the validities of the reunion and union of these genera.

MATERIALS AND METHODS

Materials used. The materials used were three *Zebrina*, two *Setcreasea* and one *Tradescantia* clones. They were (1) *Z. pendula* Schnizl. grown in the Research Institute of Agricultural Plants, Faculty of Agriculture, Kyoto University, Muko, Kyoto, (2) *Z. pendula* cv Quadricolor, (3) *Z. purpusii* Brückn., both from the Higashiyama Botanical Garden, Nagoya, (4 and 5) two different clones of *S. purpurea* Boom., one from the Faculdade de Filosofia, Ciências e Letras, Universidade de São Paulo, Ribeirão Preto, Brazil, and another from the Faculty of Science, Kyushu University, Fukuoka, and (6) *T. ohiensis* Raf. clone KU 7 having been used for detecting the genetic effects of low-level radiations at the Laboratory of Genetics, Faculty of Agriculture, Kyoto University, Kyoto, utilizing its blue/pink heterozygosity (Ichikawa, 1971) and complete sterility due to triploidy (Ichikawa et al., 1981). *T. ohiensis* was chosen for comparing with *Zebrina* and *Setcreasea* clones, because this species belongs to the series Virginianae of the section Tradescantia (Hunt, 1980) or to the *T. virginiana* group of *Tradescantia* (Martinez and Martinez, 1993), which has been regarded to be representative of *Tradescantia*.

All these materials have been propagated vegetatively since they were found or received, and each of them is thus a genetically identical clone to the original clone being maintained at each source.

Growing methods of fully rooted cuttings. Cuttings with at least two nodes were taken from potted plants grown in either a Sherer CEL 38-15 growth chamber or greenhouse, and were grown by immersing their basal nodes in aerated water kept dark in a growth room kept at 23 ± 1°C with 16-h day length until they bore young roots. Rooted cuttings were then cultivated in a nutrient solution circulating system set in the same growth room until they were sufficiently rooted to permit collection of the necessary numbers of root tips. The nutrient solution used was a 1/3000 Hyponex solution.

Pretreatment and fixation. Root tips were collected from well grown young roots by cutting at 10–20 mm from their tips. The root tips were immediately pretreated with the saturated solution of α -bromonaphthalene for 3.5 h at room temperature (RT). The pretreated root tips were fixed with freshly prepared Farmer's fluid (98% ethanol : acetic acid = 3 : 1) for 24 h at RT, and the fixed root tips were stored in 70% ethanol at 4°C.

Preparation for karyotype analysis. The root tips were dipped in acetocarmine for 30–60 min at RT until they were well stained. The root tips were either processed further immediately after staining, or else they were kept in the dye at 4°C. The stained root tips were heated carefully and macerated at about 70°C, and preparations for analyzing the karyotypes were made by the squash method. Photomicrographs of well spread metaphase chromosomes were taken for measuring the relative length (RL in %; length of each chromosome relative to the total length of all chromosomes) and the arm ratio (AR; length of long arm divided by that of short arm) of each chromosome.

Giemsa C-banding. The preparations used for karyotype analyses were then deeply frozen by putting them on dry ice for at least 10 min, the cover glass of each frozen preparation was quickly removed using a razor, and the preparations were immediately immersed in 99.5% ethanol for dehydrating them overnight at RT. The preparations were then hydrolyzed in 0.2 N HCl at 60°C for 2.5 min, washed twice with deionized water, and were dipped in the saturated $Ba(OH)_2$ solution for 11 (*Zebrina*) or 15 min (*Setcreasea* and *Tradescantia*) at RT. After dipping the preparations in deionized water for 10 min at RT, they were immersed in 2 × SSC solution (0.3 M NaCl + 0.03 M $C_6H_5Na_3O_7$-$2H_2O$) for 60 min at 60°C. The preparations were stained with a 2.5% solution (pH = 7) of Giemsa (Merk & Co., Inc) for 25–60 min at RT. Photomicrographs were taken after each preparation was mounted with xylene and covered with a new cover glass to confirm the positions of C-bands.

