

Karyotype Analysis in Seven Cultivated *Juniperus chinensis* and a Cultivated *Pinus densiflora*¹

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Juniperus chinensis 7栽培種과 *Pinus densiflora*

1栽培種에 對한 核型分析¹

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ABSTRACT

This study was designed to investigate the karyotype analysis of *Juniperus chinensis* and its seven varieties, and *P. densiflora* for *multicaulis*. Following results were obtained.

1. Three varieties, *J. chinensis* v. *kaizuka*, *J. chinensis* v. *aureo-variegata* and *J. chinensis* v. *procumbens* are turned out as tetraploids.
2. Varieties having many long chromosomes and long mean relative length of chromosomes are *J. chinensis* and *J. chinensis* v. *aureo-globosa*, while varieties having short chromosomes are *J. chinensis* v. *horizontalis* and *J. chinensis* v. *globosa*.
3. Varieties with high mean ratio of long arm length to short arm are *J. chinensis* v. *globosa* and *J. chinensis* v. *kaizuka*, while a variety with the low mean ratio is *J. chinensis* v. *aureo-globosa*.
4. When chromosomes are arranged according to the total length, the most similar variety with *J. chinensis* was *J. chinensis* v. *sargentii*, *J. chinensis* v. *horizontalis*, *J. chinensis* v. *globosa* and *J. chinensis* v. *aureo-globosa*, while the least similar one was *J. chinensis* v. *procumbens*.
5. *Pinus densiflora* for *multicaulis* has shorter mean relative length of chromosome than *P. densiflora*, while the arm ratio of the former is higher than the latter.
6. When chromosomes are arranged according to the total length, six chromosomes showed the same order between the two varieties.
7. *P. densiflora* for *multicaulis* has many chromosomes with secondary constriction.

Keywords: karyotype analysis; variety.

要 約

본 연구는 *Juniperus chinensis* 의 7varieties와 *Pinus densiflora*의 한 변종에 대한 karyotype analysis의 결과로 다음과 같은 성적을 얻었다. 1) *J. chinensis* v. *kaizuka*, *J. chinensis* v. *aureo-variegata* 와 *J.*

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chinensis v. *procumbens* 는 tetraploid 이다. 2) 각 수종의 염색체에 있어 긴 평균장과 긴 염색체를 많이 가지고 있는 수종은 *J. chinensis* 와 *J. chinensis* v. *aureo-globosa* 이고 그 반대로 짧은 평균장과, 짧은 염색체를 가진 수종은 *J. chinensis* v. *horizontalis* 와 *J. chinensis* v. *globosa* 이다. 3) 각 염색체의 short arm 에 대한 long arm 의 평균비치가 큰 수종은 *J. chinensis* v. *globosa* 와 *J. chinensis* v. *kaizuka* 이고, 작은 수종은 *J. chinensis* v. *aureo-globosa* 이다. 4) 염색체를 긴 순서로 배열할 때의 배열순서가 *J. chinensis* 의 배열상과 가장 근사한 수종은 *J. chinensis* v. *sargentii*, *J. chinensis* v. *horizontalis*, *J. chinensis* v. *globosa* 와 *J. chinensis* v. *aureo-globosa* 이며, *J. chinensis* v. *procumbens* 는 배열상이 가장 다르다. 5) *Pinus densiflora* for. *multicaulis* 는 염색체 평균장이 짧고, arm ratio 는 크다. 6) 염색체를 긴 순서로 배열할 시의 longarm 과 short arm 의 배열순서가 6본의 염색체에서 같이 나타난다. 7) *P. densiflora* for. *multicaulis* 는 secondary constriction 이 많은 염색체에서 나타난다.

INTRODUCTION

Many varieties in *Juniperus chinensis* have been known, and their morphological characteristics have drawn interests and have been used as important garden and landscape trees. *Pinus densiflora* also has many varieties, and the classification of the varieties was carried out by Uyeki (1928) according to the tree forms. Among the varieties, *P. densiflora* for. *multicaulis* Uyeki has been known for its many small stems without main stem and has been planted at gardens for its high aesthetic value.

