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·研究论文·

抗叶锈病小偃麦代换系WTS135的遗传学分析与分子标记开发

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摘要 由于人工驯化与现代育种操作, 普通小麦(*Triticum aestivum*)的遗传多样性日渐狭窄, 更容易受到病虫害威胁。通过远缘杂交将野生近缘种的抗病基因导入小麦, 有助于拓宽小麦的遗传基础, 为培育抗病品种提供新抗原。十倍体长穗偃麦草(*Thinopyrum ponticum*)是小麦遗传改良中应用最广泛的近缘物种之一, 对小麦锈病等多种病害表现出良好的抗性。利用远缘杂交和染色体工程, 创制了1份小麦-长穗偃麦草种质材料WTS135, 对叶锈菌(*Puccinia triticina*)生理小种THTT表现出免疫。系谱分析表明, 其叶锈病抗性来源于长穗偃麦草外源染色体。基因组原位杂交(GISH)-荧光原位杂交分析显示, 1对十倍体长穗偃麦草染色体替换了小麦7D染色体。液相芯片分析表明, 外源染色体属于第7部分同源群, 其近着丝粒区的信号密度及丰度明显较低, 与GISH分析结果互相佐证, 因此推测WTS135是1个7St (7D)的二体异代换系。分子标记检测显示, WTS135携带的抗病基因与已知的长穗偃麦草第7部分同源群抗叶锈病基因*Lr19*和*Lr29*不同, 推测有可能为1个抗叶锈病新基因。借助Specific-locus amplified fragment sequencing技术, 开发了10个长穗偃麦草特异引物, 用于快速追踪WTS135中的外源染色质。表型调查显示, WTS135的产量与轮回亲本济麦22无显著差异, 可直接用于小麦的抗病育种。

关键词 小麦, 十倍体长穗偃麦草, 叶锈病, 远缘杂交, 代换系, 原位杂交, 分子标记

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小麦(*Triticum aestivum*, 2*n*=6*x*=42, AABBDD)是世界三大粮食作物之一, 全球种植面积超过2.17×10^8 hm^2, 产量超过7.7×10^8 t, 为人类提供了约20%的蛋白质和能量(Ibba et al., 2022)。小麦产量对世界粮食安全起着至关重要的作用。预计到2050年, 随着全球人口的不断增长, 对小麦的需求量会增加60% (Tripathi et al., 2019)。虽然近年来小麦产量有所增加, 但是长期的人工驯化和现代育种操作使得小麦的遗传基础日趋狭窄, 导致小麦抵抗真菌、病毒和细菌等病害的能力逐步下降, 影响小麦产量的进一步提升(Reynolds et al., 2012; Singh et al., 2016)。小麦野生近缘属种携带多种优良性状, 是小麦遗传改良的宝贵基因库。通过远缘杂交, 将外源物种中的抗病基因导入小麦, 是拓宽小麦遗传基础, 改良小麦抗病性的重要途径。

小麦叶锈病是一种由叶锈菌(*Puccinia triticina*)引起的小麦真菌性病害, 通常导致感病品种减产近30%, 严重时超过50% (Lin et al., 2022)。目前, 已经正式命名了80多个抗叶锈病基因, 其中近一半来自小麦的野生近缘种(Prasad et al., 2020; Mapuranga et al., 2023)。例如, Singh等(2012)将黑麦 (*Secale cereale*, RR)中的*Lr25*转移至小麦4B染色体长臂, 并筛选到1个共显性标记*Xgwm251*, 距离*Lr25*位点3.8 cM。Pirseyedi等(2015)将易变山羊草(*Aegilops peregrina*, $U^pU^pS^pS^p$)的*Lr59*转移至小麦1A染色体长臂, 经诱导同源群配对, 筛选出抗叶锈病小片段易位系*Lr59-151*, 并且开发了*DUPW217*标记用于辅助育种。Friebe等(1993)通过C-分带、原位杂交以及同工

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酶分析, 对3个含有*Lr38*的小麦-中间偃麦草(*Thinopyrum intermedium*, JJJ^{S}J^{S}StSt)衍生系W44、W49和W52进行鉴定, 发现抗叶锈病、条锈病和秆锈病的W44与W52分别为7Ai-2(7D)和7Ai-2(7A)的代换系, 而抗叶锈病的W49仅是1个T2AS·2AL-7Ai-2L的易位系, 因此证实*Lr38*位于7Ai-2染色体的长臂末端。尽管已发现不少抗叶锈病基因, 但随着叶锈菌生理小种的不断进化, 多个抗性基因, 如*Lr10*、*Lr16*、*Lr26*、*Lr33*、*Lr35*以及*Lr50*对大部分*Pt*生理小种已失去抗性, 因此迫切需要寻找新的小麦叶锈病抗原(Zhang et al., 2020; 段振盈等, 2021)。

长穗偃麦草(*Thinopyrum ponticum*, 2*n*=10*x*=70, E^{e}E^{e}E^{b}E^{b}E^{x}E^{x}StStStSt或JJJJJJJ^{s}J^{s}J^{s}J^s)是一种多年生牧草, 根系发达, 生长旺盛, 对锈病、白粉病和赤霉病等多种病害均具有抗性, 是小麦育种的重要基因库。目前, 已知的长穗偃麦草抗叶锈病基因有*Lr19* (Sharma and Knott, 1966; Friebe et al., 1996)、*Lr24* (Smith et al., 1968)和*Lr29* (Sears, 1973, 1977)。早在1966年, *Lr19*基因就已被转移至普通小麦中, 现在仍对大部分叶锈病生理小种具有抗性, 但是由于其紧密连锁1个黄色素基因, 限制了其应用(Knott, 1968)。随后, Zhang等(2005)利用37个7D-7E#1重组体, 将*Lr19*基因定位于7E长臂末端的*Xwg420*与*Xmwg2062*标记之间。Jiang等(1994)发现了1个抗叶锈病和秆锈病的小麦-长穗偃麦草易位系Amigo, 其亲本Teewon是由小麦-长穗偃麦草的第三同源群附加系与普通小麦杂交得到。经原位杂交(genomic *in situ* hybridization, GISH)以及荧光原位杂交(fluorescence *in situ* hybridization, FISH)分析, 发现Amigo的易位片段来源于Teewon, 证实了Amigo的叶锈和秆锈病抗性分别来源于长穗偃麦草3Ae#1染色体上的*Lr24*与*Sr24*。随后, Li等(2024)通过GISH、Non-denaturing FISH和PCR-based landmark unique gene标记分析, 借助4个含有3Ag长臂的易位系, 将*Lr24*定位于3AgL上的0.7–0.85 cM区间。Tar等(2002)利用81个随机扩增多态性DNA (random amplified polymorphic DNA, RAPD)引物对*Lr29*的近等基因系进行鉴定, 开发出1个*Lr29*的特异性标记*OPY10*$_{950bp}$, 用于快速选择抗叶锈病小麦品种。

