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学位論文全文に代わる要約

Extended Summary in Lieu of Dissertation

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Name

学位論文題目： *Habenaria* as genetic resources of floriculture（花き遺伝資源としての*Habenaria*）

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Dissertation Summary

Orchidaceae is one of the largest family angiosperm plants. Orchids are popular in many countries both in domestic and international flower markets because they have a variation of plant and flower appearances, long shelf life of flowers and some species have year-round flowering. There are several genera having economic values, such as *Cymbidium, Dendrobium, Oncidium, Phalaenopsis, Vanda*, etc. (Kaewphalug et. al., 2017). The orchid markets continue growing due to increasing people demands both of cut flowers and pot plants (Thammasiri, 2016; Paul and Kumalia, 2017). Moreover, orchid breeding is active to produce new hybrids because the demands are increasing every year (De, et. al., 2015). Terrestrial orchids are not popular in commercial markets. They are only available as commercial pot plants for limited number of interested persons. In this study, I focused on small *Habenaria* species to improve their commercial values.

Habenaria is one of terrestrial orchids including more than 800 species. They have variations in plant forms, flower shapes and flower colors (Batista et al., 2013; Jin et al., 2014; Kurzweil, 2009; Pedron et al., 2012; Pridgeon, 1992). Not only the number of *Habenaria* species in flower market is limited but also the number of this plants in theirs habitat is decreasing from environmental changes and overcollection by people (Barnes et. al., 2013; Mitsukuri et. al., 2009). In this study, I focused on *Habenaria radiata* and *Habenaria rhodocheila* complex. Since *H. rhodocheila* and related species are closely related, they are combined to one group named *H. rhodocheila* complex. The aim of this study consists of four parts. Characteristics of two *Habenaria* (*H. radiata* and *H. rhodocheila* complex) were investigated under cultivated condition on growth and development and flower pigment. Second, the efficient of propagation methods of two *Habenaria* species were investigated. Third,

the interspecific hybrids between the two species were produced. And forth, preservation method of *Habenaria* genetic resources was investigated.

The morphological characteristics of *H. radiata* and *H. rhodocheila* complex were studied. The outstanding characteristic of these two plants is their flowers. *H. radiata* had white two lateral petals and a lip with two fine side lips and one central lip. The Japanese name of *H. radiata* is Sagisou due to flower shape is similar to bird heron flying. They had some green leaves and 2 to 4 flowers on the inflorescence. The flower is small but can be used as a cut flower (Sinumporn et. al., 2015). *H. radiata* produced some round shaped tuber at the top of stolon during growing season. There are some other genotypes of *H. radiata* having different plant morphological characteristics, such as, akatsuki, ginga, kinsei ohushyu, etc. *H. rhodocheila* complex is a group of plants having similar plant characteristics but they are different in flower color that is pink, red, orange, and yellow. *H. rhodocheila* complex (red genotype 1 and yellow) had crown shaped tuber and the others were round shapes. Lips of *H. rhodocheila* complex had different epidermal cell shapes and pigments depending on genotype. The epidermal cell shapes of *H. rhodocheila* complex (yellow and pink flower genotype) were dome shape. Other genotypes had conical papilate epidermal cells. All genotypes showed unique pigmentation in the lips. Histological observation revealed that the particles was present on the vacuole surface showing theirs unique plant pigmentation. The lips of each genotype of *H. rhodocheila* complex contained at least two types of carotenoids, and yellow flavonoids. Pink, orange, and red flower genotypes contained unknown anthocyanin like pigments in the lips.

Establishment of micropropagation is an essential condition for commercialization of orchid. The success of micropropagation makes it possible to supply the plants in commercial scale. The development of culture protocol including suitable media for each orchid is required. *In vitro* propagation of two *Habenaria* species was investigated. First, the process of fertilization and seed development of *H. rhodocheila* complex was observed from day 0 to 8 weeks after pollination. The results showed that pollen tube behavior of *Habenaria* is similar to that of other orchid species. Seed germination occurred in cultured ovary harvested 4 weeks after pollination (WAP), suggesting that fertilization would have occurred before 4 WAP. Furthermore, the number of germination increased with the number of WAP. The suitable *in vitro* medium for *H. radiata* and *H. rhodocheila* complex seeds and protocorms was Malmgren modified terrestrial orchid medium (MM). The seeds can germinate and develop to plantlets on MM but the acclimatization step is still problematic in *Habenaria in vitro* propagation.

Production of interspecific hybrids between *H. radiata* and *H. rhodocheila* was investigated. The

advantages of interspecific hybridization are expanding of biological diversity. The hybrids increased commercial value would prevent parents picking from their natural habitats. Six reciprocal cross combinations were made. The pod set frequencies varied depending on both cross combinations and the ovule parents. The obtained progenies were evaluated their hybridity by PCR-RFLP. The progenies of *H. radiata* × *H. rhodocheila* (orange, yellow) contained both true hybrids and apomicts. The true hybrids showed intermediate morphological characteristics of parents. The flower was characterized by lips having slight fringed lateral lobes with pale yellow color. Leaf had lanceolate light green leaves. The hybrids had tubers with intermediate morphological characteristics of the parents. The reciprocal cross *H. rhodocheila* (orange, pink) × *H. radiata* was not successful. The progenies of *H. rhodocheila* (yellow) × *H. radiata* were apomicts. This study showed that interspecific hybrids could be produced in completely different *Habenaria* species from Japan and Thailand. This is the first success on interspecific crossing of *H. radiata* and *H. rhodocheila* complex. This achievement open the way for the development of new *Habenaria* resources.

Preservation of genetic resources is an important issue for any plant breeding program. The numbers of both *H. radiata* and *H. rhodocheila* complex are decreasing in their natural habitats due to urban expanding, environmental changing, agricultural area expanding, etc. (Mitsukuri et al., 2009; Stewart and Kane, 2006; Tanaka et al., 2015). Cryopreservation is a reliable way to keep plant genetic resources for a long period. Present study demonstrated the possibility of cryopreservation of *H. radiata* and *H. rhodocheila*. *H. radiata* and *H. rhodocheila* complex seeds can be cryopreserved by use of cryo-plate vitrification method. *H. radiata* seeds showed high viability after immersion in LN without PVS2 treatment. On the other hand, *H. rhodocheila* seeds can be cryopreserved with 20 to 30 min PVS2 treatment. Moreover, the possibility of cryopreservation of *Habenaria* protocorms was showed. Although there was no consistent tendency in the change of the survival rate with the time of PVS3 treatment, 90% of the protocorms survived after immersion LN by 120 min PVS3 treatment, suggesting cryopreservation of *Habenaria* protococorms was possible if sufficient dehydration was achieved.

This study clarified the basic information of proliferation methods and its problem in the two high ornamental valued *Habenaria* species, created the interspecific hybrids, and revealed preservation method of the genetic resources. The results will greatly contribute for the utilization of *Habenaria* as a horticultural plant.

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