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博 士 学 位 论 文

光线和赤霉素对菟丝子寄生关系建立的影响及其蛋白质组学研究

Effects of Different Lights and GA on the Parasitism between Dodder and Its Hosts and Study of Its Proteome

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摘　　要

菟丝子属 (*Cuscuta spp*) 隶属于旋花科 (*Convolvulaceae*)，为寄生草本植物。成熟菟丝子的根、叶已退化，依靠茎缠绕寄主，然后，通过形成吸器与寄主的维管组织相连并从寄主体内获取养分。种子萌发后，菟丝子幼苗的生长主要依靠种子中储存的营养物质或进行有限的光合作用，但通常只能维持不超过 3 周的时间，因而，尽早发现寄主并与之建立寄生关系对其生存至关重要。在此过程中，缠绕发生和吸器形成分别作为与寄主连接和侵入寄主体内建立寄生关系的标志，成为当前寄生机制研究中的热点。

本研究首次采用具有特定波长的 LED 作为照射光源，通过光照生理实验，研究了不同光照条件下南方菟丝子幼苗的弯钩打开、缠绕发生及吸器形成的规律，以及不同光照条件下 GA_3 及 GAs 生物合成抑制剂 paclobutrazol 对菟丝子弯钩打开与缠绕发生的影响。

光照是诱导菟丝子与寄主建立寄生关系的必要条件，如果没有合适的光照条件，即使能满足其它可能存在的条件，菟丝子幼苗也不会发生缠绕。在合适的光照条件下，没有与其它物体接触的菟丝子幼苗也不会发生缠绕，显示触觉也可能是缠绕发生过程中的一个必要条件。而化学信号则可能在上述过程中起着一定的促进作用。

波长为 460 nm 的蓝光在菟丝子弯钩打开和缠绕发生中表现出不同的作用机制。在一个连续的蓝光照射过程中，菟丝子幼苗可以相继完成弯钩打开和缠绕发生两个生理过程。已证明弯钩打开过程是由光敏色素 Pfr 调节控制的，而缠绕发生过程则是由光敏色素 Pr 调节控制的。

本研究所用波长为 879 nm 的远红光无法促使光敏色素 Pfr 向 Pr 的转换。红光诱导弯钩打开的菟丝子幼苗，如果立即使用远红光照射，几乎无法诱导缠绕发生；然而，在远红光照射前，如果将材料先在暗处放置一定时间，则可以显著诱导缠绕的发生。证明在菟丝子中存在有 Pfr 向 Pr 的暗转换过程。

红光诱导弯钩打开的菟丝子幼苗，如果在远红光照射前使用蓝光照射 5 min，即可显著诱导缠绕的发生，而蓝光诱导弯钩打开的菟丝子幼苗，如果在远红光照射前使用红光照射 5 min，则可以明显抑制缠绕的发生，以上结果不仅显示出在

上述过程中存在有光敏色素与隐花色素的相互作用，而且波长为 879 nm 远红光的作用方式还显示出，在菟丝子缠绕发生过程中，除了光敏色素或隐花色素的光调控途径外，还需要有其它的光反应参与。

一定浓度的 GAs 生物合成抑制剂 paclobutrazol 可以抑制蓝光诱导的菟丝子缠绕发生，而通过在上述培养基中添加不同浓度 GA_3 可以恢复或部分恢复蓝光对菟丝子缠绕发生的诱导，证明 GA_3 参与了对菟丝子缠绕发生的诱导调控过程。但是，GA_3 对菟丝子弯钩打开过程则没有明显的作用效果。

首次运用蛋白质组学方法研究了菟丝子缠绕发生相关蛋白变化，试图寻找与缠绕机制发生有关的蛋白。

提取蓝光和白光照射下不同缠绕发生状态的菟丝子幼苗全蛋白，通过2－DE技术获得了具有良好重复性和灵敏性的蛋白表达图谱。每张凝胶中都分离、检测出大约1800个左右的蛋白点；通过比对分析，挑选出47个显著变化的差异表达蛋白；

