8 Cryptomeria Japonica

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8.1 Introduction

8.1.1 Brief History of the Crop

The first author to attempt to divide *Cryptomeria japonica* into distinct lines or varieties was Murai (1947), who recognized two lines, Omote-sugi and Ura-sugi, based on differences in their leaf morphology. Broadly speaking, Omote-sugi grows in the region facing the Pacific Ocean, and Ura-sugi in the region facing the Japanese Sea. Yasue et al. (1987) found similar general geographical differentiation, with some exceptions, based on diterpene hydrocarbon constituents. However, little differentiation among natural populations of *C. japonica* has been detected in analyses of isozyme (Tomaru et al. 1994) and cleaved amplified polymorphic sequence (CAPS) markers (Tsumura and Tomaru 1999). Even the genetic differentiation between populations on the mainland and Yaku Island, located far from the mainland, appears to be very small. Differences between the two lines recognized by Murai were reflected in differences between their 6PGD isozymes in a study by Tomaru et al. (1994), but the distinction was much less clear in a subsequent CAPS marker analysis (Tsumura and Tomaru 1999).

As there is a long history of cultivation of *C. japonica*, many varieties have been developed. Miyajima (1983) classified them into two kinds of varieties. One is a geographical race that has not been improved artificially; the other is a cultivar that has been improved artificially. The first cultivars were selected in the 16th century. Afforestation with the species began in the early 18th century, at which time the main traditionally used cultivars were selected by foresters on Kyushu Island. Since then many cultivars have been developed and most of them have been maintained by propagating cutting.

8.1.2 Botanical Descriptions

C. japonica belongs to the family Taxodiaceae. Although *C. fortunei* was found later as a second species of this genus, no apparent differences from *C. japonica* have been reported. *C. japonica* and *C. fortunei* have almost the same genetic sequences, and *Cryptomeria* forms a clade with *Glyptostrobus* and the *Taxodium* group, based on the sequences of four chloroplast genes, in Taxodiaceae (Kusumi et al. 2000). *C. japonica* grows in warm temperate and cool temperate zones, its natural distribution extending from Yaku island, 30°15' N, to the north end of Honshu island, 40°42' N (Maeda 1983). *C. japonica* prefers habitats with high humidity and rich soil. It is a monoecious plant that bears diclinous flowers in a flowering season extending from March to April. Its needles are lozenge-shaped in cross-section.

The chromosome complement of *C. japonica* consists of 11 pairs of metacentric or submetacentric chromosomes with gradual variations in length (Sax and Sax 1933; Mehra and Khoshoo 1956). Secondary constrictions are found on chromosomes 6 and 10. Chromosome 10 always has secondary constrictions on its short arm, whereas chromosome 6 may occur either with or without secondary constrictions (Toda 1979a, b; Kondo et al. 1985). The heteromorphy of chromosome 6 indicates that heteromorphic chromosomes do not necessarily disrupt meiotic processes (Hizume et al. 1989). These secondary constrictions have been clearly visualized by fluorescent banding with chromomycin A_3 (Kondo and Hizume 1982). The fluorescent bands coincide in position with the secondary constrictions and in number with the maximum number of nucleoli per cell. The fluorescent band of chromosome 10 is larger than that of chromosome 6. The nucleolus formed in chromosome 10 is also larger than that of chromosome 6 (Kondo et al. 1985). Fluorescent signals generated by in situ hy-

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bridization with the wheat 18S-5.8S-26S rDNA genes appear at the same position as the fluorescent bands generated with chromomycin A_3 (Hizume et al. 1998). Furthermore *Arabidopsis*-type telomere sequence repeats (TTTAGGG)n can hybridize to both ends of each chromosome (Hizume et al. 2000). Compared with the chromosomes of *Pinus*, those of *C. japonica* seem to have a simpler, less rearranged morphology.

The genome size of *C. japonica* has been determined by flow cytometry of isolated nuclei stained with propidium iodide using *Hordeum vulgare* nuclei as an internal standard (Hizume et al. 2001). The resulting value (mean for five plants) was 22.09 pg/2C, corresponding to ca. 10 Gbp (1×10^{10} bp) according to conversion factors published by Mukai (1998), equivalent to 100 times more than the *Arabidopsis* genome.

