

柑橘轮斑病抗性鉴定方法的建立

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摘要:【目的】探究柑橘轮斑病最适宜接种条件, 建立一种快速鉴定柑橘轮斑病抗性的方法。【方法】以柑橘轮斑病菌为接种体, 运用离体叶片接种法, 在室内不同温度下对离体柠檬叶片的不同部位进行接种, 探讨柑橘轮斑病的最适宜接种条件。随后采用前期确立的接种体系对27个主栽柑橘品种进行轮斑病抗性评价。【结果】针刺接种易发病, 最适宜的接种温度为10℃, 最适宜接种部位为叶片背部。27个柑橘品种中沙田柚为免疫品种, 091无核沃柑最易感病。【结论】研究建立了叶片背面针刺、10℃条件下保湿培养28 d的抗性鉴定体系。并利用此技术体系鉴定出柚类抗病力最强, 杂柑类相对最感病, 而橙类、柑类与橘类抗性处于柚类与杂柑类中间。

关键词:柑橘轮斑病; 柑橘轮斑病菌; 接种方法; 抗性评价

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Establishment of an identification method of citrus resistance to target spot

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Abstract:【Objective】Citrus target spot is becoming increasingly severe in China, damaging the young leaves, shoots and fruit in more and more citrus varieties. This study was carried out in order to explore the most suitable inoculation conditions for establishing a rapid, simple and reliable method to identify resistance to the disease. 【Methods】*In vitro* leaf inoculation method was used to inoculate the pathogen to leaves under 5, 10, 15, or 20℃ in a moisture box and the diameters of the speckles were recorded at different time after inoculation. Then, 27 different citrus varieties were inoculated with a typical strains Pc-W ZBY1 and kept in a humidified box using the established inoculation method and the severity of the symptom was recorded. The resistance was evaluated based on the average diameter of the speckle. 【Results】At 5, 10, 15, and 20℃ with a humidity of 90%, no lesions were found on adaxial or abaxial leaf surface without artificial wound, while lesions occurred in all inoculated leaves with wounds. The wounded leaves were more likely to be infected by *Ps. citricarpa* at different tested temperatures, and the most suitable inoculation site was the abaxial side of leaves. At 28 days after inoculation in all temperatures, the lesion hardly expanded and the size of the lesions differed significantly among treatments. Therefore, 28 days after inoculation was the best time for identification of disease resistance. Lesions developed much faster on the abaxial side than the adaxial side of the leaves. Different temperatures affected the disease development greatly. At 10℃, the diameter of lesion spots was the largest, significantly larger ($p < 0.05$) than under the other temperatures. Therefore, 10℃ was the optimal for

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inoculation and incubation for resistance identification in laboratory. Evaluation of resistance to *Ps. citricarpa* among the citrus varieties showed that most varieties were susceptible. Pc-WZBY1 strain showed different pathogenicity in different cultivars with significant differences ($p < 0.05$) among some varieties. The results showed that Shatianyou had an immune phenotype. The other varieties of *Citrus grandis* could be classified into highly resistant type. In *Citrus limon*, the average diameter of the speckle of Eureka Lemon was 5.9 mm, and the diameter of lesion spot in some leaves was as large as 12 mm; seedless Orah 091 produced the largest lesion spot of 14 mm and was the most susceptible variety. Jinqiu Shatangju showed the strongest resistance in hybrids. In other varieties, Fukumoto navel orange was the most susceptible variety in *Citrus sinensis*, and *C. sinensis* ‘Karakara’, Yubei licheng and Tarocco had a similarly higher resistance to *Ps. citricarpa*. Compared with the aforementioned two pomelo varieties, disease spots in most pomelo varieties were hard to see, suggesting excellent performance in disease resistance. In all tested varieties, Shatianyou was immune, and Meyer lemon was of high resistance to *Ps. citricarpa*. The high incidence of citrus target spot in the other tested varieties showed the strong virulence of the pathogen which is casting high risk in citrus production. 【Conclusion】A method leaf inoculation to identify resistance to citrus target spot disease was optimized with inoculating on the abaxial side of leaves and incubating at 10 °C for 28 days in moisture box. Shatianyou is immune, and Meyer lemon has high resistance to *Ps. citricarpa*. Orah 091 was the most susceptible variety and Jinqiu Shatangju most resistant in hybrids, which are mostly susceptible. *C. grandis* is more resistant compared with *C. sinensis* and *C. reticulata*.