RESULTS

Karyotypes of *Zebrina* clones. The results of the karyotype analyses in three clones of *Zebrina* examined (*Z. pendula*, *Z. pendula* cv Quadricolor and *Z. purpusii*) are shown as idiograms in Fig. 1A. *Z. pendula* and *Z. purpusii* had 24 chromosomes each, whereas *Z. pendula* cv Quadricolor had 23 chromosomes. Classifying their chromosomes by AR values, each clone was found to have three or four groups of chromosomes, i.e., large metacentric (M), subtelocentric (ST) and telocentric (T) chromosomes in all clones, and one short acrocentric (SA) chromosome only in *Z. pendula* cv Quadricolor. Following the grouping of chromosomes, the chromosomal constitutions of the three clones could be expressed as 2n = 4 M + 6 ST + 14 T for *Z. pendula* and *Z. purpusii*, and 2n = 6 M + 5 ST + 11 T + 1 SA for *Z. pendula* cv Quadricolor.

One clear difference found between *Z. pendula* and *Z. purpusii* was the number of satellite chromosomes. Namely, while only the longest subtelocentric chromosome had satellites at the short arm in *Z. pendula*, the two longest subtelocentric chromosomes of *Z. purpusii* had satellites at their short arms, as shown in Fig. 1A. In *Z. pendula* cv Quadricolor, on the other hand, only the unique short acrocentric chromosome had satellites at the long arm, none of the five subtelocentric chromosomes having satellites, as shown in this figure. It was also found in *Z. pendula* cv Quadricolor that the shortest metacentric chromosome has a larger AR value than the values close to 1 of the other five metacentric chromosomes of this clone and also of the four metacentric chromosomes each of *Z. pendula* and *Z. purpusii*.

Karyotypes of *Setcreasea* clones. The results of karyotype analyses in two clones of *S. purpurea* (Brazil and Fukuoka) are shown as idiograms in Fig. 1B. Both clones had 24 chromosomes with AR values ranging from about 1.0 to 1.6. In the present study, the chromosomes were classified into metacentric (M; AR = 1.0–1.1), nearly metacentric (M'; AR = 1.1–1.3) and submetacentric (SM; AR = 1.4–1.6) chromosomes, because *T. ohiensis* had no chromosome of the nearly metacentric type (see below), and also because the shortest metacentric chromosome with the larger AR value of *Z. pendula* cv Quadricolor mentioned above was only exception in *Zebrina*. Both clones had identical chromosomal constitution of 2n = 8 M + 8 M' + 8 SM, and no clear difference was found between the karyotypes of these two clones.

Karyotype of *T. ohiensis* clone KU 7. The result of karyotype analysis in *T. ohiensis* clone KU 7 is shown as an idiogram in Fig. 1C. This triploid clone has 18 chromosomes as described earlier (Ichikawa et al., 1981). The chromosomes were either metacentric (M) or submetacentric (SM), and the chromosomal constitution was found to be symmetric being 2n = 9 M + 9 SM. This clone therefore seems to have three genomes, each consisting of 3 M + 3 SM chromosomes. Satellites were observed not infrequently at the short arms of three submetacentric chromosomes, but they were not always observed and thus are not shown in Fig. 1C.

C-banding patterns of *Zebrina* clones. The Giemsa C-banding patterns of the three *Zebrina* clones are shown in Fig. 2A. Besides clear centromeric bands observed in all chromosomes of the three clones, each clone showed a banding pattern differing from the others.

In *Z. pendula*, a clear telomeric band at the short arm of the shortest subtelocentric chromosome, a clear satellite band in the only one satellite subtelocentric chromosome, and clear single interstitial bands near the centromeres of eight telocentric chromosomes were observed. A thin terminal band at the long arm of one metacentric chromosome as well as thin single interstitial bands near the centromeres at the long arms of the two shortest subtelocentric chromosomes and in one telocentric chromosome were also detected in this clone, as shown in Fig. 2A.

