Chapter 6

Conclusions

Part I Studies on factors influencing growth and *in vitro* tuberization.

A series of experiments was carried out to find the effects of some factors influencing growth and tuberization of *Pecteilis sagarikii* seedlings *in vitro*. It showed that the *in vitro* tuberization could be formed spontaneously, while the various tested factors played important roles as a promoter and/or inhibitor for shoot and tuber growth and development. Sucrose played a major role as important sources of carbon and energy for tuber growth and starch accumulation, and also affected the osmotic potential of the culture medium, influencing tuber growth and development. Coconut water played a role as a source of plant growth promoter and carbon source, and probably as a weak osmoticum. In addition, the coconut water had acted synergistically with BAP; when only coconut water was added, it showed the effect as the tested cytokinin BAP, while including with BAP, it reduced BAP effectiveness in decreasing oval-shape tuber formation, tuber growth and development, and also the formation of protocorm-like bodies. The coconut water also helped to increase starch accumulation in tuber. The cytokinin, BAP at low concentrations from 0.01 - 0.10 mg/l played an important effect in decreasing tuber starch accumulation. While BAP at higher levels from 0.5 - 2.0 mg/l strongly suppressed tuber formation, but promoted the formation of protocorm-like bodies. The auxin, IAA had no significant effect on shoot and tuber growth, but reduced the formation of the oval-shape tuber. While the highest concentration, i.e. 1.0 mg/l, helped to increase starch accumulation in the protocorm, shoot and tuber. Illumination played an important role in the tuber formation and growth. The plantlets formed the highest percentages of the oval-shape tuber in the dark condition. The artificial lighting acted as a source of energy for photosynthesis to produce the chemical energy, the photosynthetic sucrose, and starch which could be used to compensate some external sucrose supplement. While the supplemented sucrose was major sources of carbon skeletons and energy for plant growth and development. An increase in sucrose level gave rising in starch accumulation in all plant tissues.

In conclusion, the achievement of the *in vitro* tuberization must be considered on both tuber quality and tuber quantity. The high quality producing tuber must be big in size, natural-like oval-shape forming and also contains sufficient starch reservoirs enables the tuber to survive through dormancy period and capable to produce a healthy plant in the next growing season. According to the study results, a dark condition supported the oval-shape tuber forming and starch accumulation in tuber. When cultured in a dark condition in the absence of coconut water, an optimal sucrose concentration for tuber growth was 3 %. Increasing sucrose concentration combined with coconut water at 15 % gave high starch accumulation in tuber. BAP, IAA and organic substances had no significant effect on tuber growth, but gave a decrease in oval-shape tuber forming percentage and also starch accumulation. Thus,

the suitable conditions for *in vitro* tuberization of the protocorm-derived seedlings of *Pecteilis sagarikii* should be cultured in the dark condition in CMU_1 medium supplemented with sucrose at 3 % plus coconut water at 15 %, without IAA, BAP and organic substance.

Part II Histological study on tuberization.

Histological study on the first *in vitro* tuberization of the germinated protocorms and *in vivo* tuberization of the mature plants showed that the tuberization processes were similar, which could be divided into 4 stages. However, they were differed in the origins of the tuber primordia. The first *in vitro* tuberization of a protocorm-derived plantlet originated from its apical meristem with a new-formed tuber tip primordium at the lower meristematic tissue of the apical meristem, whereas the *in vivo* tuberization originated from an adventitious bud occurring at the basal part of the mother plant monopodial stem. Afterward, the tuber primordia of both origins, produced youngest leaf primordia, grew in the geotropic direction and became concave to form a deep cup as a narrow hollow in their forming tuber stalks. Thereafter, the upper meristem produced new leaf primordia to cover it until a new tuber-shoot bud was completely formed. The lower meristem simultaneously showed cell division to increase the tuber length and tuber width, until a complete tuber was obtained. In addition, it was found that the *in vivo* tuberization was related to the floral bud initiation and development.

Part III Studies on the changing of internal macro elements, free amino acids and some growth regulators in mature plants during tuberization.

The analyses on the changing in the concentrations of macro elements in tubers and shoots at different developing stages showed that the nitrogen, potassium, calcium and magnesium increased in their concentrations from the dormancy stage to the vegetative stage. The nitrogen level slightly decreased in the pre-flowering stage, the same as the potassium level in tuber, while the potassium level in shoot had slightly increased. But the levels of calcium and magnesium did not distinctly change. In the case of phosphorus, it slightly increased in concentration when the tuber was sprouting, and then decreased continuously until the plant developed into the pre-flowering stage.

The study on the changing of free amino acid contents in various organs at different developing stages showed that the asparagine was an important dominant free amino acid in all plant organs at different stages of development. Arginine and the anserine were second dominant free amino acids in some organs.

For the analysis of free IAA and free ABA contents, it showed that free IAA and free ABA in shoots decreased in concentrations from the vegetative stage to the pre-flowering stage. While the analyses of both substances from the tuber samples in different stages of plant development could not be detected from the used technique.