Questions have been arised whether the morphological variations are related to the morphological or numerical variations in chromosomes of each species. The present article reports some relationships between morphological variation and chromosomal variation in the two species and their varieties.

MATERIALS AND METHODS

Juniperus chinensis and its seven varieties have different morphological characters shown in Table 1. Chromosomes were observed in root - tips of big trees of *J. chinensis* and *J. chinensis* v. *kaizuka* and of rooted cuttings of the other varieties from April to June. Chromosomes of *P. densiflora* and its variety were observed from root-tips of germinating seeds. Chromosome observation was carried out primarily followed by the methods of Saylor (1964). The root-tips were pretreated in 8-hydroxyquinoline (0.3g/l) for 24 hours at 18°C fixed in Farmer's fluid for 24 hours, and stained in acetocarmine. Measurements of the chromosome length were made with photographs of each chromosome at 1800 x magnification.

RESULTS AND DISCUSSION

Table 1. Morphological characters in seven varieties of *Juniperus chinensis* L.

Scientific name	Characteristics
1. <i>J. chinensis</i> L.	Tree, conical tree form, long needles (L.N.) and scale-type leaves (S.T.L.).
2. <i>J. chin.</i> var. <i>horizontalis</i>	No main stem, stem and branch ascendent form, L.N. and S.T.L.
3. <i>J. chin.</i> var. <i>sargentii</i>	Decumbent tree form, L.N. and S.T.L.
4. <i>J. chin.</i> var. <i>globosa</i>	No main stem, shrub, globose tree form, S.T.L. are many produced.
5. <i>J. chin.</i> var. <i>aureo-globosa</i>	No main stem, shrub, globose tree form, S.T.L. with yellow color are variegated.
6. <i>J. chin.</i> var. <i>procumbens</i>	No main stem or shrub, decumbent tree form, L.N. and S.T.L.
7. <i>J. chin.</i> var. <i>aureo-variegata</i>	Main stem, L. N. and S.T.L. with yellow color.
8. <i>J. chin.</i> var. <i>kaizuka</i>	Main stem, spiral branch, S.T.L. leaves color is dark than other <i>Juniperus</i> species.

A. *Juniperus chinensis* L. and its varieties

J. chinensis and its seven varieties have morphological differences in having main stem, tree form branch and needle shape, color of leaf and others, as shown in Table 1. Number of chromosomes (Table 2) was not the same among varieties; *J. chinensis* and four other varieties have $2n = 22$ normal diploids, while *J. chinensis v. kaizuka*, *J. chinensis v. aureo-variegata* and *J. chinensis v. procumbens* have $2n = 44$ tetraploids. A few chromosomes of each variety have first constriction and secondary constriction.

Mean size of guard cells of $2n$ varieties was 44.7μ . However, mean size of guard cells of $4n$ varieties was 52.9μ , showing general characteristics of larger stomata in $4n$ varieties than those of $2n$ varieties (Mergen, 1958; Kim, 1975) (Fig. 1).

Natural tetraploids in gymnosperms are very rare, as Mehra (1960) pointed out. Reports of tetraploids in *Juniperus* are tetraploids of *J. chinensis* and *J. squamata* (Mehra, 1960) and triploids of *J. virginiana* by Gustafsson (1960). However, reports on natural tetraploids of *Cryptomeria japonica* are numerous in recent year (Somego, 1981; Kikuti, 1985). In addition to above facts, selected elite trees are found as triploids. Therefore, the possibility of usable tri- and tetraploids in gymnosperm