Z. pendula cv Quadricolor showed a unique banding pattern markedly different from that in *Z. pendula*. Namely, clear telomeric bands were observed at the long arms of all the six metacentric chromosomes, as well as a clear satellite band at the long arm of the unique short acrocentric chromosome, and clear single interstitial bands near the centromeres of six telocentric chromosomes. Also detected in this clone were thin terminal bands at the short arms of two metacentric and three subtelocentric chromosomes as well as thin single interstitial bands near the centromeres at the short arms of two metacentric chromosomes, as shown in Fig. 2A.

The banding pattern observed in *Z. purpusii* also differed, but not greatly, from that in *Z. pendula*. Namely, both of the two satellite subtelocentric chromosomes had clear satellite bands, the long arm of the longest metacentric chromosome had one clear interstitial band near the centromere, two telocentric chromosomes had two clear interstitial bands each, while eight other telocentric chromosomes had clear single interstitial bands each, and no clear terminal band was detected, as shown in Fig. 2A. The orders of RL of the eight telocentric chromosomes with clear single interstitial bands were same as in *Z. pendula*.

It should be noted that several thin terminal bands were observed, but not always, in *Z. pendula* and *Z. purpusii*, and they are not shown in Fig. 2A because it was difficult to identify the chromosomes possessing these thin terminal bands.

C-banding patterns of *Setcreasea* clones. The Giemsa C-banding patterns of the two *Setcreasea* clones are shown in Fig. 2B. Adding to clear centromeric bands

