高通量测序技术在害虫和害螨抗药性研究中的应用

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摘要：螨类是陆生动物中仅次于昆虫的第2大类群。长期以来，对害螨抗药性的研究都落后于害虫。近年来，随着高通量测序技术的出现，开辟了害虫和害螨抗性机理研究和抗性监测的新局面。本文概述了高通量测序技术应用于害虫和害螨抗药性机理研究已取得的进展，介绍了利用高通量测序技术开发的抗药性监测新方法，并提出了害虫和害螨抗药性机理研究和田间种群抗性水平监测的发展方向。

关键词：高通量测序技术；抗药性机理；抗药性监测；害虫；害螨

Application of high-throughput sequencing in the study of acaricide resistance

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Abstract: Mites are the second-largest group of terrestrial animals following insects. The research in acaricide resistance of mites, however, has been substantially lagged behind. In recent years, the advent of genomics era has afforded researchers with high-throughput sequencing technologies to address outstanding biological questions. In this review, we summarized the current progresses in mechanistic studies in acaricide resistance of pest insects and mites and resistance monitoring using high-throughput sequencing approaches. Finally, we concluded this review by sharing our perspectives on future research avenues.

Key words: high-throughput sequencing; acaricide resistance mechanism; resistance monitoring; insect pests; mites

自化学农药问世以来，化学防治始终是有害生物防治的重要手段。然而化学农药盲目、大量、连续和单一使用导致害虫抗药性的发生，成为当前病虫害防治工作中最大的障碍。因此，害虫抗药性形成的内在机理一直是昆虫毒理学的研究热点（Li et al.，2007；Heckel，2012）。以前关于害虫抗药性机理的研究仅局限在单一基因或单一机制，高通量测序技术的出现使科研人员能系统地研究害虫抗药性产生的分子机理，避免了单一基因和单一机制的局限性和盲目性；同时，利用高通量技术分析害虫抗药性分子标记能快速、准确、便捷地监测田间种群的抗药性水平（Riga et al.，2017；陈龙飞等，2020；李飞，2021）。随着高通量测序技术成本的不断下降，利用高通量测序技术研究害虫抗药性机理和监测田间种群抗药性

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水平已逐渐成为害虫抗药性治理的新理念和新策略。

1 害虫和害螨对杀虫剂的抗性机理

害虫对杀虫剂的抗性机理主要体现在3个方面:第一,药剂在害虫表皮的穿透率下降导致抗药性的产生,主要是由于害虫表皮相关因子表达量的增加阻遏了药剂的快速渗透(韩召军等,1995; Matsumura,2010;Zhu et al.,2013);第二,害虫体内解毒代谢酶活力升高而引起抗药性的产生,主要是因为相关代谢酶的表达量上升(Bajda et al.,2015;Feyereisen et al.,2015);第三,害虫体内药剂直接作用的靶标分子产生突变,使得药剂对作用靶标敏感性降低而使昆虫表现出抗性(Carvalho et al.,2012;Cassanelli et al.,2015;Mermans et al.,2017)。

2 高通量测序技术在抗药性机理研究中的应用

最初,人们对害虫抗药性机理的关注点仅局限于某一个基因上,然而,越来越多的研究表明大多数抗药性是多个基因、多重机制参与的复杂生物进化现象,很少存在某一个或一类已知基因能够完整阐明抗药性机制(van Leeuwen & Dermauw,2016;Riga et al.,2017)。因此,全面系统寻找抗药性相关基因并探寻其参与抗药性的机制是有害生物防治环节中亟待解决的问题。近年来,随着现代生物技术的发展,高通量测序技术的出现使大规模筛选抗药性相关基因、从整体层面研究抗药性机理成为可能。利用昆虫基因组挖掘大量与抗药性相关的基因,再结合转录组进行抗药性基因功能研究和抗药性水平检测,能够加深对昆虫抗药性机制的理解,更全面地揭示昆虫抗药性机理和现状。

