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申 请 年 度：2025

4、 外语能力证书

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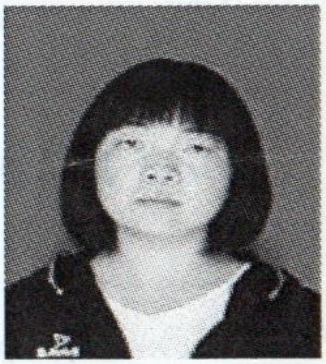

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5、 学术能力证明材料

ACTA AGRICULTURAE BOREALI-SINICA 华北农学报 · 2022,37(3):8-18

蓖麻赤霉素氧化酶基因的全基因组鉴定和表达分析

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(云南省农业科学院 经济作物研究所,云南 昆明 650205)

摘要:赤霉素氧化酶基因(GAox)是赤霉素合成和调控的关键酶,其通过调节植物活性GA水平调控植物株高。为了解析蓖麻赤霉素氧化酶基因(RcGAox),利用生物信息学分析方法对RcGAox进行全基因组鉴定,并分析其理化性质、保守结构域、系统发育、基因上游2 kb启动子区域顺式作用元件预测,通过蓖麻表达数据库以及外源赤霉素和多效唑处理2个蓖麻品种的顶端嫩茎转录组测序分析RcGAox基因表达模式。结果表明:蓖麻共有30个赤霉素氧化酶基因,其中7个RcGA2ox、4个RcGA3ox、19个RcGA20ox,蛋白质分子质量在26.12~44.31 ku,等电点预测值在5.06~7.82,内含子个数为1~2个;蛋白结构域分析保守基序Motif 1、Motif 2、Motif 4存在30条蛋白序列中;系统进化分析将RcGAox基因分为5个不同的亚群:Ⅰ、Ⅱ、Ⅲ、Ⅳ和C20 GA2ox,其中Ⅰ、Ⅱ、Ⅲ分别对应GA2ox、GA3ox、GA20ox;启动子顺式作用元件预测光反应相关的顺式元件数量最多,且在预测区域均匀分布,18个基因含1~2个赤霉素相关元件;蓖麻RcGAox在胚乳、雄花、叶片中特异表达的基因分别有7,2,1个,转录组测序结果有5个基因在嫩茎中表达,推测*RcGA2ox7*、*RcGA20ox1*和*RcGA20ox14*可能是参与赤霉素合成途径来调控蓖麻株高的主要基因,且通过调控植物体内活性赤霉素水平来响应外源激素对株高的作用。

关键词:蓖麻;赤霉素氧化酶;分子特征;生物信息学;基因表达

中图分类号:Q78;S563.03 **文献标识码**:A **文章编号**:1000-7091(2022)03-0008-11

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Genome-wide Identification and Expression Analysis of Gibberellin Oxidase Gene in *Ricinus communis* L.

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Abstract: Gibberellin oxidase gene(GAox) is a key enzyme in the synthesis and regulation of gibberellin, and effect on plant height through regulating active GA level. To analyze the gene of gibberellin oxidase in castor, we identified gibberellin oxidase gene(RcGAox) from the castor bean whole genome by bioinformatics, and analyzed physicochemical properties, conserved domain, phylogeny, promoter cis-acting element. The RcGAox gene expression pattern was analyzed by tissue specific expression of online database and apical tender stem transcriptome sequencing. A total of 30 gibberellin oxidase genes were identified from the castor genome, including 7 RcGA2ox, 4 RcGA3ox, and 19 RcGA20ox. The molecular weight was ranged from 26.12 to 44.31 ku, and the isoelectric point was ranged from 5.06 to 7.82. Gene structure analysis showed that the number of introns was ranged from 1 to 2. Protein conserved domain analysis showed that all the genes shared conserved Motif 1, Motif 2 and Motif 4. Phylogenetic analysis showed that RcGAox genes were clustered into five subfamilies Ⅰ, Ⅱ, Ⅲ, Ⅳ and C20 GA2ox, and subfamilies Ⅰ, Ⅱ, Ⅲ correspond to GA2ox, GA3ox and GA20ox respectively. Promoter cis-acting element prediction showed that light-related elements had largest number and uniform distribution in predicting region, and 18 genes had 1 to 2 gibberellin-related element. There were 7, 2, and 1 RcGAox genes specifically expressed in endosperm, male flowers and leaves respectively. Transcriptome sequencing showed that 5 genes were expressed in tender stems. It was supposed that *RcGA2ox7*, *RcGA20ox1* and *RcGA20ox14* might be the main gene involved in gibberellin synthesis pathway to regulate castor plant height. These results might provide theoretical basis for further studies on the RcGAox

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Dai M Y，Gao M，Hu Z H，Hu X L，Yang J，Li W C. Transcription analysis of elongated and shortened stem node induced by exogenous gibberellin and paclobutrazol in *Ricinus communis*[J]. Southwest China Journal of Agricultural Sciences，2024，37(12)：2669－2682. DOI：10. 16213/j. cnki. scjas. 2024. 12. 012.

