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姓　　名：吕燕荣

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Article

Identification the Pathogens Causing Rot Disease in Pomegranate (*Punica granatum* L.) in China and the Antifungal Activity of Aqueous Garlic Extract

Xingqi Li †, Xinyue Lu †, Yiheng He, Mengting Deng and Yanrong Lv *

College of Horticulture, Northwest A&F University, Yangling 712100, China; 15664663605@163.com (X.L.); 18291703884@163.com (X.L.); hyh930111@163.com (Y.H.); mengtingd2019@163.com (M.D.)

* Correspondence: rongly@nwafu.edu.cn; Tel.: +86-181-9102-0381

† The authors contribute equally to this article.

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Abstract: Rot disease is a serious disease in pomegranate (*Punica granatum* L.) plantations in China. This disease usually weakens tree vigor, and seriously reduces the ornamental value, fruit yield, and quality. A better understanding of the pathogen that causes a disease is important for its control. Thus, the aims of this study were to isolate and identify the pathogen causing rot disease and to explore substances for its biological control. In this study, the morphology of the hyphae and spores of the pathogens was observed, and the pathogens were identified by morphological characteristics and the internal transcribed spacer (ITS) regions of their rDNA. Furthermore, the activity of an aqueous garlic extract as antifungal treatment for the identified pathogens was assessed. The results showed that the pathogens causing soft rot and dry rot in 'Xinjiang Big Seed' pomegranate were most probably *Aspergillus niger* and *Botryosphaeria dothidea*, respectively. In addition, the pathogenicity of *A. niger* was stronger than that of *B. dothidea.* The aqueous garlic extract had a strong antifungal effect on both pathogens by inhibiting mycelium growth in vitro, and the minimum inhibitory concentrations against *A. niger* and *B. dothidea* were 7.5 mg/mL and 10 mg/mL, respectively.

Keywords: pomegranate; rot disease; pathogen identification; antifungal activity; garlic extract

1. Introduction

Pomegranate (*Punica granatum* L.) trees have a long cultivation history. This species has strong adaptability and a wide cultivation area. They are widely planted in Shandong, Shaanxi, Henan, and Xinjiang provinces in northern China, and there is also a certain amount of pomegranate cultivation in southern China [1]. The pomegranate tree is one of the preferred trees for landscape greening and has high ornamental value. The pomegranate fruit, which is usually consumed either as fresh fruit or fruit juice, is nutritious with a high content of carbohydrates, flavonoids, vitamins, folic acid, potassium, iron, and other mineral substances [2,3]. In addition, the pomegranate fruit has relatively high amounts of pharmacologically and medicinally bioactive compounds, which contribute to the hypolipidemic, antioxidant, antiviral, anticancer, antibacterial, and vascular protection effects [3–6]. A recent study has also shown that the pomegranate metabolite Urolithin A(UroA) can alleviate inflammatory bowel disease [7]. Therefore, pomegranate fruit is increasing popular with consumers, and the market demand has recently risen. Nevertheless, pomegranate trees and fruit are susceptible to either bacterial or fungal diseases during growth. Of these diseases, rot disease is possibly the most serious one in China. The rot disease weakens tree vigor and leads to lower ornamental and greening value. In addition, the fruit yield and quality can be seriously reduced in the pomegranate cultivations. On the whole, the ecological and economic benefits of pomegranate trees are severely restricted by rot disease

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检索报告

根据委托人吕燕荣委托，通过网络检索，吕燕荣发表的1篇论文被《科学引文索引》扩展版（SCI-Expanded）数据库收录。数据库具体检索结果如下：

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作者:Li, XQ (Li, Xingqi)[1] ; Lu, XY (Lu, Xinyue)[1] ; He, YH (He, Yiheng)[1] ; Deng, MT (Deng, Mengting)[1] ; Lv, YR (Lv, Yanrong)[1]

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通讯作者地址: Lv, YR (通讯作者)

Northwest A&F Univ, Coll Hort, Yangling 712100, Shaanxi, Peoples R China.