液相芯片技术是一种借助目标探针与靶向序列互补结合, 进而实现定点捕获与测序的技术, 在农业育种领域展现出巨大的应用价值。为培育优良农作物品种, 科研人员会对农作物材料进行检测。首先构建基因组DNA文库, 再依据DNA互补原理设计针对农作物重要农艺性状相关SNP位点的探针并标记(Leng et al., 2015)。通过液相芯片技术, 可快速筛选出具有抗病和高产等优良性状基因的植株, 加速育种进程。

本研究报道了1个高抗叶锈病的小麦-长穗偃麦草异代换系WTS135, 结合GISH、mc-FISH (multi-color-FISH)和液相芯片技术, 明确了其基因组构成及外源染色体的部分同源群, 借助系谱分析推断叶锈病的抗性来源, 通过田间调查考察其综合农艺性状, 并利用特异性位点扩增片段测序(specific-locus amplified fragment sequencing, SLAF-seq)技术开发了外源特异性分子标记, 用于抗病染色体的快速追踪。

1 材料与方法

1.1 植物材料

小偃麦种质材料WTS135的杂交组合为: 小偃81/4/小偃81/3/小偃81//中农28/R431/5/济麦22/6/济麦22/7/济麦22/8/济麦22。首先, 以小麦品种中农28为母本, 长穗偃麦草品系R431为父本, 通过远缘杂交获得杂种F_1。随后, 将F_1与小麦品种小偃81杂交并回交2次, 将BC_2F_1再次与小麦品种济麦22杂交并回交2次, 自交后分离得到WTS135。WTS135及其亲本均保存在中国科学院遗传与发育生物学研究所李振声研究组。

1.2 叶锈病抗性评估

WTS135及其亲本的叶锈病抗性鉴定在河北农业大学植物保护学院进行。轮回亲本济麦22作为感病对照, THTT生理小种在众多叶锈菌生理小种中比较流行, 且毒性较强, 故抗病鉴定使用叶锈菌生理小种THTT (Yang et al., 2023b)。当供试材料长至两叶一心时, 采用扫叶法接种, 接种过的材料立即放入温度为20°C、相对湿度为100%的温室中黑暗培养16小时。随后移至温度为22–25°C、相对湿度为70%、光周期为12小时光照/12小时黑暗的温室中培养。当感病对照济麦22叶片出现大量夏孢子堆时, 进行抗病性调查。按照Roelfs等(1992)的分级标准, 将抗病等级分为0–4级, 0–2级表示抗病, 3–4级表示感病。

1.3 细胞学鉴定

参照Han等(2006)的方法进行小麦根尖细胞有丝分裂中期染色体制备。首先，将饱满的种子放在铺有湿润滤纸的培养皿中，23°C恒温培养2天，剪取约2 cm的根，在N_2O中处理1.5小时。随后，用90%乙酸固定8分钟，去离子水漂洗，切取根尖，放入含有1%纤维素酶和2%果胶酶的混合液中，37°C水浴1小时。然后，在75%乙醇中将根尖碾碎，离心收集细胞，加入100%乙酸悬浮细胞，吸取10 μL悬浮液滴加至湿盒中的载玻片上。在显微镜下检查，挑选分裂相好的染色体中期分裂相，置于紫外交联仪上，1 250 μJ·cm$^{2-1}$下交联3次，24°C保存备用。

1.4 原位杂交(GISH)分析

原位杂交(GISH)流程参照Fu等(2012)的方法进行并稍做改动。采用CTAB法提取长穗偃麦草和中国春的基因组DNA (Saghai-Maroof et al., 1984)。以TEXAS Red®-5-dCTP标记的十倍体长穗偃麦草DNA为杂交探针，沸水浴30分钟的中国春基因组DNA作封阻。按照探针与封阻的质量比为1:200配制GISH杂交液，每张载玻片上滴加10 μL杂交液，沸水浴5分钟，随即放入55°C恒温培养箱孵育过夜。用2×柠檬酸钠(saline-sodium citrate, SSC)缓冲液清洗盖玻片，晾干后滴入含4',6-二脒基-2-苯基吲哚(4',6-diamidino-2-phenylindole, DAPI)的抗褪色剂(H-1200, Vector)，荧光显微镜(BX53, Olympus)下观察，选择分裂相好并且外源信号清晰的细胞拍照，使用软件CellSens Standard 1.12 (Olympus)合成图片。将拍照完成的载玻片，放入2× SSC中浸泡30分钟，取出吹干后，随即放在强光下淬灭荧光备用。使用Alexa Fluor 488-5-dUTP (Invitrogen)标记的拟鹅观草(*Pseudoroegneria stipifolia*, 2*n*=2*x*=14, StSt) DNA为探针，沸水浴30分钟的二倍体长穗偃麦草(*Thinopyrum elongatum*, 2*n*=2*x*=14, EE)作封阻，按照探针与封阻的质量比为1:70配制杂交液，再次进行原位杂交，后续步骤同上所述。