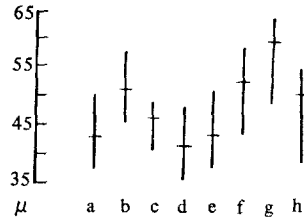


Fig. 1. Comparisons on the length of guard cells in seven varieties of *J. chinensis*. The long vertical line represents the range and the horizontal line represents the mean. a - *J. chinensis*, b - *J. chin. v. horizontalis*, c - *J. chin. v. sargentii*, d - *J. chin. v. globosa*, e - *J. chin. v. aureo-globosa*, f - *J. chin. v. procumbens*, g - *J. chin. v. aureo-variegata*, h - *J. chin. v. kaizuka*.

also seems to be high. Therefore, three tetraploids of *J. chinensis* varieties found in this study may be used as good materials for induction of artificial triploids of this species in future.

Though little morphological variation was found in auto tri- and tetraploids reported in above papers, tetraploids in this study show big differences in morphology, as shown in Table 1. The morphological differences among varieties of *J. chinensis* may partly come from the differences in polyploids. Colchitetraploids of *Robinia pseudoacacia* L.

Table 2. Ratio in percentage of length of each chromosomes to length of the longest chromosomes in seven varieties of *J. chinensis*

<i>J. chinensis</i> (2n)	<i>J. chin. v. horizontalis</i> (2n)	<i>J. chin. v. sargentii</i> (2n)	<i>J. chin. v. globosa</i> (2n)	<i>J. chin. v. aureo-globosa</i> (2n)	<i>J. chin. v. kaizuka</i> (4n)	<i>J. chin. v. aureo-variegata</i> (4n)	<i>J. chin. v. procumbens</i> (4n)	
Chromosomes	Relative length	Relative length	Relative length	Relative length	Chromosomes	Relative length	Chromosomes	Relative length
1	100.0	100.0	100.0	100.0	1,2	100.0	as	100.0
2	97.1	75.0	96.8	77.8	3,4	97.8	in	86.7
3	91.4	70.8	90.3	72.2	5,6	93.3	kaizuka	80.0
4	91.4	70.8	90.3	72.2	7,8	88.9	kaizuka	76.7
5	85.7	69.2	87.1	69.4	9,10	80.0		73.3
6	85.7	63.3	83.9	69.4	11,12	77.8		70.0
7	80.0	62.5	83.9	69.4	13,14	71.1		66.7
8	80.0	60.8	74.2	61.1	15,16	62.2		66.7
9	77.1	60.8	71.0	58.3	17,18	48.9		63.3
10	71.4	58.3	54.8	47.2	19,20	40.0		60.0
11	62.9	52.5	38.7	38.9	21,22	33.3		50.0
Mean	83.9	67.6	79.2	66.9		72.1		72.1

Table 3. Ratio of the long arm to the short arm in seven varieties of *J. chinensis*

<i>J. chinensis</i> (2n)		<i>J. chin.</i> <i>v. hori-</i> <i>zontalis</i> (2n)	<i>J. chin.</i> <i>v. sar-</i> <i>gentii</i> (2n)	<i>J. chin.</i> <i>v.</i> <i>globosa</i> (2n)	<i>J. chin.</i> <i>v. aureo-</i> <i>globosa</i> (2n)	<i>J. chin. v.</i> <i>kaizuka</i> (4n)		<i>J. chin. v.</i> <i>aureo-variegat</i> (4n)		<i>J. chin. v.</i> <i>procumbens</i> (4n)	
Chrom- osomes	Arm ratio	Arm ratio	Arm ratio	Arm ratio	Arm ratio	Chrom- osomes	Arm ratio	Chrom- osomes	Arm ratio	Chrom- osomes	Arm ratio
1	1.06	1.18	1.58	2.27	1.00	1,2	1.37	as in	1.14	as in	1.19
2	1.83	1.00	1.14	1.15	1.15	3,4	1.00	<i>kaiz-</i> <i>uka</i>	1.17	<i>kaiz-</i> <i>uka</i>	1.21
3	1.00	1.13	1.00	1.36	1.08	5,6	1.47		1.18		1.14
4	1.29	1.83	1.15	1.60	1.45	7,8	1.00		1.88		1.00
5	1.00	1.20	1.70	1.08	1.00	9,10	1.00		1.00		1.08
6	1.50	1.31	1.00	1.50	1.18	11,12	1.19		1.33		1.70
7	1.33	2.00	1.17	1.78	1.09	13,14	1.29		1.22		1.36
8	1.80	1.42	1.09	1.75	1.20	15,16	1.80		1.50		1.40
9	1.45	1.64	1.20	1.63	1.20	17,18	2.67		1.71		1.30
10	1.78	1.00	1.83	2.40	1.44	19,20	1.00		1.25		1.44
11	1.20	1.27	1.40	1.00	1.00	21,22	2.75		1.14		2.17
Mean	1.39	1.36	1.30	1.59	1.16		1.50		1.32		1.36