外源赤霉素和多效唑诱导蓖麻伸缩茎节的转录组分析

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摘　要：【目的】旨在探索调控蓖麻株高生长的关键代谢通路及基因，以期为通过分子育种技术培育出理想株型的蓖麻新品种提供基因资源与理论依据。【方法】以外源赤霉素诱导蓖麻伸长茎节和多效唑诱导缩短茎节，通过转录组测序分析赤霉素处理后（GA vs CK）和多效唑处理后（PAC vs CK）的差异表达基因。【结果】赤霉素处理显著诱导 1539 个基因的差异表达，其中 619 个基因上调，920 个基因下调；而多效唑处理则导致 786 个基因的差异表达，包括 380 个上调基因和 406 个下调基因。GO 富集分析表明，这些差异表达基因主要富集于细胞组分构建、生物合成过程、信号传导及转录调控等关键生物学过程。特别值得注意的是，赤霉素氧化酶基因 RcGA2ox7、RcGA2ox3 在赤霉素处理下显著上调，而 RcGA20ox3 和 RcGA20ox4 则在多效唑处理下上调，这些基因通过调控体内活性赤霉素浓度，响应外源赤霉素和多效唑作用。KEGG 富集分析结果显示，两组差异表达基因的主要富集通路包括植物激素信号传导途径、淀粉和蔗糖代谢途径以及苯丙烷类生物合成途径。在植物激素信号传导途径中，生长素、细胞分裂素、赤霉素、脱落酸、乙烯等多种激素相关基因均表现出显著的差异表达。在苯丙烷类生物合成途径中，催化单木质醇聚合为大分子木质素的过氧化物酶基因 28320. t000063 和 30015. t000008 响应赤霉素处理下调表达，响应多效唑处理上调表达。GA 处理导致类黄酮合成途径的主要基因上调表达，PAC 处理与之相反，GA 处理促进类黄酮生物合成增加，导致木质素合成减少。【结论】通过转录组测序分析，成功筛选出一批可能参与蓖麻株高调控的关键基因，包括赤霉素氧化酶基因、生长素信号传导基因、木质素合成过程中的过氧化物酶基因以及类黄酮合成基因。这些发现不仅为揭示蓖麻株高调控的分子机制提供重要依据，也为未来通过基因编辑或分子育种技术优化蓖麻株型奠定基础。另外，由于外源赤霉素会干扰蓖麻生长发育，产生不利影响，蓖麻生产中应避免施用赤霉素。

关键词：蓖麻；株高；转录组分析；激素信号传导；苯丙烷类生物合成

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Transcription analysis of elongated and shortened stem node induced by exogenous gibberellin and paclobutrazol in *Ricinus communis*

DAI Meng-yuan，GAO Mei，HU Zun-hong，HU Xue-li，YANG Jin，LI Wen-chang
（Industrial Crops Institute of Yunnan Academy of Agricultural Sciences，Kunming 650205，China）

Abstract：【Objective】 The purpose of the study was to explore the key metabolic pathway and genes that regulated the castor plant height，in order to provide genetic resources and theoretical basis for breeding new varieties of ideotype by molecular breeding technology.【Method】 The exogenous gibberellin (GA) inducing castor elongate stems and paclobutrazol (PAC) inducing curtate stems were used for RNA-seq and the differentially expressed genes (DEGs) between GA vs CK and PAC vs CK were analyzed respectively.【Result】Our analysis yielded 1539 significant DEGs in the GA vs CK comparison，comprising 619 upregulated and 920 downregulated genes. Similarly，the PAC vs CK comparison identified 786 significant DEGs，with 380 upregulated and 406 downregulated genes. Gene Ontology (GO) enrichment analysis underscored the significant enrichment of these DEGs in pathways related to cellular component organization and biogenesis，signal transducer activity，and transcription factor activity. Furthermore，we observed that GA treatment upregulated the expression of gibberellin oxidase genes *RcGA2ox7* and *RcGA2ox3*，whereas PAC treatment upregulated *RcGA20ox3* and *RcGA20ox4*. These four gibberellin oxidase genes played a

