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Effects of mycorrhizal fungus *Mycena* sp. on the growth and polysaccharide properties of *Dendrobium officinale*

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Dear Editor,

Dendrobium officinale Kimura et Migo (Orchidaceae), an endangered species, is a medicinal plant and the sole origin plant of Dendrobii Officinalis Caulis (Tiepishihu), a rare Chinese herbal medicine. Several photosynthetic orchids have recently been confirmed as mixotrophic, with the ability to recover carbon from mycorrhizal fungi associated with their roots (Selosse and Roy, 2009). Although some studies have focused on the positive effect of mycorrhizal fungi on *D. officinale* seedling growth, there is no literature on the use of these beneficial fungi in the artificial cultivation of the plant. Our preliminary research has demonstrated that a larger number of pelotons existed in the root cortical cells of M2-infected *D. officinale*, and the infected seedlings grew more efficiently with flourishing roots than the uninfected plants after 4 months of dual culture (Zhang et al., 2012). Therefore, we further investigated its effects on the growth and polysaccharide properties of *D. officinale* in a cultivation base. The experiment was conducted in Zhangpu County, Fujian Province, from June 2013 to January 2015. Solid cultures of M2 were inoculated at the roots of sterile seedlings during transplantation.

The relationship between the plant dry weight (PDW) (Y) and the growth time (x) of the treatment (T) and control (CK) groups showed that the growth rate of T was lower than that of CK in the initial stage; however, the growth rate of T increased, surpassing that of CK (Figure 1A). The model expression of T was: $Y=0.446e^{0.139x}$ ($P<0.01$, $R^2=0.981$), and CK was: $Y=0.613e^{0.084x}$ ($P<0.01$, $R^2=0.929$). The change in stem dry weight (SDW) of the two groups with growth time was similar to that PDW. After 19 months of growth, PDW and SDW of T were (6.766±1.110) and (3.758±0.603) g clump^{-1}, respectively, which were 2.69 and 2.87 times the PDW and SDW, respectively, of CK (Table S1 in Supporting Information). The number of seedlings (NS) also changed differently in the two groups. NS T increased obviously in T in the second cultivation year in March 2014 (after 9 months of growth); whereas similar cases in CK were delayed by three months. No difference was detected in NS between the two groups. In the third cultivation year in January 2015 (after 19 months of growth), the NS of the two groups both decreased; however, NS in CK decreased considerably more than that in T (Figure 1B, Table S1 in Supporting Information). In addition, M2 affected the dry matter distribution ratio in the vegetative organs of the plant. The percentage of root dry weight (PRDW) of T decreased gradually with growth time and reached (20.7±4.8) % after 19 months of growth, which was significantly lower than that of CK ($P<0.01$). In contrast, the PRDW of CK fluctuated, but was consistently >30% (Figure 1C, Table S2 in Supporting Information).

These results implied that M2 inhibited plant growth in the early stages of interactions between the two species and played a positive role after establishing symbiosis by inducing the plant to germinate new shoots in advance in the spring, and increasing the survival rate of seedlings in the

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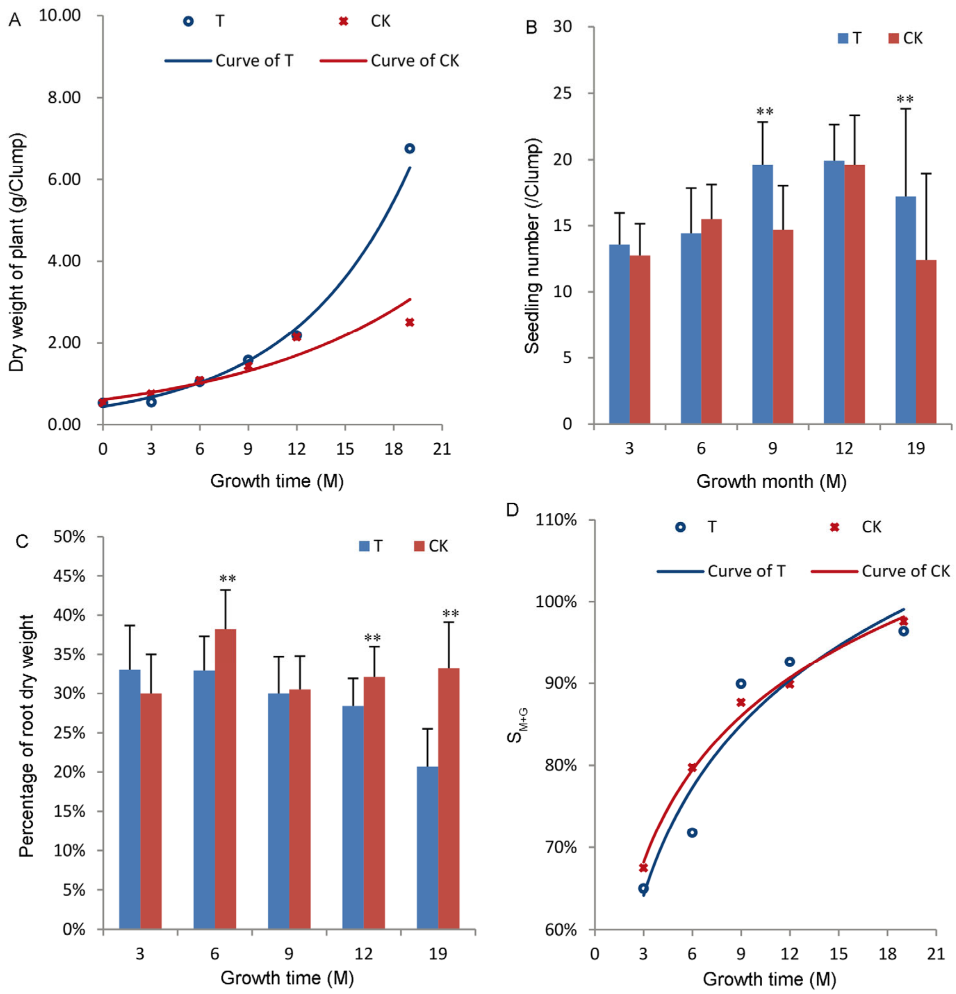

