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水稻卷叶突变体基因 *shallot like1-Fuhui673* 鉴定、克隆与序列分析

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摘要 叶片是水稻进行光合作用的重要器官, 其形态直接影响光合作用的强弱. 叶片适度卷曲可以使叶片保持直立, 改善群体内部透光状况, 提高光能利用率, 进而提高水稻产量. 本研究通过Co_{60}-γ辐照诱变杂交水稻恢复系福恢673, 获得一个卷叶突变体, 命名为*sll1-fh673*(*shallot-like1 from Fuhui673*). 经遗传学分析表明, 该突变体*sll1-fh673*卷叶性状受1对隐性基因控制, 结合混合群体(BSA)和隐性群体(RCA)分离分析法, 将该基因*ssl1-fh673*精细定位于水稻第9染色体的inDel标记Ri9-20与Ri9-7之间52 kb的区域内. 测序结果表明, *sll1-fh673*(LOC_Os09g23200)的核苷酸序列在第1个外显子第447 bp处由C替换为A, 447 bp后缺失5个碱基, 导致编码的氨基酸序列从第148位起发生改变并移码, 造成蛋白功能异常. 因此, 推测*sll1-fh673*是*SLL1*的新等位基因, 该新等位基因的挖掘丰富了*SLL1*基因的等位资源, 有助于深入研究叶片卷曲的形成机理.

关键词 水稻, 卷叶, *sll1-fh673*, 图位克隆, 序列分析

矮化育种和杂种优势利用使水稻的产量连上台阶后, 育种家们又在寻求能够大幅度提高水稻产量的新方法. 目前, 育种家比较公认的育种方法就是超高产育种, 袁隆平[1]在超高产株型设计中对叶片的要求是向内微卷. 卷叶水稻与展叶水稻相比, 优势主要表现在: (1) 可显著降低叶片的披垂程度, 减少叶片投影面积, 使叶片正、反两面受光, 增加群体上层叶面积的比例, 改善群体内部透光状况[2,3]; (2) 中后期叶片更抗衰老, 提高中后期光能利用率, 增加在中、高密度下群体光合速率、群体干物质生产以及产量[4]; (3) 有利于发挥在高肥水平下的产量潜力[5]; (4) 有利于提高稻米加工品质(如提高整精米率、降低垩白率和垩白度等)、理化品质和米饭质地等[6].

目前, 在水稻中已经克隆了一系列形成卷叶的基因, 主要分为4类: 第一类是调控泡状细胞的数目或大小发生改变的基因, 如*ACL1*[7], *LC2*/*OsVIL3*[8,9], *OsCOW1*/*NAL7*[10,11], *NRL1*/*ND1*/*sle1*/*DNL1*/*OsCD1*[12~17], *OsHox32*[18], *OsMYB103L*[19], *OsZHD1*[20], *REL1*[21],

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REL2[22], *RL14*[23], *ROC5*[24], *SRL1*[25]和*YABBY1*[26]等; 第二类是调控近轴/远轴面发育的不平衡引起的基因, 如*ADL1*[27], *OsAGO7*[28], *OsAGO1a*[29,30], *SLL1*/*RL9*[31,32]等; 第三类是调控厚壁组织发育异常导致叶片卷曲的基因, 如*SLL1*/*RL9*[31,32], *SRL2*/*AVB*/*NRL2*[33~35]; 第四类是调控角质层发育异常导致叶片卷曲的基因, 如*CFL1*[36].

本研究利用浙江省农业科学院作物与核技术利用研究所的钴60-γ射线辐照自育恢复系福恢673干种子, 获得1个葱状卷叶突变体, 该突变体叶片形状主要表现为叶片向内卷, 植株半矮化, 籽粒充实度差. 通过对该突变体的遗传学分析和基因精细定位、克隆, 结合生物信息学分析并预测候选基因, 为后续基因的功能互补验证和研究水稻卷叶的分子机理奠定基础.