8.1.3 Economic Importance

C. japonica is one of the most important timber species in Japan, favored for its straight bole and rapid growth, and has been planted on 4.53 million ha, corresponding to 45% of the artificial forest in Japan. In the Kanto region, which covers central parts of Honshu island including Tokyo, the average height, DBH (diameter at breast height), and volume per hectare of artificial stands are typically 18.8 m, 24.9 cm, and 428.7 m^3 at 40 years of age, and 21.5 m, 29.2 cm, and 515.7 m^3 at 50 years of age, respectively (Ohtomo 1983).

8.1.4 Breeding Objectives

Systematic breeding of *C. japonica* began in the late 1950s. A Tree Breeding Station network has been established under the Forestry Agency of the Ministry of Agriculture and Forestry, covering the whole of Japan. The main initial breeding objective was to improve its growth. Later, resistance to Sugi bark borer and snow damage was added, and recently the breeding objectives have become diverse. As the wood of *C. japonica* is softer than that of imported timber, improvement of wood strength is an urgent goal to promote domestic forestry. Reductions in pollen production, increased rates of CO_2 fixation, and shade tolerance have also been added to the objectives, to address issues associated with pollinosis, global warming, and multistoried forests, respectively.

8.1.5 Classical Mapping Efforts

Linkage was first evaluated in relation to morphological characters when Ohba et al. (1974) investigated relationships among the dominant *twisted-leaf* gene and two recessive genes (an albino gene and a gene responsible for green coloration in winter) but found no linkage among them. However, Kuromaru et al. (1983) found linkage between two loci encoding peroxidase isozymes with a recombination value of 0.167 in repulsion phase. In addition, Kuramoto et al. (1996) found a linkage relationship between a dwarf gene and a gene associated with leaf whitening in summer, with a recombination value of 0.315 in coupling phase. Furthermore, Kuramoto et al. (1997) found several linkage relationships among five isozyme loci and the dwarf gene. Because of the presence of lethal genes, the segregation ratio was distorted in some progeny families, especially selfed families. Although morphological traits and isozymes were studied, only their linkage relationships were examined; no linkage maps were constructed because of the limited number of markers.

8.1.6 Classical Breeding Achievements

As *C. japonica* is the most important tree species in Japan, ca. 3,500 plus tree clones of the species, more than for any other major species bred in Japan, have been selected for mass selection breeding. As mentioned above, the main initial breeding objective was to improve its growth. Compared to local varieties, we have obtained a 15% increment in volume. Problems caused by the sugi bark borer, *Semanotus japonicus*, whose larvae feed on bark and xylem, have also been addressed. To help prevent damage by this insect, an inoculation test has been established and a resistant variety has already been released. A further problem is that crooked trees are often found in regions with heavy snowfall, caused by the pressure exerted by snow sliding down slopes. Two clones, which grow straight even in heavy snow regions, have already been developed after field trials in a region with heavy snowfall.

8.2 Construction of Genetic Maps

8.2.1 Brief History of Mapping Efforts

Since it is the leading species in Japanese forestry and more genetic information has been gathered since the early linkage studies, Japanese tree geneticists and breeders have been eager to construct a linkage map of *C. japonica*. This has been a major challenge for the scientists involved, who have struggled to keep up with advances in breeding major food crop species, such as wheat and rice, and model species (especially *Arabidopsis thaliana*). Mukai et al. (1995) constructed the first linkage map, mainly based on RFLP markers. Although this map provided limited coverage, it was well constructed. CAPS markers were added to it by Iwata et al. (2001), and it was finally integrated, in a consensus map, by Tani et al. (2003). In addition, linkage maps based on dominant RAPD and AFLP markers were constructed by Kuramoto et al. (2000) and Nikaido et al. (2000), respectively. The linkage map by Kuramoto et al. (2000) was designed to facilitate QTL analysis of wood strength. The distances covered by these maps were longer than those based on RFLP or CAPS markers.