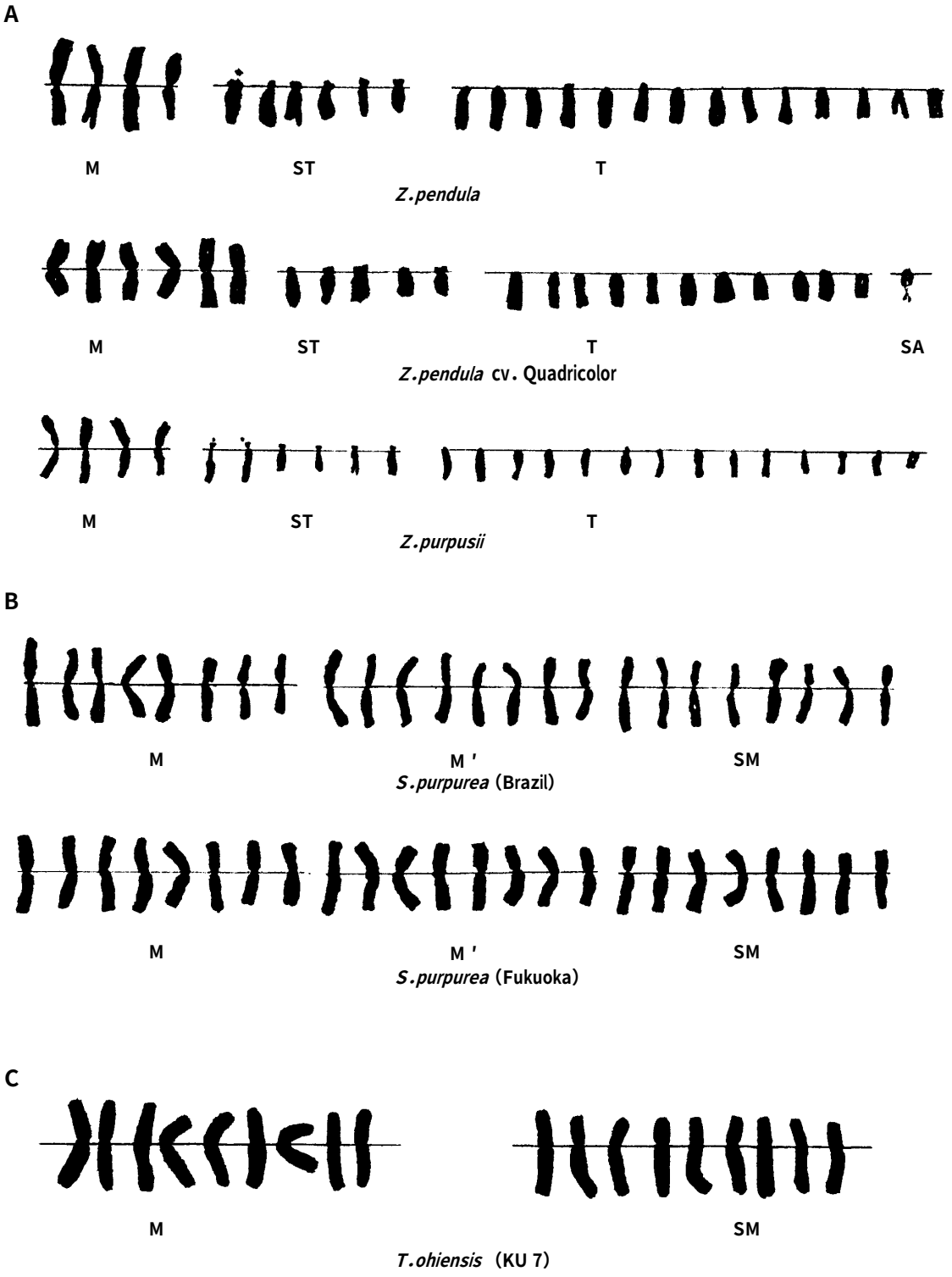

Fig. 1. Idiograms of the chromosomes of (A) *Z. pendula* (2n = 24), *Z. pendula* cv Quadricolor (2n = 23) and *Z. purpusii* (2n = 24), (B) clones Brazil and Fukuoka of *S. purpurea* (2n = 24), and (C) *T. ohiensis* clone KU 7 (2n =18). The horizontal lines show the positions of centromeres. Chromosomes are grouped by their AR values, i.e., into metacentric (M), subtelocentric (ST), telocentric (T) and short acrocentric (SA) chromosomes in *Zebrina* clones; metacentric, nearly metacentric (M'; see text) and submetacentric (SM) chromosomes in *Setcreasea* clones; and metacentric and submetacentric chromosomes in *T. ohiensis* clone KU 7; and they are arranged from left to right in the order of their RL values within each group of chromosomes. The satellites observed not infrequently at the short arms of three submetacentric chromosomes of *T. ohiensis* clone KU 7 are not shown (see text).

