

# A new aseptic cultivation method to accelerate growth

TOMOAKI OYAMADA, AKIRA HIRATSUKA and SIGEKAZU KURAKAKE reveal their technique for achieving flowering one year after sowing *Cypripedium macranthos* var. *speciosum*



**T**HE USUAL TECHNIQUE for aseptic germination of *Cypripedium macranthos* var. *speciosum* is to sow the seeds in a sealed container on a growth medium made of gelling agents such as agar or Gellan gum and stiffened with a solidifying agent. While this results in successful germination, the subsequent growth rate of seedlings is poor on this medium. With a view to remedying this problem, we re-examined the non-sugar method of cultivation.

First, we reconsidered the question of substrate and developed Paper Lite medium (PL medium), which is made from a mixture of paper pulp and vermiculite. Second, we produced an aseptic air ventilation filter and placed it on the top of the growth container. We germinated seeds on a special medium prescribed and patented by Tomoaki Oyamada, then transplanted young cultured plants onto the PL medium. Immediately after, we gave them low temperature treatment, then moved them to a bright place, maintained these conditions for 120 days, and fed them with a special solution every four weeks. The solution is similar to the one used for germination, but this solution does not contain sucrose, potato cubes, activated carbon or gellan gum.

### The results

As a result of this technique, the height and number of leaves increased, and so did the number and development rate of dormant buds (see left). After 150 days of this treatment, and just prior to their acclimatisation and potting, plants grown-on using to the non-sugar method were compared with others produced in the usual way. The former surpassed the latter in total weight, height and number of leaves, number of roots, number and development of dormant buds,



**Above** A plant of *Cypripedium macranthos* var. *speciosum* cultivated using the non-sugar, PaperLite (PL) medium, in flower three years after germination

and survival rate. Afterwards, they were transplanted to an outdoor examination field to assess the practicality of the new method. A year later, many of them had survived and were in flower (right).

These results indicate that we have established an aseptic cultivation method which accelerates the growth of *Cypripedium macranthos* var. *speciosum*. This method greatly eases the cultivation of a species that is generally considered to be a difficult subject. It also significantly shortens the time this species requires to reach flowering size when grown from seed.

### The future

It is hugely important to establish an artificial propagation technology quickly as a measure to conserve plants such as an endangered *Cypripedium macranthos* var. *speciosum*. As our method does not employ phytohormones, the percentage of plants exhibiting somaclonal variation

is thought to be low. Also, as our cultivation method is applied after germination from crossing, genetic heterogeneity is preserved.

For amateur growers and nurseries the PL cultivation method will greatly help to secure a shorter and more stable production period. ■

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