8.2.2 First-Generation Maps

The first linkage map was constructed by Mukai et al. (1995) using an F_2 progeny from a cross between two cutting cultivars, Kumotooshi and Okinoyama. A total of 91 (77 RFLP, 12 RAPD, and 1 isozyme) markers were distributed among 13 linkage groups (LGs), covering 887.3 cM (Table 1). The average interval between adjacent markers was 12.3 cM. Thirty-five markers distributed in six clusters showed distorted segregation, presumably due to the presence of (an) embryonic lethal gene(s) (Ohba 1979) or other deleterious genes, the effects of which would appear mainly in selfed progeny. LG 5 was assigned to chromosome 10 using trisomics (Suyama et al. 1996). Iwata et al. (2001) developed CAPS markers and applied them to the same population (Mukai et al 1995). In the revised linkage map, a total of 167 markers (46 CAPS, 101 RFLP, 17 RAPD, and 2 isozyme markers and 1 dwarf gene) were distributed among 15 LGs, covering 1,109.1 cM, with an average interval between adjacent markers of 8.7 cM. As CAPS markers were added to the map, 30 markers consisting of RFLP, RAPD, and isozyme markers without confirmed map positions in the previous map were mapped to precise positions. Thus, the linkage map was extended to 1,109.1 cM and its density was increased. The cited authors developed 217 CAPS markers, and the polymorphisms of the CAPS markers were found to be associated more strongly with intron than with exon regions. The CAPS markers proved to be very useful in the integration of different maps.

Kuramoto et al. (2000) constructed a linkage map of RAPD markers using an F_1 progeny between two cutting cultivars, Boka and Iwao, for QTL analysis of wood strength. As a pseudotestcross mapping strategy (Grattapaglia and Sederoff 1994) was adopted, linkage maps of both parents were obtained. In the linkage map of Iwao (Fig. 1), 119 RAPD markers were distributed among 21 LGs, covering 1,756.4 cM, with an average interval between adjacent markers of 14.8 cM. In the linkage map of Boka (Fig. 2) 84 RAPD markers were distributed among 14 LGs, covering 1,111.9 cM, with an average interval between adjacent markers of 13.2 cM. The genome length was initially estimated as ca. 2,800 cM using the moment estimator method according to Hulbert et al. (1988). All these maps were constructed using the MAPMAKER/EXP 3.0 computer program (Lander et al. 1987; Lincoln et al. 1992a). Nikaido et al. (2000) constructed longer linkage maps based on AFLP markers and a small number of CAPS markers using an F_1 progeny from reciprocal crosses between two cutting cultivars, Kumotooshi and Haara, and (again) a pseudotestcross mapping strategy. In the linkage map of Kumotooshi a total of 132 (123 AFLP and 9 CAPS) markers were distributed among 23 LGs, covering 1,992.3 cM, with an average interval between adjacent markers of 17.9 cM. In the linkage map of Haara a total of 91 markers, consisting of 83 AFLP and 8 CAPS markers, were distributed among 19 LGs, covering 1,266.1 cM, with an average interval between adjacent markers of 16.0 cM. Segregation distortion of AFLP markers was found in half of them, attributed to the presence of lethal genes and fragment-complexes originating from different loci.

8.2.3 Second-Generation Maps

Tani et al. (2003) integrated linkage maps of two unrelated F_2 populations using JoinMap 3.0 software (Van

