5. Conclusions

Somewhat changed silvicultural methods may be needed when using grafted clones in forestry. Choice of proper spacing is then important and research on the effect of competition and on the method at plant propagation is needed. On the whole the variation between plants within the seedling material was larger than between grafts within the clones except for straightness *(Figure 3 a—g)*. The reason could be that genetic differences within the seedling material caused more variation than the genetic differences in the root part and the physiological differences resulting from the grafting procedure.

It seems to be possible to decrease variation, especially for volume production, by conducting trials with grafted clones, but then competition must be low. Clones could thus be a great help in breeding work. A greater number of clones must be tested to give information of interaction between genotype and spacing.

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The somatic chromosomes of Cryptomeria japonica with special reference to the marker chromosomes[1])

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Summary

The diploid chromosome number of *Cryptomeria japonica* was found to be 2n = 2x = 22, which concurs with previous reports. The majority of chromosomes were found to have median kinetochores. One submetacentric chromosome pair has an unusually long primary constriction which consists of chromomeres connected by the kinetonema. The possibility that this unusual region is of a compound nature, containing the nucleolar organizer and microtubule attachment sites, is discussed. Observations of the present study were compared with results of previous studies in order to provide a better understanding of the chromosome morphology in *Cryptomeria.*

Key words: Cryptomeria, Taxodiaceae, marker chromosomes, kinetochore, kinetonema.

Zusammenfassung

Die diploide Chromosomenzahl von *Cryptomeria japonica* hat sich als 2n = 2x = 22 herausgestellt, was mit früheren Berichten übereinstimmt. Die Mehrzahl der Chromosomen zeigten mittelgroße Kinetochoren. Ein submetazentrisches Chromosomenpaar hat eine ungewöhnlich lange primäre Constriktion, die aus Chromomeren besteht, welche durch Kinetonemen verbunden sind. Die Möglichkeit, daß diese ungewöhnliche Region zusammengesetzter Natur ist und einen nukleolaren Organisator und mikrotubulare Anknüpfungsstellen enthält, wird diskutiert. Beobachtungen der gegenwärtigen Untersuchung wurden mit den Ergeb-

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nissen früherer Untersuchungen verglichen, um ein besseres Verständnis der Chromosomen-Morphologie bei *Cryptomeria* zu erlangen.

Introduction

Cryptomeria japonica D. Don, Japanese Sugi, is a monotypic genus in the redwood family, *Taxodiaceae*. The conifer is widely planted throughout the world but thought to be endemic only to Japan since the Pliocene era (Florin, 1963). Morphologically, *Cryptomeria* is distinct within *Taxodiaceae*. The genus is invariably classified in a monotypic tribe (Hida, 1962; Eckenwalder, 1976) or even separated from *Taxodiaceae* into a monotypic family (Hayata, 1932).

The chromosome number of *Cryptomeria*, 2n = 2x = 22, was initially reported by Sax and Sax (1933) and Matsumoto (1933) in independent studies. Chromosome counts in later studies have confirmed the initial observations (Zinnai, 1947; Kanazawa, 1949; Chiba, 1950; Zinnai and Chiba, 1952; Saito and Hashizume, 1958; Shibata *et al.*, 1959; Matsuda and Miyajima, 1976; Schlarbaum and Tsuchiya, 1981). Chromosome morphology was studied in detail by Sax and Sax (1933), Matsumoto (1933), Stiff (1952), Mehra and Khoshoo (1956), Kuroki (1969), Toda (1977, 1979 a, b, 1980, 1981 a, b), Mehra and Anand (1979) and Somego and Kikuti (1980). However, descriptions of the *Cryptomeria* karyotype are conflicting among these previous studies. The present authors, therefore, undertook a study of the somatic chromosomes of *Cryptomeria* to make accurate observations on the chromosome complement and discuss these observations in relation to previous studies. Additionally, the study was undertaken to investigate for the presence of marker chromosomes with unusual structures which have been previously reported to occur in taxodiaceous tree species (Schlarbaum and Tsuchiya, 1975, 1981, 1984; Schlarbaum, 1980; Schlarbaum *et al.*, 1983).

Materials and Methods

A single clone was selected for study from three *Cryptomeria* populations, respectively, located in Kyushu, Aomori Prefecture and Kumamoto Prefecture. Somatic cells in the root tip meristematic tissue were isolated and analyzed.