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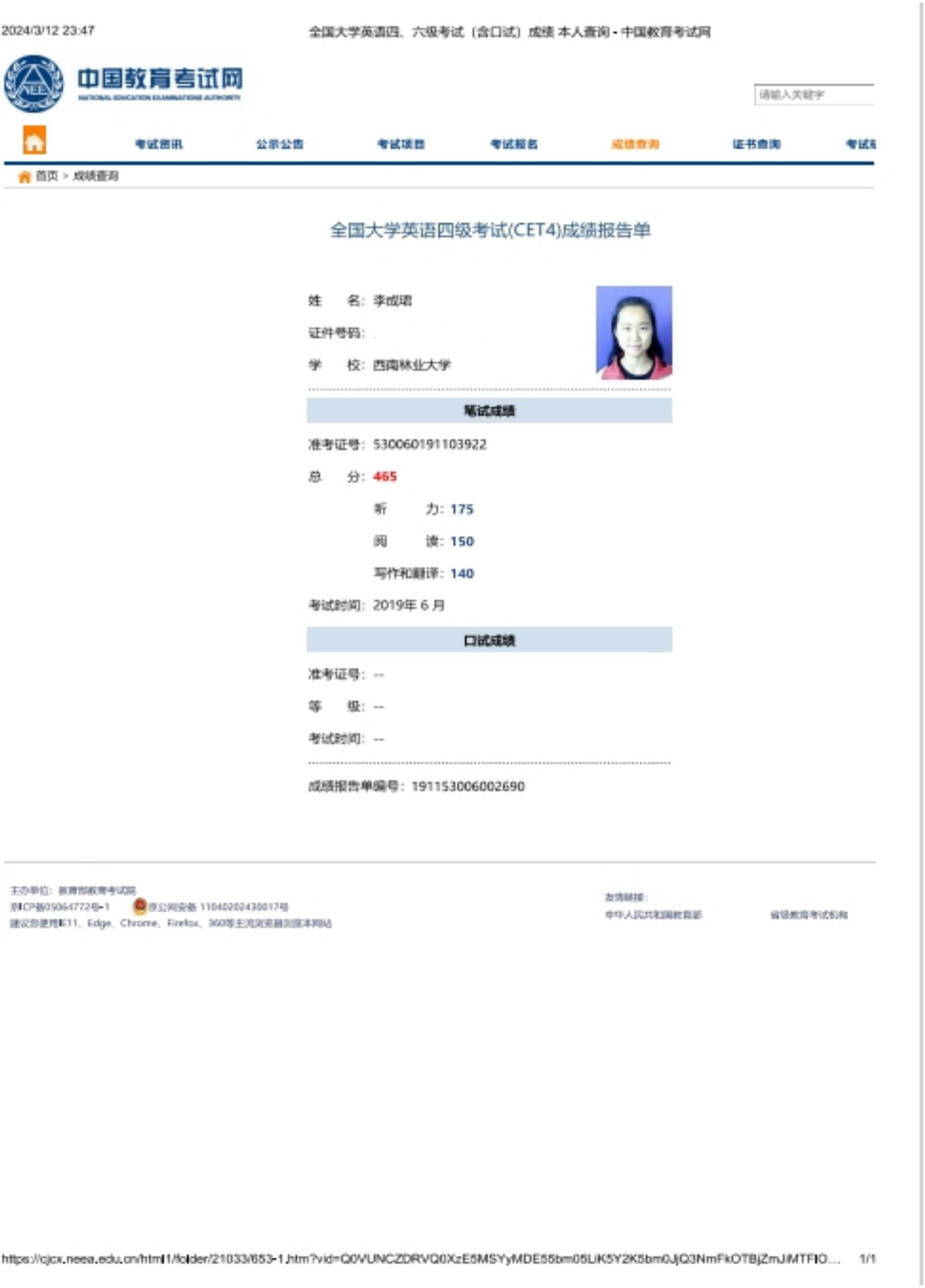
申 请 年 度：2025