地址:[1] Northwest A&F Univ, Coll Hort, Yangling 712100, Shaanxi, Peoples R China

电子邮件地址:18291703884@163.com; 15664663605@163.com; hyh930111@163.com; mengtingd2019@163.com; rongly@nwafu.edu.cn 研究方向:Forestry

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第一作者李星奇，第一署名单位西北农林科技大学园艺学院。

通讯作者吕燕荣，署名单位西北农林科技大学园艺学院。

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Effect of ozone application on bioactive compounds of apple fruit during short-term cold storage

Yanrong Lv[a,b,*], Ibrahim I. Tahir[b], Marie E. Olsson[b]

[a] College of Horticulture, Northwest A&F University, Yangling, Shaanxi, 712100, China
[b] Department of Plant Breeding, Swedish University of Agricultural Sciences, P.O. Box 101, SE-23053 Alnarp, Sweden

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ABSTRACT

Recently ozone has been used to decrease postharvest disease in different fruits. The effects of different pre-storage ozone treatments combined with cold storage on concentrations of triterpenes, phenolic compounds as well as quality attributes in two apple cultivars, 'Amorosa' and 'Santana', were investigated. The results showed that overall the changes caused by ozone treatment were within the range of fluctuations normally occurring in untreated apples during storage. Ursolic acid concentration in the apple peel of both cultivars was not affected by any ozone treatment, while oleanolic acid showed cultivar-specific changes. After one month of storage, the concentration of total phenols in the peel of both cultivars was decreased 11 to 16% by gaseous ozone as well as ozonated water treatment, mainly due to decreased total flavonols concentration; while no differences were found in the concentration of total phenols in the apple flesh by ozone treatments. Procyanidin B2 and (-)-epicatechin were only detectable in 'Amorosa' but not in 'Santana'.

1. Introduction

In recent years, there has been growing interest in health benefits from plant food and increasing number of investigations of plant bioactive compounds (Barbieri et al., 2017; Pandey et al., 2017). Apples (*Malus domestica* Borkh), as one of the most popular fruits in the world, contain different kinds of phytochemicals, including triterpenes and various phenolic compounds (Andre et al., 2012; Rysman et al., 2016). In apple peel, oleanolic acid and ursolic acid account for 0.28% and 1.43% of the dry weight respectively (Jäger et al., 2009). A study on 109 cultivars of apples, originating from both New Zealand and Luxembourg, showed that the polyphenolics in apple peel analyzed by high-performance liquid chromatography (HPLC) ranged from 29 to 7882 mg kg^{-1} fresh weight; while in the flesh, the polyphenolics concentration was in average lower (733–4868 mg kg^{-1} fresh weight) (Andre et al., 2012). Many studies have linked dietary intake of polyphenols and triterpenes to reductions in the risk of different chronic diseases, such as different types of cancers and cardiovascular disease (Del Rio et al., 2013; Koeberle et al., 2018; Leem et al., 2018). Different investigations have shown that the concentration of phenolics and triterpenes in apples varies with cultivar, the part of the fruit, maturity stage, environmental conditions during growth, storage and processing conditions (Kevers et al., 2011; Carbone et al., 2011; Boyer and Liu, 2004; Lv et al., 2016).

Ozone is a strong oxidant and antimicrobial agent (Kim et al., 1999) and it has been approved for use as a food additive by the Food and Drug Administration (USFDA) in the United States (USFDA, 2001). Previously, it has been suggested that ozone can be used as an alternative sanitizer to replace the traditional chlorine (Bott, 1991). The advantages of ozone include a more effective function against a wide range of microorganisms and the fact that it does not result in any chemical residues (Graham, 1997; Xu, 1999); therefore, ozone is accepted by many organic grower organizations (Horvitz and Cantalejo, 2014). Recent investigations have shown that suitable level of ozone in storage can control pathogenic diseases and extend the shelf life of fruits and vegetables, such as kiwifruit, mulbeery, citrus, tomato and so on. (Barboni et al., 2010; Boonkorn et al., 2012; García-Martín et al., 2018; Minas et al., 2014; Tabakoglu and Karaca, 2018). However, ozone as a highly reactive compound might cause physiological changes in crops, including tissue damage, induction of defense mechanisms, induced ethylene production and increased respiration (Forney, 2003). Besides, human might get ccute and chronic health effects when exposured to ozone (Heidi et al., 2018). Therefore, the World Health Organization (WHO) has provided a guideline value of 100 μg/m^3 as the maximum 8 h mean ozone concentration in order to protect human health (WHO, 2006). When the zone applied in the practice, the health guideline should be concerned. According to previous studies, two main application modes of ozone are treating

* Corresponding author at: College of Horticulture, Northwest A&F University, Yangling, Shaanxi, 712100, China.
E-mail addresses: rongly@nwsuaf.edu.cn (Y. Lv), ibrahim.tahir@slu.se (I.I. Tahir), marie.olsson@slu.se (M.E. Olsson).

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标题:Effect of ozone application on bioactive compounds of apple fruit during short-term cold storage

作者:Lv, YR (Lv, Yanrong)[1,2] ; Tahir, II (Tahir, Ibrahim I.)[2] ; Olsson, ME (Olsson, Marie E.)[2]

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通讯作者地址: Lv, YR (通讯作者)

Northwest A&F Univ, Coll Hort, Yangling 712100, Shaanxi, Peoples R China.

