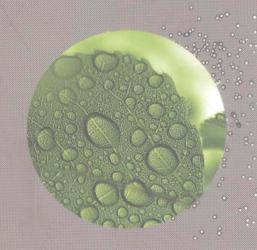
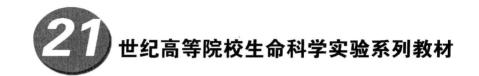
# 植物ZHIWU 生理学实验指导 英汉双语

SHENGLIXUE SHIYAN ZHIDAO YINGHAN SHUANGYU

徐晓峰 吴锦霞 莫蓓苹 编著





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## 前 言

《植物生理学》是生物科学、生物技术、农学、园艺、植物保护、农业资源与环境等专业必修的专业基础课程。随着植物生理学在微观与宏观领域的迅速发展,新知识、新技术不断更新与出现,迫切需要对传统的植物生理学教学内容进行精简与改革;同时,面向世界培养创新型人才是我国在新的时代背景下提出的人才培养新目标,也是高校的重要任务。因此,实施双语教学是培养学生具有国际合作意识、国际交流与竞争能力的国际化人才的重要途径。为了紧跟学科的发展,配合双语教学的实施,编者根据多年的教学体会,参阅大量的相关资料,编写了本教材。

本书精选了涵盖水分生理、矿质营养生理、光合作用、呼吸作用、生长发育、植物生长调节物质及抗性生理学等实验原理和技术的21个实验项目;采用中英文对照的方式编写。附录部分包括各种常用数据表及常用仪器的使用方法等,可供读者查阅。本书吸收了植物生理学研究中新的实验技术和方法,在实验内容上体现了科学性和实用性,可供农林院校有关专业的本科生阅读,也可供其他植物生理学工作者参考使用。

本书的出版得到了广东省生物科学实验教学示范中心建设专项经费的资助。本书是深圳大学生物科学实验中心的系列实验教材之一,全书由徐晓峰、吴锦霞统稿,其中实验1~2由莫蓓莘编写;实验3~4、6、8~9、11~12、14~17以及附录部分由徐晓峰编写;实验5、7、10、13、18~21由吴锦霞编写。

本书在编写过程中,参考并引用了大量的国内外相关资料,由于篇幅有限,书中不能——列出,在此一并表示衷心的感谢。本书在申请立项过程中,得到了学校设备处和学院实验中心的大力支持,在此表示衷心的感谢。

由于编者水平所限、书中错漏之处在所难免、敬请读者给予批评指正。

编 者 2012年8月

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## 基本要求

- (1) 预习。为了取得更好的实验效果,必须预习实验指导中的相关内容,这样才能熟悉所做实验的目的,以及理解将采用的实验技术的原理。
- (2) 参与。合作伙伴之间共用一套实验用具。为获取最好的实验效果,每个人都必须参与全部的实验过程,以确保每个人都不遗漏任何重要的实验技术或观察。
- (3) 出勤。学生必须参加课程安排的所有实验,教师会在实验课上检查出勤情况。如果学生没有充分的理由(如生病或其他合理的原因),实验课缺勤次数超过20%,那么,该学生该门课程的成绩为不合格。
- (4)清洁。保持实验室和实验设备的整洁。每天实验结束后, 离开前必须清理工作台,如撕去贴在玻璃器皿上的标签并将用过的 玻璃器皿洗干净,用95%的酒精洗掉用记号笔做的标记,将废弃物 丢进相应的垃圾箱里。



### **General Instruction**

- 1. Preparation. In order to get the most out of these laboratory experiments, it is ESSENTIAL that you read through the lab manual ahead of time, so that you are familiar with the goals of that day's experiment, and the principles of the techniques you will use.
- 2. Participation. You will share a set of equipments with a fellow student, and will carry out experimental work cooperatively. It is important that both you and your partner participate fully in all laboratory experiments in order to obtain the maximum benefit. You should always monitor your partner's work, to ensure that you do not miss any important experimental techniques or observations.
- 3. Attendance. Attendance at all scheduled laboratories is compulsory, and will be checked at each laboratory session. Absence from the lab for other than compelling and fully documented reasons (illness or compassionate reasons, etc.) for over 20% of the lab periods will result in your being given an INCOMPLETE grade for the course at the end of the semester.
- 4. Cleaning. You are responsible for keeping the laboratory and equipment clean. Leave your bench clean and tidy at the end of each day. Remove tape and labels from glassware and wash all used glassware in the sinks. Use 95% alcohol to wash off labels made with markers. Throw waste in the appropriate garbage.