目前,已有大量关于高通量测序技术应用于害虫体内抗性相关基因的挖掘和表达差异性分析的研究报道,主要集中在农业害虫和卫生害虫,如小菜蛾*Plutella xylostella*(He et al.,2012)、飞蝗*Locusta migratoria*(Jiang et al.,2012)、大豆蚜*Aphis glycines*(Bai et al.,2010)、烟粉虱*Bemisia tabaci*(Wang et al.,2010)、灰飞虱*Laodelphax striatellus*(Xu et al.,2013)、臭虫*Cimex lectularius*(Mamidala et al.,2012)、冈比亚按蚊*Anopheles gambiae*(Nkya et al.,2014)和家蝇*Musca domestica*(Mahmood et al.,2016)等。以此为基础,昆虫抗药性相关的新基因陆续被发现,取得了许多突破性进展。2020年,中国农业科学院蔬菜花卉研究所张友军研究员团队发现丝裂原活化蛋白激酶(mitogen-activated protein kinase,MAPK)信号途径能激活转录因子*CREB*从而调控关键抗性P450基因表达导致烟粉虱形成抗药性(Yang et al.,2020)。随后,该团队又在世界顶级期刊*Cell*上阐明了世界重大农业害虫烟粉虱利用水平基因转移方式获得植物源解毒酶基因*BtPMaT1*解毒寄主的防御物质(Xia et al.,2021)。另外,该团队还发现另一个关键的细胞素色P450基因*CYP4C64*上游5′-UTR区域的甲基化识别位点的突变使该基因过量表达,从而导致烟粉虱对新烟碱类杀虫剂噻虫嗪产生抗性(Yang et al.,2021)。南京农业大学吴益东教授团队构建了首个高质量染色体水平的甜菜夜蛾*Spodoptera exigua*参考基因组,通过基因图位克隆、基因编辑和体外功能表达等多种手段揭示了甜菜夜蛾细胞色素P450氧化酶通过单个氨基酸突变对阿维菌素类杀虫剂产生抗性的新机制(Zuo et al.,2021)。

螨类属于节肢动物门螯肢亚门蛛形纲蜱螨亚纲,是仅次于昆虫的第2大陆生动物群(van Leeuwen & Dermauw,2016)。全球节肢动物抗药性排名前10位的物种中有2种隶属于蜱螨亚纲,分别是二斑叶螨*Tetranychus urticae*和微小牛蜱*Boophilus microplus*,其中二斑叶螨抗药性排名第1(van Leeuwen et al.,2010)。与昆虫相比,螨类的抗药性机理研究一直以来都较为薄弱,主要存在以下2方面原因:第一,螨类个体微小,增加了生测试验的复杂度;第二,螨类基因资源严重匮乏。自2011年二斑叶螨的基因组公布以后,多种螨类的基因组和转录组信息相继被报道,极大地推动了螨类抗药性机理研究的发展(Grbić et al.,2011;van Leeuwen & Dermauw,2016)。

然而相对于昆虫,利用高通量测序技术对害螨的抗药性机理研究仍然较少,且主要集中在二斑叶螨上。二斑叶螨是世界范围内的重要农业害螨,寄主高达1 100多种。过去人们将二斑叶螨在很短的时间内产生广泛的抗药性归因于其体型微小、发生世代多、繁殖速度快、发育历期短以及遗传上的单倍–二倍体(未受精卵发育成雄螨,受精卵发育成雌螨)(Khajehali et al.,2011),后续研究从更多角度揭示了这一现象的根源。其中包括与昆虫类似的靶位点分子突变机理,如乙酰胆碱酯酶(acetylcholinesterase,AchE)的G119S、A201S、T280A、G328A和F331W氨基酸替换导致二斑叶螨对有机磷和氨基甲酸酯类杀虫剂产生抗性(Anazawa et al.,2003;