1.5 Mc-FISH分析

GISH分析后，按Huang等(2018)所述方法进行多彩色荧光原位杂交(mc-FISH)分析。首先，将携带染色体的载玻片在2× SSC中浸泡30分钟，随后置于强光下，淬灭荧光。将处理好的载玻片放入70%乙醇(含0.15 mol·L^{-1} NaOH)中，44°C条件下变性5分钟，接着室温下用75%乙醇脱水10分钟，最后在100%乙醇中脱水5分钟，晾干备用。Mc-FISH分析的寡核苷酸探针套由生工生物工程(上海)股份有限公司合成，包括由6-羧基四甲基罗丹明标记的pAs1-1、pAs1-3、pAs1-4、pAs1-6、AFA-3和AFA-4，以及6-羧基荧光素标记的寡核苷酸pSc119.2-1和$(GAA)_{10}$。8个探针分别加入适量ddH_2O，使浓度为100 μmol·L^{-1}，等体积混合。将去离子甲酰胺、20× SSC、鲑鱼精DNA、50%硫酸葡聚糖以及探针混合液按照7.5:1.5:0.5:2.5:1 (v/v/v/v/v)的比例混匀，100°C金属浴13分钟，随后立即放入–20°C冰箱10分钟，取出配制成杂交液备用。每张载玻片滴加10 μL杂交液，然后放入不透光的湿盒中，室温过夜。用2× SSC清洗后，使用DAPI对染色体进行复染，显微镜观察、拍照和图像处理方法同GISH分析。

1.6 液相芯片分析

使用GenoBaits®WheatplusEE液相芯片，分析异代换系基因组组成。首先，提取基因组DNA，进行物理破碎，片段峰值控制在200–300 bp之间，经末端修复后连接poly (A)尾。用连接酶为片段连接测序接头，利用磁珠对文库进行纯化，保留插入片段为200–300 bp的连接产物。加入带有Barcode的测序引物和高保真酶进行PCR扩增，再次利用磁珠进行纯化，完成测序文库的构建。随后，从测序文库中吸取500 ng，加入探针和杂交试剂，变性后置于65°C温育2小时，然后清洗杂交产物，再进行1轮PCR扩增，完成杂交捕获文库的构建。质检后上机测序。通过数据质控，去除接头以及低质量数据，经参考序列比对分析，获得clean reads。使用软件BWA-MEN将其与参考基因组序列比对，获得在参考基因组上的位置，并计算样本的比对率。最后使用R软件包制图(Wang et al., 2010; He et al., 2016)。

1.7 特异性分子标记开发

利用SLAF-seq开发长穗偃麦草特异性探针，使用限制性内切酶*Hae*III对其DNA进行酶切，选取长度为400–450 bp的片段，进行双端测序。将得到的序列与乌拉尔图小麦(*Triticum urartu*)基因组(Ling et al., 2018)和粗山羊草(*Aegilops tauschii*)基因组(Zhao et al.,

2017)进行比对，选取同源性小于50%的标签，即为长穗偃麦草特异序列标签。利用Primer3 (https:// bioinfo.ut.ee/primer3-0.4.0/)设计PCR引物，由生工生物工程(上海)股份有限公司合成。采用CTAB法提取WTS-135及其亲本长穗偃麦草、济麦22、小偃81和中农28的基因组DNA，稀释至100 ng·μL^{-1}。PCR反应体系总体积为20 μL: 含2× TSINGKE® Master Mix (Green) (北京擎科生物科技股份有限公司) 16 μL，样品DNA (100 ng·μL^{-1}) 2 μL，浓度为10 μmol·L^{-1}正向和反向引物各1 μL。PCR反应程序: 98°C预热2分钟; 98°C变性10秒，48–56°C退火15秒(视Tm值定)，72°C 延伸10秒，36个循环; 72°C延伸5分钟。扩增产物用2%琼脂糖凝胶电泳分离，使用Tanon 1600凝胶图像系统拍照。取引物扩增WTS135及其亲本，挑选长穗偃麦草和WTS135中扩增片段大小相同，且在其余亲本中无扩增或扩增片段不同的引物，即为WTS135中外源基因组特异引物。

1.8 农艺性状评价

2023–2024年，在中国科学院遗传与发育生物学研究所北京昌平试验基地(116.2°E, 40.6°N)，开展WTS135及其轮回亲本济麦22的农艺性状调查，行长2 m，行间距0.2 m，株间距0.1 m，设置3个重复。待植株成熟后，从中部挑选生长一致的5个单株，测量株高(plant height, cm)和主穗长(spike length, cm)；统计有效分蘖数(effective tiller number)、小穗数(spikelet number per spike)和主穗粒数(kernel number per spikelet)；使用万深(SC-E)外观品质扫描仪扫描总粒数(total kernel number)，称取单株产量(yield per plant, g)，并计算千粒重(thousand-kernel weight, g)。使用Excel 2016和SPSS 19.0软件分析数据。

2 结果与分析

2.1 WTS135的GISH和mc-FISH分析

当以十倍体长穗偃麦草的基因组DNA为探针，普通小麦基因组DNA为封阻时，WTS135体细胞的42条染色体中，有40条染色体呈现蓝色信号，2条染色体呈现红色信号，说明WTS135携带了20对小麦染色体和1对十倍体长穗偃麦草染色体(图1A)。当以拟鹅观草基因组DNA为探针，二倍体长穗偃麦草基因组DNA为封阻时，2条长穗偃麦草染色体近着丝粒区的绿色杂交信号较强，而2条染色体臂杂交信号较弱，推测这2条外源染色体属于十倍体长穗偃麦草中的St基因组(图1B)。对同一细胞进行FISH分析，发现2条外源染色体的短臂靠近末端区域有微弱的红色信号，长臂末端有1条明显的红色杂交带；通过与标准小麦染色体杂交信号比对(Peto, 1936)，结果显示WTS135的小麦A组和B组染色体完整正常，而D组缺失了1对7D染色体，因此推测WTS135中1对来自长穗偃麦草St基因组的染色体替换了1对小麦7D染色体(图1C)。

2.2 液相芯片分析

利用液相芯片GenoBaits®WheatplusEE分析WTS135，共获得17 529 208个原始读数，所有序列碱基类型分布均匀，且无明显的AT和GC分离现象。将数据过滤，获得17 529 208条clean reads，并且样本的Q30大于97%，说明建库质量较高，可进行后续芯片分析。构建WTS135的深度图，观察其分布及丰度(图1D)，结果显示WTS135中普通小麦的7D染色体上的检测信号微弱，而在二倍体长穗偃麦草的7E染色体上出现明显信号，且7E染色体的长臂和短臂末端信号密集，着丝粒附近位置信号较弱，由此推断其为7St染色体。