## 实验室安全规则

## 安全是每一个人的责任!

- (1) 保持实验室整洁。在实验室工作时,非实验必需的物品,如外套、书本、背包等,应放到指定的放置地点。
- (2) 只限于上该课程的学生(已受过安全教育)进入实验室。 未经老师或实验员的允许,不能带访客进入。
- (3) 在实验室中必须穿实验服,并将纽扣系好。实验服不能穿出实验室,更不能穿去上洗手间。
- (4) 进入实验室必须穿皮鞋或球鞋,不能穿凉鞋或拖鞋,以防腐蚀性液体滴到脚上;长发必须扎在脑后。
- (5) 不准在实验室吃东西;不准将午餐或饮料带进或存放在实验室;不准在实验室内使用化妆品(包括润唇膏)或戴隐形眼镜。不要咬笔!
- (6) 实验室内禁止用嘴移液。必须使用球管或移液器,或自动 移液器。
- (7) 离开实验室之前,或手接触到可能被污染的材料之后,要 用肥皂洗手。
  - (8) 开口性创伤、割伤、划伤和擦伤要用防水绷带包扎好。
- (9) 对尖锐物体,如针和刀片,要非常小心地处理。锐器和破碎玻璃要放在适当的容器里。如果打破装有细菌培养液的试管,溶液泼出,在清理之前要先消毒材料。要特别小心以免割伤或感染!必须严格按照实验步骤进行清理。



### Lab Safety Rules

### Safety is everyone's responsibility!

- 1. Keep the laboratory neat, orderly and clean. When you are working in the laboratory, store things you don't need (e. g. coats, books, backpacks) out of the way, in the places provided (wall hooks, under-bench cupboards). Do not bring unnecessary materials (e. g. extra books, clothing) to the laboratory.
- Access to the laboratory is limited to students registered in the course. Who
  have been advised of the potential hazards. Do not bring visitors without permission
  of the instructor or demonstrator.
- 3. Wear a lab coat in the laboratory, and button it up properly. Don't wear it outside the laboratory, including when you go to the washroom!
- 4. Wear shoes, not sandals, at all times in the laboratory. Corrosive reagents can easily be dropped onto unprotected toes. Tie back long hair.
- 5. Do not eat or drink in the laboratory; do not bring or store your lunch or coffee cup in the laboratory; do not apply cosmetics (e. g. lip balm) or adjust contact lenses in the laboratory. Do not chew on your pen!
- 6. Oral pipetting is prohibited in laboratory. Use a bulb or pro-pipettor, or an automated pipettor.
- 7. Wash your hands—with soap—before leaving the laboratory, and at any time after handing materials that may be contaminated.
- 8. Open wounds, cuts, scratches and grazes should be covered with water-proof dressings.
- 9. Sharp objects, such as needles and blades, should be handled with great care. Dispose of sharps and broken glass in the proper containers. If a tube of bacterial culture breaks and spills, disinfect the material before you clean up. Be extremely careful to avoid cutting and infecting yourself! Follow the laboratory procedures for clean-up.

## 实验报告的一般要求

撰写正式书面报告的目的是让学生学会如何撰写和编排真正的科学报告。每份科学杂志都有自己的格式要求,但也都遵循类似的格式,其中包括标题、作者名单、摘要或概要、材料和方法、结果(表中的数据、图表、照片和说明)、解释结果的讨论以及在稿件中所引用的参考文献等。建议大家把科学出版物作为参考例子,如《植物生理学学报》、《美国国家科学院院刊》、《自然》、《细胞》等。

撰写实验报告是学生练习书面沟通技巧的机会。此外,也能显示出学生是否很好地掌握了本实验的基本概念。

实验报告须遵循的一般准则如下:

- (1) 采用双倍行距的文本,小四号字体,边距 2.54 cm。
- (2) 不要使用人称代词,比如"我分离出叶绿素"这种表述是不恰当的,而应用"叶绿素用……方法分离出来"。
  - (3) 要用过去时态来写。
- (4) 每份实验报告会包括多个实验部分,每部分实验要列出一个标题,还有前言、材料、方法和讨论等部分。
- (5) 书写清楚简洁,使用正确的语法、拼写和标点符号。报告应遵循使其易于阅读的逻辑方式。