Khajehali et al.，2010）；电压门控钠离子通道（voltage-gated sodium channel，VGSC）的L1024V和A1215D氨基酸替换导致了其对甲氰菊酯产生抗性、F1538I和A1215D氨基酸替换使其对联苯菊酯产生了抗性（Tsagkarakou et al.，2009；Kwon et al.，2010a）；谷氨酸门控氯离子通道（glutamate chloride channel，GluCl）中，GluCl1上G314D的氨基酸替换使二斑叶螨对阿维菌素不敏感，后续的研究表明该突变加上GluCl3上G326E的氨基酸替换导致二斑叶螨对阿维菌素产生了更高的抗性（Kwon et al.，2010b；Dermauw et al.，2012）。还有一些靶位点突变造成了二斑叶螨特殊的抗药性作用模式，如细胞色素b（cytochrome b，cytb）的G126S、I136T、S141F、D161G和P262T氨基酸替换导致二斑叶螨对联苯肼酯产生了强烈的抗性（van Leeuwen et al.，2008）；几丁质合成酶1（chitin synthase 1，CHS1）I1017F突变导致螨类对生长调节剂如乙螨唑、四螨嗪及噻螨酮产生了抗性（van Leeuwen et al.，2012；Demaeght et al.，2014）；线粒体呼吸复合物I的PSST亚基上H92R突变会导致其对哒螨灵、吡螨胺和唑螨酯产生抗性，这些药剂均为线粒体电子转移抑制剂（Bajda et al.，2017）。以上研究均表明二斑叶螨靶标位点突变能导致其对杀虫杀螨剂产生抗性。另外，更重要的原因是二斑叶螨具有独特的解毒代谢基因家族。基因组数据分析表明，二斑叶螨有86个细胞色素P450（cytochrome，CYP）基因（Grbić et al.，2011），虽然数量上接近于昆虫，但是*CYP2 clan*基因特异性扩张，研究表明*CYP2 clan*基因家族的*CYP392*参与了二斑叶螨对大多数杀虫杀螨剂的抗性（Demaeght et al.，2013；Riga et al.，2014；2015）。羧酸酯酶（carboxyl/cholinesterases，CCE）基因超家族在二斑叶螨中也异于昆虫，共鉴定出71个CCE基因，包含了2个新的进化分支J’和J’’。谷胱甘肽*S*-转移酶（glutathione *S*-transferase，GST）基因超家族在二斑叶螨中也存在特异性扩张的现象，包含了扩张的delta-class GST家族基因和12个mu-class GST家族基因，后者过去被认为只存在于脊椎动物体内（Grbić et al.，2011；Pavlidi et al.，2015）。ABC转运蛋白基因在昆虫对杀虫剂产生耐药性过程中起到了关键作用（Dermauw & Leeuwen，2014），二斑叶螨基因组中鉴定出103个ABC转运蛋白基因，是所有后生动物中拥有该基因数量最多的物种（Dermauw et al.，2013a）。螨类其他参与解毒代谢的基因也与昆虫存在一定的差异，如主要促进子（major facilitator family，MFS）家族基因、内环裂解双加氧酶（intradiol ring-cleavage dioxygenase，ID-RCD）家族基因和脂钙蛋白家族基因（Dermauw et al.，2013b）。由此可见，解毒代谢相关基因家族的广泛扩张是二斑叶螨成为抗药性最严重物种的重要原因。

我国学者利用高通量测序技术通过对朱砂叶螨*Tetranychus cinnabarinus*抗药性的数据分析筛选出朱砂叶螨抗药性相关基因。Shen et al.（2014）从转录组数据中筛选出13个朱砂叶螨GST基因，并证明甲氰菊酯抗性品系中，其中3个GST基因在亚致死剂量甲氰菊酯的诱导下表达量会升高。Wei et al.（2016）筛选出23个CCE基因，并证明其中某些CCE基因参与了朱砂叶螨对甲氰菊酯和丁氟螨酯的抗性。Shi et al.（2015；2016；2017）对朱砂叶螨抗甲氰菊酯品系的P450基因进行了鉴定并对相关基因的转录调控进行了研究。Bu et al.（2015）利用转录组测序技术比较了*β*-谷甾醇处理过的朱砂叶螨与对照之间的基因表达差异，发现了对*β*-谷甾醇胁迫产生应答响应的多个基因。