The root tips were immersed in 8-hydroxyquinoline (0.002 M) for 24—36 hours at 4° C and fixed in a 3:1 mixture of ethyl alcohol and glacial acetic acid. After several days in the fixative, the root tips were hydrolyzed in 1 N HCl at 60° C for 10—15 minutes and stained with Feulgen or acetocarmine (Saylor, 1961; Schlarbaum and Tsuchiya, 1976).

The squash technique was used for slide preparation. Slides were made permanent by applying several drops of a 10:1 mixture of 45 percent acetic acid and glycerol to the edge of the cover slip (Tsuchiya, 1971).

Results

The diploid chromosome number of *Cryptomeria* was observed as 2n = 22 *(Fig. 1)* as previously reported by Sax and Sax (1933), Matsumoto (1933), and in other studies. Numerous cells (ca. 20—40 from each clone source) were present in which the diploid number was confirmed and the morphological features of the chromosomes were easily observed. There were no notable differences in chromosome morphology among the clones from the three populations.

With few exceptions, all of the chromosomes were metacentric *(Fig. 1)*. However, one chromosome pair was submetacentric and exhibited a long kinetochore region. As clearly shown at meta-anaphase *(Fig. 2 a)* and metaphase *(Fig. 2 b)*, this region was not an artifact nor an aberration but a consistent feature of the chromosome complement. The length of this kinetochore region varied in different cells at the same stage of mitosis and some variation was observed between homologous chromosomes in the same cell which may be partly ascribed to artifacts *(Fig. 2 b)*. The region apparently consists of chromomeres connexted by a darkly stained chromonema or kinetonema (Matsuura, 1941). In some slide preparations, the kinetochore region showed a spiral structure *(Fig. 2 b* - third chromosome from left). Occasionally, two large chromomeres were observed near the middle of the region as shown by several metaphase chromosomes in *Figure 2 b*. Conventional satellited chromosomes were not observed in the chromosome complement of *Cryptomeria*.

The chromosome complement of *Cryptomeria*, with the exception of the marker chromosome pair, was generally symmetrical in kinetochore position and relative size.

Figure 1. — Somatic metaphase cell of *Cryptomeria japonica* (2n = 22). Arrows indicate marker chromosomes with long kinetochore regions.

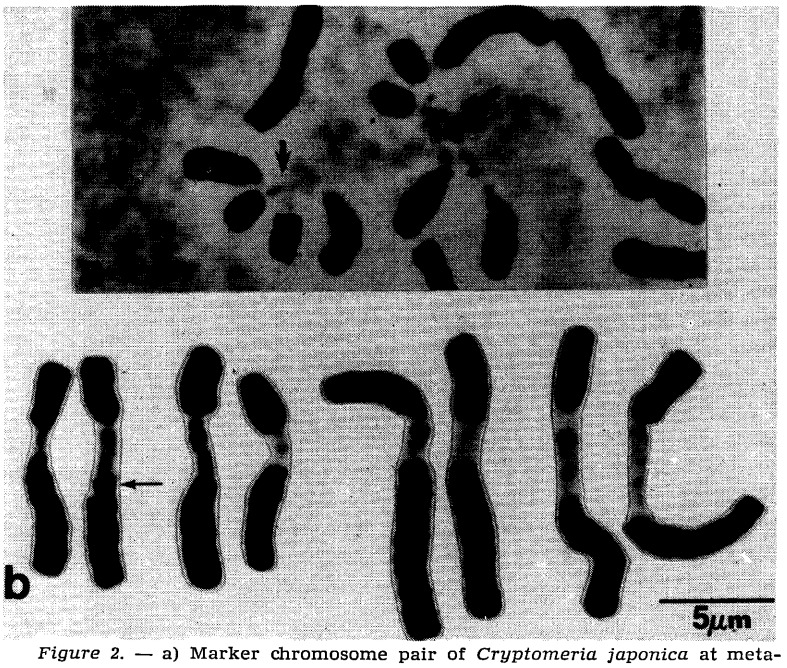

Figure 2. — a) Marker chromosome pair of *Cryptomeria japonica* at meta-anaphase. Arrows indicate long kinetochore regions. b) Isolated marker chromosome pairs at metaphase from four cells of *Cryptomeria japonica*. The "Köpfchen" is indicated by an arrow in the right chromosome of the first pair. The spiralization of the kinetonema is evident in the left chromosome of the second pair.