地址:

[1] Northwest A&F Univ, Coll Hort, Yangling 712100, Shaanxi, Peoples R China

[2] Swedish Univ Agr Sci, Dept Plant Breeding, POB 101, SE-23053 Alnarp, Sweden

电 子 邮 件 地 址 :rongly@nwsuaf.edu.cn; ibrahim.tahir@slu.se; marie.olsson@slu.se 研究方向:Agriculture

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第一作者吕燕荣，第一署名单位西北农林科技大学园艺学院。

通讯作者吕燕荣，署名单位西北农林科技大学园艺学院。

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Exogenous γ-Aminobutyric Acid Treatment That Contributes to Regulation of Malate Metabolism and Ethylene Synthesis in Apple Fruit during Storage

Shoukun Han, Yuyu Nan, Wei Qu, Yiheng He, Qiuyan Ban, Yanrong Lv,* and Jingping Rao*

College of Horticulture, Northwest A&F University, Yangling, Shaanxi 712100, China

Supporting Information

ABSTRACT: Organic acid is an important indicator of fruit quality, and malate is the predominant organic acid in apple fruit. However, the regulation of malate metabolism in postharvest fruit is rarely reported. Here, we found that, compared with a control treatment, a 10 mM γ-aminobutyric acid (GABA) treatment remarkably delayed the loss of tiftratable acidity and malate and increased the succinate and oxalate contents in "Cripps Pink" fruit stored in polyethylene bags at room temperature. The higher malate levels in GABA-treated fruit were accompanied by higher activities of cytosolic nicotinamide adenine dinucleotide-dependent malate dehydrogenase (cyNAD-MDH) and phosphoenolpyruvate carboxylase (PEPC) but lower cytosolic NAD phosphate-dependent malic enzyme (cyNADP-ME) and phosphoenolpyruvate carboxykinase (PEPCK) activities than those seen in control fruit. Notably, ethylene production was significantly reduced by GABA treatment, paralleling the downregulation of *MdACS*, *MdACO*, and *MdERF* expression. Meanwhile, GABA treatment also enhanced the activity of the GABA shunt and promoted the accumulation of GABA. This study provides new insights into the regulation of malate metabolism and reports for the first time the possible interplay between GABA and ethylene signaling pathways in apple fruit during postharvest storage.

KEYWORDS: *apple, γ-GABA, malate metabolism, ethylene, postharvest storage*

INTRODUCTION

Organic acids are important indicators of fruit taste and, together with sugars and aromatic volatiles, determine flavor quality.[1] Generally, the level of organic acids in fruits increases gradually during the process of fruit growth and development but decreases continuously during ripening and postharvest storage.[2,3] In most fruits, malate and citrate, which technically are the conjugate bases of malic acid and citrate acid and are used to refer to all physiological forms of each compound, are considered the main organic acids.[4]

Malate is the predominant organic acid in apple fruit[5] and is involved in various metabolic pathways in fruit cells, including glycolysis, the tricarboxylic acid (TCA) cycle, gluconeogenesis, the glyoxylate cycle, and the synthesis of complex secondary metabolites.[6] Previous studies showed that malate appeared to have a wide range of important functions, such as regulating cytosolic pH, controlling stomatal aperture, balancing cellular energy supply, and increasing resistance to heavy metal toxicity.[7−10] Notably, malate tends to play an important role in postharvest softening and susceptibility to bacterial infection.[11] In recent years, studies on the accumulation and metabolism of malate in fruit cells confirmed that cytosolic nicotinamide adenine dinucleotide-dependent malate dehydrogenase (cyNAD-MDH) and phosphoenolpyruvate carboxylase (PEPC) dominated malate biosynthesis by catalytic reactions, whereas malate degradation was attributed to cytosolic NAD phosphate-dependent malic enzyme (cyNADP-ME) and phosphoenolpyruvate carboxykinase (PEPCK), which would encourage phosphoenolpyruvate (PEP) formation from oxaloacetate (OAA), thereby allowing malate to synthesize OAA.[3,5,6,12,13]

Many studies have been carried out to identify the factors that influence the formation of fruit acidity. Environmental temperature, water supply, and mineral fertilization have all been shown to regulate the metabolism and accumulation of organic acids during the process of fruit growth and development.[4,14−16] However, the regulation of malate metabolism in postharvest fruit is still unclear. Liu et al.[17] and Bekele et al.[18] reported that 1-methylcyclopropene (1-MCP) treatment significantly retains the level of malate in apple fruit during storage. Similarly, exogenous ethylene application leads to an increase in malate degradation relative to the untreated apple fruit during storage, which is attributed to the increase in respiratory consumption.[19] More extensive studies are urgently needed.