高通量测序技术应用于其他螨类抗药性的研究也相继被报道，如苹果全爪螨*Panonychus ulmi*（Bajda et al.，2015）、咖啡小爪螨*Oligonychus coffeae*（Amsalingam et al.，2016）、柑橘全爪螨*Panonychus citri*（Niu et al.，2012）、羊痒螨*Psoroptes ovis*（Burgess et al.，2011）和粉尘螨*Dermatophagoides farinae*（Chan *et al.*，2015）等。

3　高通量测序技术在抗药性监测中的应用

近年来，将高通量技术应用于害虫种群抗药性检测的研究报道也相继出现。Zhu et al.（2013）利用高通量测序技术筛选了14个臭虫对拟除虫菊酯的抗性分子标记，并利用这些分子标记监测了美国中西部地区24个臭虫田间种群对拟除虫菊酯的抗性水平，为美国中西部地区臭虫对拟除虫菊酯抗性的全面治理提供了重要的参考数据。Guo et al.（2017）建立了一种用于检测AchE基因抗性突变的程序ACE，利用该程序分析了7种害虫136项研究的971份RNA-Seq数据中AchE的突变频率，结果表明，乌干达东部按蚊种群的抗药性基因位点突变频率已达到很高的水平；小菜蛾的G227A突变与其对有机磷或氨基甲酸酯类杀虫剂的抗性水平呈正相关；二化螟*Chilo suppressalis*和烟粉虱的抗药性基因突变频率也很高；而丽蝇蛹集金小蜂*Nasonia vitripennis*的抗药性基因突变频率较低。陈龙飞等（2020）以3种

重要害虫家蝇、白纹伊蚊*Aedes albopictus*和小菜蛾的8个抗性/敏感种群的转录组数据为对象，使用ACE程序检测了不同种群中的AchE抗药性突变，并利用生物信息学的方法检测其解毒酶基因的表达量变化，分析这3种害虫对有机磷类和氨基甲酸酯类杀虫剂的抗性分子基础，评估获得的3种害虫种群抗药性情况与其之前的报道基本相符；同时，研发了能够检测4种杀虫剂靶标突变和3类解毒代谢酶表达量的综合检测程序FastD，并为FastD程序构建了在线分析平台。以上2个研究表明基于转录组数据的害虫抗药性检测方法可以很好地反映害虫种群的抗药性状况，在线分析平台也为害虫种群抗药性监测提供了极大的便利。

目前，尚未有将利用高通量测序技术应用于害螨抗药性监测的报道。害螨具有一些独特的、区别于昆虫的抗药性靶标基因突变位点以及解毒代谢酶，因此利用该技术监测害螨抗药性需要开发新的平台。

4 展望

对有害生物抗药性机理研究的最终目的是为其田间抗药性治理策略的制定提供理论依据，在这个过程中，如何对其田间抗药性进行全面监测是问题的关键。不同于过去仅仅集中在个别种群或小范围地区，高通量测序技术可以实现在大范围内对田间害虫种群进行抗药性的监测，虽然目前此类报道并不多（Zhu et al.，2013；Guo et al.，2017；陈龙飞等，2020），然而该技术展现出了巨大的应用潜力。利用高通量测序技术获得有害生物抗药性的相关基因，不仅可以筛选出分子标记，实现快速、准确、大范围地抗药性监测，还可以有效鉴定未被发现的抗药性相关基因，从中获得潜在的新型杀虫剂的分子靶标。随着高通量测序技术的进一步发展和成本的不断下降，利用该技术研究田间害虫和害螨种群抗药性机理以及进行抗药性水平监测，必将成为田间抗药性治理的新思路和新方法，从而为害虫和害螨的长期可持续防治措施的制定提供理论依据。

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