

学校编码：10384

密级_____

学号：33320131151708

厦 门 大 学

硕 士 学 位 论 文

红树植物秋茄幼苗叶片应对水淹胁迫的生理响应及差异蛋白质组学分析

Physiological and Proteomic Analyses in Responses of Mangrove Plant *Kandelia obovata* Seedling Leaves to Submergence Stress

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论文提交日期：2016年5月

论文答辩时间：2016年6月

2016年5月

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缩略词

2-DE	2-D gel electrophoresis	凝胶双向电泳
4D-CS	four day complete submergence treatment	全淹4天处理
ABA	abscisic acid	脱落酸
APX	ascorbate peroxidase	抗坏血酸过氧化物酶
AsA	ascorbic acid	抗坏血酸
CBB	Coomassie brilliant blue	考马斯亮蓝
DHAR	dehydroascorbate reductase	脱氢抗坏血酸还原酶
DTT	dithiothreitol	二硫苏糖醇
FW	fresh weight	鲜重
GA	gibberellin	赤霉素
GS	glutamine synthetase	谷氨酰胺合成酶
GR	glutathione reductase	谷胱甘肽还原酶
GSH	glutathione	谷胱甘肽
GST	glutathione S transferases	谷胱甘肽S-转移酶
H ₂ O ₂	hydrogen peroxide	过氧化氢
HSPs	heat shock proteins	热激蛋白
MALDI	matrix-assisted laser desorption/ionization	基质辅助激光解吸电离
MDA	malondialdehyde	丙二醛
MW	molecular weight	分子量
NBT	nitroblue tetrazolium	硝基氮蓝四唑
PMF	peptide mass fingerprinting	肽指纹图谱
PMSF	phenylmethanesulfonylflu oride	苯甲基磺酰氟
Pn	net photosynthetic rate	净光合速率
POD	peroxidase	过氧化物酶

PSI	photosystemI	光系统I
PSII	photosystem II	光系统II
PI	isoelectric point	等电点
ROS	reactive oxygen species	活性氧
Rubisco	ribulose-1,5-bisphosphate carboxylase/oxygenase	核酮糖二磷酸羧化酶加氧酶
SOD	superoxidedismutase	超氧化物歧化酶
TCA cycle	tricarboxylic acid cycle	三羧酸循环
TOF/TOF MS	time of flight mass spectrometry	飞行时间二级质谱技术

摘要

水淹是限制多数植物生长和发育的重要限制因子。红树植物是生活在滨海湿地的一类对水淹具有较高耐性的木本植物。近年来,在对红树林湿地的围垦和鱼塘养殖的开发过程中经常会造成对红树植物的严重水淹,甚至是长时间、整株植物的被淹(特别是对幼苗)。然而,红树植物对这种长时间、全淹胁迫的响应仍有待研究。在本研究中,基于蛋白质组学技术,我们采用红树植物秋茄(*Kandelia obovata*)幼苗为实验材料,来探究红树植物秋茄耐受长时间、全淹胁迫的生理响应和分子机制。主要研究结果如下:

(1) 秋茄幼苗持续全淹处理。全淹处理秋茄幼苗 3 天后,随着水淹天数的增加,秋茄叶片色素降解,净光合速率降低,叶片光合系统破坏,7 天后植株几乎不能成活。根据所测秋茄叶片叶绿素含量、类胡萝卜素含量、叶绿素 a/b 比率、光合作用相关指标,本实验选择全淹第 4 天为处理组,以下简称为 4D-CS。

(2) 生理数据表明,4D-CS 处理可以破坏秋茄叶片细胞膜系统,导致活性氧物质(ROS)积累。4D-CS 处理下,秋茄叶片中的超氧化物歧化酶(SOD)活性和抗氧化小分子物质谷胱甘肽(GSH)和抗坏血酸(AsA)含量相对于对照组显著提高,而过氧化物酶(POD)、抗坏血酸过氧化物酶(APX)和谷胱甘肽 S-转移酶(GST)三种抗氧化酶的活性明显降低。由此我们推断,SOD、AsA 和 GSH 相对于其他一些抗氧化酶(POD, APX, GST)在响应 4D-CS 胁迫过程中可能起到更为重要的作用。

(3) 运用双向电泳技术来研究 4D-CS 处理下秋茄叶片蛋白质组的变化,我们分别在秋茄叶片的对照组和 4D-CS 处理组的蛋白质图谱上获得 500 多个蛋白质点,经 PDQust 软件分析和 MALDI-TOF/TOF-MS 鉴定,分析得到表达量变化在 2 倍以上的差异蛋白质有 46 个,其中 16 个上调表达,30 个下调表达。大多数差异表达的蛋白质亚细胞定位于叶绿体内,少数分布在线粒体、高尔基体、细胞核、细胞液、过氧化物酶体中。根据它们的生物学功能把这些差异表达蛋白质分为三大类,分别为光合作用和能量产生相关的蛋白质、物质代谢过程相关的蛋白质、氧化还原平衡和激素信号响应相关的蛋白质。

