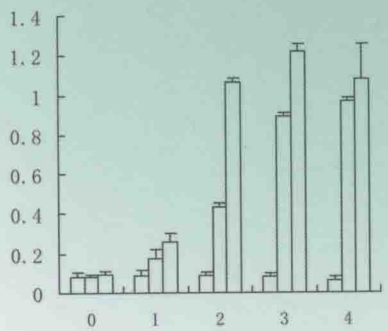
Series of Academic Creative Research Groups in the New Century

Ecological Physiology of *Catharanthus roseus*

(长春花生理生态学研究)

Series Editor: ZU Yuan-Gang
Authors: TANG Zhong-Hua YU Jing-Hua
GUO Xiao-Rui ZU Yuan-Gang



SCIENCE PRESS
www.sciencep.com

Series of Academic Creative Research Groups in the New Century

Ecological Physiology of *Catharanthus roseus*

（长春花生理生态学研究）

Series Editor: ZU Yuan-Gang

Authors: TANG Zhong-Hua YU Jing-Hua
GUO Xiao-Rui ZU Yuan-Gang

Science Press

Beijing

Responsible Editor: PANG Zaitang XIA Liang

Published by Science Press
16 Donghuangchenggen North Street
Beijing 100717, China

http://www.lifescience.com.cn

ISBN 978-7-03-018978-3

Ecological Physiology of *Catharanthus roseus*

This book shows the original research results of eco-physiology of *Catharanthus roseus*. The theory and methods of plant physiology, as well as proteomics, were conducted in *C. roseus* to investigate and reveal the physiological processes and mechanisms during development and in response to environmental cues. The physiological processes include alkaloid metabolisms, photosynthetic and respiratory response, soluble sugar and phytohormones variations induced by light, temperature, water and biotic molecules. Their biological functions and interactions were also discussed.

This book provides a reference for scientists and students on plant physiology or ecology.

长春花生理生态学研究

本书是关于长春花生理生态学原始研究结果的专著。主要运用植物生理学的理论和方法，结合蛋白质组学技术，初步研究和揭示了长春花在生长发育和响应环境变化过程中的生理学过程和机制。包括长春碱、长春质碱和文朵灵三种生物碱，果糖、葡萄糖和蔗糖三种可溶性糖，光合和呼吸作用及 ABA、GA 和 IAA 三种植物激素在长春花生长发育，响应光照、温度、水分和生物分子胁迫过程中的代谢动态和相互作用，并探讨了它们的生物学功能和相互联系。

本书可供植物生态学和植物生理学研究领域的科研教学人员和研究生参考。

Foreword to the Series

Since the Big Bang, by a mode of the random collision of particles of quarks and others at meson-cosmic level, with direction from meson-, nano-, micro-, middle-, macro- to astro-cosmic level, and with the headspring for natural evolution of the heterogeneity of movement, celestial and natural bodies including sizeable solar system, earth, life system even our human-being with high intelligent brains are evolved and developed in the cosmos.

However, the eyesight for human perceiving the nature solely limits at the macro-cosmic level. From the meson- to astro-cosmic level, people can but cognize the complexity of nature from partial, qualitative to numerable and quantitive concept step by step with help of variable tools, which undergoes a long-term course of million years, and this process therefore drives the development of science and technology from qualitative study to quantitive study to intelligence study, from mono-disciplinary study to interdisciplinary study and also regularizes our academic works from individual study to the study with an scholar group. Since the 90th of last century, quick share of global scientific and technological resources and the conformity of interdisciplinary scientific studies instead of individual ones have further strengthened the ability to entirely and completely cognize the essence of nature. Accordingly, the important breakthrough in clarifying the essence of nature has been gestating in the process of innovation studies from academic groups in the promising 21 centuries.

I began to touch life science in 1972 and began my scientific career in this filed in 1978. During the near 30 years of academic studies, I gradually realized the limitation of study in a single discipline and by an individual scientist, so, I opened my mind and set up an interdisciplinary academic group by way of organized in the key laboratory since 1990. With the advantage of grouped intelligence from scientists, we try to link the studies at macro-cosmic and micro-cosmic levels and try to understand the underlying mechanisms for the interaction between life system and environmental system. Through the efforts over decades, we have achieved original and initiative results. Here, I would like to publish them in my edited series: "Series of Academic Creative Research Groups in the New Century", wishing this will be beneficial for the academic integration and development of scientific free exploring studies.