showed five types, and they were quite different morphologically (Kim, 1975). Some tetraploids were also found among morphologically different seedlings in *P. rigida* x (*P. rigida* x *taeda*) (Hyun, et al. 1967) and *P. rigida* x *taeda* (Kim, et al. 1963).

Variation in length of chromosomes of *J. chinensis* varieties was investigated. Results are expressed

as relative length to the longest chromosome in each variety. Mean relative length of *J. chinensis* and *J. chinensis v. aureo-globosa* is long, while that of *J. chinensis v. horizontalis* and *J. chinensis v. globosa* is short. Three varieties, *J. chinensis*, *J. chinensis v. sargentii* and *J. chinensis v. aureo-globosa* have four chromosomes of relatively long ones (chromo-

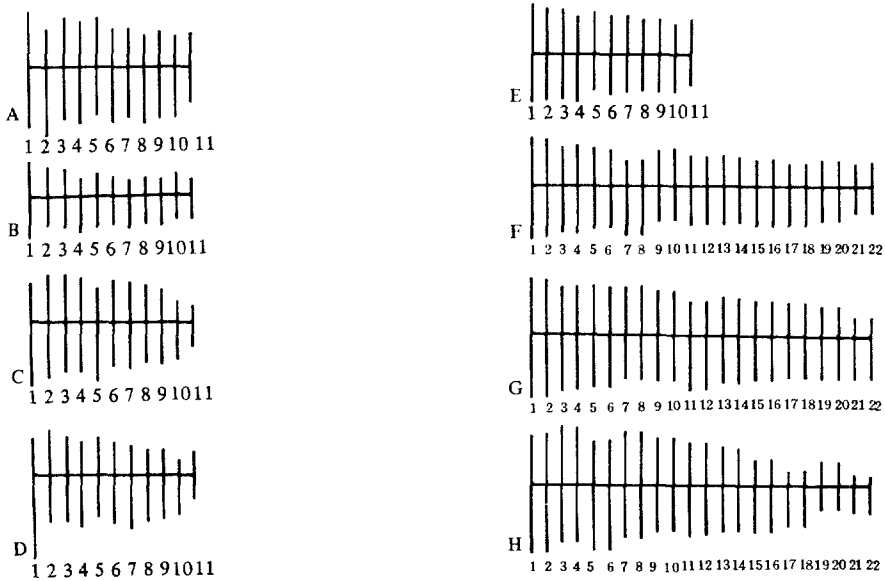


Fig. 2. Karyotype of haploid *Juniperus* cells. A - *J. chinensis*, B - *J. chin. v. horizontalis*, C - *J. chin. v. sargentii*, D - *J. chin. v. globosa*, E - *J. chin. v. aureo-globosa*, F - *J. chin. v. aureo-variegata*, G - *J. chin. v. Procumbens*, H - *J. chin. v. kaizuka*.

(Idiograms arranged in descending order of length of chromosomes.)

somes having relative length ≥ 90.0).

