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博 士 学 位 论 文

双孢蘑菇对氨基苯甲酸合酶基因在拟南芥中的功能分析

Functional analysis of *Agaricus bisporus*

para-Aminobenzoic acid synthase gene in *Arabidopsis thaliana*

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目录

目录.....	I
CONTENTS.....	V
摘要.....	IX
ABSTRACT.....	XI
缩写对照照表.....	XIV
第一章 前言.....	1
1.1 模式植物拟南芥.....	1
1.2 植物转基因技术及应用.....	2
1.2.1 植物转基因技术路线.....	3
1.2.2 常用的植物转基因技术方法.....	3
1.2.3 农杆菌介导的 floral-dip 法转基因.....	4
1.2.4 转基因技术的应用.....	5
1.3 PABA 合成代谢以及功能.....	6
1.3.1 PABA 和其它重要的苯甲酸衍生物.....	6
1.3.2 PABA 的代谢途径.....	6
1.3.3 PABA 是合成叶酸的重要前体.....	8
1.3.4 PABA 的其它功能.....	10
1.4 植物活性氧族代谢和信号转导.....	10
1.4.1 ROS 的产生机制.....	11
1.4.2 ROS 的清除机制.....	12
1.4.3 ROS 调控的生理过程.....	14
1.4.4 ROS 在信号转导中的作用.....	18

1.5 植物耐高温的分子机制.....	21
1.5.1 热休克蛋白 (Heat shock proteins, HSPs)	22
1.5.2 热激信号转导通路 (Heat shock signalings)	23
1.5.3 植物激素.....	24
1.6 植物抵御 UV 的分子机制.....	25
1.6.1 UV 辐射对植物的生理形态的影响.....	25
1.6.2 UV 辐射对次生代谢产物的影响.....	26
1.6.3 UV 辐射对抗氧化系统的影响.....	26
1.6.4 UV 辐射对 DNA 分子及基因表达调控的影响.....	27
1.7 本研究的背景、目的和意义.....	28
第二章 材料和方法.....	30
2.1 材料.....	30
2.1.1 植物材料.....	30
2.1.2 菌株与质粒.....	30
2.1.3 仪器与耗材.....	30
2.1.4 主要试剂.....	31
2.1.5 培养基的配置.....	32
2.1.6 试剂的配制.....	32
2.2. 方法.....	35
2.2.1 拟南芥的种植方法.....	35
2.2.2 分子克隆技术.....	36
2.2.3 农杆菌介导的拟南芥侵染花序转化法及转基因植株的筛选.....	41
2.2.4 转基因拟南芥总 DNA 提取.....	42
2.2.5 Trizol 法提取植物中 RNA 的提取.....	42
2.2.6 总 RNA 反转录成 cDNA 第一条链.....	43
2.2.7 实时荧光定量 PCR.....	43
2.2.8 蛋白质技术.....	44
2.2.9 拟南芥主根生理特性分析.....	46
2.2.10 GUS 活性的检测.....	46