4、 外语能力证书



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阅　　读：150

写作和翻译：140

考试时间：2019年 6 月

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5、 学术能力证明材料

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模拟氮沉降对木荷和卷荚相思种子萌发的影响

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[摘 要] 【目的】研究氮沉降对2种林木种子萌发的影响,为南方森林的经营管理提供参考。【方法】以木荷和卷荚相思种子为试验材料,采用室内试验,选择2、6和12 g/L硝态氮(KNO_3)、铵态氮($(NH_4)_2SO_4$)、混合氮(NH_4NO_3)进行氮沉降模拟试验,以蒸馏水为对照(CK),测定各处理木荷和卷荚相思种子的发芽率、相对发芽率、发芽指数、活力指数和发芽抑制率,在此基础上采用主成分分析综合评价不同处理对种子萌发的影响。【结果】随着KNO_3、$(NH_4)_2SO_4$、NH_4NO_3质量浓度的升高,木荷和卷荚相思种子的发芽率、相对发芽率、发芽指数和活力指数均呈下降趋势,而发芽抑制率逐渐提高。由上述5种发芽指标可知,同一氮源下,其不同质量浓度对木荷和卷荚相思种子萌发的促进作用均表现为2 g/L>6 g/L>12 g/L;同一质量浓度下,3种氮源中以$(NH_4)_2SO_4$对木荷和卷荚相思种子萌发的促进作用最佳。主成分分析结果显示,木荷和卷荚相思均以主成分1能够反映原始变量80%以上的信息,因此选择主成分1对不同处理进行综合排名,结果在木荷和卷荚相思种子各处理中综合排名第1的均为2 g/L $(NH_4)_2SO_4$处理。【结论】当质量浓度为2~12 g/L时,3种氮源对木荷和卷荚相思种子萌发的影响有差异,但最适宜的处理均为2 g/L $(NH_4)_2SO_4$。

[关键词] 木荷;卷荚相思;种子萌发;氮沉降
[中图分类号] S718.519 [文献标志码] A [文章编号] 1671-9387(2024)05-0048-09

Effects of simulated nitrogen deposition on seed germination of *Schima superba* and *Acacia cincinnata*

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Abstract:【Objective】This study investigated the effects of nitrogen deposition on seed germination of 2 tree species to provide reference for forest management in southern China.【Method】*Schima superba* and *Acacia cincinnata* seeds were selected for laboratory experiments with simulated nitrogen deposition of 2, 6 and 12 g/L nitrate nitrogen (KNO_3), ammonium nitrogen ($(NH_4)_2SO_4$) and mixed nitrogen (NH_4NO_3). Distilled water was used as control (CK). The germination rate, relative germination rate, germination index, vitality index and germination inhibition rate of *S. superba* and *A. cincinnata* seeds were determined. On this basis, principal component analysis was used to comprehensively evaluate the effects of different treatments on seed germination.【Result】With the increase of nitrate nitrogen (KNO_3), ammonium nitrogen ($(NH_4)_2SO_4$) and mixed nitrogen (NH_4NO_3) amounts, the germination rate, relative ger-

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申 请 学 院：（001）林学院

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4、 外语能力证书

Open Access Article IF 4.9 SCIE JCR Q1 生物学2区

Integrated Metabolomics and Transcriptomics Analyses Reveal the Regulatory Mechanisms of Anthocyanin and Carotenoid Accumulation in the Peel of *Coffea arabica*

by Zuquan Wang †, Chun Xie †, Yihong Wu, Haobo Liu, Xuesong Zhang, Huabo Du, Xuejun Li * and Chuanli Zhang *

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Abstract

The color of coffee fruits is influenced by several factors, including cultivar, ripening stage, and metabolite composition. However, the metabolic accumulation of pigments and the molecular mechanisms underlying peel coloration during the ripening process of *Coffea arabica* L. remain relatively understudied. In this study, UPLC-MS/MS-based metabolomics and RNA sequencing (RNA-seq)-based transcriptomics were integrated to investigate the accumulation of anthocyanins and carotenoids in the peel of *Coffea arabica* at different ripening stages: green peel (GP), green-yellow peel (GYRP), red peel (RP), and red-purple peel (RPP). This integration aimed at elucidating the molecular mechanisms associated with these changes. A total of ten anthocyanins, six carotenoids, and thirty-five xanthophylls were identified throughout the ripening process. The results demonstrated a gradual decrease in the total carotenoid content in the peel with fruit maturation, while anthocyanin content increased significantly. Notably, the accumulation of specific anthocyanins was closely associated with the transition of peel colors from green to red. Integrated metabolomics and transcriptomics analyses identified the GYRP stage as critical for this color transition. A weighted gene co-expression network analysis (WGCNA) revealed that enzyme-coding genes such as 3AT, BZ1, and lcyE, along with transcription factors including MYB, NAC, and bHLH, which interact with PHD and SET TR, may regulate the biosynthesis of anthocyanins and carotenoids, thereby influencing peel pigmentation. These findings provide valuable insights into the molecular mechanisms underlying the accumulation of anthocyanins and carotenoids in *Coffea arabica* peel during fruit maturation.