On the other hand, only one (two in tetraploid varieties) chromosome is long in *J. chinensis* v. *horizontalis*, *J. chinensis* v. *globosa*, *J. chinensis* v. *aureo-variegata* and *J. chinensis* v. *procumbens*. Particularly, chromosome 11 of *J. chinensis* v. *sargentii*, *J. chinensis* v. *globosa* and *J. chinensis* v. *kaizuka* was very short.

Ratio of the length of long arm to that of short arm is shown in Table 3. Mean values of the ratio in *J. chinensis* v. *globosa* and *J. chinensis* v. *kaizuka* are relatively high, while that in *J. chinensis* v. *aureo-globosa* is exceptionally low. Position of centromere of each chromosome is shown in Fig. 2 and Table 4. Position of centromere is classified arbitrarily in four groups with the ratio of the length of long arm to short arm shown in Table 3. Nine chromosomes of *J. chinensis* v. *aureo-globosa* have median centromeres, while only three chromosomes of *J. chinensis* v. *globosa* have it. On the other hand, *J. chinensis* v. *globosa* has five terminal centromere chromosomes, while *J. chinensis* v. *aureo-globosa* has none. The other five varieties have similar number of median and terminal centromere chromo-

somes with *J. chinensis*. Position of centromere of each chromosome is compared to the same number of chromosomes of *J. chinensis*. Five chromosomes of *J. chinensis* v. *globosa* and *J. chinensis* v. *aureo-variegata* have same position of centromere with *J. chinensis*, while three chromosomes of *J. chinensis* v. *horizontalis*, *J. chinensis* v. *sargentii* and *J. chinensis* v. *kaizuka* have.

When chromosomes of each variety are arranged in descending order of total length, the order of short arm and that of long arm disagreed (see Fig. 2). Table 5 shows exceptional chromosome numbers in descending order of length of short arm and long arm, and chromosome numbers showing same arrangement with *J. chinensis* by the short arm and the long arm. Final column in Table 5 shows the chromosome numbers showing same arrangement with *J. chinensis* by both arms. *J. chinensis* v. *sargentii* has the most similar chromosomes with *J. chinensis* in regard to the order of short and long arm, having six chromosomes in same order with *J. chinensis*. The next most similar one is *J. chinensis* v. *horizontalis*, *J. chinensis* v. *globosa* and *J. chinensis* v. *aureo-globosa*, having five chromo-

Table 4. Comparisons of the position of centromere in seven varieties of *J. chinensis*

Species	Chromosomes											Number of chromosomes having same position of centromere with <i>J. chinensis</i>	Number of chromosomes with median(m) and terminal centromeres(t)
	1	2	3	4	5	6	7	8	9	10	11		
<i>J. chinensis</i>	m	t	m	sm	m	st	sm	t	st	t	m		
<i>J. chin. v. horizontalis</i>	<u>m</u>	m	<u>m</u>	t	<u>m</u>	sm	t	st	t	m	sm	3	m ; 5, t ; 3
<i>J. chin. v. sargentii</i>	st	m	<u>m</u>	m	t	m	m	m	m	<u>t</u>	sm	2	m ; 7, t ; 2
<i>J. chin. v. globosa</i>	t	m	sm	st	<u>m</u>	<u>st</u>	t	<u>t</u>	t	<u>t</u>	<u>m</u>	5	m ; 3, t ; 5
<i>J. chin. v. aureo-globosa</i>	<u>m</u>	m	<u>m</u>	st	<u>m</u>	m	m	m	m	st	<u>m</u>	4	m ; 9, t ; 0
<i>J. chin. v. kaizuka</i>	sm	m	st	m	<u>m</u>	m	<u>sm</u>	<u>t</u>	t	m	t	3	m ; 5, t ; 3
<i>J. chin. v. aureo-variegata</i>	<u>m</u>	m	<u>m</u>	t	<u>m</u>	sm	<u>sm</u>	st	t	sm	<u>m</u>	5	m ; 5, t ; 2
<i>J. chin. v. procumbens</i>	<u>m</u>	sm	<u>m</u>	m	<u>m</u>	t	<u>sm</u>	sm	sm	st	t	4	m ; 4, t ; 2