2.3 抗病性鉴定

为评估WTS135及其亲本的苗期叶锈病抗性水平，对各材料接种叶锈菌生理小种THTT。大约14天后，当感病对照济麦22完全发病时(IT=4)，进行抗病性调查。WTS135及其亲本十倍体长穗偃麦草的叶片上均无任何夏孢子堆出现，且出现过敏性反应引发的坏死斑，说明二者对THTT小种近免疫(IT=;)；其它亲本品种，如小偃81、中农28和济麦22的叶片上均出现大而多的夏孢子堆，表现出高感(IT=4) (图2A)。上述结果表明，WTS135的叶锈病抗性来源于亲本十倍体长穗偃麦草。接着，我们又利用3个叶锈菌生理小种(PHTT、THTS和DGJJ)对WTS135及其亲本进行抗病性鉴定，结果显示WTS135对这3个生理小种均表现出近免疫(IT=;)，亲本济麦22、小偃81和中农28表现出中感至高感(IT=3–4)，而长穗偃麦草同样表现出近免疫(IT=;) (附图1)。这表明WTS135所携带的抗叶锈病基因具有广谱抗性，开发潜力很大。

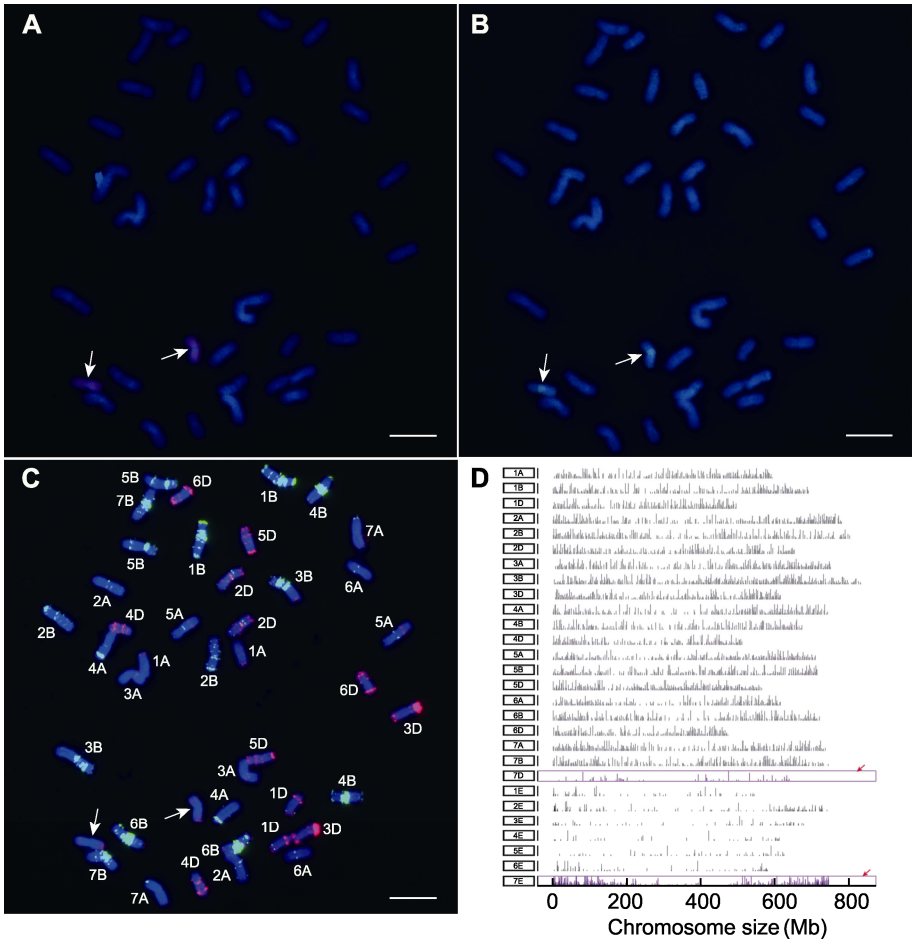

图1 WTS135的GISH、mc-FISH及液相芯片分析

(A) 以十倍体长穗偃麦草基因组DNA为探针，中国春基因组DNA作封阻的GISH结果; **(B)** 以拟鹅观草基因组DNA为探针，二倍体长穗偃麦草基因组作封阻的GISH结果; **(C)** 使用寡核苷酸探针套作探针的mc-FISH结果; **(D)** WTS135液相芯片结果。白色箭头表示外源染色体，紫色框代表染色体的增加或缺失。Bars=20 μm。

Figure 1 GISH, mc-FISH, and liquid chip analysis of WTS135

(A) GISH analysis using *Thinopyrum ponticum* gDNA as a probe and Chinese Spring gDNA as a block; **(B)** GISH analysis using *Pseudoroegneria stipifolia* gDNA as a probe and *Th. elongatum* gDNA as a block; **(C)** Mc-FISH analysis using combined oligo probes; **(D)** The liquid chip analysis of WTS135. The white arrows indicate exogenous chromosomes, purple frames indicate chromosome additions or deletions. Bars=20 μm.