Keywords: *Coffea arabica*; fruit peel coloration; ripening process; anthocyanins; carotenoids; metabolomics; transcriptomics

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完成时间：2024 年 11 月 4 日

一、项目的技术要求

张传利发表论文被 SCI 数据库收录、影响因子、中科院分区等情况。

二、检索数据库

1.数据库：

（1）Science Citation Index Expanded（SCI-E）

（2）中国科学院文献情报中心期刊分区表

2.检索策略：题名　作者　单位　期刊名称

三、检索结论

依照用户委托，通过以上数据库检索，张传利发表的 1 篇论文：1 篇被 SCI 数据库收录，详细情况见附表（SCI 数据库收录情况）。

检索员(签字)：　　检索员职称：

云南农业大学图书馆

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2024 年 11 月 4 日

附表　SCI 数据库收录情况

序号	论文题录信息	作者排名	作者单位	文献类型	SCI 影响因子(年份)	中科院分区(大类/年份)	Top 期刊
1	Wang, ZQ (Wang, Zuquan); Xie, C (Xie, Chun); Wu, YH (Wu, Yihong); Liu, HB (Liu, Haobo); Zhang, XS (Zhang, Xuesong); Du, HB (Du, Huabo); Li, XJ (Li, Xuejun)*; **Zhang, CL (Zhang, Chuanli)***. Integrated Metabolomics and Transcriptomics Analyses Reveal the Regulatory Mechanisms of Anthocyanin and Carotenoid Accumulation in the Peel of Coffea arabica[J]. INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES, 2024, 25(19): 10754.	通讯作者	云南农业大学	Article	4.9 (2023)	生物学 2 区 (2023)	否

注：*代表通讯作者。

5、 学术能力证明材料

报告编号：(2024)YNAU00877

论文和期刊收录/引用检索报告

项目名称：张传利发表论文被数据库收录情况

委托人：云南农业大学　张传利

委托时间：2024 年 11 月 4 日

检索机构：云南农业大学图书馆

完成时间：2024 年 11 月 4 日

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Integrated Metabolomics and Transcriptomics Analyses Reveal the Regulatory Mechanisms of Anthocyanin and Carotenoid Accumulation in the Peel of *Coffea arabica*

by Zuquan Wang †, Chun Xie †, Yihong Wu, Haobo Liu, Xuesong Zhang, Huabo Du, Xuejun Li * and Chuanli Zhang *

College of Tropical Crops, Yunnan Agricultural University, Pu'er 665000, China

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† These authors contributed equally to this work.

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Abstract

The color of coffee fruits is influenced by several factors, including cultivar, ripening stage, and metabolite composition. However, the metabolic accumulation of pigments and the molecular mechanisms underlying peel coloration during the ripening process of *Coffea arabica* L. remain relatively understudied. In this study, UPLC-MS/MS-based metabolomics and RNA sequencing (RNA-seq)-based transcriptomics were integrated to investigate the accumulation of anthocyanins and carotenoids in the peel of *Coffea arabica* at different ripening stages: green peel (GP), green-yellow peel (GYRP), red peel (RP), and red-purple peel (RPP). This integration aimed at elucidating the molecular mechanisms associated with these changes. A total of ten anthocyanins, six carotenoids, and thirty-five xanthophylls were identified throughout the ripening process. The results demonstrated a gradual decrease in the total carotenoid content in the peel with fruit maturation, while anthocyanin content increased significantly. Notably, the accumulation of specific anthocyanins was closely associated with the transition of peel colors from green to red. Integrated metabolomics and transcriptomics analyses identified the GYRP stage as critical for this color transition. A weighted gene co-expression network analysis (WGCNA) revealed that enzyme-coding genes such as 3AT, BZ1, and lcyE, along with transcription factors including MYB, NAC, and bHLH, which interact with PHD and SET TR, may regulate the biosynthesis of anthocyanins and carotenoids, thereby influencing peel pigmentation. These findings provide valuable insights into the molecular mechanisms underlying the accumulation of anthocyanins and carotenoids in *Coffea arabica* peel during fruit maturation.