1 材料与方法

(ⅰ) 试验材料与种植. 通过辐射诱变福恢673干种子, 获得1份水稻卷叶突变体, 命名为*sll1-fh673*(*shallot like1-Fuhui673*). 经过连续几代自交, 卷叶性状能稳定遗传. 2013年在福建省农业科学院水稻研究所沙县夏茂基地配制*sll1-fh673*/9311的杂交组合, 收获F_1种子, 2014年在海南三亚藤桥福建省南繁基地种植F_1种子并套袋自交收获F_2种子, 2014年在福建省农业科学院水稻研究所沙县夏茂基地种植F_2, 在分蘖盛期调查单株表型, 并进行表型的统计学分析. 2015年在福建省农业科学院水稻研究所沙县夏茂基地配制*sll1-fh673*/日本晴杂交组合, 2015年在海南三亚藤桥福建省南繁基地种植F_1并收获F_2种子, 2016年在福建省农业科学院水稻研究所沙县基地种植F_2, 并进行表型的统计学分析.

(ⅱ) 水稻叶片DNA的提取. 采用CTAB法提取水稻叶片DNA[37].

(ⅲ) PCR标记及其分析. SSR标记和InDel标记的引物序列由福州尚亚生物技术有限公司合成. PCR反应和电泳程序参考罗曦等[38]方法.

(ⅳ) 卷叶基因*sll1-fh673*的初步定位. 采用混合分离分析法(bulked segregant analysis, BSA)和隐性群体(recessive-class analysis, RCA)分析法, 在*sll1-fh673*/9311的F_2分离群体中分别随机选择平展叶与卷叶各10株的叶片, 等量混成平展叶池和卷叶池; 用本实验室保存有的平均分布在12条染色体上的148对SSR标记对野生型、卷叶突变体、平展叶混合池、卷叶混合池进行多态性分析, 寻找连锁标记, 初步定位卷叶基因*sll1-fh673*.

(ⅴ) 卷叶基因*sll1-fh673*的精细定位. 根据初步定位的结果, 在网站http://ensembl.gramene.org寻找目的基因区段日本晴与9311之间的碱基插入或者缺失, 利用 Primer Premier 5.0软件设计InDel标记. 用野生型和卷叶突变体有多态性的InDel标记对*sll1-fh673*/日本晴的F_2群体隐性单株进行分析, 精细定位卷叶基因*sll1-fh673*.

(ⅵ) 候选基因预测、克隆和序列分析. 通过生物信息学网站Gramene (http://www.gramene.org) 和RGAP (http://rice.plantbiology.msu.edu/index.shtmL)对目标基因精细定位的两侧标记所在染色体区段进行基因预测, 找到可能的候选基因, 根据候选基因的序列设计引物, 克隆相关候选基因, 根据已发表的文献分析候选基因的功能.

2 结果与分析

2.1 卷叶突变体*sll1-fh673*的叶片表型观察

与野生型相比, 突变体在全生育期内均表现为叶片高度卷曲成葱状, 沿中脉纵向内卷(图1(a), (b)). 该突变体的株高变矮, 只有90.6 cm, 是野生型的69.9%(图1(a)); 突变体的穗形畸形, 穗长变短, 粒形变小, 种子发育不良(图1(c), (d)). 突变体*sll1-fh673*的表型与已报道的*SLL1*的突变体表型相似.

2.2 卷叶基因*sll1-fh673*的遗传学分析和精细定位

日本晴与卷叶突变体杂交的F_1植株叶片表现为平展叶, 说明卷叶性状为隐性性状. F_2群体(总共363个单株)平展叶273株, 卷叶90株. 经卡方测验, 平展叶与卷叶的表型符合3∶1分离比($\chi^2=0.33<\chi^2_{0.05,1}=3.84$), 说明突变体的卷叶性状是受1对隐性核基因控制.

利用本实验室保存的均匀分布在水稻12条染色体上的148对SSR引物进行两亲本(9311和卷叶突变体)的多态性分析, 其中的43对SSR标记在9311和卷叶突变体之间具有多态性. 利用BSA基因池对43对SSR引物进行多态性分析, 发现第9号染色体RM553在BSA基因池里表现多态, 进一步用RM553对卷叶池的10个隐性单株进行分析, RM553在10个单株中有2个发生了交换. 所以将卷叶基因*sll1-fh673*初步定位在水稻第9号染色体上, 并与RM553有连锁(图2).