2.4 分子标记分析

使用SLAF-seq技术获得十倍体长穗偃麦草特异序列标签，设计引物扩增WTS135及其亲本。PCR扩增结果显示，*Thp32*、*Thp39*、*Thp40*、*Thp94*、*Thp115*、*Thp124*、*Thp242*、*Thp251*、*Thp328*和*Thp374*十个标记在WTS135与十倍体长穗偃麦草中扩增出相同大小的条带，而在其它亲本中无特异性扩增或扩增出大小不同的条带，表明这些标记为WTS135中7St染色体的特异标记(图3A；表1)。这些引物有助于定位7St染色体上的叶锈病抗性基因及用于小麦的抗病性遗传改良。

2.5 农艺性状评价

考察WTS135及其轮回亲本济麦22的重要农艺性状。结果显示，WTS135的株高(82.25 cm)、分蘖数(23.75)和株粒数(755.75)均极显著(P<0.01)高于济麦22的株高(69.00 cm)、分蘖数(13.33)以及株粒数(517.33)；主穗长和主穗粒数与济麦22无明显差异；

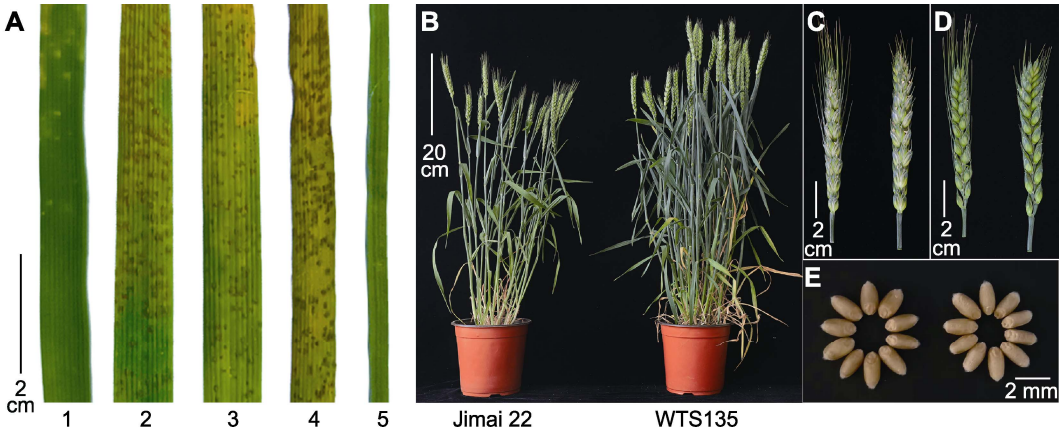

图2 WTS135及其亲本的叶锈病抗性评价及WTS135和济麦22的农艺性状
(A) WTS135及其亲本的叶锈病抗性评价(1: WTS135; 2: 小偃81; 3: 济麦22; 4: 中农28; 5: 十倍体长穗偃麦草); **(B)** 成株; **(C)** 主穗正面观(WTS135 (右), 济麦22 (左)); **(D)** 主穗侧面观(WTS135 (右), 济麦22 (左)); **(E)** 成熟籽粒(WTS135 (右), 济麦22 (左))。

Figure 2 Evaluation for leaf rust resistance in WTS135 and its parents, and agronomic traits of WTS135 and Jimai 22
(A) Evaluation for leaf rust resistance in WTS135 and its parents (1: WTS135; 2: Xiaoyan 81; 3: Jimai 22, 4: Zhongnong 28, 5: *Thinopyrum ponticum*); **(B)** Adult plants; **(C)** Front view of the main spike (WTS135 (right), Jimai 22 (left)); **(D)** Lateral view of the main spike (WTS135 (right), Jimai 22 (left)); **(E)** Matured seeds (WTS135 (right), Jimai 22 (left)).

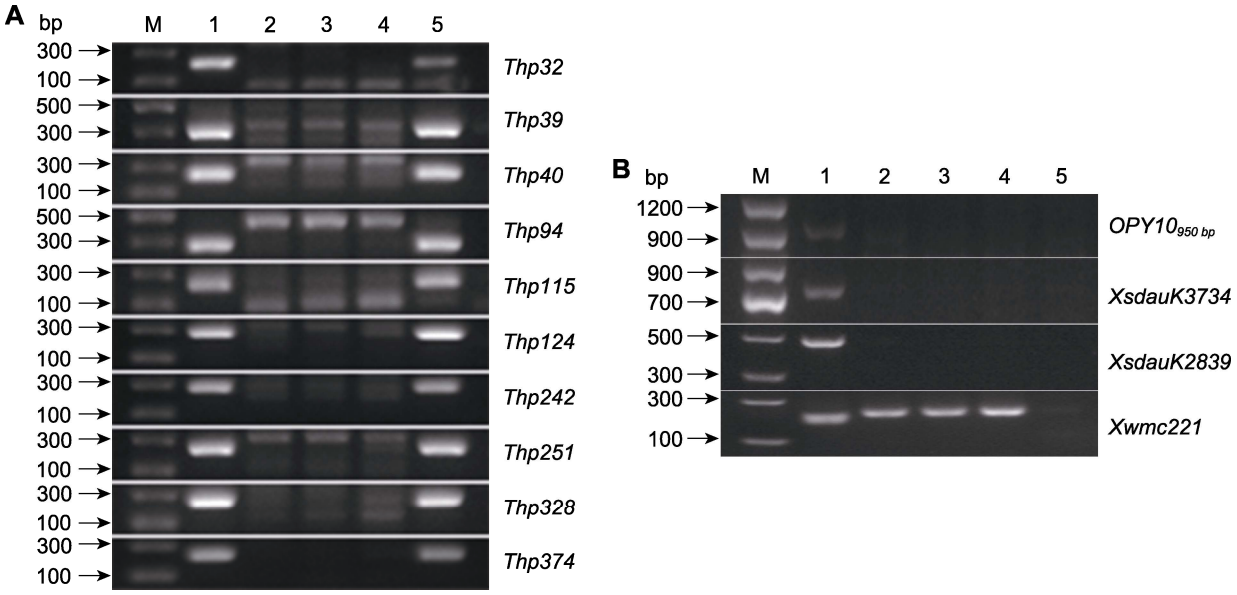

图3 10对长穗偃麦草特异引物的扩增结果**(A)**及*Lr19*和*Lr29*基因分子标记检测结果**(B)**
M: Marker; 1: 长穗偃麦草; 2: 小偃81; 3: 济麦22; 4: 中农28; 5: WTS135

Figure 3 Amplification results of 10 pairs of *Thinopyrum ponticum*-specific primers **(A)** and the results of molecular marker detection of *Lr19* and *Lr29* **(B)**
M: Marker; 1: *Thinopyrum ponticum*; 2: Xiaoyan 81; 3: Jimai 22; 4: Zhongnong 28; 5: WTS135