Key words: Citrus target spot; *Pseudofabraea citricarpa*; Inoculation method; Resistance evaluation

柑橘轮斑病(*Citrus target spot*)是由*Pseudofabraea citricarpa*引起的一种柑橘真菌性病害^[1-2],2004年在陕西城固首次报道^[3],2018年冬季于重庆万州突然暴发,造成当地尤力克柠檬树大量感病,呈现由北向南加速传播的趋势^[4]。2021年已有湖北宜昌和湖南吉首等地的发病报道^[5],与笔者此前预测的该病在国内的适生区范围一致^[6]。近年来柑橘轮斑病在我国发生愈加频繁,一旦发生造成枯枝落叶、大量落果甚至绝产,威胁着柑橘产量和柑橘品质。目前主要通过化学药剂和一些农事操作防治柑橘轮斑病菌^[4,7],这往往伴随着经济、环境、安全方面的压力以及效果不显著等问题。相反,抗病品种的合理利用则是一种高效且环保的绿色防控手段。

目前关于柑橘轮斑病的研究仍处于初级阶段,主要集中于病原鉴定方法、致病因子鉴定及病害适生区预测等方面。柑橘轮斑病原菌特异SCAR标记引物的开发也为该病害的早期鉴定提供了快速、简便的途径^[8]。此外,已有研究采用整合转录组学和分泌蛋白组学结合的方法发现植物细胞壁降解酶、植物-病原互作蛋白以及萜类生物合成途径在*Ps. citricarpa*致病性中发挥关键作用^[1]。最新研究表明,柑橘轮斑病在我国处于高度风险等级,对长江上

中游及鄂西-湘西两大柑橘产区潜在威胁较大^[6]。但是,柑橘轮斑病的抗性鉴定并没有一套完善可行的标准化体系,这也导致柑橘轮斑病抗性种质资源筛选及抗病品种选育进程缓慢。

为此,笔者以病斑直径平均值为抗感性评价标准^[9],同时以发病率致病力评价标准^[10],根据病斑直径和发病率鉴定不同柑橘品种抗病性,构建抗性评价体系;选择在当地发病严重的尤力克柠檬叶片作为离体接种的材料,参考林月莉等^[11]的离体叶片接种法并加以改良,作为抗性鉴定接种方法。建立柑橘轮斑病菌的抗性鉴定体系,进而采用构建好的抗性鉴定体系,研究抗性鉴定的适宜接种部位、接种温度。在最适宜的温度和接种部位下,将病原菌接种到离体叶片,评价病原菌致病力,研究不同柑橘品种的抗病性,筛选出抗耐病品种。

1 材料和方法

1.1 材料

供试菌株:Pc-WZBY1分离自重庆市万州区白羊镇发病柠檬果园,经过形态学和PCR鉴定为柑橘轮斑病(*Ps. citricarpa*),在20 °C于马铃薯葡萄糖琼脂(PDA)培养基避光培养,直至菌丝体覆盖约四分

之三的平板,4℃保存、备用。

供试柑橘种质27份来自于重庆市三峡农业科学院柑橘种质资源圃,详见表1。

表1 试验所用柑橘品种

Table 1 *Citrus* cultivars used in this study

编号 Code	品种 Cultivar
1	沙田柚 Shatianyou
2	北京柠檬 Meyer lemon
3	卡拉卡拉 Cara cara Navel Orange
4	渝北梨橙 Yubei pear orange
5	中柑1号 Zhongan No. 1
6	塔罗科血橙 <i>Citrus sinensis</i> ‘Tarocco’
7	98-1长叶橙 98-1 long leaf orange
8	龙都早香柚 Longduaoxiangyou
9	丽朵血橙 Liduo Blood Orange
10	沃柑 Orah
11	强德勒红心柚 Chandler Pummelo
12	94-1长叶橙 94-1 long leaf orange
13	伦晚 Lane late Navel Orange
14	葡萄柚 <i>Citrus paradisi</i>
15	奉晚 Fengwan Navel Orange
16	津香橙 Jinxiang orange
17	爱媛30号 Aiyuan No. 30
18	口之津 Koizumi
19	金秋沙糖橘 Jinqushatangju
20	琯溪蜜柚 Guanximiyou
21	尤力克 Eureka lemon
22	W-默科特 W. Murcott Afourer
23	南香 Nankou
24	金钱橘 Clementines
25	温州蜜柑 Satsuma mandarin
26	福本 Fukumoto Navel Orange
27	091无核沃柑 Orah 091

