

学位論文要旨
Dissertation Abstract

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

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Habenaria is a large genus in the family Orchidaceae, consisting of more than 800 species distributed in tropical and subtropical areas. Although, *Habenaria* species show diverse plant forms, flower shapes, and petal colors, but only a few species are commercialized. The aims of this study are to evaluate the basic characteristics of the two *Habenaria* species, and to investigate propagation methods, production of interspecific hybrids, and cryopreservation of genetic resources.

At first, the morphological characteristics of two *Habenaria* were investigated to evaluate their horticultural potential; cultivation condition, growth and development and flower pigment. In this study, I focused on two *Habenaria* species: *H. radiata* and *H. rhodocheila*. *H. radiata* is a species native to wetlands of Japan. This species is low-temperature tolerant. The flowers have white petals looks like a white egret bird. This species has been used as ornamental pot plants but it can be used as cut flowers. *Habenaria rhodocheila* is found in Southeast Asia. The flowers of *H. rhodocheila* have a large lip showing a wide range of color such as orange, pink, red, and yellow. In this study, I adopted the name *H. rhodocheila* complex and distinguished the genotypes only by the color of the petals. Lips of *H. rhodocheila* complex contained at least two types of carotenoids showing λ_{\max} 445 to 450 or 470 to 475 nm, and yellow flavonoids. Lips of pink, orange, and red flower genotypes contained unknown anthocyanin like pigments in the lips.

Establishment of aseptic seedling is essential to meet commercial demands of *H. rhodocheila*. The utility of MM was confirmed by use of *H. radiata*, and then adopted well to *H. rhodocheila*. Ovary culture was useful to shorten the period from pollination to seed germination of *H. rhodocheila*. The inorganic nitrogen source in the medium had to significant effect during the early stage of protocorm development, but ammonia nitrogen had negative effect on subsequent growth of *H. rhodocheila* seedlings.

Six reciprocal cross combinations between *H. radiata* and *H. rhodocheila* complex 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. This is the first on successful interspecific crossing of *H. radiata* and *H. rhodocheila* complex.

Preservation of genetic resources is an important issue for any plant breeding program. Cryopreservation is a reliable way to keep plant genetic resources for a long period. Cryopreservation of *H. radiata* and *H. ridocheila* complex seeds were investigated by use of cryo-plate vitrification method. The seeds of *H. radiata* showed high viability after immersion in LN without PVS2 treatment. On the other hand, *H. ridocheila* 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* protocorms was possible if sufficient dehydration was achieved.

In this study, I investigate the basic characteristics of two *Habenaria* species, investigate the proliferation method, and revealed the problems. Further attempts were made to produce interspecific hybrids and succeeded. I also clarified the method of cryopreservation of these genetic resources. This result has greatly contributed to the utilization of *Habenaria* as a horticultural plant.