

Restoration of *Habenaria radiata* Grown from Seeds in Vitro to the Native Habitat⁴

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Habenaria radiata, which belongs to Orchidaceae, grows indigenously in wet land of a temperate region of Japan. The plant is a perennial and blooms in summer. Since the flower looks like an egret with its unique shape and white color, the plant is liked by many people in Japan. Recently, the plant has been decreasing because of many kinds of development in rural regions and secret stealing. A marsh in Takanabe, Miyazaki prefecture in southern Japan is a typical place where the plant has been decreasing because of theft. We are now trying to restore the plants to the marsh, a native habitat, to protect it from extinction.

Undehisced pods taken from *H. radiata* growing indigenously in the marsh were harvested in Autumn 1998. After the surface of the pods was sterilized with ethanol, the seeds were scattered on the hormone-free Hyponex medium. The medium contained 3% sucrose and was solidified with agar. The pH was adjusted at 5.2 or 5.75 before autoclaving. The seeds germinated into green round protocorms. After 3 to 4 months of culture, bulbs often developed on root tips in the medium.

We recognized three methods of regeneration of the plant via the bulbs in this culture system. The first is the regeneration via the bulbs formed in vivo from the plant during acclimatization. The second is the regeneration via bulbs formed in vitro. The third is the regeneration via bulbs stored for 2 months at low temperature (approximately 1°C). The plants regenerated from these methods were grown in the sphagnum medium in a plastic pots. The time of transplantation to the marsh was 16 Sept. and 12 Oct. 1999. The total number of plants transplanted was 203, and 89% of them survived. At the end of November they withered and new plants germinated from bulbs in the next spring (2000). As a whole, the number of new plants was double that of those planted before winter. The rate of propagation of the plant transplanted in September was far higher than that transplanted in October. This is considered to be due to the difference in the amount of photosynthesis between the plants of the two groups. Flower stalks began to be observed in mid July, attaining maximum number at mid August. The flowering started 1 month later than the formation of the flower stalks. The number of flower increased gradually in the same manner as the increase in flower stalks. This unevenness in the formation of flower stalks and anthesis shows the diversity of genes of plants in the colony which is a characteristic of the wild-type plant.