Table 1. Linkage maps of Cryptomeria japonica

Authors	Population*	Markers	Linkage groups	Total map distance (coverage %)	Marker interval	Genome length
Mukai et al. (1995)	F_2 (Kumotooshi × Okinoyama)	91 markers (77 RFLP, 12 RAPD, 1 isozyme, 1 dwarf gene)	13 groups and 6 pairs	887.3 cM	12.3 cM**	Not estmated
Iwata et al. (2001)	Same population as Mukai et al. (1995) F_2 (Kumotooshi × Okinoyama)	167 markers (46 CAPS, 101 RFLP, 17 RAPD, 2 isozyme, 1 dwarf gene)	15 groups	1,109.1 cM	8.7 cM	Not estimated
Nikaido et al. (2000)	F_1 (Haara, Kumotooshi) reciprocal crosses	Kumotooshi: 132 markers (123 AFLP, 9 CAPS) Haara: 91 markers (83 AFLP, 8 CAPS)	Kumotooshi: 23 groups Haara: 19 groups	Kumotooshi: 1,992.3 cM (80%) Haara: 1,266.1 cM (50%)	Kumotooshi: 17.9 cM Haara: 16.0 cM	Length of 2,500 cM was used
Kuramoto et al. (2000)	F_1 (Boka × Iwao)	Iwao: 119 RAPD markers Boka: 84 RAPD markers	Iwao: 21 groups Boka: 14 groups	Iwao: 1,756.4 cM (62%) Boka: 1,111.9 cM (45%)	Iwao: 14.8 cM Boka: 13.2 cM	2,800 cM
Tani et al. (2003)	Two populations • Same population as Mukai et al. (1995) F_2 (Kumotooshi × Okinoyama) • F_2 from sibcross of F_1 (Yabukuguri × Iwao)	438 markers (172 CAPS, 200 RFLP, 37 microsatellite, 5 SNP, 22 RAPD, 1 isozyme, 1 dwarf gene)	11 large groups and 1 small group	1,372.2 cM	3.0 cM	• F_2 (Kumotooshi × Okinoyama): 1,395.5 cM • F_2 from sibcross of F_1 (Yabukuguri × Iwao): 1,810.1 cM, 2,168.5 cM

* All parents were cutting cultivars such as Kumotooshi, Okinoyama, Haara, Iwao, Boka