**封面** 封面应写清标题、姓名、学号、实验室搭档姓名、课程编号、实验小组号。

**摘要** 摘要中须概述背景、结果和获取这些结果的方法,以及结论。写摘要的基本方法是分别用一到两句话来总结每个部分(引言、材料和方法、结果和讨论)。这一部分内容通常最后来写。

**引言** 引言是为在报告其余部分出现的研究提供一个背景。此外,引言部分还应介绍 实验的目的。

**材料和方法** 该部分不能照搬实验指导中的材料和方法,而是要适当地参考实验指导,概述实际实验中所用的方法,包括对实验指导中的方法有所改变的部分。

**结果** 结果部分陈述最终的数据和观察资料,但不讨论数据。结果部分的文字应该是 将图和表格所显示的结果介绍给阅读者。图可以是照片或绘制的图,表格要用三线表。

讨论 讨论部分可以看出实验者是否完全理解了实验中所学技术的背景知识,是否盲目按照实验指导操作。此部分要对所得结果以及该结果所说明的问题进行分析。讨论中的每一个结论都必须有实验结果的支持(参考图示或引用表中的数据)。如果实验不成功,还要分析产生该结果的可能原因(例如使用的技术或使用方法不准确)。讨论应以一个总结整份报告最终实验结果和主要结论的简短句子来结尾。

- 绘制图表的注意事项:
- (1) 报告中的图要有一个恰当的标题。
- (2) 标题应该是一个独立的清晰表述,图和图标应能独立说明问题。



- (3) 结果和讨论中要引用到图所提供的数据。
- (4) 在水平向的x 轴或横坐标上绘制规律性变化的已知量(自变量),如时间、底物浓度,温度等。未知的或测量出的量(因变量),例如吸光度、酶活力等,则在垂直向的y 轴或纵坐标上标出。
- (5) 每一组数据都使用明确定义的符号(①, $\diamondsuit$ , $\spadesuit$ , $\square$ , o···)而不是x,+或一个小点。

### General Instructions For All Lab Reports

Each student will be responsible for writing all the lab reports throughout the semester. The following general guidelines must be followed for each lab report.

The purpose of writing formal lab reports is for you to learn how to write and compile proper scientific reports. Each scientific journal has its own formatting requirements; however, they all follow a similar format with a title, a list of authors, an abstract or summary, materials and methods, results (data in tables, figures, pictures and description), a discussion which interprets results, and a list of references cited in the body of the manuscript. It is suggested that you look through a few journals for examples of scientific publications. Some suggested journals are: Plant Physiology, Proceedings of the National Academy of Sciences (PNAS), Nature, and Cell.

Remember that the lab reports are an opportunity for you to practice your written communication skills. Additionally, it is a great opportunity for you to demonstrate your knowledge of laboratory concepts.

#### **General Guidelines**

- (1) Double-spaced text and a 12-point font size. One inch margins.
- (2) Do not use personal pronouns, i. e., "I isolated the chlorophyll" is unacceptable. Rather, use "chlorophyll was isolated by  $\cdots$ ".
  - (3) Write in the past tense.
- (4) Each lab report will contain multiple exercises. Combine these into one unit. Therefore, you should have one title, introduction, materials and methods sections, results and discussions.
- (5) Write clearly and concisely using proper grammar, spelling and punctuation. The report should follow in a logical manner making it easy to read.

#### Title Page

A cover page which includes a title, name and identification number, lab partner's name, the course number, group number.

#### Summary/Abstract

A summary paragraph which clearly states the background, results with methods to obtain those results, and a conclusion. A basic method to construct the abstract is to summarize each of the sections (Introduction, Materials and Methods, Results and Discussion) into 1 to 2 sentences each. This section is often written last.

#### Introduction

The introduction presents a background to the research that is presented in the remainder of the report. Additionally, the introduction presents the objectives of the study that was conducted.

#### Materials and Methods

In this course, we are not requiring you to rewrite the materials and methods presented in the Course Manual. Rather, reference the sources appropriately and include any changes from the methods outlined in the Course Manual.

#### Results

The Results section presents final data and observations, but the data are not discussed here. The text in the results section should guide the reader through the figures and tables. Figures would include graphs and photographs of gels, whereas tables should be organized in three-line format.