ZU Yuan-Gang
January 2004
Harbin

丛书序言

自从宇宙大爆炸以来，自然天体即在介观的水平上，以夸克等粒子的随机碰撞为基本的能量运动形式，由介观向纳观、微观、中观、宏观、宇观方向，以运动的异质性为自然演化的源泉，以无限性的宇量规模演化成太阳系、地球、生命系统直至形成具有高度发达大脑的人类。

然而，人类直观认知自然界的视野仅限于宏观水平，对于从介观到宇观无限性宇量规模的认知，人类也只能借助于各类观测工具由局部、定性、可数计量开始逐渐加深对自然界复杂性的认知，其间经历了数万年的发展历程，因而也推动着科学技术由定性研究到定量研究向智能研究，由单一学科到学科交叉向学科融合的方向发展，也规范着科学研究的行为由个体化向群体化方向发展。进入 20 世纪 90 年代，人类开始迅捷共享全球科技资源，科学研究的群体化整合进一步增强了科学家在整体观上全面认知自然界本质的凝聚力，因而酝酿着人类在 21 世纪通过学术团队创新来实现对自然界整体本质认知的重大突破。

我于 1972 年开始接触生命科学研究，1978 年开始从事生命科学研究，在大约 30 年的学术生涯中，逐渐认识到单一学科和个体化研究的局限性。为此，我于 1990 年开始，下决心以重点实验室的形式组建学术团队，发挥集体智慧的优势，试图将宏观研究与微观研究结合，来全面揭示生命系统与环境系统相互作用的内在机理。经过十几年的努力，积累了一些原始创新性的研究成果，现以《新世纪学术创新团队著作丛书》的形式陆续刊出，以有利于自由探索式学术交流和集成发展。

祖元刚

2004 年 1 月于哈尔滨

Foreword

The cultivation of medical plants in China has been of long history and widely distributed. There is a misunderstanding during cultivation and biomass of medical plants, rather than active substances, were considered as the most important criterion for evaluating their quality. Low content of objective compounds accumulates in cultivated individuals in contrast to wild ones and this leads to the gap existing in cultivation production and requirement in international market. To solve this question, the correlations between metabolisms of natural products and environmental fluctuations in *Camptotheca acuminata* and *Acanthopanax seneicosus* from 1992 were investigated in this book. The results showed that endogenous contents of active compounds were tightly regulated by environmental factor, development program. These findings revealed in part the physiological and ecological foundations of secondary metabolite, providing instruction to cultivations.

Vinbalstine and vincristine are two most important anti-cancer medicine approved by FDA produced by *Catharanthus roseus*, which was widely cultivated in Hainan province of China. To improve the efficiency of cultivation of *C. roseus*, the research group including doctor student Tang. Zhonghua, Yu Jinghua and postgraduate Guo Xiaorui, was constructed to investigate the ecological physiology and its molecular mechanisms of *C. roseus* in Hainan province and green house in Harbin from 2002 under my instruction. Dr. Tang were mainly dedicated in illustrating metabolisms and environmental mechanisms of vinblastine, catharanthine, and vincristine, consequently showing the properties of alkaloid metabolisms regulated by growth and environmental cues, namely temperature, light intensity and water supply. The roles of photosyntheis, respiration, plant hormones, and carbohydrates played during plant interactions with environment were also discussed compared with alkaloid metabolisms by Dr. Tang. In the aspect of molecular basis for physiological ecology of *C. roseus*, proteomics technique was used by Dr. Yu to demonstrate the mechanisms involved with alterations of physiology affected by circumstances. While Master candidate Guo was engaged in studying the effects of heat shock on protein changes of *C. roseus*, especially. Based on these work, Mr.Tang and Miss Guo completed their Ph. D. thesis and Master thesis, respectively.

From 2003, our research group unceasingly concentrated on physiology of *C. roseus* and started to prepare for this book. This monograph particularly focused in the metabolisms of vindoline, catharanthine and vinblastine and their biological functions during interactions with environments. The increasing evidence later demonstrated that

alkaloid metabolism was closely related with photosynthesis, respiration, plant hormones and carbohydrates. The theory and methods of plant physiology, as well as proteomics, were conducted in *C. roseus* to investigate and reveal the physiological processes and mechanisms during development and in response to environmental cues. The physiological processes include alkaloid metabolisms, photosynthetic and respiratory response, soluble sugar and phytohormones variations induced by light, temperature, water and biotic molecules. Their biological functions and interactions were also discussed.