2.2.11 PABA 提取与浓度测定 ^[21]	47
2.2.12 SA 提取与浓度测定 ^[21]	47
2.2.13 拟南芥根中 H ₂ O ₂ 水平检测.....	48
2.2.14 AtMPK 抗体制备.....	48
2.2.15 抗氧化酶活测定.....	49
2.2.16 单细胞凝胶电泳.....	50
第三章 结果与分析.....	52
3.1 <i>Pabs</i> 转基因拟南芥筛选和鉴定.....	52
3.1.1 <i>Pabs</i> 转基因拟南芥 T ₁ 代筛选和鉴定.....	52
3.1.2 <i>Pabs</i> 转基因拟南芥纯合子筛选和鉴定.....	55
3.1.3 <i>Pabs</i> 转基因拟南芥 PABS 蛋白的检测和 PABA 含量的测定.....	56
3.2 过表达 <i>Pabs</i> 基因对拟南芥生长发育的影响.....	58
3.2.1 过表达 <i>Pabs</i> 基因对拟南芥形态的影响.....	58
3.2.2 T-DNA 插入拟南芥 PABA 合酶基因导致突变株不育.....	60
3.2.3 外源 PABA 抑制拟南芥主根的生长.....	65
3.2.4 PABA 促进拟南芥根毛的生长发育.....	67
3.2.5 PABA 可以激活根部的生长素信号途径.....	68
3.3 过表达 <i>Pabs</i> 基因诱导 ROS 的产生.....	69
3.3.1 过表达 <i>Pabs</i> 基因导致根尖 ROS 水平升高.....	69
3.3.2 过表达 <i>Pabs</i> 诱导拟南芥 <i>PR-1</i> 基因的表达.....	70
3.4 拟南芥中 <i>Pabs</i> 基因耐温功能的鉴定.....	72
3.4.1 <i>Pabs</i> 转基因拟南芥在高温胁迫下的形态变化.....	72
3.4.2 <i>Pabs</i> 转基因拟南芥在高温胁迫下的 MAPK 变化.....	74
3.4.3 <i>Pabs</i> 转基因拟南芥在高温胁迫下 HSP 的变化.....	74
3.5 过表达 <i>Pabs</i> 基因增强拟南芥的 UV-C 耐受性.....	75
3.5.1 <i>Pabs</i> 转基因拟南芥对 UV-C 敏感性减弱.....	76
3.5.2 <i>Pabs</i> 转基因拟南芥根尖具有高水平的 ROS.....	78
3.5.3 <i>Pabs</i> 转基因拟南芥具有更高的抗氧化酶活性.....	79
3.5.4 UV-C 诱导拟南芥 PABA 的积累.....	81

3.5.5 过表达 <i>Pabs</i> 基因减轻 UV-C 诱导的 DNA 损伤.....	82
3.5.6 UV-C 处理后 DNA 损伤标记基因的表达分析.....	83
3.5.7 过表达 <i>Pabs</i> 基因诱导 <i>DDM1</i> 基因的表达.....	85
第四章 讨论.....	86
4.1 双孢蘑菇 <i>Pabs</i> 基因可以在拟南芥中稳定地表达出具有生物活性蛋白质	86
4.2 拟南芥 <i>adcs</i> 是合成 PABA 的关键酶.....	86
4.3 PABA 可以调节拟南芥根的生长发育.....	87
4.4 PABA 在拟南芥高温防御信号转导中的作用.....	88
4.5 拟南芥中 PABA 防御 UV-C 胁迫的作用机制.....	89
第五章 展望.....	92
参考文献.....	93
附录.....	108
致谢.....	111

Contents

Chinese Contents	I
English Contents	V
Chinese Abstract	IX
English Abstract	XI
Abbreviations	XIV
Chapter 1 Introduction	1
1.1 The model plant <i>Arabidopsis</i>	1
1.2 Transgenic plant technology and its application	2
1.2.1 Technical routes of transgenic plant technology.....	3
1.2.2 General methods of transgenic plant technology.....	3
1.2.3 Floral dip method for <i>Agrobacterium</i> -mediated transformation.....	4
1.2.4 The application of transgenic plant technology.....	5
1.3 The synthesis and function of PABA in plants	6
1.3.1 PABA and other important benzoic acid derivatives.....	6
1.3.2 PABA metabolism.....	6
1.3.3 PABA is an important precursor for the synthesis of folate.....	8
1.3.4 The other function of PABA.....	10
1.4 Reactive oxygen species metabolism and signal transduction in plants	10
1.4.1 The mechanism of ROS generation.....	11
1.4.2 The mechanism of ROS scavenging.....	12
1.4.3 Physiological processes mediated via ROS.....	14
1.4.4 The role of ROS in signal transduction pathway.....	18
1.5 Molecular mechanism of heat tolerance in plants	21