(4) 参与光合作用和能量产生相关的蛋白质主要有核酮糖-1,5-二磷酸羧化

酶/加氧酶 (Rubisco)、Rubisco 结合蛋白、二磷酸-果糖醛缩酶 (FBP)、醛缩酶家族、NAD-脱氢酶、醌氧化还原酶、NADP-铁氧还蛋白还原酶 (FNR)、放氧复合体蛋白 2 (OEE 2)、ATP 合成酶、ATP 酶等。参与物质代谢过程相关的蛋白质主要有环化酶蛋白家族、谷氨酰胺合成酶(GS)、磷酸丙糖异构酶同工酶(TPI)、苹果酸脱氢酶 (MDH)、ATP 依赖的 Clp 蛋白水解酶亚基 (ClpA)、驱动蛋白 4、1-磷酸-L 肌醇合酶 (MIPS)、UDP-L-阿拉伯糖变位酶、20S 蛋白水解酶 β 亚基 D1 等。与氧化还原平衡和激素信号相关的蛋白质有乳酸酰谷胱苷肽裂解酶、ABA-胁迫-成熟诱导蛋白(ASR)、抗坏血酸过氧化物酶(APX)、叶绿体内的 32KD 干旱诱导-胁迫-蛋白 (CDSP 32)、热激蛋白 (HSPs)、NAD(P)结合的罗斯曼折叠超家族蛋白等。另外, 还有一个重要的转录因子 myc2 bHLH 被鉴定了出来。

(5) 在 4D-CS 处理下, 多数与光合作用暗反应阶段相关的蛋白质表达量下调, 造成秋茄幼苗叶片光合作用减弱。与此相反, 与光合作用光反应阶段和光合磷酸化过程相关的蛋白质表达量多数上调, 这可能是秋茄应对全淹胁迫的一种可能机制。

(6) 在 4D-CS 处理下, 我们发现与脱落酸 (ABA) 相关的一个重要蛋白质 CDSP 32 和另一个重要转录因子 myc2 bHLH 表达量均下调。结合其他相关代谢相关蛋白质的表达量变化, 因此我们认为秋茄可能通过 ABA 信号转导途径调控植株采取“逃离策略 (Escape strategy)”以提高其水淹耐受性。

关键词: 秋茄; 水淹; 红树植物; 植物生理; 蛋白质组学

Abstract

Submergence is a severe constraint on growth and development for most of plants. Mangroves are a kind of woody plant distributed along coastal wetland with high waterlogging tolerance. In the recent years, over-exploration of mangrove wetland as fishing pond and tideland reclamation usually resulted severe submergence, even long-lasting and complete submergence for mangrove plants, particularly for seedlings and saplings. However, how mangrove plants respond to continuous and complete submergence is still unknown yet. Here, based on proteomic approaches, we used the seedlings of *Kandelia obovata*, a mangrove plant species, to investigate the physiological responses and molecular mechanisms of *K. obovata* to long-lasting and complete submergence. The main results are listed as follows:

(1) *K. obovata* seedlings were completely submerged in water constantly. After 3 day of complete submergence, the pigments were degraded, Pn was depressed and the photosynthetic systems were destructed in the leaves of *K. obovata* seedlings with the submergence lasting. Furthermore, the seedlings of *K. obovata* almost can not survive after 7 days of submergence. According to the content of chlorophyll, carotenoid, chlorophyll a/b and parameters of photosynthesis, we used 4 day as the suitable submergence time for the subsequent experiments. The treatment of 4 days complete submergence was then abbreviated as 4D-CS.

(2) Physiological results demonstrated that 4D-CS destroyed membrane system, led to ROS accumulation in *K. obovata* leaves. It was found that the contents of ascorbic acid (AsA), glutathione (GSH) and superoxide dismutase (SOD) were significantly increased under 4D-CS, meanwhile, the activities of three antioxidant enzymes including ascorbate peroxidase (APX), glutathione S-transferase (GST) and peroxidase (POD) were decreased. We concluded that SOD, GSH and AsA may play more important role in respond to 4D-CS than some other antioxidative enzymes such as POD, APX and GST.

(3) 2-DE was performed to explore the proteome changes in *K. obovata* leaves under 4D-CS. More than 500 protein spots were detected on each of CK and 4D-CS gel. After carefully analysis by PDQuest and by MALDI-TOF/TOF-MS, we successfully identified 46 differentially expressed proteins with more than 2-fold changes in their abundance, including 16 up-regulated proteins and 30

down-regulated proteins in 4D-CS leaves compared to the control. Among them, most of proteins locate in chloroplast, some proteins locate in mitochondria, Golgi apparatus, nucleus, cytoplasm and peroxisome. According to the biological functions, these identified differentially expressed proteins can be classified into three groups including the proteins related to photosynthesis and energy production, the proteins related to cellular processes and metabolism, the proteins related to redox homeostasis and hormone signaling response.