** Marker interval was calculated by authors

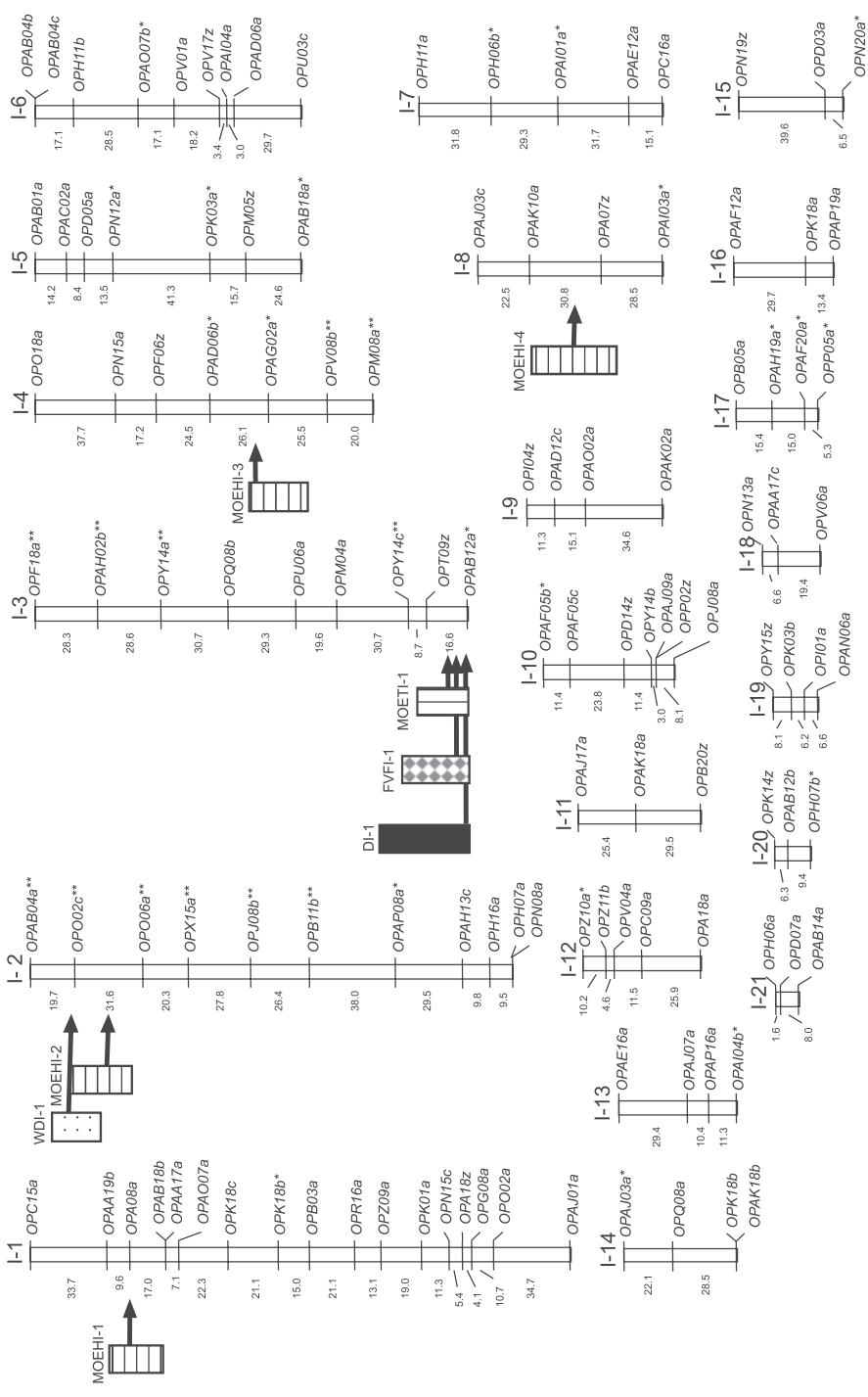

Fig. 1. Linkage map and estimated locations of QTLs in Iwao-sugi, using RAPD markers. A total of 177 linked markers were distributed among 25 LGs. Four LGs that consisted of only two markers were not included in this figure. The markers are listed on the *right* and map distances in centiMorgan are shown on the *left*. One *asterisk* (*) and two *asterisks* (**) designate markers with distorted segregation ($0.01 < P < 0.05$ and $P < 0.01$, respectively). One hundred nineteen RAPD markers with confirmed map positions were assigned to 21 LGs, covering 1,756.4 cM. *Bars* to the *left* of the LGs correspond to 2.0 LOD support intervals for the QTL locations. *Arrows* extending from bars indicate the most likely QTL positions estimated using MAPMAKER/QTL analysis (Table 1). MOEH, modulus of elasticity measured by the hanging method; MOET, modulus of elasticity measured by the tapping method; FVF, fundamental vibration frequency; WD, wood density in green condition; D, diameter (Kuramoto et al 2000, courtesy Can J For Res)