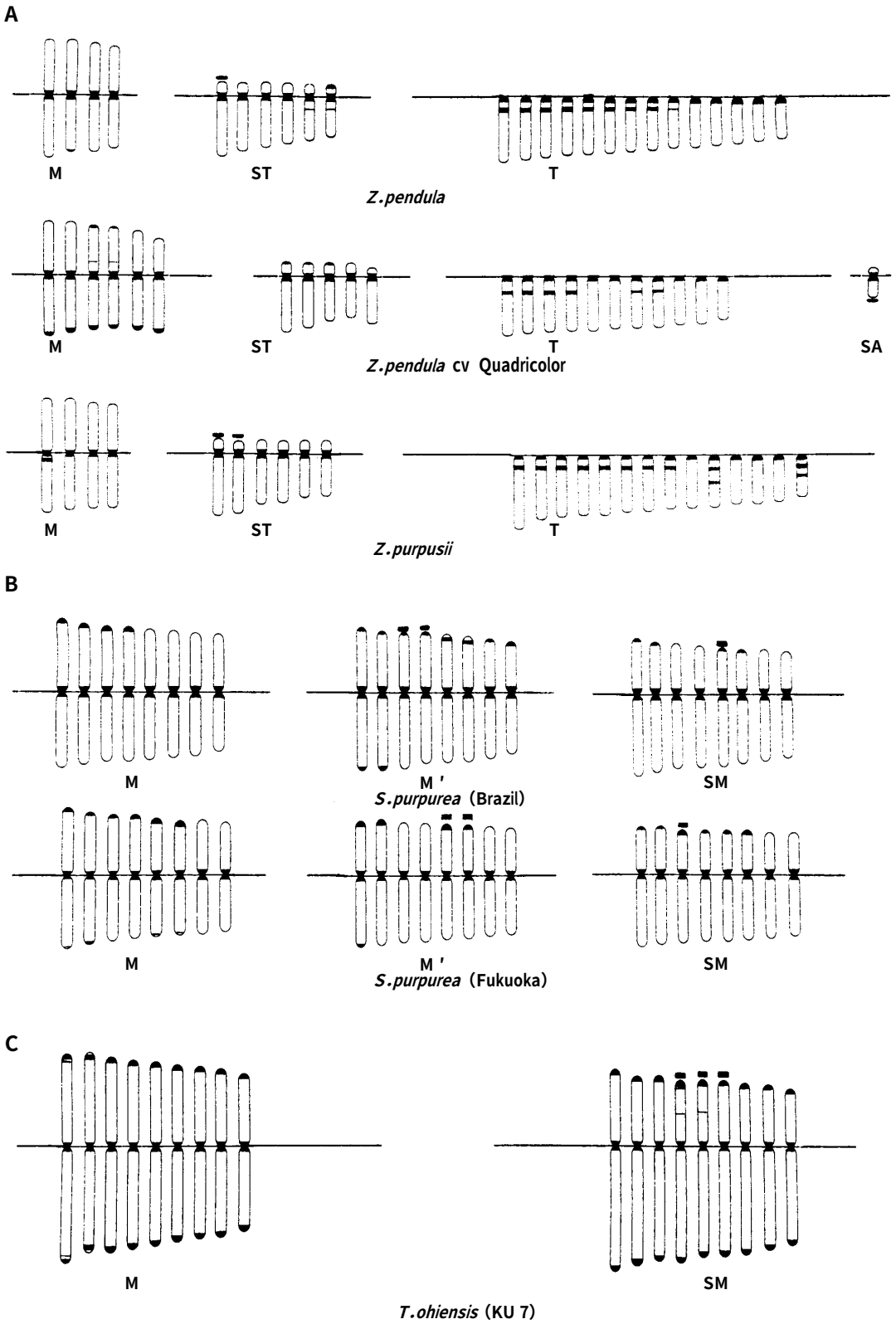

Fig. 2. Giemsa C-banding patterns observed in the chromosomes of (A) *Z. pendula*, *Z. pendula* cv Quadricolor and *Z. purpusii*, (B) clones Brazil and Fukuoka of *S. purpurea*, and (C) *T. ohiensis* clone KU 7. The horizontal lines show the positions of centromeres. Chromosomes are grouped by their AR values, and are arranged in the order of their RL values within each group of chromosomes, as in Fig. 1. Several thin terminal bands observed but not always in *Z. pendula* and *Z. purpusii* are not depicted, because it was difficult to identify the chromosomes.

observed in all chromosomes, clear telomeric bands in many of metacentric, nearly metacentric and submetacentric chromosomes and clear satellite bands at the short arms of two nearly metacentric and one submetacentric chromosomes were observed in both clones. However, the banding patterns of the two clones were somewhat different from each other.