γ-Aminobutyric acid (GABA), a nonprotein amino acid, is recognized as a plant signaling molecule.[20] GABA is synthesized via the GABA shunt pathway, which extends from the TCA cycle. To date, studies have shown that glutamate decarboxylase (GAD), GABA transaminase (GABA-T), and succinate semialdehyde dehydrogenase (SSADH) are all involved in GABA metabolism.[21,22] In the cytosol, GABA synthesis from glutamate is irreversibly catalyzed by GAD, and GABA is then transported into the mitochondria, where it is oxidized by GABA-T and SSADH to produce succinate, which finally flows back to the

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作者:Han, SK (Han, Shoukun)[1] ; Nan, YY (Nan, Yuyu)[1] ; Qu, W (Qu, Wei)[1] ; He, YH (He, Yiheng)[1] ; Ban, QY (Ban, Qiuyan)[1] ; Lv, YR (Lv, Yanrong)[1] ; Rao, JP (Rao, Jingping)[1]

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通讯作者地址: Lv, YR; Rao, JP (通讯作者)

Northwest A&F Univ, Coll Hort, Yangling 712100, Shaanxi, Peoples R China.

地址:[1] Northwest A&F Univ, Coll Hort, Yangling 712100, Shaanxi, Peoples R China

电子邮件地址:rongly@nwafu.edu.cn; raojingpingxn@163.com

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第一作者 韩寿坤，第一署名单位西北农林科技大学园艺学院。

通讯作者吕燕荣，署名单位西北农林科技大学园艺学院。

通讯作者饶景萍，署名单位西北农林科技大学园艺学院。

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五. 项目执行期间，甲、乙双方各自取得的研究成果及其相关知识产权归各自单独所有，但甲方有权因非商业目的（如：政府性会议、报告、文件、统计资料等）使用乙方信息。甲乙双方共同实质性研究形成的成果（专利、论文、奖励等）由双方另签协议约定知识产权共享。

六. 项目执行过程中，乙方任何重大调整（如任务考核指标调整、经费预算调整等）都应及时通知甲方，报项目承担单位和专业机构批准后签署补充协议。补充协议应对调整后的各方责任义务进行约定，与本协议具有同等效力。

七. 本协议自双方盖章（签字）之日起生效，有效期至课题验收合格之日。协议一式肆份，项目负责人、课题负责人、子课题负责人和甲方科技管理部门各执一份，具有同等法律效力。未尽事宜由双方协商解决。

甲方（单位盖章）：

法人代表：

课题负责人：雷玉山

2020 年 11 月 23 日

乙方（单位盖章）：

法人代表：

子课题负责人：吕燕东

2020 年 11 月 23 日

资助类别： 一般项目（青年）

申请代码： C.生命科学-C15.园艺学与植物营养学-C1505.园艺作物采后生物学

项目编号： 2019JQ-476

9051307370l2

陕西省自然科学基础研究计划

项目合同（任务）书

项目名称： UV-B照射对苹果果实三萜合成关键酶OSC的分子调控

承担单位： 西北农林科技大学 （盖章）

项目负责人： 吕燕荣

起止时间： 2019-01-01至2020-12-31

填报日期： 2019年05月13日

西北农林科技大学

正式版

陕西省科学技术厅 制

八、本合同签约各方

甲方：陕西省科学技术厅

业务处室负责人（签章）：

业务处室经办人（签章）： 电话：

通讯地址：

邮编：

（盖章）

陕西省科学技术厅 科研合同专用章

2019-05-29

乙方：西北农林科技大学

单位负责人（签章）：

项目负责人（签章）：

通讯地址：陕西省杨凌示范区邰城路3号 电话：029-87080002

邮编：712100

（盖章）

2019年6月10日

正式版

计划类别： 青年人才农业推广实践   项目编号：TGZX2018-40

西北农林科技大学

试验示范站（基地）科技成果推广项目

合 同 书

项目名称：苹果果实成熟度检测APP的研发与推广

承担单位：园艺学院

依托基地：宝鸡千阳苹果试验示范站

项目负责人：吕燕荣

联系电话：18191020381

电子信箱：rongly@nwafu.edu.cn

起止年限：2018年10月— 2020年9月

西北农林科技大学科技推广处印制

西北农林科技大学校长办公室文件

办发〔2019〕11号

关于公布2019年本科生全英文教学课程建设项目评审结果的通知

各学院（系、部）：

根据《关于开展2019年本科生全英文教学课程建设项目申报工作的通知》要求，经教师申请、学院推荐、学校组织专家评审、校内公示，共遴选出《果蔬营养与分析》等15门课程为2019年本科生全英文教学课程立项建设项目，现将结果予以公布（见附件）。