#### Discussion

The Discussion section is an opportunity for you to show that you understand the background principles to the techniques you learned in the exercises, and did not blindly follow the protocols outlined in the manual. The discussion presents an analysis of your results and the significance of your findings. Each item you discuss must be clearly supported by the results (refer to figures or quote values in tables, etc.). If your experiments did not work, include some possibilities as to why (e. g., technique used or inaccuracy in methodology). The discussion should end with a short concluding sentence summarizing the entire report with final results and major conclusions.

#### **Graphs and Diagrams**

- (1) A proper title is extremely important.
- (2) The title must be self-explanatory so that the figure may stand on its own.
- (3) The figure must be referred to within the text prior to its presentation.
- (4) Plot the known quantity being systematically changed (independent variable), such as time, substrate concentration, temperature etc., on the horizontal x-axis or abscissa. The unknown or measured quantity (dependent variable), such as absorbance, enzyme activity etc., is plotted on the vertical y-axis or ordinate.
- (5) Use clearly defined symbols ( $\bigoplus$ ,  $\diamondsuit$ ,  $\spadesuit$ ,  $\square$ , o...) and not x, + or a small point '.', for each set of data.



## 实验 1 质壁分离法测定植物组织渗透势

#### 【实验目的】

了解植物体内不同组织和细胞之间、植物与环境之间水分的转移与植物组织渗透势的 关系、学习测定植物组织渗透势的基本方法。

#### 【实验原理】

植物细胞的渗透势主要取决于液泡的溶质浓度,因此又称溶质势。植物细胞的渗透势是反映植物水分代谢、生长发育及抗逆性的重要指标。在干旱、盐渍等许多逆境条件下,一些植物常表现为细胞内主动积累溶质,以降低其渗透势,增加吸水能力,在一定程度上维持膨压,保障细胞的生长和气孔的开放,这种现象叫做渗透调节作用。渗透调节能力的大小可以用逆境条件下细胞渗透势的降低值来表示,因此,在水分生理与抗逆性生理研究中渗透势是必不可少的测定指标。渗透势的测定方法可分为两类:一类是液相平衡法(质壁分离法),另一类是气相平衡法(热电偶湿度计法、露点法、压力室法等)。质壁分离法是目前测定植物组织渗透势常用的方法,其主要原理是:将植物组织放入一系列不同浓度的蔗糖溶液中,经过一段时间,植物细胞与蔗糖溶液间将达到渗透平衡状态。如果在某一溶液中细胞脱水达到平衡时刚好处于初始质壁分离状态,则细胞的压力势( $\Psi_{\rm p}$ )将下降为零。此时细胞液的渗透势( $\Psi_{\rm s}$ )等于外液的渗透势( $\Psi_{\rm s}$ 0)。此溶液称为该组织的等渗溶液,其浓度称为该组织的等渗浓度。

根据下述公式即可计算出细胞液的渗透势  $\Psi$ :

$$\Psi_{\cdot} = \Psi_{\cdot 0} = -icRT$$

式中  $\Psi_{0}$ ——供试溶液的渗透势, MPa;

i——溶质的解离系数、蔗糖为1:

c——供试溶液的物质的量浓度,  $mol \cdot L^{-1}$ , 以水作溶剂;

R——摩尔气体常数、0.008314 MPa·L·mol<sup>-1</sup>·K<sup>-1</sup>:

T———执力学温度、 $K_{\circ}$ 

实际测定时,初始质壁分离状态难以在显微镜下直接观察到,所以一般均以质壁分离的最低浓度与不发生质壁分离的最高浓度的平均值作为判断等渗浓度的标准。

#### 【实验材料】

洋葱鳞茎或鸭跖草。

#### 【设备与试剂】

显微镜、载玻片、盖玻片、温度计、尖头镊子、刀片、小培养皿(直径为6 cm)、试剂瓶、烧杯、容量瓶、量筒、吸管、吸水纸、0.03%中性红溶液、蔗糖系列标准溶液。

蔗糖系列标准溶液的配制: 蔗糖预先在 60 ℃ ~ 80 ℃ 下烘干, 配制 0. 30, 0. 35, 0. 40, 0. 45, 0. 50, 0. 55, 0. 60, 0. 65, 0. 70 (mol·L<sup>-1</sup>) 等一系列不同浓度的蔗糖溶液, 具体