ZU Yuan-Gang

July 2006

Harbin

序　言

药用植物栽培在我国具有悠久的历史和广泛的分布，但一直存在只追求表面生物量大小，忽视植物在初生代谢，特别是次生代谢过程中生物合成活性成分含量高低的问题，极大地降低了栽培的效率，也不符合国际市场的需求。有鉴于此，我自 1992 年对我国特有药用植物——喜树及刺五加的内在活性物质代谢的环境调控进行研究，发现次生代谢产物的合成与植物所处的发育阶段、环境扰动及其他生理活性分子均有密切的关系。这些发现不但揭示了次生代谢产物的生理生态学基础，还对它们的高效栽培起到了指导作用。这使我认识到，对药用植物进行生理生态学研究具有重要的理论和实践意义。

长春花含有的长春碱和长春新碱都是美国 FDA 认可的重要抗癌原料药，在我国海南省广泛栽培。为了进一步提高长春花的栽培和使用效率，我和我指导的博士研究生唐中华、于景华，硕士研究生郭晓瑞于 2002 年组成研究小组，以长春花为研究对象，分别在海南岛野外和东北林业大学室内进行观察测试，重点研究长春花的生理生态学特性及其分子基础。其中，唐中华重点围绕文朵灵、长春质碱和长春碱的次生代谢动态和环境调控机制开展工作，试图揭示它们在生长发育过程中及其在温度、光照和水分调控下的次生代谢规律。同时，也探讨长春花初级代谢与环境条件的相互作用规律，尤其是长春花的光合作用、呼吸作用、植物激素和植物碳水化合物代谢及其环境条件对初级代谢的深刻影响。在我们的研究小组中，于景华在环境条件对长春花生理代谢影响的研究原理和测试方法方面，特别是以蛋白质组学为内容的长春花生理生态特征的分子基础研究的研究原理和测试方法方面，发挥了重要的作用。此外，郭晓瑞重点开展 ABA 对长春花耐受高温胁迫的作用及其蛋白质组学研究。在此基础上，唐中华完成了他的博士学位论文，郭晓瑞完成了她的硕士学位论文。

唐中华和郭晓瑞的博士和硕士学位论文通过答辩后，我和唐中华、于景华、郭晓瑞一起，进一步整理了我们研究小组的研究结果，撰写出《长春花生理生态学研究》英文书稿，内容包括长春碱、长春质碱和文朵灵等三种生物碱，果糖、葡萄糖和蔗糖等三种可溶性糖，长春花光合和呼吸作用及 ABA、GA 和 IAA 三种植物激素在长春花生长发育，响应光照、温度、水分和生物分子胁迫过程中的代谢动态和相互作用，并探讨了它们的生物学功能和相互联系，初步揭示了长春花在生长发育和响应环境变化过程中的生理生态学过程和机制。此英文书稿又经我们多次的研讨和修改，进一步整理出此专著。现将此专著收录于我主编的《新世纪学术创新团队著作丛书》中，不足之处，殷盼指正。

祖元刚

2006 年 7 月于哈尔滨

Preface

The researches on physiological processes of medical plants during developments and in response to environmental cues are of great significance. *Catharanthus roseus* is a tropic plant and has been widely cultured all around the world due to their anti-cancer activity of vinblastine and vincristine. It is well known that it can normally grow under high temperature conditions over 40℃ and is characteristic of strong tolerance to abiotic stress.

Our tutor, Prof. Zu yuangang, has been developing ecophysiological studies on medical plants, including *Camptotheca acuminata, Acanthopanax senticosus* etc from 1992. These results provided detailed insight into the physiologically responsive processes of medical plants under different cultured conditions. Regulation rules and mechanisms focusing on primary and secondary metabolisms were also demonstrated and give instruction to efficient cultivation of them. From 2000, we were organized into one group focusing on physiological ecology of *C. roseus* to provide technical instructions for *C. roseus* planters. Under the elaborate instruction of Prof. Zu, we were engaged in this research work for more than 3 years, partly in Hainan and obtained some original results, some parts of which were collected into this book. In these results, the theory and methods of plant physiology, as well as proteomics, were conducted in *C. roseus* to investigate and reveal the physiological processes and mechanisms during development and in response to environmental cues. The physiological processes include alkaloid metabolisms, photosynthetic and respiratory response, soluble sugar and phytohormones variations induced by light, temperature, water and biotic molecules. Their biological functions and interactions were also discussed.

This work was supported by Key Project of Chinese Ministry of Education (03061) and (104191). I am particularly grateful to postgraduate Yang lei, Sun yanfei, Zhao xiaoju, Jiao yan and Gao yang, cooperating to participating parts of research work in this book.