Note, 1. long arm : short-arm ratio(m; 1.00 - 1.20, sm; 1.21 - 1.40, st; 2.41 - 1.60, t ; ≥ 1.61)

2. - indicates chromosomes having same position of centromere with *J. chinensis*

Table 5. Features of karyotypes obtained by arranging the chromosomes according to the total length in seven varieties of *J. chinensis*

Species	Chromosomes showing exceptions to the descending order in shorter arm lengths	Chromosomes arranged as in <i>J. chinensis</i> in order of the shorter arm	Chromosomes showing exceptions to the descending order in longer arm lengths	Chromosomes arranged as in <i>J. chinensis</i> in order of the longer arm	Chromosomes arranged as in <i>J. chinensis</i> in order of the shorter and longer arm
<i>J. chinensis</i>	3,4,5,9,11		2,4,6,7,8,9,10		
<i>J. chin. v. horizontalis</i>	5,6,8,9,10,11	1,2,5,7,9,11	4,7,9	1,3,4,5,7,9,11	1,5,7,9,11
<i>J. chin. v. sargentii</i>	2,3,4,5,8	1,3,4,5,6,7,10	4,6,7	1,3,4,5,6,7,11	1,3,4,5,6,7
<i>J. chin. v. globosa</i>	2,5,11	1,5,6,7,8,10	4,6,7,8	1,3,4,5,6,7,8	1,5,6,7,8
<i>J. chin. v. aureo-globosa</i>	5,11	1,2,5,6,7,8,10,11	4,6,10	1,3,4,5,6,10,11	1,5,6,11
<i>J. chin. v. kaizuka</i>	2(3,4), 4(7,8), 5(9,10),9(17,18)	1,4,5,6,7,8,9,10	3(5,6),6(11,12), 11(21,22)	1,5,6	1,5,6
<i>J. chin. v. aureo-variegata</i>	5(9,10),6(11,12), 7(13,14),10(19,20)	1,2,5,8	4(7,8),6(11,12), 8(15,16),9(17,18)	1,3,4,5,6,8,9,11	1,5,8
<i>J. chin. v. procumbens</i>	7(13,14)	1,2,6,8,10	6(11,12),7(13,14)	1,3,5,6,7,11	1,6

somes. On the contrary, *J. chinensis v. procumbens* has only two chromosomes in same order with *J. chinensis*.

Judging by all karyological characteristics mentioned above on *J. chinensis* varieties, *J. chinensis v. sargentii*, *J. chinensis v. aureo-globosa* and *J. chinensis v. aureo-variegata* seem to be close varieties with *J. chinensis*, while *J. chinensis v. kaizuka* seem to be the farthest variety from *J. chinensis*. However, further studies are needed for more clear

conclusion on relationships among *J. chinensis* varieties. Differences in number and morphology of chromosomes among varieties in *J. chinensis* are confirmed by this study.

B. *Pinus densiflora* and *P. densiflora* for. *multicaulis*

P. densiflora for. *multicaulis* is a variety having many small stems without main stem. The needle length of this variety is shorter and the cone sone is

Table 6. Ratio in percentage of length of each chromosomes to length of the longest chromosomes and arm ratio in *p. densiflora* and its variety