差异表达蛋白经胰蛋白酶分解以及MALDI-TOF/MS分析，得到了所有47个差异蛋白的PMF；通过 Mascot 2.1 软件 (MASCOT 2.1, Matrix Science, UK) 提交每个蛋白的PMF进行数据库检索，得到了相应蛋白的鉴定结果。

差异蛋白整体变化状况基本上反映了与之相联系的菟丝子在缠绕发生与吸器形成过程中剧烈的内、外部形态结构变化和生理变化。光敏色素及其信号传导蛋白、细胞壁降解相关蛋白、细胞形态结构及极性变化相关蛋白与蓝光诱导的菟丝子缠绕发生与吸器形成有关；此外，在菟丝子缠绕发生与吸器形成过程中，差异表达的蛋白还包括物质能量代谢以及核酸代谢有关蛋白。

本研究首次建立了中国菟丝子的组织培养体系。愈伤组织诱导培养基为 MMS + NAA 1 mg L^{-1} + BA 1 mg L^{-1} ，分化培养基为 MMS + 1 mg L^{-1} BA。完成了由农杆菌介导的基因转化条件的优化。

关键词：菟丝子；寄生机制；差异蛋白质组学

Abstract

Dodder (*Cuscuta* spp.) is a holoparasitic higher plant in the family *Convolvulaceae*. It is comprised of twining stems without root and leaves. Although some species contain a small amount of chlorophyll, they have little or no photosynthetic activity of their own and are therefore depend on their hosts for survival by entwining the stem or leaves of the hosts and uniting their vascular system with haustoria for obtaining water, minerals and nutrition. The seedlings of *Cuscuta* spp are self-sufficient, but for little more than 3 weeks, so it is essential to detect and attach to potential hosts for their survival. Furthermore, the ability of dodders which can invade such a large range of species indicates that they must have highly adaptable mechanisms for host attachment. Unfortunately, the nature of the mechanism(s) is unknown. In these processes, the occurrence of twining and haustoria formation is regarded as the sign for dodders to parasite the hosts successfully and thus became the focus of studies on host-parasite interactions.

With different kinds of light-emitting diode (LED) as light sources, the effects of different lights on hook opening, twining and haustoria development of the seedlings of *Cuscuta australis* were studied in the present study, and the effects of GA_3 and GAs biosynthesis inhibitor paclobutrazol on hook opening and twining of the seedlings were also studied in different light conditions.

Light is a necessary factor for dodders to parasite the hosts successfully. Even if other factors were satisfied, dodders still did not wrap around a potential host if no appropriate light existed. The fact that no coils could be induced if the seedlings hadn't made contact with small wooden rods, plastic rods or other seedlings of dodders in the present study suggested tactile pressure is also a necessary factor to parasite hosts successfully. Chemical signals might facilitate host recognition and twining response.

Blue light with 460 nm wavelength displayed different roles for controlling hook opening and twining response respectively. The fact that blue light could induce hook opening response which was proved to be controlled by Pfr of phytochrome indicate

the occurrence of a synergistic interaction between phytochromes (Pfr) and cryptochromes, and that blue light could induce twining response which was proved to be controlled by Pr of phytochromes indicate the occurrence of antagonistic interaction between Pfr and cryptochromes. Interestingly, under continuous blue light the seedlings of *C. australis* could be induced to accomplish hook opening and twining step by step.

Far-red light with 879 nm wavelength didn't show it could transfer Pfr into Pr in the present study. That far-red light (879 nm) could hardly induce twining response of the seedlings immediately after hook opening induced by red light unless the seedlings of dodders had been put in darkness for 8 h firstly suggested that there existed dark conversion of Pfr to Pr.