图 1 (网络版彩色)野生型和突变体的株型、叶片形态、穗型和粒型. (a) 野生型和突变体的株型; (b) 野生型和突变体的叶形; (c) 野生型和突变体的穗型; (d) 野生型和突变体的粒型

Figure 1 (Color online) The phenotype of plant, leaf, spikle and grain between wild type and mutant. (a) The plant type between wild type and mutant; (b) the leaf type between wild type and mutant; (c) the spikle type between wild type and mutant; (d) the grain type between wild type and mutant

为了进一步定位卷叶基因, 在RM553附近设计并合成了60对SSR引物, 通过连锁分析, 将*sll1-fh673*基因定位于标记RM3769与RM3700之间. 接着在该区间又设计并合成了50对SSR引物, 进一步把该基因定位在SSR标记RM24218与RM24315之间, 遗传距离为7.0 cM. 由于9311与卷叶突变体同为籼稻, 遗传差异比较小, 以至于在初步定位区间难以获得多态性标记, 因此构建了日本晴与卷叶突变体杂交的F_2群体用于卷叶基因*sll1-fh673*的精细定位. 根据日本晴和9311的序列差异, 在SSR标记RM24218与RM24315之间, 开发了23对新的InDel标记(Ri9-1~23), 其中Ri9-7, 8, 9, 10, 11, 13, 16, 17, 19, 20, 21, 22, 23在亲本之间多态性较好(表1). 用这些标记分析日本晴/卷叶突变体F_2群体(363个单株)的90个卷叶单株, 结果



图 2 RM553 标记在BSA池中的电泳图. 1, 9311; 2, 卷叶亲本; 3, 平展叶混合池; 4, 卷叶混合池; 5, 20 bp DNA marker

Figure 2 The electrophoretogram of marker RM553 with gene pool of bulked segregant analysis (BSA). 1, 9311; 2, parent of rolled leaf; 3, mixing pool of flat leaf; 4, mixing pool of rolled leaf, 5, 20 bp DNA marker

表明卷叶基因与Ri9-17, -21, -22, -23共分离, 其中一侧与Ri9-20有2个交换, 另一侧与Ri9-7有1个交换, 结合Map/maker3.0软件分析, 将卷叶基因*sll1-fh673*精细定位在标记Ri9-20与Ri9-7之间的52 kb区域内(图3).

2.3 卷叶基因的功能预测

根据MSU (http: //rice.plantbiology.msu.edu/)网站提供的基因注释信息, 在定位区域内包含有3个注释基因(LOC_Os09g23180, LOC_Os09g23190, LOC_Os09g23200)(表2), 其中LOC_Os09g23200基因是编码一个SHAQKYF的类MYB转录因子, 它通过调控水稻叶片远轴面厚壁组织细胞的程序化死亡来调控水稻叶片的形状[31]. 所以, 首先对LOC_Os09g23200基因进行了测序, 测序结果表明, 卷叶突变体中的LOC_Os09g23200基因在第1个外显子第447 bp处由C替换为A, 447 bp后缺失5个碱基, 突变导致氨基酸序列第148位起发生变化, 并在第255位处提前终止(图3, 4), 造成蛋白功能异常. 针对候选基因的5 bp缺失重新设计一对InDel引物Ri9-30(表1), 在F_2群体(日本晴/卷叶)中所有卷叶单株与Ri9-30共分离(图5), 进一步证实了由于碱基缺失导致了LOC_Os09g23200 基因的突变.

表 1 新发展的InDel标记

Table 1 Primer sequences of the polymorphic InDel markers

标记	正向引物序列(5′→3′)	反向引物序列(5′→3′)	扩展片段大小(bp)	正向引物扩增位置(对应日本晴序列)
Ri9-13	GACCAGCCTTGGTTGAAAAA	AAGGGCAAACGACATAGTGC	113	9: 13339379~13339398
Ri9-16	CGCATGATCGATGCTGTACT	GGCCATGGACCACAGTAAAA	167	9: 13656150~13656169
Ri9-19	CTTCATTTGACAAGGCACCA	GTGCATAGCTGCAAGCCAAT	152	9: 13692067~13692086
Ri9-20	AAAGTTTCGCACGAAATTAACA	TCGATCGACAGGTTGATTAGC	146	9: 13717568~13717589
Ri9-21	CCACACGATTGGATGCATTA	CCGTCATTGGTTAATTGGAAA	193	9: 13736269~13736288
Ri9-17	GACTATTGTGGGTTTCCCAGT	ACCTGCATGATGAATGACGA	277	9: 13738281~13738301
Ri9-22	CTTGCTCTAACACGGCCATT	AACTAAACACGGCCTCCATC	129	9: 13755998~13756017
Ri9-23	TGAGACAAAGATGCCAATGC	CACATCCCCAAACATGTCAC	105	9: 13760490~13760509
Ri9-7	AAATGGTCTATGCCGTCAGC	TCATATGGCGCTTGAGATTG	205	9: 13770295~13770314
Ri9-8	GTGTATGCGCGACATGAGAT	CTCCGTGTGAAGCACTGAAG	147	9: 14018067~14018086
Ri9-9	TACGTACGTGTCGCGTGATT	CGATGGTTTGCTATTGACGA	130	9: 14151697~14151716
Ri9-10	TGCAAACTCTTTTATCAATGCTG	AACCATGCTGGCAAGAAGAT	155	9: 14384273~14384295
Ri9-11	TCATCTGGCATCAATGCTTC	GAATGGTTAAACTTTACAAGAAAAAGC	113	9: 14586618~14586633
Ri9-30	GCTCAACGGCATGCTCTC	TGCTCGTCCAGCGCATGC	206	9: 13759921~13760126