范围可根据材料不同而加以调整、配好后贮干试剂瓶中、瓶口加塞以防蒸发。

#### 【实验步骤】

#### 1. 配制溶液

取干燥、洁净的培养皿9套并编号,将配制好的不同浓度的蔗糖溶液按顺序加入各培养皿中成一薄层,备用。

#### 2. 取材染色

用镊子撕取(或用刀片刮取)供试材料的表皮,大小以 0.5 cm² 为宜,迅速投入各种浓度的蔗糖溶液中,使其完全浸入,每种浓度放 4~5 片,同时记录室温。为了便于观察,先将表皮置于 0.03%的中性红溶液内染色 5 min 后,吸去水分,再浸入蔗糖溶液中。如果不染色即能区别质壁分离,可以不经染色直接镜检观察。

#### 3. 徒手制片

5~10 min 后,取出表皮薄片放在滴有同样蔗糖浓度溶液的载玻片上,盖上盖玻片。

#### 4. 显微观察

在低倍显微镜下观察,如果所有细胞都产生质壁分离现象,则取低浓度溶液中的制片作同样观察,并记录质壁分离的相对程度。如果在两个相邻浓度的切片中,一个切片没有发生质壁分离,另一个切片发生质壁分离的细胞数超过50%,则这两个浓度的平均值为等渗浓度。每一个制片观察的细胞不应少于100个。检查时可先从高浓度开始。在找到上述浓度极限时,用新的溶液和新鲜的叶片重复进行几次,直至有把握为止。在此条件下,细胞的渗透势与两个极限溶液浓度的平均值的渗透势相等。

#### 5. 记录结果

将实验结果记录于表1-1中。

表 1-1 植物细胞渗透势测定记录表

实验人	日期	材料名称	实验室温度	<del>.</del>
蔗糖浓度 (mol·L <sup>-1</sup> )	质壁分离的机	目对程度(作图表示)	视野中发生质壁分离的百	分率 (%)
0.30				
0. 35				
0. 40				
0. 45				
0. 50				
0. 55				
0. 60				
0. 65				
0. 70				

注:视野中发生质壁分离的百分率以大于50%或小于50%表示。

由所得到的等渗浓度和测定的室温,用  $\Psi_s = \Psi_{s0} = -icRT$  计算渗透势( $\Psi_s$ ),即为细胞的渗透势。



6. 注意事项

撕下的表皮组织必须完全浸没于蔗糖溶液中,浸没时间不能过短,否则会影响实验结果。

#### 【讨论】

- 1. 发生细胞质壁分离时,植物细胞的水势由什么组成?
- 2. 哪些情况下可能发生细胞质壁分离?采用什么措施才能使发生质壁分离的细胞复原?

## **Experiment 1** Measuring Osmotic Potential of Plant Tissues by Plasmolysis Approaches

#### Goals

To understand the relation of water movement, including the movement between different tissues or cells, and between plant and environment, with the water potential of plant tissue.

To learn the general methods of measuring osmotic potential of plant tissues.

#### Introduction

Water potential ( $\Psi$ ) defines the energy content of the water in the cell or plant and provides a convenient summation of all factors influencing the water status of that tissue. It is expressed in terms of pressure (bars or, more correctly, MPa, 1 MPa = 10 bars). The water potential of pure water is zero bars. The addition of salts, as in plant cells, lowers the energy content of the water (it can do less work), i. e., it lowers the  $\Psi$ . This effect represents one component of  $\Psi$ , the osmotic potential ( $\Psi_{\pi}$ ). With no external pressure or suction on the water,  $\Psi = \Psi_{\pi}$ . Adding pressure greater than atmospheric, as in the case of turgor in most plant cells, will increase the energy content of the water (it can do more work), i. e., it raises the  $\Psi$ . Likewise, adding suction or tension on the water lowers the  $\Psi$ . This represents another component of the total  $\Psi$ , the pressure potential ( $\Psi_p$ ). Other factors also influence the energy content ( $\Psi$ ) of the water in the cell, but these two are the main ones. It is important to realize that these are only components of the total water potential, and it is the total  $\Psi$  which determines the water status of the tissue and, hence, the directionality of water movement (always high to low energy; high to low  $\Psi$ ).

In the experiments described below, the principle of water movement until equilibration of  $\Psi$  will be employed as indicators of  $\Psi$  and  $\Psi_{\pi}$  tissues at unknown  $\Psi$  will be placed in sugar solutions of known and varying  $\Psi$ . Since water always moves from high to low  $\Psi$  (energetically downhill),