(4) Proteins involve in photosynthesis and energy production are mainly including ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (Rubisco), Rubisco subunit binding-protein beta subunit, fructose-bisphosphate aldolase (FBP), plastidic aldolase family protein, NAD-dependent dehydrogenase 2, quinone oxidoreductase-like protein, NADP ferredoxin reductase (FNR), oxygen-evolving enhancer protein 2 (OEE 2) and ATP synthase and so on. Proteins involve in metabolism are mainly including cyclase family protein, glutamine synthetase (GS), triosephosphate isomerase isoform (TPI), malate dehydrogenase (MDH), ATP-dependent Clp protease proteolytic subunit (ClpA), kinesin-4-like, L-myo-inositol-1-phosphate synthase (MIPS), UDP-L-arabinose mutase-like protein and 20S proteasome beta subunit D1. Proteins involve in redox homeostasis and hormone signaling response are mainly including lactoylglutathione lyase family protein, abscisic acid stress ripening protein (ASR), ascorbate peroxidase (APX), chloroplastic drought-induced stress protein of 32 kD (CDSP 32), heat shock proteins (HSPs), NAD(P)-binding Rossmann-fold superfamily protein and NAD(P)-linked oxidoreductase superfamily protein isoform 2. An important transcription factor myc2 bHLH protein was also identified.

(5) Under 4D-CS treatment, most of identified Calvin cycle related proteins were down-regulated lead to photosynthesis reduction in the leaves of *K. obovata* seedlings. On the contrary, most of proteins related to photosynthetic light-reaction including electron transfer and photophosphorylation were up-regulated. This result implied the possible adaptive mechanism of *K. obovata* to the complete submergence.

(6) Under 4D-CS treatment, it was found that two proteins related to abscisic acid (ABA) including CDSP 32 and one important transcription factor myc2 bHLH were both down-regulated. Combined with the changes of other differentially expressed proteins involving in metabolism, we concluded that *K. obovata* may adopt “escape strategy” to adapt complete submergence through ABA signaling pathways.

Keywords: *Kandelia obovata*; submergence; mangrove; plant physiology; proteomics

厦门大学博硕士学位论文摘要库

第1章 前言

1.1 植物耐水淹研究概况

1.1.1 水淹的类型及危害

水淹在全球范围内都是植物所遭受并限制植物生长和生产的主要因子^[1]。近几十年来,由于全球气候变化所导致的全球范围内洪涝事件发生频率的增加和严重性的加剧,对植物来讲,水淹在全球范围内已经成了一种不容忽视的非生物胁迫^[2](见图 1.1)。

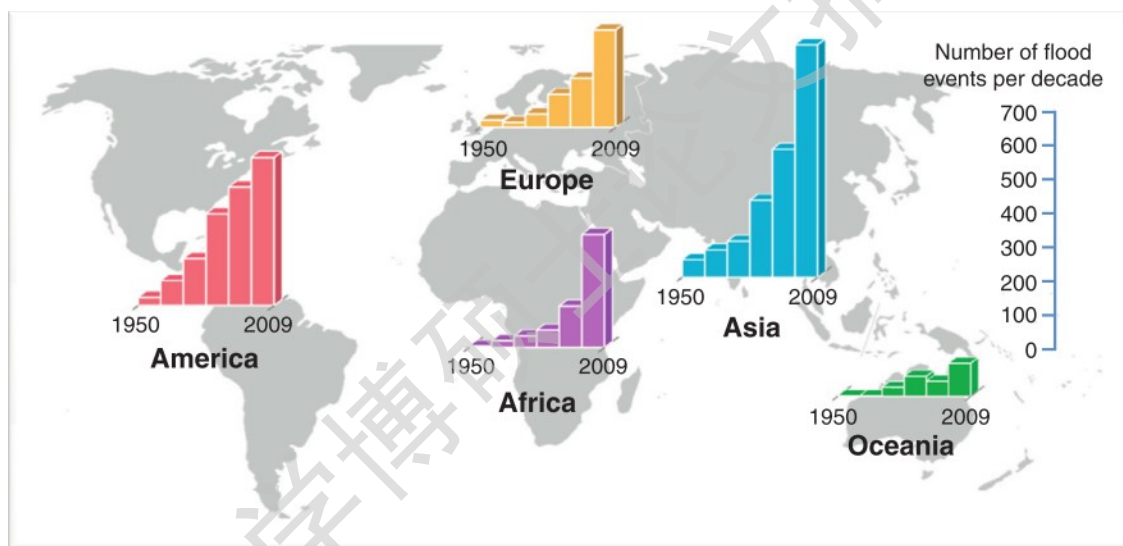


图 1.1 全球洪涝灾害事件在过去的六十年间发生频率增加^[2]。图中显示的洪涝事件次数是被比利时鲁汶大学国际灾害数据库通过不同地理区从 1950 到 2009 年期间归类为洪涝灾害的数据^[3]。此类事件包括河流或海岸洪水爆发、急速化雪、大雨和其他一些能够引发显著的社会或经济损害的洪涝灾害事件。

Figure 1.1 Numbers of floods have increased in each of the past six decades across the globe. Graphs show the number of floods classified as a disaster in the International Disaster Database of the University of Louvain, Belgium for the period from 1950 through 2009 by geographical region. Events include river or coastal floods, rapid snow melts, heavy rainfall and other occurrences that caused significant social or economic hardship.

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