

# 丹参冠瘿组织的生长和总丹参酮的积累动态

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丹参(*Salvia miltiorrhizae*)是我国传统常用中药。开展丹参的组织培养研究有可能大规模生产丹参药物<sup>[1]</sup>。作者已建立了丹参冠瘿组织培养体系并筛选获得了生长与生产的培养基<sup>[2]</sup>。本文报道在不同培养基中丹参冠瘿组织的生长和总丹参酮的积累动态。

## 实验部分

### 1 实验材料

实验采用文献<sup>[2]</sup>中所获得的生长较快的丹参冠瘿组织 Ca 株系。

### 2 培养方法

以 MS<sup>[3]</sup>, 67-V<sup>[4]</sup> 液体培养基为基本培养基, 接种量分别为  $6 \text{ g} \cdot \text{L}^{-1}$  和  $4 \text{ g} \cdot \text{L}^{-1}$ 。培养条件:  $(25 \pm 1) \text{ }^\circ\text{C}$ , 黑暗, 摇床转速  $150 \text{ r} \cdot \text{min}^{-1}$  (PYB 型普通摇床, 中国科学院武汉科学仪器厂), 500 mL 圆底烧瓶中加入培养液 100 mL, pH 5.71。共培养 35 d, 每 5 d 收获 1 次, 每次收获 3 瓶, 分别称取鲜干重, 当培养液颜色发生变化时, 用氯仿萃取其中脂溶性成分, 并分析培养物与培养液中总丹参酮含量<sup>[5]</sup>。

## 结果

### 1 在 MS 培养液中丹参冠瘿组织的生长动态

丹参冠瘿组织 Ca 株系在 MS 培养液中的生长动态曲线见图 1。图 1 表明, 第 5 ~ 30 d 为丹参冠瘿

组织的对数生长期(logarithmic phase), 第 30 ~ 35 d 为其生长的静止期(stationary phase)。第 25 ~ 30 d 是丹参冠瘿组织的生理活性和生长状况最佳时期, 在这时用做实验材料较好。在 MS 培养液中没有观察到总丹参酮的分泌。

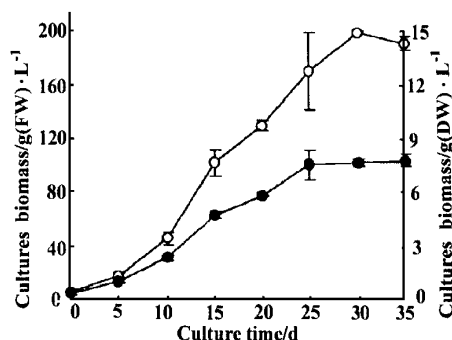


Fig 1 Dynamics of growth of crown gall cultures in *Salvia miltiorrhiza* in MS liquid medium  
○—○ Fresh weight; ●—● Dry weight

### 2 在 67-V 培养液中丹参冠瘿组织的生长和总丹参酮的积累动态

丹参冠瘿组织 Ca 株系在 67-V 培养液中的生长动态曲线(图 2)表明, 第 10 ~ 25 d 为丹参冠瘿组织的对数生长期, 第 25 ~ 35 d 为其生长的静止期。说明冠瘿组织 Ca 株系接种到 67-V 培养液后延迟期较接种到 MS 培养液后长 5 d, 而且提前 5 d 进入静止期。

图 3 显示丹参冠瘿组织 Ca 株系在 67-V 培养液中总丹参酮的积累动态。第 15 d 开始培养物中已检测到微量的总丹参酮, 至第 25 d 培养物中总丹参酮的含量一直较低, 且分泌到培养液中的总丹参酮也较少。第 25 d 后, 即培养物对数生长期结束后进入静止期时, 总丹参酮开始在培养物和培养液中大量积累。至第 35 d 收获时, 总丹参酮生产总量达 60

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mg·L<sup>-1</sup>, 此时分泌到培养液的总丹参酮占总丹参酮生产总量的大约 66%。实验 1, 2 组重复 2 次, 结果与上述基本一致。