请各学院（系、部）按照《西北农林科技大学本科教学质量工程项目管理办法》的有关规定，加强项目管理，提供必要的支

持和服务，确保项目顺利执行。课程负责人认真对照课程建设要求，把握好工作进度，合理使用经费，保证按期高质量完成课程建设任务。

附件：2019 年本科生全英文教学课程建设项目立项名单

校长办公室

2019 年 7 月 11 日

抄送：校领导。

西北农林科技大学校长办公室　　　　2019 年 7 月 11 日印发

2019年本科生全英文教学课程建设项目拟立项名单

序号	学院	课程中文名称	课程英文名称	课程类型	修课方式	课程负责人		课程参与人					备注
						姓名	职称						
1	园艺学院	果蔬营养与分析	Nutrition and Analysis of Fruits and Vegetables	专业课	选修	吕燕荣	讲师	饶景萍	何玲	刘翠华	周会玲		
2	动科学院	动物营养学	Animal Nutrition	学科基础课	必修	杨小军	教授	姚军虎	曹阳春	任周正	雷新建		
3	动医学院	动物病理解剖学	Animal Anatomic Pathology	学科基础课	必修	黄勇	教授	童德文	赵晓民	常玲玲	杜谦		
4	动医学院	免疫生物学	Immunobiology	专业课	选修	南雨辰	副教授	武春燕	周恩民	赵钦			
5	园林学院	盆景艺术	Bonsai Art	专业课	选修	宋军阳	副教授	弓弼	刘雅莉	刘建军	李厚华		
6	资环学院	生态学	Ecology	学科大类基础课	必修	蒋锐	副教授	李志	顾江新	杨晓梅	张建国		
7	水建学院	工程项目管理	Engineering Project Management	专业课	选修	孟秦倩	副教授	陈俊英	刘志明				
8	生命学院	发酵工程	Fermentation Engineering	专业课	选修	张江波	讲师	史鹏	贾志华	舒敦涛			
9	生命学院	生物反应工程	Bioreaction engineering	学科基础选修课	选修	侯文洁	讲师	秦宝福					
10	理学院	大学物理(农林生物类)	College Physics (Biological Specialty)	通识类	必修	朱杰	副教授	党亚爱	杜光源	胥建卫	陈莹莹		
11	化药学院	有机化学（I）	Organic Chemistry(I)	学科大类基础课	必修	陈淑伟	副教授	谢卫青	常明欣	尹霞	杨芳	王正审	
12	经管学院	农产品贸易与政策	Agricultural products trade and policy	学科基础课	必修	张寒	副教授	李敏	马红玉	刘军弟			
13	经管学院	管理沟通与谈判	Management communication and negotiation	专业课	必修	梁洪松	副教授	张晓妮	李桦	刘超	王谊		

14	人文学院	公共政策学	Public Policy	学科基础课	必修	梁运娟	讲师	赵丹	刘利鸽	张世勇	陈航英		
15	葡酒学院	葡萄酒工程学	Enological Engineering	专业课	必修	杨继红	副教授	李华	陶永胜	来疆文			

CULSC

第二届全国大学生生命科学竞赛

获奖证书

获奖项目： 石榴腐烂病病原菌鉴定及抑菌研究

获 奖 者： 逯新月 李星奇 张凡 王嘉萱

指导教师： 吕燕荣

获奖单位： 西北农林科技大学

获奖等级： 一等奖

证 书 号： CULSC201801031

教育部高等学校大学生物学课程教学指导委员会

教育部高等学校生物科学类专业教学指导委员会

教育部高等学校生物技术、生物工程类专业教学指导委员会

《高校生物学教学研究(电子版)》

全国大学生生命科学竞赛委员会 CULSC

二〇一八年十一月

李星奇同学完成的《UV-B 照射对苹果三萜含量及重要合成酶基因表达的影响》毕业论文被评为 2020 届本科"校级优秀毕业论文"。

特发此证

西北农林科技大学

教务处

2020 年 6 月 20 日

荣誉证书

吕燕荣 同志被评为西北农林科技大学2018年度大学生社会实践优秀指导教师。

特发此证，以兹鼓励。

中共西北农林科技大学委员会

二〇一八年十二月二十日

荣誉证书

吕燕荣　老师被评为2018年度大学生创新创业优秀指导老师。

特发此证，以兹鼓励。

中共西北农林科技大学委员会

二〇一八年十二月