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*

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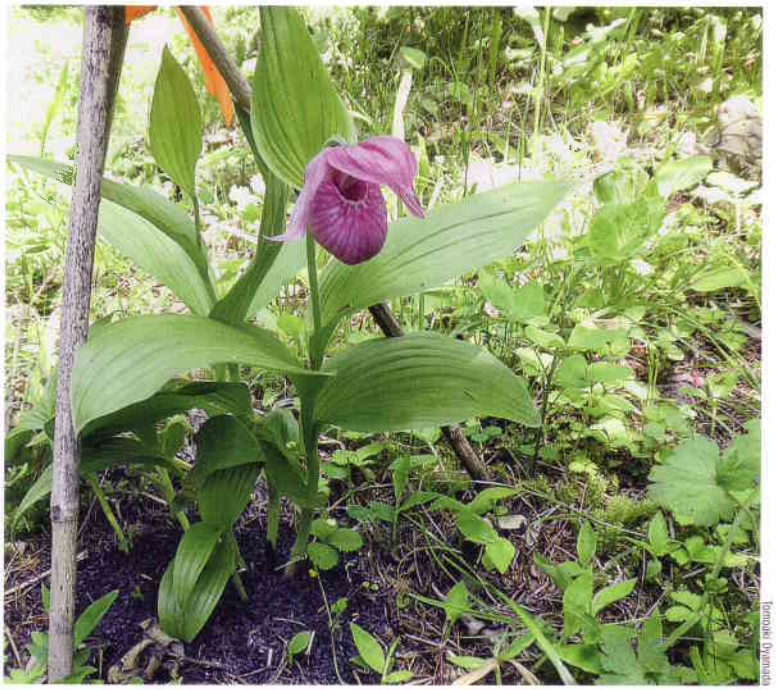
There was an increase in both the height and number of leaves

THE 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, 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).



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

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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アツモリソウ無菌培養苗の生育促進法の開発

アツモリソウの無菌播種法は，密封された培養容器中に寒天やゲランガムなどのゲル化材を培地固形剤で固めた培地を充填し，この培地上に種子を播いて発芽・生育させる．この方法では発芽において効果が期待されるが，その後に成長不良が発生する．本研究では，発芽後の生育促進を行なうために無糖培地培養法の検討を行なった．はじめに無糖培地培養法用の専用培地の検討を行い，パルプペーパーとバーミキュライトと混合した Paper Lite 培地（PL 培地）を開発した．次に，無菌通気フィルターを作製し，培養容器の上部に設置した．ＰＬ培地に培養幼植物体を移植し，苗の生育を調査した．ＰＬ培地に移植した直後に低温処理を行ない，その後，明所条件に移して 120 日間培養した結果，草丈，葉数および越冬芽形成が増加した（fig.1）．培養から 150 日後，順化・鉢上げ時の培養苗の生育を従来の培養法と比較したところ，苗の草丈，葉数，根数，苗の全重量，越冬数，生存率において無糖培地培養法が高い効果を示した．順化・鉢上後１年経過した苗の草丈，葉数，生存率を比較したところ無糖培地培養法が高い効果を示した．無糖培地培養法の実用性を確認するために，野外試験地に定植して１年後の生存と確認した結果，多くの苗が生存し，開花が確認された（fig.2）．

絶滅の危機に瀕しているアツモリソウについては,種の保存策の一つとして人工増殖技術の確立が急がれている．本研究で開発した方法は，植物ホルモンを一切使用しないため，体細胞変異（ソマクローナル変異）を生ずる確率は低いと推測される．交配後の種子を発芽させての培養なので,遺伝的多様性は保たれており,今後はアツモリソウの自生地復元において活用が期待される．現在まで，日本の種苗登録制度においてアツモリソウ属植物を交配親として開発された園芸品種の登録はない．日本人が開発したアツモリソウ交配種のサンダーズリスト（ラン科植物交配種の国際登録リスト，英国王立園芸協会）への登録もわずかである．これは育種試験のための交配親の確保，交配によって得られた種子からの苗の安定生産と育苗，登録に必須の品種固定の確認や生育特性調査用の苗の確保など，大量の苗が必要となる育種過程に対応できる生産技術が確立されていないことに大きな原因がある．PL 培養法は園芸・育種分野において，苗の安定生産と育種年限の短縮化に効果を発揮し，地域資源としてのアツモリソウの利用を可能とするだろう．

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