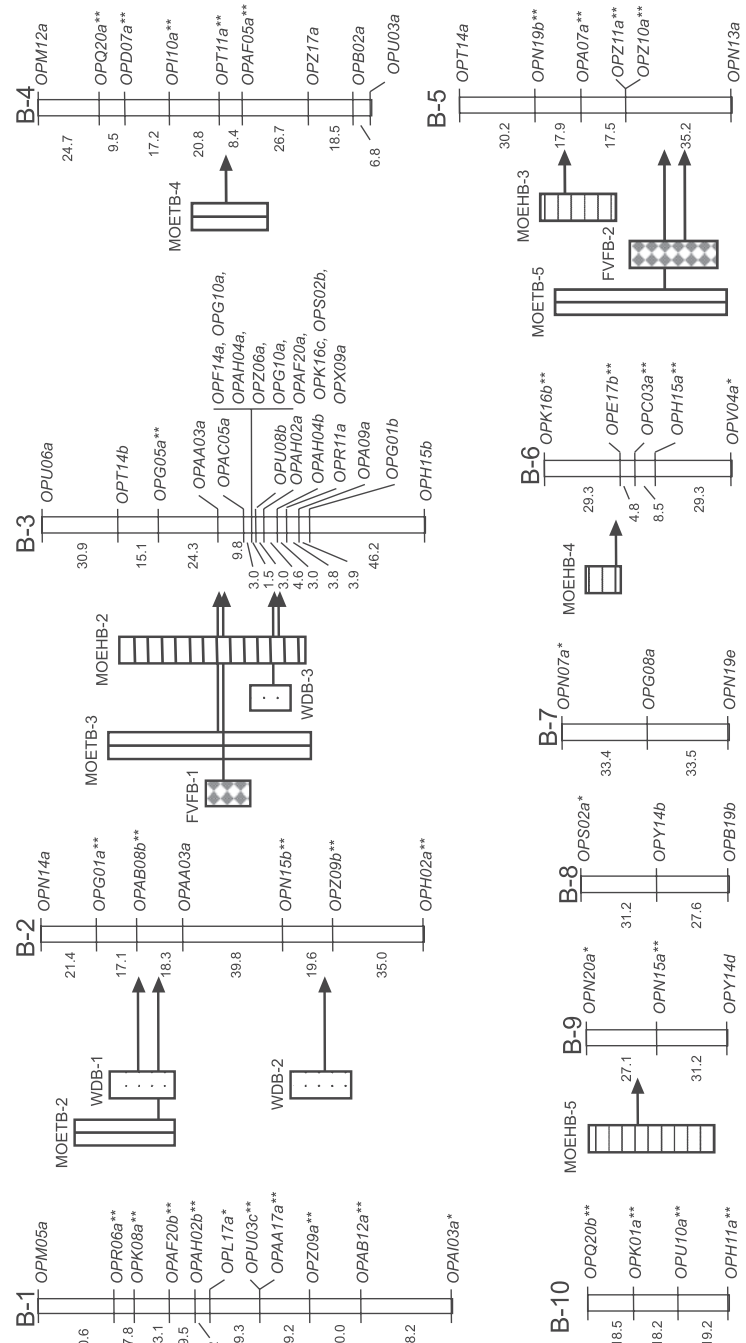

Fig. 2. Linkage map and estimated locations of QTLs in Boka-sugi, using RAPD markers. A total of 117 linked markers were distributed among 21 LGs. Seven LGs that consisted of only two markers were not included in this figure. The markers are listed on the *right* and map distances in centiMorgan are shown on the *left*. One *asterisk* (*) and two *asterisks* (**) designate markers with distorted segregation ($0.01 < P < 0.05$ and $P < 0.01$, respectively). Ninety-eight RAPD markers with confirmed map positions were assigned to 14 LGs, covering 1,111.9 cM. *Bars* to the *left* of the LGs correspond to 2.0 LOD support intervals for the QTL locations. *Arrows* extending from *bars* indicate the most likely QTL positions estimated using MAPMAKER/QTL analysis (Table 2). MOEH, modulus of elasticity measured by the hanging method; MOET, modulus of elasticity measured by the tapping method; FVF, fundamental vibration frequency; WD, wood density in green condition (Kuramoto et al 2000, courtesy Can J For Res)

Table 2. Gene mapping in Cryptomeria japonica

Reference	Trait	Population	Marker type	Gene symbol	Linkage group	Flanking marker	Distance
Mukai et al. (1995)	Dwarf	F_2 progeny from a cross between Kumotooshi and Okinoyama	RAPD, RFLP	MT-d	2	RAPD marker, K08b RFLP marker, CD0461R	8.1 cM 33.5 cM
Tani et al. (2003)	Dwarf	F_2 progeny from a cross between Kumotooshi and Okinoyama	CAPS, RFLP, SNP, microsatellite	MT dwarf	KO7	RAPD marker, K08b RFLP marker, CD0461R	6.8 cM 13.5 cM
		Two unrelated F_2 populations, F_2 from a cross between Kumotooshi and Okinoyama, and F_2 population from a sibcross of F_1 (Yabukuguri × Iwao) (integrated map)	CAPS, RFLP, SNP, microsatellite	MT dwarf	YI5&KO7	RFLP marker, CD0195R Microsatellite marker, CJG0083M	1.7 cM 4.7 cM
Goto et al. (2003)	Allergen, Cry j 1	F_1 progeny between Boka and Iwao	RAPD	CRYJ1-352	I-18	RAPD marker, OPN13a	0 cM
Tani et al. (2003)	Allergen, Cry j 1	F_2 progeny from a cross between Kumotooshi and Okinoyama	CAPS, RFLP, SNP, microsatellite	Cry j 1C	KO7	Microsatellite marker, CJS0002M RFLP marker, CC1778R	2.7 cM 2.6 cM
		Two unrelated F_2 populations, F_2 from a cross between Kumotooshi and Okinoyama, and F_2 population from a sibcross of F_1 (Yabukuguri×Iwao) (integrated map)	CAPS, RFLP, SNP, microsatellite	Cry j 1C	YI5&KO7	CAPS marker, CC2989C Microsatellite marker, CJS002M	0.5 cM 2.1 cM
Tani et al. (2003)	Allergen, Cry j 2	F_2 progeny from a cross between Kumotooshi and Okinoyama	CAPS, RFLP, SNP, microsatellite	Cry j 2C	YI2	RFLP markers, CD0440R RFLP markers, CC1488R1	3.7 cM 4.2 cM
		Two unrelated F_2 populations, F_2 from a cross between Kumotooshi and Okinoyama, and F_2 population from a sibcross of F_1 (Yabukuguri × Iwao) (integrated map)	CAPS, RFLP, SNP, microsatellite	Cry j 2C	YI2&KO11,12	CAPS marker, CC2657C RFLP marker, CD0344R	14.7 cM 10.8 cM