TANG Zhong-Hua, YU Jing-Hua and GUO Xiao-Rui
June 2006
Harbin

前　言

对药用植物的生长发育和环境调控进行植物生理学研究具有重要的理论和实践意义。长春花作为原产于马达加斯加群岛的一种热带植物，由于其产生的长春碱和长春新碱是国际上重要的抗癌原料药，已在世界上得到了广泛的栽培和引种。同时，该植物常常生长在高温达到40℃以上的地区，具有很强的抗逆性，在生理生态学研究中具有重要的理论意义。

我们的导师祖元刚教授自1992年以来带领他的学生对喜树、刺五加等药用植物进行了深入的生理生态学研究，揭示了药用植物在不同栽培条件下的生理学响应，阐述了次生代谢和初生代谢的环境调控规律和机制，为其高效栽培提供了理论指导。当我们分别进入博士或硕士学习阶段时，祖元刚教授针对长春花栽培中缺乏生理生态学理论和技术指导的现状，为我们组建了“长春花生理生态学”专门研究小组。在祖元刚教授的精心指导下，我们经过三年多的努力，多次赴海南省进行实地调查和观测，结合在东北林业大学温室内的实验研究，重点研究了文朵灵、长春质碱、长春碱三种生物碱，果糖、葡萄糖、蔗糖三种可溶性糖，光合和呼吸作用，以及ABA、GA和IAA三种激素在长春花生长发育和响应不同环境变化过程中的代谢动态及分子机制，着重探讨了上述生理活性分子之间的相互作用以及在响应环境变化中的生物学功能。上述研究成果，进一步丰富了我国植物生理生态学的理论，同时也对长春花的高效栽培具有指导意义。

本项研究工作受到教育部重点项目(03061)和(104191)的资助，特此致谢。特别感谢杨蕾、孙艳斐、赵晓菊、焦琰、高杨、李晓微等各位硕士研究生对本书部分研究工作的大力协助。衷心希望各位读者不吝指教、提出宝贵意见。

唐中华　于景华　郭晓瑞

2006年6月于哈尔滨

Contents

1 Introduction

1.1 Plant physiological ecology

Plants are fixed in one special surroundings exerted by variable light, temperature, water supply, and the other stimuli. A plant individual must cope with two questions during its whole life cycle. The first one is to acquire the resource for establishment and growth from a sometimes hostile and meager environment. The other one is to struggle with competing neighbors of the same of different species. But the mechanisms involved with either capture of resource from environments or allocation of these captured resource to growth and storage in the plant are, in essence, questions of plant physiology affected by environments, which was called plant physiological ecology or ecophysiology. Then, plant physiological ecology is an experimental science that seeks to describe the involved mechanisms that underlie ecological observations in physiological level. This science aims to provide understanding into the physiological process of growth, reproduction, survival, abundance about plants due to the interaction between plants with their biotic and abiotic environments (Lamber et al., 1998). The questions involved in eco originate from ecology in widest sense because most of them were elicited by agriculture, forestry, and horticulture. So, researches on plant physiological ecology, on the one hand are the need of theory field, providing insight into physiological mechanisms of ecological difference, on the other hand are in favor of changing growing, reproductive characteristics artificially for human.

There have been two impetuses for rapid development of plant physiological ecology, including investigation of the underlying physiological mechanisms involved with observed morphology difference and improvement of agriculture yield. Development of portable equipment for measuring leaf gas exchange enabled ecologists to measure rates of carbon gain and loss by individual leaves (Lambers et al., 1998). Developmental physiology of plants has achieved striking success in elucidating the mechanisms underlying endogenous and induced processes of growth and morphogenesis. These achievements undoubtedly provide more insight into carbon assimilation and allocation, taking up and transport of water and mineral nutrition, chemical role of secondary metabolites, regulation of plant hormones on growth and development, etc (Larcher, 2003).

1.2 Main questions involved with plant physiological ecology

With the rapid development of branches of plant biology, newly developed methods were more and more introduced into the research work of plant physiological

ecology and there are more and more combination of this science with molecular biology and developmental biology. The main questions involved with plant physiological ecology include photosynthesis, respiration, long-distance transport, water supply and use, plant hormones, secondary metabolites, antioxidants and interactions of plants with other organisms.