Furthermore, if the seedlings above-mentioned were illuminated by blue light for 5 min or 1 h before far-red light, twining could be induced obviously. If the seedlings after hook opening induced by blue light were first illuminated by red light for 5 min or 1 h, the effect of far-red light on twining was restrained obviously. The results showed that there not only existed mutual interactions between phytochromes and cryptochromes, but another light reaction was involved in the process of twining besides photo-reversible phytochromes considering the function of far-red light with 879 nm wavelength.

The fact that the negative effect of paclobutrazol (10^{-3} μM) on twining response under blue or far red light was reversed by addition of GA_3 suggested GA_3 is involved in the twining response of the seedlings. But application of exogenous GA_3 or paclobutrazol has no distinct effect on hook opening of the seedlings of *C. australis*.

It is the first time that proteome of the seedlings of *Cuscuta autralis* with respective blue light or white light treatment has been investigated in order to find out proteins involved in twining response induced by blue light.

After twining response of the seedlings was induced by blue light, total proteins were

extracted from the seedlings of *Cuscuta autralis* treated with blue light and white light and separated by two-dimensional gel electrophoresis (2-DE). Approximately 1,800 protein spots were detected on each 2-D gel after staining with silver nitrate. Among them, 47 up- and down-regulated protein spots at least 1.5-fold increase in abundance were selected.

For protein identification, spots were manually excised from the 2-D gel and digested with trypsin. The resulting tryptic fragments were analyzed by MALDI-TOF/MS. Each of PMFs of 47 protein spots was submitted for cross-species protein identification using an in house database search engine (MASCOT 2.1, Matrix Science, UK).

The fluctions of differential proteins mirrored situation of inner/outer morphologic variations and physiological alteration associated with the process of twining and haustoria development.

The results suggested we could associate the responses of twining and haustoria development of the seedlings with proteins involved in phytochromes and light signal transmission, proteins involved in degradation of cell wall, proteins involved in alteration of cell structure and cell polarity, and proteins associated with material and energy metabolism and metabolism of nucleic acid.

An efficient culture method for callus induction, its long term maintenance and shoot regeneration was achieved firstly in the present study for *Cuscuta chinensis*. The MMS medium containing 1 mg L^{-1} NAA and 1 mg L^{-1} BA was optimal for callus indution. The MMS medium containing 1 mg L^{-1} BA was suitable for shoot regeneration. Genetic transformation conditions of *C. chinensis* mediated by *Agrobacterium* have been optimized in the present study.

Keywords: *Cuscuta* spp.; parasitic mechanism; differential proteomics.

缩略 Abbreviations

ABA：Abscisic acid 脱落酸

AP：Ammonium persulfate 过硫酸氨

Avr：Avirulence 无毒性

CaM：Calmodulin 钙调素

Cb：Carbenicillin Na_2 羧苄青霉素

KT：Kinetin 激动素

DTT：Dithiothreitol 二硫苏糖醇

ESI-Q MS/MS：Electrospray ionization tandem quadrupole mass spectrometry
电喷雾电离串联四极杆质谱

EST：Expressed sequence tags 表达序列检签

GA：Gibberellin 赤霉素

HER：High energy reaction 高能量反应

Hyg：Hhygromycin B 潮霉素

IEF：Isoelectric focusing 等电聚焦

IPG：Immobilized pH gradiednt 固相pH梯度

Kan：Kanamycin 卡那霉素

LED：Light-emitting diode 发光二极管

LRR：Leucine-rich repeat 近C端富含亮氨酸的重复序列

Lx：Lux 勒克斯

MALDI-TOF MS：
Matrix-assisted laser-desorption ionization-time of flight mass spectrometry
基质辅助激光解析电离飞行时间质谱

NAA：Naphthalene acetic acid 萘乙酸

NBS：Nucleotide binding site 近N端核苷酸结合位点

Pfr：Far-red light-absorbing form 光敏色素远红光吸收型

Pr：Red light-absorbing form 光敏色素红光吸收型