只有千粒重(30.07 g)极显著(P<0.01)低于济麦22 (41.67 g) (表2; 图2)。虽然WTS135的千粒重极显著低于济麦22, 但是由于其分蘖数显著增多导致株粒数增加, 平衡了千粒重明显降低的不利影响, 使得WTS135单株产量与济麦22并无明显差异, 表明WTS135可直接用于小麦的叶锈病抗性育种。

表1 WTS135中长穗偃麦草特异引物

Table 1 *Thinopyrum ponticum*-specific primers in WTS135

Primer name	Primer sequence (5'→3')	Fragment size (bp)	Annealing temperature (°C)
Thp32	F: TTGCAGCAGATCGAATCAAG R: CCTTCTTTCCCCGTTACTGTT	237	51
Thp39	F: GCATCATCTGCATTGTCGTC R: TCTGCACATGATACCCCAGA	290	52
Thp40	F: GACCATGTAGGTGCAACGTG R: AATCACAAAGCCCCTCCTTT	270	52
Thp94	F: CCAAACCAACAAGCACATTG R: AGCACCTTTTGGATGACTGC	285	51
Thp115	F: ACAAGCAGACGACAATGCAA R: TGAGTATTTCGAGGGTTGTGG	220	52
Thp124	F: AGGCTGGATGACCGAGTATG R: GATCCAGTCGTGGAAGGTGT	295	55
Thp242	F: CTGCATGAGCAGAGTCTGGA R: GAACTCCATTCACAGCAGCA	290	54
Thp251	F: TTTTCTTTGCTGCCTTCGTT R: GCTTGTGGTGAAGCAAATCA	260	51
Thp328	F: ATTTTCGCCACTCGTCATTC R: CTCTTGAAGGGGTCCAGACA	270	51
Thp374	F: GCCCAGCAGACAGGTAAGTT R: CAGTGACGAACATCCCCTTT	255	53

表2 WTS135与济麦22的农艺性状对比

Table 2 Comparison of agronomic traits between WTS135 and Jimai 22

Traits	Jimai 22	WTS135
Plant height (cm)	69.00±3.00	82.25±1.26**
Effective tiller number	13.33±1.53	23.75±1.71**
Spike length (cm)	8.80±0.56	8.83±0.57
Spikelet number per spike	20.67±0.58	19.50±1.00
Kernel number per spikelet	43.67±2.89	47.50±3.87
Total kernel number	517.33±62.05	755.75±15.17**
Yield per plant (g)	21.56±2.66	22.73±0.50
Thousand-kernel weight (g)	41.67±1.12**	30.07±0.11

表中数据为平均值±标准误(*n*=3), *t*检验。** *P*<0.01

Data in the table are means ± SE (*n*=3), *t*-test. ** *P*<0.01

3 讨论与结论

作为小麦的三级基因库，长穗偃麦草是小麦遗传改良中应用最成功的野生近缘物种之一。1920–1930年间，自Tsitsin (1965)成功实现小麦与长穗偃麦草的远缘杂交开始，育种家们一直致力于通过远缘杂交和染色体工程，创制小麦-长穗偃麦草新种质，将外源优异抗病基因转移至普通小麦(Li et al., 2008)。其中，小偃麦代换系是将1对或多对长穗偃麦草染色体替换掉相同数目的小麦染色体所形成的重要中间材料。完整的外源染色体虽然携带了优良基因，但也携带了很多不利基因，导致代换系往往难以直接用于小麦育种。通过物理辐射或化学诱变剂处理代换系，创制含有目标基因的小片段易位系，将极大减少遗传累赘，提高优良基因的遗传改良作用(Zhu et al., 2017; Wang et al., 2022; Zhang et al., 2024)。例如, Sharma和Knott (1966)对小麦-长穗偃麦草7E1 (7D)代换系的花粉进行辐射，并将其回交到普通小麦中，筛选到1个携带*Lr19*基因的易位系K11695。Xu等(2023)利用2个抗性不同的小麦-长穗偃麦草代换系组成双亲群体，将*Lr19*定位于长穗偃麦草7E1染色体长臂的0.3 cM区间内。此外，他们还通过中国春*Ph1b*突变体与K11695杂交，筛选到2个携带*Lr19*的小片段易位系，并创制了不含黄色素基因的小片段易位系，极大提高了*Lr19*基因的利用价值。Yang等(2023a)利用^{60}Co-γ射线辐射高抗白粉病的小麦-长穗偃麦草4Ag (4D)代换系的花粉，然后将辐射过的花粉授给易感白粉病小麦品种小偃81，创制了一系列易位系，通过细胞遗传学分析、抗病性鉴定、特异性引物扩增和序列比对，将抗性基因定位于4Ag短臂的3.79–97.12 Mb区间。不仅如此，他们还从中挑选出1个高抗白粉病的易位系WTT146，通过与济麦22进行杂交并回交，在BC_2F_2代中筛选到1个纯和的易位系J146，抗病性鉴定显示J146的白粉病抗性与WTT146相近，且农艺性状表现良好，可直接用于小麦的白粉病抗性育种。本研究创

制的代换系WTS135与济麦22相比, 虽然WTS135单株产量与济麦22差别不大, 但是其千粒重明显低于回交亲本济麦22, 根本原因在于WTS135是整条染色体的代换, 在携带抗叶锈病基因的同时还携带大量不利基因。为提高WTS135的千粒重, 我们对其进行了辐射诱变, 后续有望获得既携带抗病基因又不影响农艺性状的小片段易位系, 进而培育出农艺性状优良的抗叶锈病小麦品种。

Deng等(2024)开发了一款高分辨率小麦-长穗偃麦草液相芯片GenoBaits®WheatplusEE。该芯片包含80 000个二倍体长穗偃麦草特异基因组探针和10 000个小麦基因组探针, 是小麦染色体工程研究的有力工具。借助这款芯片, 研究者可快速分析小麦-偃麦草远缘杂交后代, 确定小麦和偃麦草染色体(或染色体片段)的拷贝数, 识别小麦-偃麦草易位染色体片段的断点以及检测小麦染色体的变异。Zhang等(1996)提出着丝粒及附近区域是多倍体中亚基因组分化的核心区域, 并建议用StStE^{e}E^{b}E^{x}作为十倍体长穗偃麦草基因组组成。本研究利用液相芯片分析, 发现WTS135中的十倍体长穗偃麦草染色体与二倍体长穗偃麦草7E染色体的相似度在着丝粒和近着丝粒区较低, 与原位杂交结果相互印证, 证实WTS135为7St (7D)二体代换系, 并说明可通过液相芯片的信号丰度及密度推测渗入小麦中的外源染色体来源。