1.2 接种方法

选取大小、长势基本一致的健康,成熟的尤力克柠檬叶片,用75%(φ)乙醇表面消毒晾干后放入铺有灭菌纱布的无菌托盘,加蒸馏水浸湿纱布。把直径5 mm的菌饼接种至托盘内的叶片上,菌丝面紧贴叶片。每叶片放置2个菌饼,覆上保鲜膜确保湿润度,每隔24~48 h补充无菌水1次。接种21 d后,每隔2 d测量1次病斑直径并记录数据,直至病斑不继续扩展。

1.3 柑橘轮斑菌离体接种条件探究

1.3.1 创伤对接种效果的影响 采用1.2节接种方法对离体尤力克柠檬叶片接种,采用无伤接种和针刺接种2种处理,每个处理10枚叶片。根据前期开

展的预试验以及朱丽^[7]针对柑橘轮斑病菌开展的前期研究,选择将接种后的叶片分别置于5℃、10℃、15℃、20℃恒温培养箱内黑暗保湿培养。以无菌PDA块接种叶片作为对照,3次重复。

1.3.2 接种温度及部位对接种效果影响 采用1.2节接种方法对离体尤力克柠檬叶片接种,分别对叶片正面、背面接种两种处理,每个处理10枚叶片。将接种后的叶片分别置于5℃、10℃、15℃、20℃恒温培养箱内黑暗保湿培养。以无菌PDA块接种叶片为对照,3次重复。

1.4 不同柑橘品种抗性鉴定

依据1.3部分确定的最适接种条件,采用离体接种方法对27个柑橘品种叶片进行抗性鉴定,每个处理10枚叶片,3次重复。接种21 d后测量不同品种叶片的病斑直径。

1.5 数据分析

试验数据采用SPSS统计分析,处理间的差异性分析采用多重比较应用中的最小显著差法(LSD)^[8]。

2 结果与分析

2.1 创伤对接种效果的影响

在5℃、10℃、15℃、20℃温度下分别对离体叶片正面、背面无伤接种无菌PDA菌丝块,叶片均不发病,而针刺有伤条件下接种病菌时均发病。叶片接种病菌22 d后,刺伤部位症状表现明显(图1-A)。各温度下发病情况不同,病斑均随接种时间延长表现不同程度扩展,且接种病菌28 d后,发病情况保持稳定,病斑大小几乎不再扩展,此时各接种条件下病斑差异表现显著,接种病菌28 d是整个观测期内抗性鉴定的最佳时间,也是品种抗性鉴定时观测的最佳时间(图1-B)。除15℃外,其他温度下,叶片背面针刺病斑长度大于叶片正面针刺病斑长度。5℃叶片病斑呈黑色,边缘油渍状,随着接种时间延长病斑颜色变淡,刺伤部位产生白色条状菌丝。10℃叶片病斑呈褐色,具晕圈,随着接种时间延长,病斑老化且中央产生黑色凸起。15℃叶片病斑呈红褐色,随后逐渐扩大呈深褐色,刺伤部位产生白色条状菌丝。20℃叶片病斑呈深褐色,一些病斑表面覆盖白色菌丝,随着病斑老化其表面产生黑色凸起(图1-A)。