表 2 水稻第 9 染色体定位区间内基因及其推测功能

Table 2 Gene names and their putative functions in the target interval

基因名称	推测功能
LOC_Os09g23180	expressed protein
LOC_Os09g23190	transposon protein, putative, CACTA, En/Spm sub-class, expressed
LOC_Os09g23200	KANADI1, putative, expressed

3 讨论

研究水稻叶片卷曲的分子调控机制, 对提高水稻产量具有重要意义. 目前, 一系列卷叶基因的克隆对揭示水稻卷叶的分子机理起了重要的作用. 但由于叶片结构的复杂性, 其厚壁组织、泡状细胞、木质部、维管束、叶肉细胞等其中之一任何一个结构发生改变都会导致叶片形态的变化. 因此, 形成卷叶的分子机理还有待继续深入研究.

目前已报道的*SLL1*等位基因有4个[31,32], *sll1-1*和*sll1-2*是Nipponbare EMS诱变来的, *rl9-1*和*rl9-2*是中花11经过钴60辐照诱变来的, 以上4个等位基因都是由粳稻突变来的, 而*sll1-fh673*是由籼稻福恢673突变来的, 目前为止, *sll1-fh673*是该等位基因唯一1个籼稻遗传背景的突变体. 此外, *sll1-fh673*的突变位点与*sll1-1*, *sll1-2*, *rl9-1*和*rl9-2*均不相同. *sll1-1*在第1个外显子发生单碱基替换, *sll1-2*在第3个外显子发生单碱基替换, *rl9-1*在第1个外显子有5个碱基缺失, *rl9-2*在第1个内含子和第2个外显子之间的发生单碱基替换. *sll1-fh673*在第1个外显子第447 bp处C替换为A, 447 bp后缺失5个碱基. 因此, 本研究中的卷叶突变体基因*sll1-fh673*是*SLL1*的新等位基因, 丰富了*SLL1*基因的等位资源, 有助于育种上对卷叶基因的利用.

*SLL1*是一个KANADI类转录因子, 此前关于*SLL1*的报道由于分子生物学技术受限, 主要是从叶片形态剖析及生理特征方面进行研究, 关于*SLL1*具体如何调控叶片卷曲的分子机理仍未被阐述. 而近十年分子技术得到迅猛发展, 许多针对转录因子的研究方法新出现或者得到优化, 如EMSA, ChIP, 酵母单杂交, 互作板芯片微孔技术等, 都有助于研究蛋白质与DNA的相互作用, 后期我们可以尝试通过这些方法预测*SLL1*的结合位点并寻找下游的互作蛋白, 进而对*SLL1*具体如何调控叶片卷曲的分子机理进行研究.