Chromosomes	Relative length		Arm ratio	
	<i>P. densiflora</i>	<i>P. densiflora</i> for. <i>multicaulis</i>	<i>P. densiflora</i>	<i>P. densiflora</i> for. <i>multicaulis</i>
1	100.0	100.0	1.05	1.04
2	97.4	90.9	1.00	1.00
3	97.4	90.9	1.53	1.27
4	92.3	89.0	1.00	1.04
5	89.7	83.6	1.19	1.09
6	87.2	83.6	1.00	1.00
7	82.1	78.2	1.13	1.05
8	79.5	74.5	1.21	1.05
9	76.9	65.5	1.00	1.57
10	66.7	61.8	1.17	1.83
11	64.1	56.4	1.78	1.38
12	59.0	47.3	1.09	2.25
Mean	82.7	76.8	1.18	1.30

Table 7. Comparisons of the position of centromere in *P. densiflora* and its variety

Species	Chromosomes												Number of chromosomes having same position of centromere with <i>P. densiflora</i>	Number of chromosomes with median(m) and terminal centromeres(t)
	1	2	3	4	5	6	7	8	9	10	11	12		
<i>P. densiflora</i>	m	m	st	m	m	m	m	sm	m	m	t	m		m ; 9, t ; 1
<i>P. densiflora</i> for. <i>multicaulis</i>	m	m	sm	m	m	m	m	m	st	t	sm	t	6	m ; 7, t ; 2

Note, see Table 4.

Table 8. Features of karyotypes obtained by arranging the chromosomes according to the total length in *P. densiflora* and variety

Species	Chromosomes showing exceptions to the descending order in shorter arm lengths	Chromosomes arranged as in <i>P. densiflora</i> in order of the shorter arm	Chromosomes showing exceptions to the descending order in longer arm lengths	Chromosomes arranged as in <i>P. densiflora</i> in order of the longer arm	Chromosomes arranged as in <i>P. densiflora</i> in order of the shorter and longer arm
<i>P. densiflora</i>	4,5,6,9,12		3,5,11		
<i>P. densiflora</i> for. <i>multicaulis</i>	4,6,11	1,3,4,5,6,7,8, 10,12	3,9,10	1,2,3,4,6, 7,10	1,3,4,6,7, 10

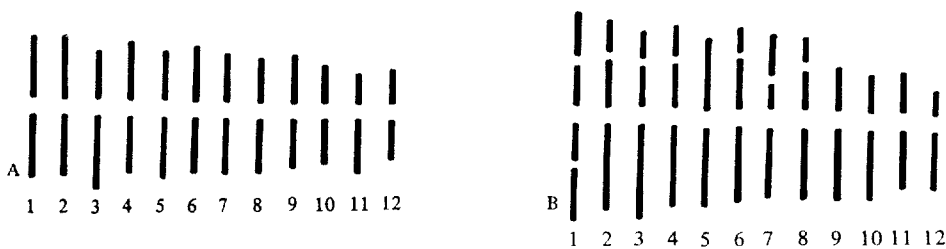


Fig. 3. Idiograms of *P. densiflora* (A) and *P. densiflora* for. *multicaulis* (B). The chromosomes are arranged according to the total length.

smaller than *P. densiflora*.

Mean relative length of chromosomes of *P. densiflora* for. *multicaulis* is shorter than that of *P. densiflora* by 7 percent (Table 6). Mean ratio of length of the long arm to that of the short arm is 1.30, which is bigger than that of *P. densiflora*.

No big differences were observed on the position of centromere. Number of chromosomes with median or terminal centromere is similar between the two varieties. Six chromosomes out of twelve have same position of centromere (Table 7).

When chromosomes are arranged according to the total length from the longest one in descending order, chromosomes 1, 3, 4, 6, 7 and 10 of *P. densiflora* for. *multicaulis* are similar with *P. densi-*

flora in the short and long arm (Table 8).

The most remarkable difference of chromosome morphology of *P. densiflora* for. *multicaulis* and *P. densiflora* is secondary constriction on many chromosomes of the former variety (Fig. 3).

Many papers reports similar results in many species and varieties (Gustafsson, 1960; Mergen, et al. 1963; Kim, 1963; Saylor, 1964; Simak, 1964; Shidei, et al. 1965; Baranec, 1979).

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