2.2 不同温度及接种部位对接种效果的影响

采用十字交叉法测量病斑直径,在以尤力克柠

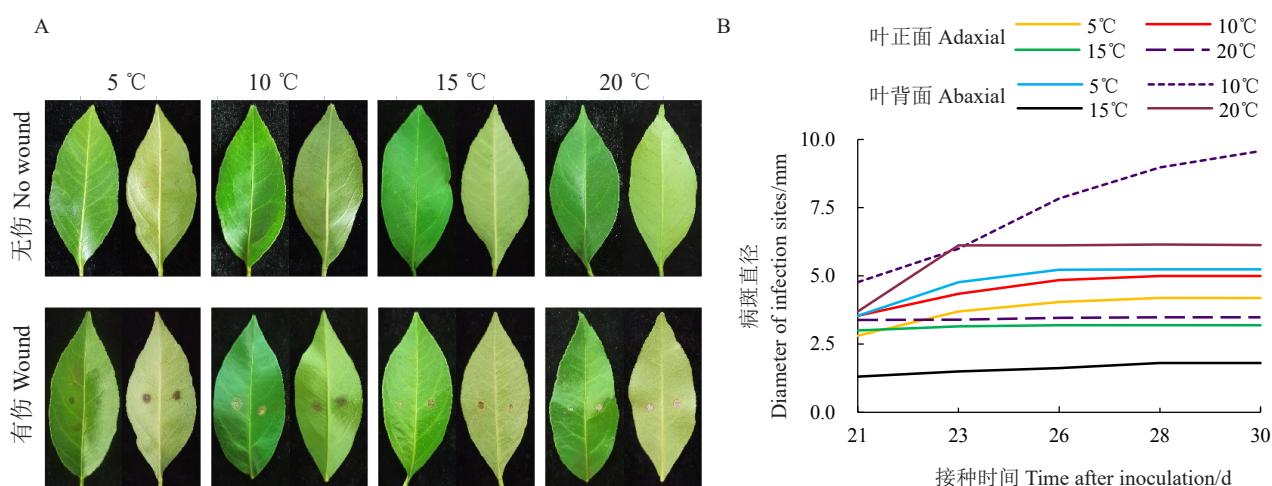


图 1 离体柠檬叶片接种病菌后病斑扩展情况

Fig. 1 The comparison of lesion expansion on detached leaf inoculated with *Pseudofabracea citricarpa*

檬为材料的抗性鉴定试验中,叶片接种病菌28 d调查发现,在10 °C、20 °C下,叶片背面针刺接种产生的病斑直径大于叶片正面且表现显著性差异,而5 °C、15 °C下叶片正面、背面病斑大小有差异但并未达到显著性水平。10 °C下叶片背面接种形成的病斑直径最大,且与其他各温度下叶片正面、背面接种形成的病斑直径存在显著性差异($p < 0.05$)。因此,10 °C下叶片背面接种是室内抗性鉴定的最适接种条件(图2)。

2.3 不同柑橘品种对柑橘轮斑病的抗性差异

从图3可知,10 °C条件下对不同供试柑橘品种的离体叶片背面进行伤口接种后产生的病斑直径和发病率存在不同程度的差异(图3),部分柑橘品种甚至与其他品种的病斑直径达到显著性差异水平($p < 0.05$)。其中,沙田柚抗性最强,表现为完全免疫。尤力克柠檬平均病斑直径为5.9 mm,部分叶片

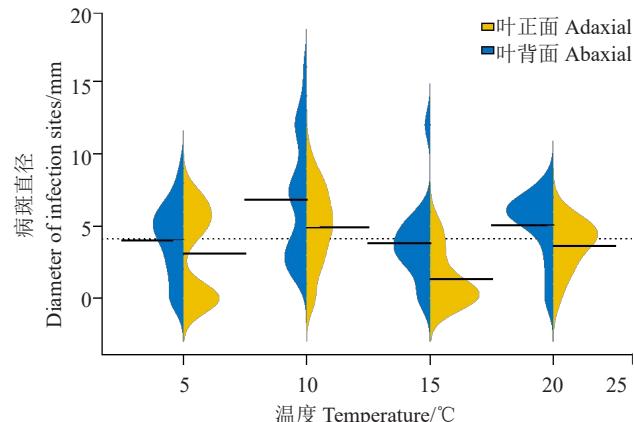


图 2 不同温度下接种离体叶片正、背面平均病斑直径

Fig. 2 Average lesion diameter on the detached leaves inoculated with *Pseudofabracea citricarpa* at different temperatures