In clone Brazil, clear telomeric bands were observed at both arms of the two longest nearly metacentric chromosomes and at the short arms of the four longer metacentric, four nearly metacentric and four submetacentric chromosomes, and two other nearly metacentric chromosomes had clear single bands very close to the telomeres at their short arms, as shown in Fig. 2B. In clone Fukuoka, on the other hand, clear telomeric bands were observed at both arms of the longest nearly metacentric chromosome and at the short arms of six longer metacentric, three nearly metacentric and six longer submetacentric chromosomes, and four metacentric chromosomes had thin terminal bands at the long arms, as shown in Fig. 2B. Another difference between the two clones was that their two nearly metacentric and one submetacentric chromosomes which had clear satellite bands as shown in Fig. 2B (although no clear satellites were detected by karyotype analyses; see Fig. 1B) were found to be placed at different orders of RL in the respective chromosome groups.

C-banding pattern of *T. ohiensis* clone KU 7. The Giemsa C-banding pattern of *T. ohiensis* clone KU 7 is shown in Fig. 2C. All of the metacentric and submetacentric chromosomes were found to have clear centromeric and telomeric bands, the latters being observed at both arms, and three submetacentric chromosomes had clear satellite bands at their short arms. Thin interstitial bands were observed at about the midpoints of the short arms of the two submetacentric chromosomes with satellite bands and near the telomeric band at the short arm of the longest submetacentric chromosome with satellite band, and also near the telomeric bands at both arms of the longest metacentric chromosome. The longest satellite chromosome had six bands in total, and the longest metacentric and another satellite chromosomes had five bands each.

DISCUSSION

Phylogenetic relationships among the three genera. *Zebrina* and *Setcreasea* have been described to be closely related, mainly based on morphological (Brenan, 1966) and cytological studies (Jones and Jopling, 1972). *Setcreasea* had been divided from *Tradescantia* and included in the section Zebrineae together with *Zebrina* (Pichon, 1946). It was proposed later that *Setcreasea* should be reunited with *Tradescantia* based on morphological comparisons (Hunt, 1975), and *Zebrina* has also been proposed to be united with *Tradescantia* (Hunt, 1986). In fact, Evans (1995) found a close relationship between *Zebrina* and *Tradescantia* at the molecular level, i.e., by comparing the base sequences of the chloroplast-encoded gene, *rbc*L.

However, the results obtained in the present study show that there are distinct cytological differences at least between *Zebrina* and the other two genera studied. The chromosomal constitution of 4 M + 6 ST + 14 T commonly possessed by *Z. pendula* and *Z. purpusii* and also that of 6 M + 5 ST + 11 T + 1 SA of *Z. pendula* cv Quadricolor (Fig. 1A) agree well with two of the four karyotypes reported by García (1984). It should be noted that *Z. purpusii* had been regarded as a variety of *Z. pendula* by Matuda (1955). These chromosomal constitutions of *Zebrina* obviously differ from those of 8 M + 8 M' + 8 SM of the two clones of *Setcreasea* (Fig. 1B). The lengths of the 28 fundamental chromosome arms of these *Zebrina* clones (both arms of metacentric, one arm each of subtelocentric and telocentric chromosomes) are roughly comparable with those of the 48 chromosome arms of *S. purpurea* (Fig. 1), but these *Zebrina* clones are cytologically different from *S. purpurea*, which has the chromosomal constitution typical of a tetraploid with a basic chromosome number of six consisting of 2 M + 2 M' + 2 SM chromosomes.

On the other hand, such six chromosomes constituting the basic number of *S. purpurea* are much closer to the 3 M + 3 SM chromosomes constituting the basic number of six of *T. ohiensis*, although the latter has larger chromosomes than the former (Fig. 1). Size and constitution of chromosomes similar to those of *S. purpurea* clones have been observed in *T. crassifolia* Car. (clone BNL 2021; 2n = 24) (Ichikawa and Sparrow, 1967), while those similar to *T. ohiensis* clone KU 7 have been observed in *T. virginiana* L. cv Purple Dome (clone BNL 2031; 2n = 24) and in the hybrid clone BNL 02 (2n = 12) which had been thought to be derived from a hybrid between *T. occidentalis* (Britt.) Smith. and *T. ohiensis* (Ichikawa and Sparrow, 1967). *Setcreasea* has also been described as being chemotaxonomically close to the *T. crassifolia* group of *Tradescantia* (Martinez and Martinez, 1993).