Keywords: *Coffea arabica*; fruit peel coloration; ripening process; anthocyanins; carotenoids; metabolomics; transcriptomics

王祖权, 谭玉龙, 郭银楠, 等. 代谢组学分析揭示成熟前后小粒咖啡果皮黄酮和类胡萝卜素差异 [J]. 食品工业科技, 2025, 46(7): 32−41. doi: 10.13386/j.issn1002-0306.2024080277
WANG Zuquan, TAN Yulong, GUO Yinnan, et al. Metabolomic Analysis Reveals Differences in Flavonoids and Carotenoids in the Peel of *Coffea arabica* L. before and after Ripeness[J]. Science and Technology of Food Industry, 2025, 46(7): 32−41. (in Chinese with English abstract). doi: 10.13386/j.issn1002-0306.2024080277

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代谢组学分析揭示成熟前后小粒咖啡果皮黄酮和类胡萝卜素差异

王祖权[1,2], 谭玉龙[1,2,+], 郭银楠[1,2], 谢　纯[1,2], 李学俊[1,2], 杜华波[1,2], 俞思莹[1,2], 张传利[1,2,*]
（1.云南农业大学热带作物学院, 云南普洱 665099;
2.云南省咖啡重点实验室, 云南普洱 665099）

摘　要: 探究小粒种咖啡果皮在成熟前后黄酮和类胡萝卜素代谢产物的变化及其在着色机制中的作用。采用超高效液相色谱-串联高分辨质谱代谢组学分析方法，并结合主成分分析、正交偏最小二乘判别分析和聚类分析，对成熟前后果皮中的黄酮和类胡萝卜素代谢产物进行特征分析。共鉴定出 234 种黄酮类代谢物和 40 种类胡萝卜素代谢物，其中黄酮醇（81 种）和叶黄素（34 种）分别为主要类型。成熟后果皮中类胡萝卜素含量显著下降，总黄酮含量变化不大，但花色苷积累模式相反。叶黄素和 *β*-类胡萝卜素在早期积累与绿色着色相关，而矢车菊素衍生物则促进成熟果皮的红色着色。此外，八氢番茄红素和六氢番茄红素在成熟果皮中特有积累。本研究解析了小粒种咖啡果皮成熟前后黄酮和类胡萝卜素积累与着色之间的关系，为咖啡果皮着色机制提供了新视角，并为咖啡副产品的加工利用及健康功能食品的开发提供了理论依据。

关键词: 小粒种咖啡, 咖啡果皮代谢组学, 代谢物积累, 黄酮类化合物, 类胡萝卜素积累
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Metabolomic Analysis Reveals Differences in Flavonoids and Carotenoids in the Peel of *Coffea arabica* L. before and after Ripeness

WANG Zuquan[1,2], TAN Yulong[1,2,+], GUO Yinnan[1,2], XIE Chun[1,2], LI Xuejun[1,2], DU Huabo[1,2], YU Siying[1,2], ZHANG Chuanli[1,2,*]
（1.College of Tropical Crops, Yunnan Agricultural University, Pu'er 665099, China;
2.Yunnan Provincal Key Laboratory of Coffee, Pu'er 665099, China）

Abstract: This study examined the changes in flavonoid and carotenoid metabolites in the peel of *Coffea arabica* L. in mature and immature stages and their roles in coloration. Ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS) was employed to analyze the metabolite profiles, in combination with principal component analysis (PCA), orthogonal partial least squares discriminant analysis (OPLS-DA), and clustering analysis. A total of 234 flavonoid metabolites and 40 carotenoid metabolites were detected, with flavonols (81 species) and lutein (34 species) being the most abundant. The total carotenoid content in the peel decreased significantly after ripening, while the total flavonoid content remained relatively stable, although anthocyanin accumulation showed the opposite pattern. Lutein and *β*-carotene accumulation in unripe peels were linked to green coloration, while cyanidin derivatives contributed to red

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*** 通信作者:** 张传利（1980−）, 男, 硕士, 教授, 研究方向: 热带作物品质形成机制及产品内在机理研究, E-mail: 2007078@ynau.edu.cn。

1. Metabolomic Analysis Reveals Differences in Flavonoids and Carotenoids in the Peel of Coffea arabica L. before and after Ripeness

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Author affiliation: (1) College of Tropical Crops, Yunnan Agricultural University, Pu'er; 665099, China; (2) Yunnan Provincal Key Laboratory of Coffee, Pu'er; 665099, China
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4、 外语能力证书

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Integrated transcriptome and metabolome analyses reveal the mechanism by which bagging treatment affects peel reddening in Orah mandarin

Ke Wen [a], Xulin Li [a], Tuo Yin [a], Chaoying Chen [b], Yinqiang Zi [b], Ke Zhao [b], Jinan Pu [c], Wenxiu Yan [c], Xuemei Wang [c], Xianyan Zhou [d,*], Xiaozhen Liu [a,*], Hanyao Zhang [b,*]

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[b] Key Laboratory of Biodiversity Conservation in Southwest China, National Forest and Grassland Administration, Southwest Forestry University, Kunming, Yunnan 650224, China
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Keywords:
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ABSTRACT