*Lr19*和*Lr29*是来源于长穗偃麦草的第7部分同源群染色体的抗叶锈病基因(Niu et al., 2014)。为验证WTS135是否携带*Lr19*或*Lr29*, 我们利用与目标基因紧密连锁的分子标记扩增WTS135及其亲本(图3B)。*Lr29*相关标记扩增结果表明, RAPD标记*OPY10*仅在十倍体长穗偃麦草中扩增出1条950 bp的目标条带, 而在WTS135及其小麦亲本中无此特异性条带, 说明WTS135不含抗叶锈病基因*Lr29*。*Lr19*相关标记扩增结果显示, 与*Lr19*紧密连锁的标记*XsdauK3734*和*XsdauK2839*仅在十倍体长穗偃麦草中分别扩增出约750和460 bp的条带, 而在WTS135及其小麦亲本中无特异性扩增(Xu et al., 2023); 与*Lr19*有共显性的标记*Xwmc221*仅在长穗偃麦草中扩增出200 bp的目标片段, 而在普通小麦亲本济麦22、小偃81和中农28中均扩增出220 bp的条带, 在WTS135中则无特异性扩增(Gupta et al., 2006)。上述结果表明, WTS135也不含抗叶锈病基因*Lr19*。因此, 我们推测WTS135有可能携带1个新的抗叶锈病基因, 对小麦抗叶锈病育种具有很大的应用潜力。

Wang等(2020)通过远缘杂交得到1个兼具抗成株期条锈病和白粉病的材料CH10A5, 利用原位杂交、分子标记及小麦15K芯片分析, 表明CH10A5是1个1J^{S} (1D)二体代换系, 并开发了49个STS分子标记, 用于追踪长穗偃麦草的遗传物质。Li等(2021)将八倍体小偃麦SNTE20与普通小麦杂交, 获得1个兼具抗叶锈病和白粉病的材料SN19647, 经过细胞遗传学以及分子标记鉴定, 证实SN19647是1个1J^{S} (1B)二体代换系。通过对比SN19647和已知的1J^{S} (1D)代换系CH10A5的引物扩增结果证实, 尽管SN19647与CH-10A5中外源染色体部分同源群和基因组相同, 但仍有较大差异。Mago等(2019)首先将1个含有*SrB*基因的小麦-长穗偃麦草6Ae#3 (6D)代换系W3757与1个含有*Sr26*基因的T6AS·6AL-6Ae#1易位系WA-5杂交, 通过限制性片段长度多态性分析其后代, 得到1个既携带6Ae#3 L末端又携带6Ae#1 L亚端部的外源重组染色体片段T6Ae#1-6Ae#3。随后, 他们通过分析抗病性与外源片段的连锁关系, 确认*SrB*基因转移到小麦6A染色体长臂, 并证明6Ae#1-6Ae#3重组体的秆锈病抗性明显强于6Ae#3附加系。上述结果说明, 长穗偃麦草的相同部分同源群的染色体可能携带不同的抗病基因, 通过染色体工程聚合这些抗病基因, 能提高小麦抗病性的有效性和持久性。长穗偃麦草第7部分同源群携带丰富的抗病基因, 如赤霉病抗性基因*Fhb7*、叶锈病抗性基因*Lr19*和*Lr29*及抗秆锈病基因*Sr43* (周俭民, 2020)。本研究中, WTS135携带1个新的来源于第7部分同源群的抗叶锈病基因, 有望通过染色体工程实现外源抗病染色体片段重组, 从而增加小麦的抗病范围并提高其抗性强度和广度。

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Genetic Analysis and Molecular Marker Development for the WTS135–a Common Wheat-*Thinopyrum ponticum* Substitution Line with Leaf Rust Resistance

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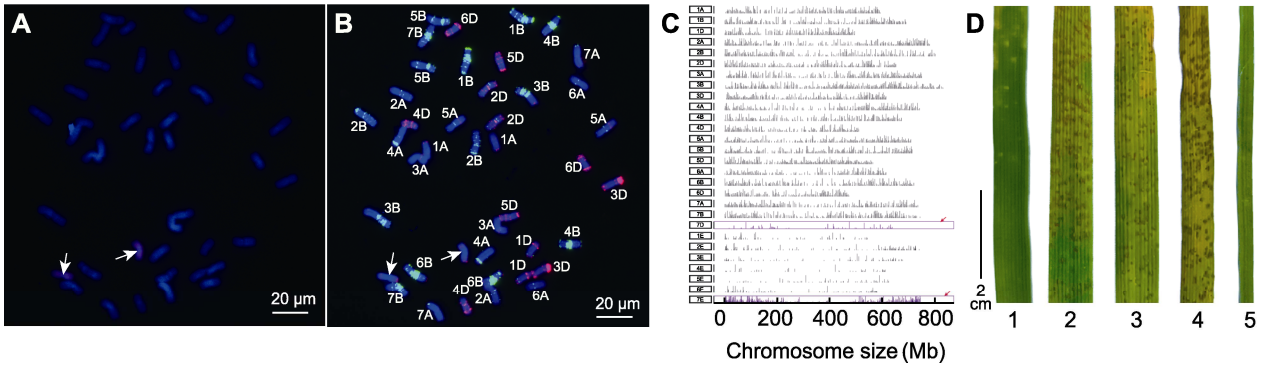
INTRODUCTION: The genetic diversity of common wheat (*Triticum aestivum*) has decreased sharply due to the domestication and modern breeding operations, making it more vulnerable to the threats from pests and pathogens. Leaf rust, caused by the fungal pathogen *Puccinia triticina* (*Pt*), is a devastating disease in wheat. Over 80 leaf rust resistance (*Lr*) genes have been identified, with nearly half originating from wheat wild relatives. However, the rapid evolution of *Pt* has rendered many *Lr* genes ineffective against prevalent *Pt* races. Consequently, identifying novel sources of resistance in wild relatives of common wheat remains an urgent priority for sustainable wheat breeding.