1.5.1 Heatshock proteins (HSPs).....	22
1.5.2 Heat shock signalings.....	23
1.5.3 Plant hormone.....	24
1.6 Molecular mechanism of UV resistance in plants.....	25
1.6.1 Physiological and morphological changes of plants upon UV radiation.....	25
1.6.2 Effects of UV radiation on secondary metabolites.....	26
1.6.3 Effect of UV radiation on oxidation system.....	26
1.6.4 Effects of UV radiation on DNA repair.....	27
1.7 The background and significance of this thesis.....	28
Chapter 2 Materials and methods.....	30
2.1 Materials.....	30
2.1.1 The plant materials.....	30
2.1.2 Bacterium strains and plasmids.....	30
2.1.3 Instruments.....	30
2.1.4 Common reagents.....	31
2.1.5 Preparation of the cultures.....	32
2.1.6 The preparation of the reagents.....	32
2.2. Method.....	35
2.2.1 Plant growth of <i>Arabidopsis</i>	35
2.2.2 The techniques of Molecular cloning.....	36
2.2.3 The method of infection of <i>Arabidopsis</i> inflorescence by <i>Agrobacterium</i> and the screening of transgenic lines.....	41
2.2.4 Total DNA extraction from transgenic <i>Arabidopsis thaliana</i>	42
2.2.5 Extraction of plant RNA by Trizol method.....	42
2.2.6 Reverse transcription.....	43
2.2.7 Real-time fluorescence quatification PCR.....	43
2.2.8 The techniques related to protein.....	44
2.2.9 Analysis of physiological characteristics of <i>Arabidopsis</i> root.....	46
2.2.10 Detection of GUS activity.....	46

2.2.11 PABA extraction and concentration determination ^[21]	47
2.2.12 SA extraction and concentration determination ^[21]	47
2.2.13 Detection of H ₂ O ₂ levels in <i>Arabidopsis</i> roots.....	48
2.2.14 Preparation of AtMPK antibody.....	48
2.2.15 Measure the antioxidant enzyme activity.....	49
2.2.16 Single cell gel electrophoresis.....	50
Chapter 3 Results and analysis.....	52
3.1 Screening and identification of <i>Pabs</i> transgenic <i>Arabidopsis</i>.....	52
3.1.1 Screening and identification of <i>Pabs</i> transgenic <i>Arabidopsis</i> T1 generation	52
3.1.2 Screening and identification of homozygous <i>Pabs</i> transgenic <i>Arabidopsis</i>	55
3.1.3 Identification of PABS and PABA content.....	56
3.2 PABA regulates <i>Arabidopsis</i> growth.....	58
3.2.1 Physiological changes in <i>Pabs</i> overexpressing <i>Arabidopsis</i>	58
3.2.2 <i>Adcs</i> with a T-DNA insertion leads to produce defective seeds.....	60
3.2.3 Exogenous PABA inhibited root growth of <i>Arabidopsis thaliana</i>	65
3.2.4 PABA promotes the development of <i>Arabidopsis</i> root hairs.....	67
3.2.5 PABA can activate the auxin signaling pathway in root.....	68
3.3 Overexpression of <i>Pabs</i> gene induced ROS generation.....	69
3.3.1 Overexpression of <i>Pabs</i> gene results in an increased ROS level in roots....	69
3.3.2 Overexpression of <i>Pabs</i> gene induces the expression of <i>PR-1</i> gene in <i>Arabidopsis thaliana</i>	70
3.4 Identification the heat tolerance of <i>Pabs</i> gene in <i>Arabidopsis thaliana</i>.....	72
3.4.1 Physiological difference of <i>Pabs</i> transgenic <i>Arabidopsis</i> under heat shock	72
3.4.2 The expression pattern of MAPKs in <i>Pabs</i> transgenic <i>Arabidopsis</i> after heat stress.....	74
3.4.3 The expression pattern of HSPs in <i>Pabs</i> transgenic <i>Arabidopsis</i> after heat stress.....	74
3.5 Overexpression of <i>Pabs</i> gene enhances UV-C tolerance of <i>Arabidopsis</i>	