Figure 1 Results of mycorrhizal cultivation of *Dendrobium officinale*. A, Fitted curves for plant dry weight to growth time. B, Effects of M2 on the seedling number. C, Effects of M2 on the percentage of root dry weight. D, Fitted curves for the sum of mannose and glucose contents in polysaccharides with growth time. **, There was a significant difference between the two groups according to *t*-test at $P<0.01$ level.

winter. Moreover, the continuous reduction of PRDW suggested the improvement of root absorption efficiency of. Mycorrhizal fungi can considerably enhance mineral nutrient absorption of the host plant to promote its growth (Behie and Bidochka, 2014). Therefore, we should further investigate the effect of M2 on *D. officinale* nutrient absorption.

Polysaccharides are the main functional components of *D. officinale*, and mannose and glucose are the most two abundant monosaccharides in the polysaccharide. After 19 months of growth, the polysaccharide content (PC) and the chromatographic peak area ratio of mannose to glucose ($R_{M/G}$) in polysaccharides of *T* were 28.97% and 3.26, respectively, which were in line with the Pharmacopoeia standard. However, the dynamic change in testing values of the two groups was different (Table S3 in Supporting Information). The chemical properties of polysaccharides of CK were formed and maintained after 12 months of growth, whereas the properties of T were stable and similar to those of CK after 19 months of growth. From 9 to 19 months of growth, the gap between the PC and $R_{M/G}$ values of the two groups increased initially and then decreased, which was possibly influenced by plant growth. We speculated that the rapid growth of T in the later stage of cultivation was not conducive to polysaccharide accumulation and prolonged the formation of the chemical properties of polysaccharides. Interestingly, the variation in the sum of mannose and glucose (S_{M+G}) content in the polysaccharides of the two groups was relatively similar (Figure 1D). The model expression of T was: $Y=0.189\ln(x)+0.434$ ($P<0.05$, $R^2=0.911$), and CK was: $Y=0.162\ln(x)+0.503$ ($P<0.01$, $R^2=0.991$). Y and x in the formulas represented S_{M+G} and growth time, respectively. The S_{M+G} of T and CK at 19 months were 96.44% and 97.62%, respectively, which were consistent with results in the literature (Chen et al., 2012).

Many laboratory experiments have shown that endophytic fungus could enhance the accumulation of the medicinal components of host plants. For example, *Gilmanella* sp. significantly promoted the volatile oil content in *Atractylodes lancea* roots after being co-cultured with sterile seedlings for seven days (Gao et al., 2012). However, our study did not report similar results, possibly owing to the difference in experimental periods and sampling intervals. The interference of the plant stress response was minimized in our study.

M2 use was optimized by an orthogonal experiment lasting 16 months with three factors at three different levels (Table S4 in Supporting Information). The supplement dosage (factor C) ($F_{2,2}$=125.58, P<0.01) and initial inoculums dosage (factor A) ($F_{2,2}$=56.72, P<0.05) of M2 significantly affected SWD. The best combination for SWD was 1.5 g/clump for initial inoculums, and 1.5 g $clump^{-1}$ for supplementary inoculums every six months, and the resulting yield was 135.3 g m^{-2} (Table S5 in Supporting Information). The best combination for PDW was the same as that for SDW. Although all tested factors had no significant effect on PC and $R_{M/G}$ (P>0.05), the orthogonal experiment showed that higher initial inoculum dosages were favorable for higher polysaccharide contents (Table S6 in Supporting Information).

Therefore, we conclude that the mycorrhizal fungus M2 could be effectively used to enhance the artificial cultivation of *D. officinale*. Simultaneously, these results may provide basis for the resource conservation and sustainable utilization of endangered medicinal orchids. Our further investigations on *D. officinale* mycorrhizal cultivation will focus on the standardization of planting techniques.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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SUPPORTING INFORMATION

Table S1 The effects of M2 on the growth of *D. officinale* (n=20)

Table S2 The effects of M2 on the dry matter distribution percentage in vegetative organs of *D. officinale* (n=20)

Table S3 Effects of M2 on the polysaccharide chemical properties of *D. officinale* (n=3)

Table S4 Factors and levels of the orthogonal experiment

Table S5 Orthogonal experiment design and the result of stem dry weight (SDW)

Table S6 Orthogonal experiment design and the result of polysaccharide content (PC)

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