Discussion

The symmetrical chromosome complement of *Cryptomeria* described in the present study concurs with previous investigations reported by KUROKI (1969), MEHRA and KHOSHOO (1956), TODA (1977, 1979 a, b, 1980, 1981 a, b), MEHRA and ANAND (1979) and SOMEGO and KIKUTI (1980). The studies of SAX and SAX (1933) and STIFF (1952) indicate conflicting viewpoints, reporting the presence of many nonmetacentric chromosomes in the complement. However, neither SAX and SAX (1933) nor STIFF (1952) presented a photograph of the chromosomes nor recognized the presence of chromosomes with unusual features. These two studies, therefore, may be considered less accurate than the present study or previous detailed studies although the possibility of intraspecific variation in the karyotypes can not be completely disregarded as *Cryptomeria* is a morphologically variable tree (DALLIMORE *et al.*, 1966).

MEHRA and KHOSHOO (1956), MEHRA and ANAND (1979), and SOMEGO and KIKUTI (1980) recognized the presence of a long primary constriction in one chromosome pair. TODA (1977, 1979 a, b, 1980, 1981 a, b) observed this unusual feature but considered it to be a secondary constriction rather than the primary constriction. TODA considered the primary constriction to be located between the long arm and a rounded, short arm referred to as a "Köpfchen", which is the short arm of an acrocentric chromosome (KIHARA *et al.*, 1936). This feature can be observed in the second chromosome from the left in *Figure 2 b*. However, the results of the present study do not agree with TODA's karyological interpretation in this particular instance. The probable position of the kinetochore in the marker chromosomes is indicated by the connected kinetonema within the unusual region at the last stage before chromosome separation in meta-anaphase *(Fig. 2 a)*. Inspection of TODA's figures indicates that the "Köpfchen" is a large, inconsistently appearing chromomere in the long arm or could possibly be a feature due to the results of differential reactivity to staining which has been reported to occur in *Cryptomeria* (SCHLARBAUM and TSUCHIYA, 1981).

MATSUMOTO (1933) reported two chromosome pairs with long kinetochore regions to occasionally occur in cells of *Cryptomeria*. Only one pair was observed in the different clones of the present study or reported in any of the previous studies. However, inspection of the photomicrograph presented by TODA (1981 b) shows two pairs of chromosomes with long kinetochore regions. One pair was the distinctive pair noted in many *Cryptomeria* studies while the other pair, unrecognized by TODA, had a kinetochore region which was comparatively shorter, though the region was definitely longer than a normal primary constriction. It is apparent, therefore, that some populations of *Cryptomeria* have two pairs of chromosomes with long kinetochore regions. More detailed studies are needed to determine if the second marker pair contains kinetonemas with chromomeres as in the more distinctive marker chromosomes.

Results of the present study show that the morphology of the long kinetochore regions is variable, although this variation was inconsistent among the marker chromosomes. No observations indicated that the long kinetochore regions of the individual chromosomes were appearing consistently different as in the SAT-chromosomes observed in *Secale cereale* by BOSE (1956, 1957, 1958).

No secondary constrictions were observed in the chromosomes of the different clones in the present study. However, TODA (1979 b, 1981 a, b) has shown indisputable evidence that secondary constrictions are present in some cultivars of *Cryptomeria*. Karyological studies of *Taiwania cryptomerioides* HAY. and *Metasequoia glyptostroboides* HU et CHENG, other members of *Taxodiaceae*, show the presence of marker chromosomes similar to the pair in *Cryptomeria* and an absence of chromosomes with secondary constrictions (SCHLARBAUM and TSUCHIYA, 1984, SCHLARBAUM *et al.*, 1983). Therefore, it is suspected that the long kineto-

chore region, rather than the secondary constriction, contains the nucleolar organization region in addition to the microtubule attachment site. Plant species with unusually long kinetochore regions believed to contain the microtubule attachment site and nucleolar organizer region have been previously reported (HUNZIKER, 1961; KHOSHOO and AHUJA, 1963; KURITA, 1953, 1960; LACOUR, 1950; SATO, 1942). Meiosis of *Cryptomeria* populations with and without secondary constrictions will have to be studied to ascertain the position of the nucleolus organizer region.

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Clonal selection in Larix laricina. I. Effects of age, clone and season on rooting of cuttings

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Summary

Experiments were conducted with 50 clones of tamarack *(Larix laricina* [DU ROI] K. KOCH) of 4 age classes ranging from 3 to 10 years, from which cuttings were taken and struck at 4 dates between May and August. The rooting medium was a mixture of peat and vermiculite kept in styro-blocks, and the cuttings were treated with a rooting hormone and irrigated by intermittent mist. Cuttings when struck were characterized by a distinct phenological state. Assessments after 3 months included rooting percent, percent of cuttings with major roots (more than 3 cm long), number of major roots per cutting, length of the longest root, and shoot phenology. Analysis of variance indicated

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