Orah mandarin is a late-ripening citrus variety that is popular with consumers because of its red peel when ripe. However, the Orah mandarin from the Yunnan production area has difficulty reddening its peel. In this study, we applied different bagging treatments to Orah mandarin at the end of fruit expansion, and by analyzing the fruits for color difference values and intrinsic qualities, we found that the white bagging (W) treatment had the best color effect and contributed to the TSS, TA, SAR of the fruits. To further investigate the reasons for the changes in the skin color of Orah mandarin, we conducted a comprehensive analysis of W treatment and unbagging fruits via transcriptomic and metabolomic techniques. Forty-nine carotenoid metabolites, in which violaxanthin laurate, apocarotenal, and β-citraurin were the main substances responsible for the red color of Orah mandarin peels, were detected via UPLC-APCI-MS/MS targeted metabolomics analysis. Eighty-one structural genes related to carotenoid biosynthesis were screened via RNA-Seq, and the transcript levels of *LYCB2*, *LUT5–6*, *ZEP9*, *ZEP5*, and *NXS2* were positively correlated with the red carotenoid content. Correlation analysis revealed that the expression levels of *HSF2*, *MYB2*, and *WRKY2* were positively correlated with those of genes and metabolites ($R^2 > 0.95$). The qRT-PCR results also verified the expression of some of the genes and transcription factors. In addition, we identified a regulatory mechanism that promotes the red color trait in the peel of Orah mandarin, where bagging treatment increased the transcript levels of genes such as *LYCB2*, *LUT5–6*, *ZEP9*, *ZEP5*, and *NXS2*, which, in turn, increased the content of apocarotenoids and carotenoids in the peel, and *HSF2*, *MYB2*, *WRKY2*, and other transcription factors (TFs) interact with the above genes and metabolites, which further positively regulate carotenoid biosynthesis. The present study provides new insights into the effects of bagging on Orah mandarin fruits to offer valuable guidance for research on fruit color and carotenoid regulation.

1. Introduction

Citrus is one of the most popular commercially available fruits on international markets because of its multiple nutritional and health benefits to humans as well as its significant economic value (Huang et al., 2023a, 2023b; Sun et al. (2024)). Data from the National Bureau of Statistics (http://www.stats.gov.cn/) show that in 2022, China's citrus planting area was 2995.81 thousand hectares, with an output of more than 60 million tons, and that the citrus industry played a vital role in the development of the national economy and rural revitalization. Owing to its characteristics such as late maturity and high sugar content, Orah mandarin (*Citrus reticulata* Blanco) has become one of the fastest-growing citrus varieties in China in recent years; it is also the prime source of income for farmers in the main producing areas (He et al., 2022; Liu et al., 2023). Citrus fruits are rich in vitamin C, phenolic compounds, minerals, essential oils, pectin, carotenoids, flavonoids, and dietary fiber, which are crucial for preventing and treating a variety of diseases, including cancer, inflammation, diabetes, and cardiovascular

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Research Paper

Genome-wide identification of carotenoid cleavage oxygenase genes in Orah mandarin and the mechanism by which *CrCCD4b1* affects peel color

Ke Wen [a,1], Xulin Li [a,1], Tuo Yin [a], Chaoying Chen [b], Ling Zhu [b], Yinqiang Zi [b], Ke Zhao [b], Xianyan Zhou [c], Xiaozhen Liu [a,*], Hanyao Zhang [a,b,*]

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CrCCD4b1

ABSTRACTS

Color is a crucial component of the commercial value of citrus fruits. Carotenoid cleavage oxygenases (*CCOs*) can affect fruit color by oxidative cleavage of different carotenoid sites, resulting in various colors. This study proposed a genome-wide analysis of the Orah mandarin CCO gene family using bioinformatics methods and combined physiological, transcriptomic, and metabolomic data to analyze the gene expression levels and carotenoid accumulation mechanisms of different colored peel. A total of 14 *CCOs* were identified in the Orah mandarin genome. Phylogenetic analysis revealed that *CrCCOs* can be classified into six subfamilies, and the gene structure and conserved motifs support the above classification. GO and KEGG functional annotation revealed that Orah mandarin *CCO* genes play crucial roles in carotenoid synthesis and catabolism. Transcriptomic data showed that the expression level of *CrCCD4b1* was positively correlated with the current status of Orah mandarin red flavedo. Physiological and metabolomic studies further revealed that apocarotenal and β-citraurin were identified as the key metabolites controlling the change in flavedo color from yellow to red. Correlation analysis revealed *CrCCD4b1* as a crucial gene in the apocarotenal and β-citraurin expression network. For the first time, we proposed *CrCCD4b1* as a potential model for increasing red carotenoid accumulation in the flavedo by promoting the biosynthesis of C30 carotenoids (apocarotenal, β-citraurin) in Orah mandarin. This study will lay the foundation for further research on the causes of differences in peel color and the mining of crucial genes regulating the red trait.