The 28 fundamental arms in *Zebrina* seem to suggest that this genus may have the basic chromosome number of seven, differing from six in *Setcreasea* and *Tradescantia*. It has been reported that quadrivalent formations were occasionally observed in the meiosis of *Zebrina* species (Mattsson, 1971; García, 1984), and these reports seem to support the possibility that *Zebrina* species were originated from a tetraploid having the basic number of seven. The four pairs of telocentric chromosomes showing clear single interstitial C-bands commonly found in *Z. pendula* and *Z. purpusii* (Fig. 2A) also suggest that these species were derived from a tetraploid having the basic number of seven.

The Giemsa C-banding patterns also showed clear dif-

ferences between *Zebrina* and *Setcreasea* (Fig. 2). While distinct interstitial bands were observed in many of the 14 telocentric chromosomes of *Z. pendula* and *Z. purpusii*, no clear interstitial band was detected in *S. purpurea*, except for the clear bands which appeared very close to the telomeres at the short arms of two nearly metacentric chromosomes of clone Brazil. The banding patterns observed in *S. purpurea*, i.e., telomeric bands at both arms of one or two nearly metacentric chromosomes and at short arms of many chromosomes, satellite bands in two nearly metacentric and one submetacentric chromosomes, and no clear interstitial band detected, were apparently closer to that in *T. ohiensis* (Fig. 2). Clear differences between *S. purpurea* and *T. ohiensis* were telomeric bands at the both arms of all chromosomes and thin interstitial bands observed in the latter.

As discussed above, the results obtained in the present study show that the *Zebrina* clones studied are obviously different from *S. purpurea* and *T. ohiensis* cytologically. Considering that *T. ohiensis* belongs to the representative of the genus, i.e., the series Virginianae of the section Tradescantia (Hunt, 1980) or the *T. virginiana* group (Martinez and Martinez, 1993), and that this species indeed has chromosomes very similar to those of *T. virginiana* (Ichikawa and Sparrow, 1967), the proposal of uniting *Zebrina* with *Tradescantia* (Hunt, 1986) would not be appropriate from the cytological standpoint. On the other hand, the reunion of *Setcreasea* with *Tradescantia* (Hunt, 1975) would not necessarily be inappropriate, considering that chromosomes similar to those of *S. purpurea* have been observed in *T. crassifolia* (Ichikawa and Sparrow, 1967) and also considering the chemotaxonomic study (Martinez and Martinez, 1993) mentioned above.

Differentiation of karyotype. While *Zebrina* species are distributed mainly in Mexico and also in Central American regions, *Setcreasea* species are distributed from Texas, USA, to Mexico, and *Tradescantia* species of the series Virginianae or the *T. virginiana* group are distributed in North America. These geographical distributions indicate that *Setcreasea* has distribution areas overlapping with those of the other two genera. According to Hunt (1975), in fact, *S. pallida* Rose, which is closely related to *S. purpurea* examined in the present study, is distributed in northern to eastern Mexico (Tamaulipas, San Luis Potosi and Veracruz states from north to south), *S. brevifolia* (Torr.) Sch. & Sydow. is distributed in northern Mexico (Coahuila and Nuevo Leon states from north to south), and *S. hirsuda* (or *hirta*) Markgraf is distributed between them (Nuevo Leon and San Luis Potosi).