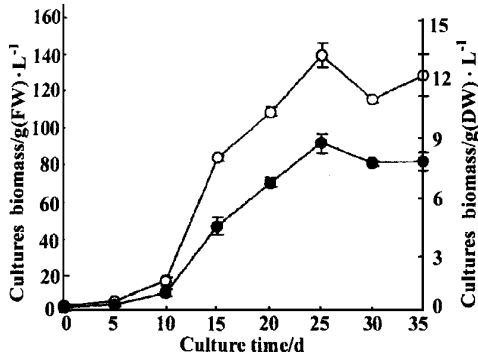


Fig 2 Dynamics of growth of crown gall cultures in *Salvia miltiorrhiza* in 67-V liquid medium  
○—○ Fresh weight ; ●—● Dry weight

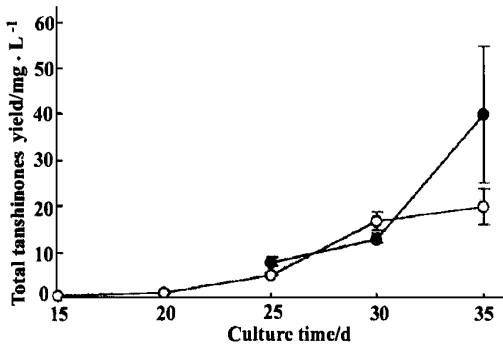


Fig 3 Dynamics of total tanshinones accumulation in crown gall cultures of *Salvia miltiorrhiza* and in 67-V liquid medium  
○—○ In the cultures ; ●—● In the medium

### 讨 论

本文研究了在 MS 培养液中丹参冠瘿组织的生长动态和在 67-V 培养液中丹参冠瘿组织的生长和总丹参酮的积累动态。因 MS 培养液有利于冠瘿组织 Ca 株系的生长<sup>[2]</sup>, 故延迟期较短(5 d), 静止期到来较晚(30 d)。67-V 培养液有利于总丹参酮的积累, 而不利于冠瘿组织 Ca 株系的生长<sup>[2]</sup>, 故延迟期

较长(10 d), 静止期到来较早(25 d)。在 MS 培养液中丹参冠瘿组织的继代时间间隔应为 25 ~ 30 d, 在 67-V 培养液中丹参冠瘿组织的生长和总丹参酮的积累不呈平行关系, 属相斥型, 即产物在细胞停止生长进入静止期后才开始大量积累。这与 Hu<sup>[6]</sup>等关于丹参毛状根的生长与总丹参酮的积累规律相一致, 而 Toivonen 等<sup>[7]</sup>认为长春花毛状根培养中生物碱的积累和培养物的生长呈平行关系。这表明, 不同基因型的植物在离体培养时细胞生长和次生代谢物积累的规律存在差异。因此, 掌握不同基因型的植物在离体培养时细胞生长和次生代谢物积累的规律, 有利于采取适当的调控措施(如添加诱导子等), 以期获得最大的活性成分生产量。我们将继续报道诱导子调控丹参冠瘿组织 Ca 株系积累总丹参酮的结果。

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## DYNAMICS OF GROWTH AND TOTAL TANSHINONES ACCUMULATION IN CROWN GALL CULTURES OF *SALVIA MILTIORRHIZA*

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**ABSTRACT:** **AIM** To determine the dynamics of growth and total tanshinones accumulation in crown gall cultures of *Salvia miltiorrhiza* in MS and 67-V liquid media. **METHODS** Fresh, dry weight and total tanshinones yields in the cultures and in the medium were determined every 5 days in crown gall suspension cultures. **RESULTS** In MS medium, the logarithmic growth phase of crown gall cultures in *S. miltiorrhiza* was from the 5th to 30th days, and the stationary growth phase was from the 30th to 35th days. From the 25th to 30th days, physiological activity of crown gall cultures was higher and their growth was better. However, in 67-V medium, the logarithmic growth phase of crown gall cultures was from the 10th to 25th days, and the stationary growth phase was from the 25th to 35th days. Total tanshinones were largely accumulated in the cultures and in the medium after 25 days. The total tanshinones yield ( $60 \text{ mg} \cdot \text{L}^{-1}$ ) was reached at the 35th day. **CONCLUSION** Knowing the regularity of the growth and total tanshinones accumulation in crown gall cultures of *S. miltiorrhiza* will be helpful to take proper regulative measures in order to obtain the maximum total tanshinones yield.

**KEY WORDS:** *Salvia miltiorrhiza*; crown gall cultures; total tanshinones