病斑直径高达12 mm,而091无核沃柑的平均病斑直径最大,可达13 mm,部分叶片病斑直径可达14 mm,抗性最弱。杂柑类种质中091无核沃柑抗性最弱,



图 3 部分柑橘品种叶片接种后的症状

Fig. 3 Symptoms of different citrus varieties after inoculation

中柑1号抗性最强;脐橙类种质中福本抗性较弱,卡拉卡拉、渝北梨橙、塔罗科血橙抗性相当且不易感病;相比柑类、脐橙类,柚类病斑直径较小,发病不明

显,发病率低,抗病力强;除沙田柚表现免疫不发病外,只有北京柠檬的发病率相对较低(20%),其他柑橘品种发病率均在30%以上(图4)。

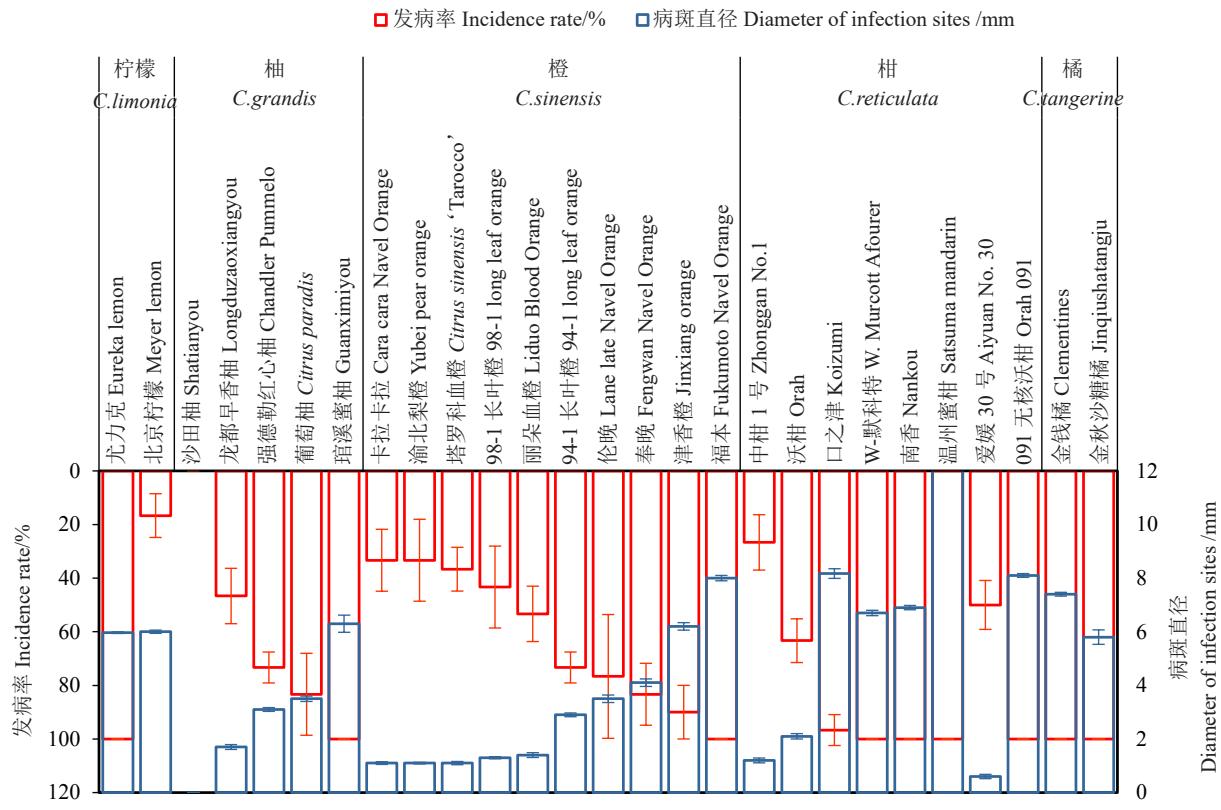


Fig. 4 The disease incidence of different citrus varieties after inoculation

3 讨 论

柑橘轮斑病发病周期较长,病情一旦加重,杀菌剂便难以有效控制,这给防治上造成很大困难。抗性品种筛选是解决防治难题的有效方法,但目前柑橘轮斑病的抗性材料鉴定及药剂筛选方面还没有一套完整的评价体系。剪根浸根法^[12]、带菌牙签活体茎秆穿刺接种法^[13]、离体叶片喷雾注射法^[14]、活体叶片法^[15]是目前已有一些植物病害抗性鉴定接种方法,笔者在本研究中采取离体叶片针刺接种法,该法操作性强,不伤害植株,单株可重复,数据收集简便。除此之外,相较于病情指数法^[16]和系统聚类分析法^[17-18],根据病斑直径和发病率进行抗性评价,排除了不同严重度分级下病情指数及致病率的差异造成的试验偏差。笔者建立的抗性鉴定体系不但为高效筛选耐病抗病品种提供了技术方案,还可用于药剂筛选,也为其他柑橘病害的抗性鉴定提供了一种