<i>thaliana</i>	75
3.5.1 <i>Pabs</i> transgenic plants were hyposensitive to UV-C.....	76
3.5.2 <i>Pabs</i> transgenic plants had a higher level of ROS in <i>Arabidopsis</i> roots.....	78
3.5.3 <i>Pabs</i> transgenic plants can reduce oxidative damage induced by UV-C by enhancing the activity of antioxidant enzymes.....	79
3.5.4 UV-C induced an accumulation of PABA in <i>Arabidopsis thaliana</i>	81
3.5.5 Overexpression of <i>Pabs</i> protects <i>Arabidopsis</i> DNA from UV-C damage...	82
3.5.6 Analysis the expression pattern of DNA damage response gene under UV-C irradiation.....	83
3.5.7 Overexpression of <i>Pabs</i> gene induces the expression of <i>DDMI</i> gene.....	85
Chapter 4 Discussion.....	86
4.1 <i>Pabs</i> gene was stably and correctly expressed in <i>Arabidopsis</i>	86
4.2 <i>Arabidopsis</i> ADCS is a key enzyme in the synthesis of PABA.....	86
4.3 PABA is a regulator of root development in <i>Arabidopsis thaliana</i>	87
4.4 Role of PABA in thermotolerance signal transduction in <i>Arabidopsis thaliana</i>	88
4.5 Mechanism of PABA in <i>Arabidopsis thaliana</i> against UV-C stress.....	89
.....	89
Chapter 5 Outlook.....	92
References.....	93
Appendix.....	108
Acknowledgment.....	111

摘要

双孢蘑菇 (*Agaricus bisporus*) 是一种产量高、消费量大、栽培广泛的食用菌。不耐高温是限制其人工栽培的主要因素。因此, 深入了解双孢蘑菇的耐温机制和培养新型种质优良的耐高温菌株, 具有十分重要的生产价值和理论意义。本实验室通过比较耐温型双孢蘑菇 02 菌株和常规双孢蘑菇 8213 菌株之间的转录组表达差异, 筛选出一个与耐温相关的基因, 对氨基苯甲酸 (PABA) 合酶基因 (*Pabs*)。该基因编码的 PABA 合酶 (PABS) 参与双孢蘑菇中 PABA 的生物合成。PABA 是合成叶酸的一个重要前体, 而叶酸是生物体内一碳单位的重要受体和供体, 在一碳单位 (C1 代谢) 转运过程中起辅酶作用; PABA 还可以显著提高双孢蘑菇的耐温特性, 然而其作用机制还不清楚。

我们通过转基因技术获得 *Pabs* 基因过表达的转基因拟南芥, 主要研究了过表达 *Pabs* 基因对拟南芥生理形态的影响, 以及探讨 *Pabs* 基因在拟南芥防御逆境胁迫的信号通路中所起的作用。

通过根癌农杆菌将双孢蘑菇 *Pabs* 基因导入拟南芥植株, 该基因整合到拟南芥基因组 DNA 中并稳定遗传至后代, 表达出具有生物活性的 PABS 蛋白, 促进植物中 PABA 的合成。我们的实验结果表明 *Pabs* 转基因拟南芥中总 PABA 浓度可达到野生型拟南芥中总 PABA 浓度的 3 倍。在拟南芥中对 *Pabs* 基因进行生物学功能分析具有一定的理论依据。