Ooijen and Voorrips 2001). One F_2 population originated from the cross between Kumotooshi and Okinoyama used by Mukai et al. (1995) and Iwata et al. (2001). The other originated from a sibcross of an F_1 progeny (Yabukuguri × Iwao). In the integrated map a total of 438 markers (172 CAPS, 200 RFLP, 37 microsatellite, 5 SNP, 22 RAPD markers, 1 isozyme marker, and 1 dwarf gene) were distributed in 11 large LGs and 1 small group, covering 1,372.2 cM in total. The average interval between adjacent markers was 3.0 cM. Although large numbers of markers were mapped (including microsatellites), resulting in a high-density map, the total distance covered by it was thought to be less than half of the total genome length, and it was still shorter than maps constructed with RAPD markers (Kuramoto et al. 2000) and AFLP markers (Nikaido et al. 2000). Tani et al. (2003) speculated that their map did not cover regions where genes were sparsely distributed and that it would be helpful to add extensive microsatellite markers or random genetic markers, such as AFLP and RAPD markers, to fill in the less dense regions (Tani et al. 2003). As the libraries were derived from limited sources, 3-day imbibed embryos and inner-bark tissues, it should be noted that this map may not have covered all of the gene-dense regions. Further integration of the maps by Kuramoto et al. (2000) and Nikaido et al. (2000) is likely to extend the coverage toward saturation. A saturated linkage map is essential for accurate QTL analysis and effective marker-assisted selection (MAS). Over 200 CAPS markers have already been developed and should facilitate the integration of different maps (Iwata et al. 2001). Thirty-seven CAPS markers have been used as bridges to integrate the two maps thus far, but this is too few (fewer than 3 bridging CAPS markers per LG for 9 out of the 12 LGs) for full integration. Thus, the CAPS markers (thus far developed, at least) were not as powerful as hoped, and the development of other kinds of markers for integration is awaited to overcome current limitations.

8.3 Gene Mapping

In the first linkage map by Mukai et al. (1995) a dwarf gene, *MT-d*, was mapped using the F_2 progeny from a cross between two cutting cultivars, Kumotooshi and Okinoyama. *MT-d* was flanked by an RAPD marker, K08b, and an RFLP marker, CD0461R, at distances of 8.1 cM and 33.5 cM, respectively. These distances were shortened to 6.8 cM and 13.5 cM, respectively, in the revised map by adding CAPS, RFLP, SNP, and microsatellite markers (Tani et al. 2003). Furthermore, *MT-d* was flanked by an RFLP marker, CD0195R, and a microsatellite marker, CJG0083M, at distances of 1.7 cM and 4.7 cM, respectively, in the integrated map. Although the distance between *MT-d* gene and CD0195R was the smallest at 1.7 cM compared with other markers, in the previous map it was 34.6 cM.

