

## CHAPTER 2

### Development of Micropropagation Technique

#### for *Castanopsis cuspidata* var. *sieboldii*

#### 2.1 Abstract

Micropropagation of *Castanopsis sieboldii* was attempted using immature zygotic embryos as the explant. *In vitro* somatic embryogenesis was achieved from immature embryo-derived callus and the morphological course of events during embryo development was evaluated by scanning electron microscopy technique (SEM). Embryogenic callus could be initiated from immature embryos obtained from actively growing acorns and optimum induction of callus was observed on Murashige and Skoog (MS) medium supplemented with 10  $\mu\text{M}$  2,4-dichlorophenoxyacetic acid (2,4-D) and 1  $\mu\text{M}$  benzylaminopurine (BAP). On the other hand, white and friable calli were initiated from embryos obtained from fully-grown acorns. Somatic embryo development was occurred on half-strength basal MS medium supplemented with 2  $\mu\text{M}$  BAP and 0.25  $\mu\text{M}$  naphthaleneacetic acid (NAA). Somatic embryo maturation was accomplished using 1  $\mu\text{M}$  zeatin and 0.25  $\mu\text{M}$  indolebutyric acid (IBA). SEM observations indicated development of an extracellular material at an early stage of differentiation and a course of differentiation leading to development of somatic embryos.

## CHAPTER 3

### **Evaluation of Applicability of Characterized Microsatellite Primers to Study Genetic Variation of *Castanopsis cuspidata* var. *sieboldii* and 4 Species in the Genus *Quercus***

#### **3.1 Abstract**

Use of microsatellite markers to study genetic variation within and between plant populations has been increasing recently, as microsatellite markers reveal higher levels of polymorphism compared to other marker systems. Fourteen microsatellite primers which were previously characterized for *C. sieboldii* and *Quercus petraea* were evaluated for their applicability to study genetic variation of *C. sieboldii* and to amplify with 4 species in the genus *Quercus*: *Q. dentata*, *Q. glauca*, *Q. mongolica* var. *grosseserrata* and *Q. salicina*.

Five out of the seven primers described for *C. sieboldii* were successfully amplified. Two out of the 7 tested primers that were described for *Q. petraea* amplified with all the 5 species and, indicating possibility of using them for study genetic relatedness between species. Six of the primers were selected to study genetic variation of *C. sieboldii*.

## CHAPTER 4

### Genetic Diversity of *Castanopsis cuspidata* var. *sieboldii* Distributed in Ryukyu Islands

#### 4.1 Abstract

*Castanopsis sieboldii* dominates evergreen broad-leaved forests in most of the Nansei Islands of Japan. Nevertheless one of most economically important tree species, less information is available on genetic variation of *C. sieboldii*. In this study genetic variation was examined, using 6 microsatellite loci and 255 mature trees, from 11 populations distributed in three islands, namely Okinawa, Ishigaki and Iriomote Islands. All the loci were highly variable, with the number of alleles per locus ranging from 17 to 34 (mean 26) and the expected heterozygosities ( $H_E$ ) ranged from 0.863 to 0.940. Observed heterozygosity showed significant deviation (0.1 percent level) from Hardy-Weinberg expectations at 5 of the 6 loci, and relatively high miss-amplification was observed at 3 loci (12.5%, 10.6% and 7.5%). Since the  $H_O$  of 7 populations showed significant deviation from Hardy-Weinberg equilibrium with positive  $F_{IS}$  values, it could be suggested that some level of self-pollination occur. Level of genetic variation of each population over all loci as  $H_O$  was 0.573 (ranged from 0.441 to 0.652). Majority of genetic variation was observed within the populations resulting lower but significant variation between populations ( $F_{ST} = 0.025$ ,  $P < 0.001$ ). The deviations may be due to apparent deficit of heterozygotes caused by the self-pollination, miss-amplification of

## CHAPTER 5

### Genetic Variation and Differentiation of Isolated Populations of *Castanopsis cuspidata* var. *sieboldii* Distributed in Satsunan Islands and Kagoshima

#### 5.1 Abstract

Genetic variation of 5 populations of *C. sieboldii* distributed Satsunan Islands which are located north east of Okinawa Island, and 1 population at Osumi Peninsula of Kyushu was investigated using microsatellite markers. High level of polymorphism at all six microsatellite loci was, as expressed by number of alleles per locus (mean = 30), expected heterozygosity (mean  $H_E = 0.939$ ) and observed heterozygosity (mean  $H_O = 0.683$ ).

Observed heterozygosity of all 6 populations (mean = 0.683) were also comparatively high and increased levels of heterozygote individuals were observed. Three of the 5 populations showed significant deviation from Hardy-Weinberg equilibrium indicating some level of inbreeding in those populations. The numbers of alleles distributed in populations of the Satsunan Islands and Osumi Peninsula were ranged between 23 and 38. Expected heterozygosities were ranged from 0.864 to 0.915, and observed heterozygosities were ranged between 0.623 and 0.749. Inbreeding coefficients of these populations were not high, 0.179 – 0.295, especially those values of Takakuma and Yakushima were 0.179 and 0.185 respectively. It suggested that trees of both Takakuma and Yakushima populations were well cross-pollinated within populations.