During photosynthesis, radiant energy is absorbed and transformed into the energy of chemical bonds. For every gram atomic weight of carbon taken up, 479 kJ of potential energy are obtained. This process is influenced by internal and external factors and thus may limit the efficiency of the overall process. During the flowering and fruiting phases an increase in photosynthetic capacity has been observed. In this case regulators of development play a critical role, as they determine the temporal and spatial distribution of carbohydrates in the whole plant. The CO_2 uptake of leaves increases initially linearly with increasing radiation but then levels off before it reaches its maximum value. Photosynthesis responds to temperature changes in the forms of an optimum curve. Water is also an essential substance for photosynthesis. However, it is not the small amount of water necessary for photosynthesis that makes water a limiting factor, but rather the large amount of water necessary to maintain the high hydration state of the protoplasm.

1.3 Introduction of *Catharanthus roseus* (L.) G. Don

Catharanthus roseus (L.) G. Don is a tropical plant. It is known as the common or Madagascar periwinkle. This periwinkle is a perennial, evergreen herb in the dogbane family (Apocynaceae) that was originally native to the island of Madagascar. It has been widely cultivated for hundreds of years and can now be found growing wild in most warm regions of the world due to its pharmaceutical use. The plant has historically been used to treat a wide assortment of diseases. It was used as a folk remedy for diabetes in Europe for centuries. In India, juice from the leaves was used to treat wasp stings. In Hawaii, the plant was boiled to make a poultice to stop bleeding. In China, it was used as an astringent, diuretic and cough remedy. In Central and South America, it was used as a homemade cold remedy to ease lung congestion and inflammation and sore throats. Throughout the Caribbean, an extract from the flowers was used to make a solution to treat eye irritation and infections. It also had a reputation as a magic plant; Europeans thought it could ward off evil spirits, and the French referred to it as violet of the sorcerers. The plants grow one or two feet high, have glossy, dark green leaves (1-2 inches long) and flowers all summer long. The blooms of the natural wild plants are a pale pink with a purple 'eye' in their centers (Figure 1.1).

Western researchers finally noticed the plant in the 1950's when they learned of a tea Jamaicans were drinking to treat diabetes. They discovered the plant contains a motherlode of useful alkaloids (more than 100 kinds in all at last count). Some, such as

catharanthine, vindoline and vindolinine lower blood sugar levels (thus easing the symptoms of diabetes). Others lower blood pressure, others act as hemostatics (arrest bleeding) and two others, vincristine and vinblastine, have anticancer properties.

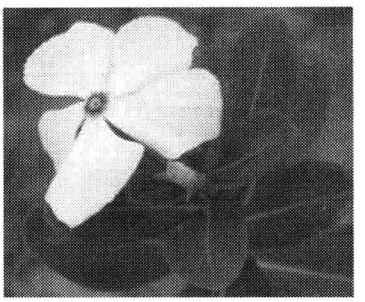


Figure 1.1 Morphology of periwinkle bloom

1.4 Previous studies on *C. roseus*

The previous studies on *C. roseus* mostly focused on alkaloid metabolism, including alkaloid synthesizing pathway, regulation mechanisms, transporting way and storage sites (De Luca, 2000). It has been shown that the alkaloids produced by *C. roseus* belong to monoterpenoid indole alkaloids that are derived from the thikimate and the deoxyxylulose phosphate pathways. The biosynthesis of the indole moiety requires tryptamine, which is derived from tryptophan by the action of tryptophan decarboxylase (TDC). The biosynthesis of the terpenoid moiety requires secologanin, which is derived from graniol via a series of enzymatic conversions (St-Pierre et al., 1999). Because the most significant alkaloid vinblastine accumulates only in trace amounts in the plants much research has been devoted to the improvement of alkaloid production in *C. roseus*. For example, various plant signaling molecules, such like ABA, MeJA, GA, were used to application in *C. roseus* to determine the regulatory mechanisms of alkaloids (Aerts et al., 1996; Vazquez-Flota and De Luca, 1998; Datta and Srivastava, 1997). In situ RNA hybridization and immunocytochemistry were used by St-Pierre et al., (1999) to reveal multicellular compartmentation of *C. roseus* alkaloid biosynthesis and intercellular translocation of a pathway intermediate in detail (St-Pierre et al., 1999).

But there are few reports investigating the physiological processes affected by environmental factors except alkaloid metabolisms. In this book, the primary results about physiological response, together with proteomics methods, of *C. roseus* to light, temperature, water supply, etc. were described. This will provide new understanding of physiological processes correlated with development, biotic stress, abiotic stress of *C. roseus*.