Cry j 1 and Cry j 2 are major allergens involved in *C. japonica* pollinosis. Goto et al. (2003) mapped the gene encoding Cry j 1 using a CAPS marker derived from isoform information acquired using F_1 progeny from a cross between two cutting cultivars, Boka and Iwao, which had already been used in QTL analysis of wood strength (Kuramoto et al. 2000). The CAPS marker cosegregated with (and thus was located in the same position, or very close to) an RAPD marker, OPN13a. Tani et al. (2003) also mapped the genes encoding Cry j 1 and Cry j 2 in their integrated map, but did not describe how they were mapped. According to Tani et al. (2003) Cry j 1 was flanked by a microsatellite marker, CJS0002M, and an RFLP marker, CC1778R, at distances of 2.7 cM and 2.6 cM, respectively. Cry j 2 was flanked by two RFLP markers, CD0440R and CC1488R1, at distances of 3.7 cM and 4.2 cM, respectively. The locations of these genes in the integrated map are shown in Table 2.

8.4 Detection of Quantitative Trait Loci

Improvement of the wood strength of *C. japonica* is an urgent objective. Kuramoto et al. (2000) analyzed QTLs associated with wood strength using a linkage map derived from RAPD markers in the F_1 progeny of a cross between Boka and Iwao. Effective QTLs were associated with several traits related to wood strength, such as the modulus of elasticity (MOE; an indicator of wood strength), wood density, fundamental vibration frequency, and stem diameter using MAPMAKER/QTL 1.1 software (Paterson et al. 1988; Lincoln et al. 1992b). Five and ten QTLs for MOE were detected in the linkage maps of Iwao and Boka, respectively (Figs. 1 and 2). Since these QTLs explained about 45% of the total phenotypic variance, they were thought to be sufficiently effective for use in breed-

ing programs. MOE was evaluated by two methods, which gave similar values but were based on different principles. In this analysis some QTLs detected by one method did not overlap with those detected by the other one. It was postulated that the differences in the mapping positions of QTLs related to MOE might reflect differences in the bases of these methods. Furthermore, QTLs associated with wood density and fundamental vibration frequency overlapped with some of the QTLs associated with MOE. Therefore, it was concluded that the QTL analysis revealed important components of wood strength, and relationships among them, via their positions on the linkage maps.

Yoshimaru et al. (1998) analyzed QTLs associated with the juvenile growth, flower bearing, and rooting ability of *C. japonica* using the linkage map constructed by Mukai et al. (1995) on the basis of RFLP, RAPD, and isozyme loci using the F_2 progeny of a cross between Kumotooshi and Okinoyama. Very effective QTLs were detected for all the traits, using the MAPMAKER/QTL 1.1 program as a free model of QTL effects (Paterson et al. 1988; Lincoln et al. 1992b). Since major QTLs for early growth were detected near the dwarf gene in LG 2, the authors considered that these QTLs are pleiotropic effects of the dwarf gene. In LG 2 effective QTLs for male and female flower bearing were also detected. Another QTL associated with female flower bearing was also detected near the dwarf gene in LG 2. This QTL was thought to have some genetic relationship with QTLs for early growth. A less effective QTL for female flower bearing was detected in LG 5.

8.5 Advanced Works

The cDNA libraries have been derived from the inner bark and strobili (male and female) of *C. japonica*. From the inner-bark library, 1,583 out of 2,231 clones were assigned to putative functions (Ujino-Ihara et al. 2000). The remaining 648 clones did not show significant homology to any known sequences. Sequences representing genes concerned with cell wall formation and stress responses were abundant. From the cDNA clones 67 sequence tagged site (STS) markers were developed (Ujino-Ihara et al. 2002). In addition, 1,210 expressed sequence tags (ESTs) representing 1,173 transcripts were obtained from male and female strobili (Ujino-Ihara et al. 2003), 807 of which were assigned to putative functions, including those of genes expressed in developing flower tissues of other plant species, such as *CONSTANS* and the genes encoding MADS-domain proteins.

8.6 Future Scope of Works

As the genome size of *C. japonica* is huge as compared to *Arabidopsis*, it will be laborious and costly to undertake comprehensive standard genome analyses, such as making a complete physical map and reading all the sequences. Therefore, it may be more efficient to adopt two other strategies. One is to increase the density of the linkage map and to map useful genes on it using conventional test crosses. Another is to isolate functional genes from the DNA library according to information obtained from intensively examined plants such as *Arabidopsis*, which would require the establishment of a DNA library that covers the whole genome.

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