1. Introduction

Citrus is one of the world's four main fruits. In 2022, China had a citrus planting area of more than three million hectares, an output of more than 60 million tons, and a peel color as a crucial indicator of the quality of the consumer's most intuitive sensory experience, which directly determines the economic benefits of the fruit (M.J. Rodrigo et al., 2013; Sun et al., 2023). The peels of mature citrus fruits are sometimes red and sometimes yellow or orange, which affects the market popularity of the fruits. In the production process, researchers have found significant differences in fruit appearance and coloration between different fruiting parts of trees, in which light conditions may be the main influencing factor (Jia et al., 2021).

The development of citrus fruit color is physiologically related to chlorophyll, carotenoid, and anthocyanin metabolism, of which the content and composition of carotenoids are the main factors affecting coloration in most citrus (Huang et al., 2022). Carotenoids are formed with 40 carbon atoms as the fundamental skeleton and isoprene as the basic unit connected by multiple conjugated double bonds, and the number of conjugated double bonds is closely related to the color of carotenoids (Sun et al., 2022). Carotenoid cleavage oxygenase (*CCO*) genes are widely involved in fruit color regulation (Ni et al., 2023). *CCOs* are vital enzyme-encoding genes in the carotenoid degradation process that can oxidatively cleave carotenoids at different sites, thereby

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Genome-wide analysis of pentatricopeptide repeat genes in castor and their potential functions in seed development

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ABSTRACT

Pentatricopeptide repeat (PPR) proteins, defined by tandem 35 amino acids helical motifs, are pivotal regulators of RNA recognition and processing in plant organelles, especially plastids and mitochondria, thereby shaping plant development. However, their structural diversity and functional roles remain poorly understood, particularly in non-model oilseed crops. Castor (*Ricinus communis* L.), a non-edible yet industrially important oilseed species, provides an ideal system to explore *PPR* gene family characteristics. In this study, we identified 434 *RcPPR* genes and comprehensively analyzed their motif composition, RNA-binding site preferences, and subcellular localization. Although conserved amino acid patterns were observed across different PPR subclasses, the canonical residues at positions 5 (N) and 35 (D/N), previously considered critical for nucleotide recognition, showed notable variation, implying flexibility in RNA-binding specificity. Chromosomal mapping revealed that *RcPPR* were frequedtly clustered in transposon-rich regions, suggesting transposable element-mediated gene family expansion. Phylogenetic and evolutionary analyses indicated that *RcPPR* are conserved across angiosperms but have experienced rapid evolution, as evidenced by elevated *Ks* values, likely driven by functional diversification. Subcellular localization predictions and experimental validation in castor leaf protoplasts confirmed predominant targeting of RcPPR proteins to mitochondria and plastids. Expression profiling revealed strong tissue specificity, with 116 *RcPPRs* exhibiting seed-specific expression and co-expression with genes involved in seed development and oil biosynthesis. Collectively, this study provides new insights into the structure, evolution, and functional relevance of PPR proteins in castor, enriching our understanding of organelle RNA regulation in developing oilseed crops.

1. Introduction

Pentatricopeptide repeat (PPR) proteins represent one of the largest families of RNA-binding proteins and play essential crucial roles in divers developmental processes in plants (Kwok van der Giezen et al., 2024). These proteins primarily regulate gene expression in plant organelles—namely plastids and mitochondria—at the post-transcriptional level, involving RNA stabilization, splicing, cleavage, editing, and translation (Prikryl et al., 2011; Small et al., 2020; Huynh et al., 2023; Chen et al., 2019; Wang et al., 2022).

PPR proteins are characterized by tandem arrays of helical motifs, each approximately 35 amino acids (AAs) in length, typically occurring in 2–27 times per protein. These motifs adopt an antiparallel α-helical fold structure that forms a helix-turn-helix architecture, which collectively generates a superhelical conformation with a central groove for RNA-binding (Hayes et al., 2012). Each motif recognizes specific nucleotides through key AAs at positions 5 and 35 (Wang and Tan, 2024). Based on structural differences, PPR proteins are classified into two major subfamilies: P and PLS (Kwok van der Giezen et al., 2024). The P subfamily comprises canonical tandem P motifs, whereas the PLS subfamily contains additional L (longer) and S (shorter) variants of PPR motifs. Based on their C-terminal domains, PLS proteins are further

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