PABA 可以调节拟南芥的生长发育。拟南芥氨基脱氧分支酸合酶基因 (*adcs*) 是 PABA 合成关键基因, 该基因突变会导致胚乳败育。过表达 *Pabs* 基因对成熟拟南芥植株的表型没有明显的改变, 但是会轻微抑制拟南芥幼苗主根的生长, 表明了 PABA 参与了拟南芥根的生长调控。与之相应, 外源 100 $\mu\text{mol/L}$ PABA 能显著抑制拟南芥根的生长, 同时促进根毛的发育。另外, PABA 会导致根尖生长素的积累。进一步研究表明, 过表达 *Pabs* 基因导致根尖 ROS 水平上升, 特别是在侧根冠 (lateral root cap) 和淀粉粒细胞 (COL) 中积累了大量的 ROS。我们推测 *Pabs* 基因可能通过促进 ROS 的产生来调节植物根的生长发育。

ROS 处于各种信号转导网络的中枢位置, ROS 动态平衡的改变不仅可以调节生物体的生长和发育, 还可以提高植物体抵御生物胁迫和非生物胁迫的能力。在

拟南芥中，我们发现过表达 *Pabs* 基因会诱导 *PR-1* 的表达，但是在 *Pabs* 转基因植株中水杨酸的含量没有增加。

我们实验室之前的研究发现，在双孢蘑菇中过表达 *Pabs* 基因或者添加 PABA 可以增强双孢蘑菇对高温的抵抗能力。因此我们进一步研究了 *Pabs* 基因对拟南芥耐热特性的影响。在热激条件下，*Pabs* 转基因拟南芥下胚轴和根的生理形态和野生型相比没有明显的差异。但是在正常条件下，过表达 *Pabs* 基因会轻微诱导 HSP101 和 HSP70 的表达；而且在高温胁迫下，过表达植株中 HSP70 表达提高幅度要明显大于野生型。我们的结果表明过表达 *Pabs* 基因所导致的 HSPs 变化似乎不会引起拟南芥产生耐热的表型。

我们还发现 *Pabs* 转基因拟南芥对 UV-C 的敏感性减弱。在 UV-C 处理后 *Pabs* 转基因植株系表现出较高的相对根长和较少的白化子叶。UV-C 辐射会导致 PABA 的积累，表明 PABA 参与到植物响应 UV-C 胁迫的信号通路。过表达 *Pabs* 基因可以保护植物的 DNA，增强植株对 UV-C 的防御能力。我们认为 PABA 是从多个方面来提高植物对 UV-C 抗性。PABA 可以直接吸收 UV-C，减少 UV-C 对细胞的伤害；也可以介导 ROS 生成从而提高植物抗氧化酶 SOD 和 APX 的活性，最终减少 UV-C 诱导的 ROS 积累；最后 PABA 还诱导 *DDMI* 基因的表达，维持拟南芥 DNA 的稳定。

综上所述，本学位论文研究发现，双孢蘑菇 PABA 合酶基因 *Pabs* 能够在拟南芥中稳定遗传、表达，提高拟南芥的 PABA 水平；植物中 PABA 除了作为叶酸合成中的重要前体，还可能作为一种新型的次级代谢产物通过调节 ROS 的平衡来参与植株生长、发育和抗逆等过程，对农作物品种改良具有一定的参考意义。

关键词：对氨基苯甲酸；活性氧；紫外线

Abstract

Agaricus bisporus is a kind of edible mushroom. It is widely cultivated as it has high yield property and is consumed largely. Temperature is the main restricted factor for its growth. It thus has great economic and ecological values to study its thermotolerance mechanism and to breed a novel thermotolerant strain. Comparing the differentially expressed genes between thermal-insensitive *A.bisporus* 02 and conventional *A.bisporus* 8213 by integrative transcriptomic, *para*-aminobenzoic acid synthase gene (*Pabs*) was screened in our laboratory. It encodes a protein regulating the biosynthesis of *para*-aminobenzoic acid (PABA) in mushroom. PABA is a precursor of folate synthesis to maintain the fungi growth. Significantly, it also can enhance heat tolerance of mushroom. PABA as a mediator of environmental stress is rarely reported.