RATIONALE: As one of the most widely used relatives in the genetic improvement of wheat, decaploid *Thinopyrum ponticum* shows excellent resistance to multiple diseases including leaf rust. By distant hybridization and chromosome engineering, we created a wheat-*Th. ponticum* line WTS135. We evaluated its disease resistance with *Pt* race THTT, developed *Th. ponticum* specific markers by specific-locus amplified fragment sequencing technology and assessed its agronomic traits by phenotypic investigation. Genomic *in situ* hybridization (GISH)-fluorescence *in situ* hybridization analysis (FISH) and liquid chip analysis have been used to identify its chromosome composition.

RESULTS: WTS135 is immune to the *Pt* race THTT. Pedigree analysis showed that this resistance originated from the exogenous chromosome of *Th. ponticum*. GISH-FISH analysis revealed that the wheat chromosomes 7D were replaced by the *Th. ponticum*-derived chromosomes. Liquid chip analysis showed that the alien chromosomes belonged to the homoeologous group 7, and the density and abundance of the signals in the peri-centromeric region were significantly lower, which was consistent with the GISH results. Therefore, it is indicated that WTS135 is a 7St (7D) disomic substitution line. After detected by the molecular markers related to known *Lr* genes on wheat 7D chromosome, it is speculated that WTS135 probably carries a novel resistance gene that is different from genes *Lr19* and *Lr29*. Ten primers specific to *Th. ponticum* were developed to rapidly trace the exogenous chromatin in WTS135. Phenotypic investigation

showed that the yield of WTS135 was not significantly different from that of the recurrent parent Jimai 22, suggesting that this line can be useful for improving disease resistance in wheat.

CONCLUSION: Introducing resistance genes from wild relatives into wheat through distant hybridization can broaden the genetic base of wheat and provide new sources for breeding disease-resistant varieties. We developed a common wheat-*Th. ponticum* 7St (7D) substitution line, which possibly has a novel alien resistance gene and could be used in the breeding for enhancing wheat disease resistance.



Chromosome composition and leaf rust resistance evaluation of WTS135. (A) GISH analysis using *Thinopyrum ponticum* gDNA as a probe and Chinese Spring gDNA as a block; **(B)** Mc-FISH analysis using combined oligo probes; **(C)** The liquid chip analysis of WTS135; **(D)** Evaluation for leaf rust resistance in WTS135 and its parents (1: WTS135; 2: Xiaoyan 81; 3: Jimai 22, 4: Zhongnong 28, 5: *Th. ponticum*). The white arrows indicate exogenous chromosomes, purple frames indicate chromosome additions or deletions.

Key words wheat, *Thinopyrum ponticum*, leaf rust, distant hybridization, substitution line, *in situ* hybridization, molecular markers

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附图1 WTS135及其亲本对叶锈菌生理小种PHTT **(A)**、THTS **(B)**和DGJJ **(C)**的抗性评价
1: WTS135; 2: 济麦22; 3: 小偃81; 4: 中农28; 5: 长穗偃麦草

Appendix figure 1 Evaluation of WTS135 and its parents for resistance to *Pt* race of leaf rust PHTT **(A)**, THTS **(B)** and DGJJ **(C)**
1: WTS135; 2: Jimai 22; 3: Xiaoyan 81; 4: Zhongnong 28; 5: *Thinopyrum ponticum*

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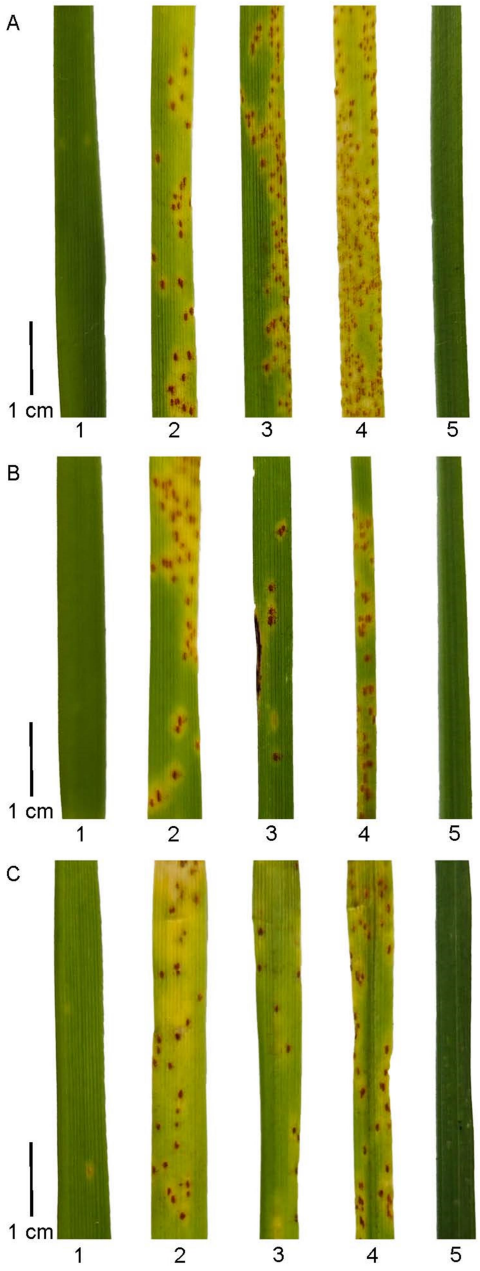

附图 1　WTS135 及其亲本对叶锈菌生理小种 PHTT **(A)**、THTS **(B)**和 DGJJ **(C)**的抗性评价
1: WTS135; 2: 济麦 22; 3: 小偃 81; 4: 中农 28; 5: 长穗偃麦草

Appendix figure 1　Evaluation of WTS135 and its parents for resistance to Pt race of leaf rust PHTT **(A)**, THTS **(B)** and DGJJ **(C)**
1: WTS135; 2: Jimai 22; 3: Xiaoyan 81; 4: Zhongnong 28; 5: *Thinopyrum ponticum*