思路。由于病原菌存在潜在的致病性分化和变异,因此培育出永远具有抗病性的品种是不可能。笔者通过室内接种试验,选择不同菌株开展寄主-病原的互作研究^[19-20],明确柑橘轮斑病菌与柑橘品种间的互作关系,在生产中合理种植柑橘品种,对病害的综合防治具有重要意义。对于研究鉴定的柑橘品种,可采用抗谱分析的方法^[21],获得抗柑橘轮斑病基因,从而利用抗性基因培育更加高抗的新品种。另外,本研究中建立的室内接种试验,还可用于病原菌侵染后孢子萌发情况、不同抗性品种的一些生理生化指标、细胞结构等的分析,以研究柑橘轮斑病菌的致病机制。

长江上中游柑橘优势区及鄂西-湘西两大柑橘产区是柑橘轮斑病发生的中高适生区,为减少经济损失,应尽量种植适宜生长且抗性较强的柑橘品种。抗性与感病的界限是模糊的,是相比较而言的,长江上中游柑橘带作为甜橙的最适宜生长区,晚熟

品种较早、中熟品种更具生长优势,需要减少福本等易感病脐橙类的种植面积,尽量栽培不易感病且发病率低的脐橙类、柠檬类、柚类,如卡拉卡拉、沙田柚、北京柠檬;鄂西-湘西柑橘带适宜宽皮柑橘的生长,主要种植温州蜜柑、橙类和一些柚类,适当减少易感病的柑类如091无核沃柑、南香及部分宽皮柑橘如金钱橘的种植面积,同时发挥其自然优势种植抗性较强的柚类如沙田柚、龙都早香柚。

植物为抵御病原菌侵染会建立一系列复杂的抗病机制,植株的形态结构特征如叶型、气孔结构等,都与植物抗病机制有关^[22-24],抗性基因是产生抗性的根本原因。根据抗病基因具有保守结构域这一特点,对保守结构域进行序列扩增是鉴定和发掘抗性基因的常用方法^[25],这一方法已应用于辣椒^[26]、大豆^[27]、小麦^[28]等作物。研究柑橘种质间抗性差异、柑橘抗病机制甚至柑橘-柑橘轮斑病菌互作的关键是研究柑橘抗性基因,黄代青等^[29]在柚的cDNA中找到10个抗病基因同源序列;谌谋华^[30]在柑橘抗病材料中找到25个抗病基因同源序列;研究不同柑橘品种抗性基因差异是了解柑橘抗轮斑病作用机制的基础,这需要今后进行更深入的研究分析。此外,研究还存在一定的局限性,离体叶片可能无法充分反映整个植物在叶龄等方面的变异性,而且一些整株植物的寄主防御反应可能在离体叶片中减弱。因此,需要今后结合田间试验或采取其他抗性鉴定方法加以验证。

笔者采用离体叶片接种法,证明10℃条件下叶片背面接种是柑橘轮斑病室内抗性鉴定的最适接种条件,且叶片接种病菌后28 d是最佳的鉴定时间。利用对27个柑橘品种抗性鉴定筛选出的抗病耐病材质,如沙田柚、北京柠檬、龙都早香柚等,同时结合柑橘轮斑病适生区,在高中风险区合理地种植和布局抗病品种,对实现农民增收,解决三农问题,迈向乡村振兴具有重大意义。

抗性鉴定方法的建立及主栽品种抗性评价不仅有利于抗轮斑病柑橘品种的鉴定和合理布局,还可用于防治柑橘轮斑病的药剂筛选、病原菌致病机制的研究,以及柑橘与轮斑菌的互作分析。

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