We obtained *Pabs* transgenic *Arabidopsis* by floral dip protocols. We further investigated the morphology changes and the tolerance-improving mechanisms of mushroom-derived *Pabs* on the perfect platform of *Arabidopsis*.

After being introduced into Col-1 mediated by *Agrobacterium tumefaciens*, *Pabs* can be integrated into the *Arabidopsis* genome DNA and stably inherited to the offspring. It can express the correspondingly active protein (PABS) which promotes the synthesis of PABA in plants. *Pabs* overexpressing plants displayed at best 3 times PABA increase compared to wild type.

PABA can regulate the growth and development of *Arabidopsis thaliana*. Aminodeoxychorismate synthase gene (*Adcs*) is a key gene of PABA biosynthesis in *Arabidopsis*. Homozygous *Adcs* mutants lead to defective embryos. In this study, overexpression of *Pabs* did not result in any obvious phenotypic alteration in mature *Arabidopsis*, except its slight inhibition on primary root growth. It suggested that PABA may affect the growth of *Arabidopsis* roots. Exogenous PABA (100 $\mu\text{mol/L}$ PABA) significantly decreases the length of *Arabidopsis* roots, and promotes the development of root hairs. In addition, PABA can lead to an accumulation of auxin in

root apical. Our further research found that overexpression of *Pabs* induced ROS generation in root apical, especially in lateral root cap and columella (COL). We proposed that *Pabs* might act as a novel regulator of the *Arabidopsis* roots growth and development via ROS.

ROS is a central messenger in a variety of signal transduction networks. The alteration of ROS homeostasis not only regulated the growth and development of organisms, but also improved the plants tolerance to biotic stress and abiotic stress. In *Arabidopsis*, we found that over expression of *Pabs* can induce the expression of Pathogenesis-related-1 gene (*PR-1*), while SA content was not changed.

Both overexpression of *Pabs* and exogenous PABA reinforced the thermotolerance of mushroom. However, there was no significant morphologic difference between *Pabs* overexpressing lines and wild type in heat stress. Interestingly, under normal conditions, overexpression of *Pabs* slightly induced the expression of HSP101 and HSP70. After heat shock, the relative increase of HSP70 expression in *Pabs* plants was significantly higher than that in wild type. Our experiments indicated that the improvement of HSPs expression induced by *Pabs* did not likely alter the phenotype of *Arabidopsis* in heat stress.

Interestingly, we also found that *Pabs* transgenic *Arabidopsis* was hyposensitive to UV-C irradiation. *Pabs* overexpression lines exhibited reduced root growth inhibition and cotyledon bleach upon UV-C stress. UV-C radiation could lead to the accumulation of PABA, which indicated that PABA was involved in response of UV-C stress. Reduced UV-C-induced DNA damage was observed in *Pabs* lines suggesting overexpression of *Pabs* may protect DNA against UV-C exposure. We assumed that PABA could enhanced plants resistance to UV-C in more than one aspect. PABA can directly absorb UV-C reducing the damage of UV-C radiation on cell. It also promotes ROS generation which in turn acts a signal to increase antioxidant enzymes SOD and APX activity in plants. Higher activity of SOD and APX prevented the accumulation of ROS induced by UV-C. Finally, PABA induced the *DDMI* expression, maintaining the stability of the genomic DNA.

In summary, this thesis showed that the PABA synthase gene of *Agaricus*

bisporus could be stably and inherited expressed in *Arabidopsis thaliana*, increasing the PABA level of *Arabidopsis*. Besides its role in folate synthesis, PABA may act as an active plant hormone to participate in the growth, development and environmental resistance of *Arabidopsis* by regulating the balance of ROS. Details of the underlying molecular mechanisms are needed to facilitate genetic improvements of crops.

Key words: *para*-amino benzoic acid, Reactive oxygen species, UV-C

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