According to García (1984), on the other hand, *Z. pendula* in Mexico, including cv Quadricolor, is distributed widely from south to north. He showed, however, that this species having 24 (4 M + 6 ST + 14 T) chromosomes (the same as *Z. pendula* in the present study; Fig. 1A) is distributed in southern to central Mexico (Veracruz, Michoacan, Mexico and Jalisco states from south to north), while that having 23 (6 M + 5 ST + 11 T + 1 SA; he described B instead of SA) chromosomes (the same as cv Quadricolor in the present study; Fig. 1A) shows a wider distribution including northern Mexico (Chiapas, Veracrus, Guerrero, Michoacan, Morelos, Puebla, Mexico, Jalisco, Guanajuato, Sinaloa and Coahuila states from south to north, and Mexico City). He also found two other types having 23 (5 M + 6 ST + 12 T) and 22 (7 M + 5 ST + 9 T + 1 SA) chromosomes in Tabasco and Jalisco states, respectively. His findings of these four different karyotypes in Mexico show that the genus *Zebrina* has differentiated by repeating fusions (or less likely separations) of chromosome arms. It should be noted that the number of fundamental arms is 28 (nf = 28) in all of the four karyotypes reported by him.

The present results of analyzing karyotypes and C-banding patterns show that, not only fusions or separations of chromosome arms (i.e., of centromeres), but also structural changes such as translocations between chromosomes must have been involved in the differentiations among the three *Zebrina* clones studied. For example, the unique short acrocentric chromosome of *Z. pendula* cv Quadricolor would not be produced without structural changes occurred between chromosomes including a satellite chromosome (Fig. 1A). García (1984) also described the possible involvement of structural changes, especially for the origin of the short acrocentric chromosome. Although the Quadricolor karyotype has a wider distribution than the 2n = 24 karyotype (García, 1984), the present results, especially the detection of clear telomeric C-bands in metacentric chromosomes and the presence of the short acrocentric chromosome only in *Z. pendula* cv Quadricolor (Fig. 2A), show that this type is rather a unique one which must have evolved differently from the others. Also, compared with the four pairs of telocentric chromosomes with clear single interstitial bands commonly observed in *Z. pendula* and *Z. purpusii*, the number of pairs of those with clear single interstitial bands in *Z. pendula* cv Quadricolor is also unique being three (Fig. 2A). The four pairs of telocentric chromosomes with clear single interstitial bands in *Z. pendula* and *Z. purpusii* not only suggest the basic chromosome number of seven, as mentioned above, but also show the unique differentiation of the Quadricolor karyotype having three (instead of four) pairs of such chromosomes. It seems worthy to refer to that *Z. pendula* cv Quadricolor has never formed even a single inflorescence under several environmental conditions tested, differing from *Z. pendula* and *Z. purpusii*.

Evidences of some structural changes of chromosomes occurred between the two *Setcreasea* clones examined were also found in the present study by comparing their C-banding patterns. Namely, the two nearly metacentric and one submetacentric chromosomes of the two clones,

which had clear satellite bands, were located in different orders of RL in the respective chromosome groups, and differences of the telomeric bands of several chromosomes were also observed (Fig. 2B).

The characteristic of the C-bandings observed in *T. ohiensis* clone KU 7, i.e., the existence of clear telomeric bands at both arms of all chromosomes (Fig. 2C), is identical to that reported for the diploid and tetraploid species of *Gibasis* (belonging to the section Gibasis of the family Commelinaceae) having the basic chromosome number of five (Kenton, 1978; Kenton et al., 1987). Telomeric bands should be detected at each end of chromosome arm, where telomeric structure must exist. The present results of detecting no clear telomeric band at the ends of many chromosome arms of *Zebrina* and *Setcreasea* may be showing a possible limitation of the Giemsa C-banding method, and uses of more advanced methods such as genomic *in situ* hybridization and/or fluorescence *in situ* hybridization may solve the problem.

Most of the genera of the family Commelinaceae are propagated vegetatively, including the three genera studied here, and thus any adaptable karyotype could be maintained in the regions where they were produced. They might have subsequently spread their distributions into neighboring areas where environmental conditions were adequate. In this sense, further studies on the relationships between cytological characteristics and geographical distributions are needed, especially for vegetatively propagating plants such as those of the family Commelinaceae.

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