4 结论

通过辐照诱变获得一个卷叶突变体*sll1-fh673*. 该突变体叶片全生育期均呈现高度卷曲成葱状, 株

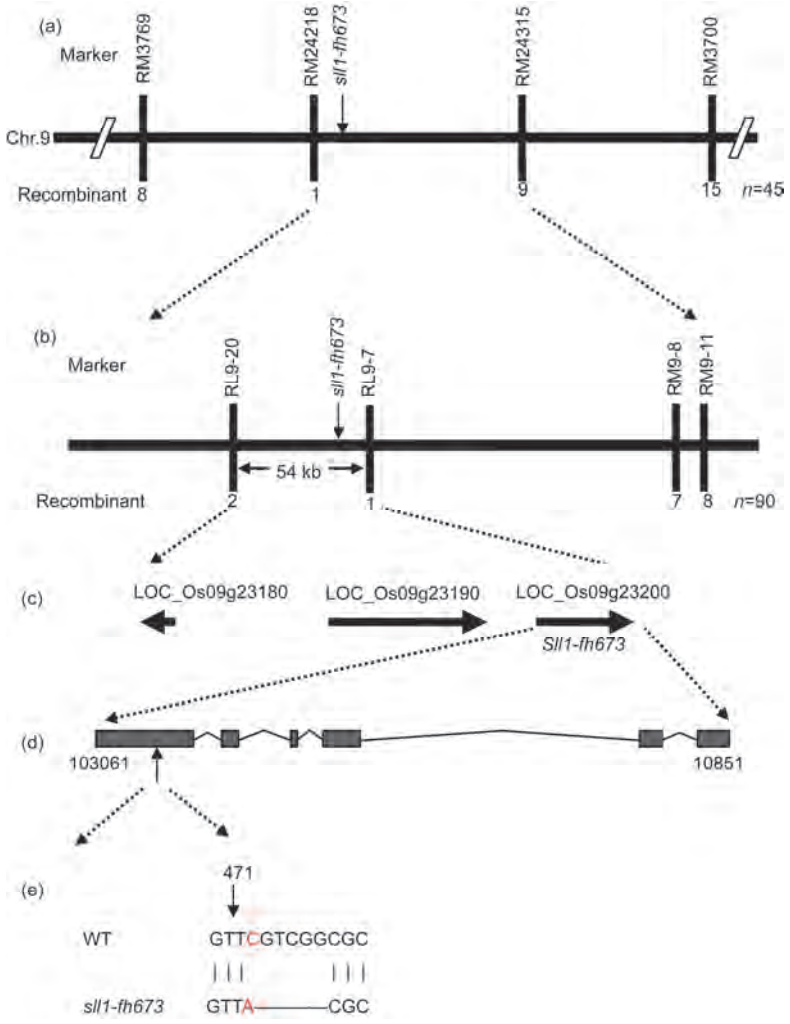


图 3 (网络版彩色)水稻卷叶基因*sll1-fh673* 的精细定位图和物理结构图. (a) *sll1-fh673* 基因的初步定位图; (b) *sll1-fh673* 目标基因区间的精细定位图; (c) 定位区间的候选基因; (d) *sll1-fh673* 基因的结构图; (e) 目标基因的突变位点

Figure 3 (Color online) The fine mapping and physical mapping of *sll1-fh673* in rice. (a) The primary mapping of *sll1-fh673*; (b) the fine mapping of *sll1-fh673*; (c) the candidate genes in the mapping regions; (d) the structure of *sll1-fh673*; (e) the mutated base between *SLL1* and *sll1-fh673*

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SLL1        MAPMMLQPLDAGGGASAPPPPIRGIPIYNGPGGFPFLQPSPTAGDVGHHHHHHHPKMGFYS  60
sll1-fh673  MAPMMLQPLDAGGGASAPPPPIRGIPIYNGPGGFPFLQPSPTAGDVGHHHHHHHPKMGFYS  60

SLL1        SYHHPSTWPSTSPSPLAAPPGAASSPLDPTAAFLSSPHHRMLSAASGRLNGMLSVSDTLR 120
sll1-fh673  SYHHPSTWPST SPSPLAAPPGAASSPLDPTAAFLSSPHHRMLSAASGRLNGMLSVSDTLR 120

                                  ▼ sll1-fh673
SLL1        SYGVPGAAAPGVIGGAHHHHHHHLHGGQPFVGALASRFMPKLPAKRSMRAPRMRWTSTLHA 180
sll1-fh673  SYGVPGAAAPGVIGGAHHHHHHHLHGGQPLRPRVPLHAQAPRQAQHARAAHALDEHPPRPL 180

SLL1        RFVHAVELLGGHERATPKSVLELMDVKDLTLAHVKSHLQMYRTVKSTDKPAASSGPADGG 240
sll1-fh673  RPRRRAPRRPREGDAQVGAGAHGRQGSDASACQEPPPDVSHREEH-

SLL1        SGDEEFAGGGQAASGGGDSMCLRGGGGGGVAAAAFAEHGRSASEGAASSVGGGGGGDMDQ 300
sll1-fh673

SLL1        SSAGNTSTTRWSNSSRDPWLSSNSCNMDAHRSVGLSSPIENLEPCRSSSSQVSNHELSSP 360
sll1-fh673

SLL1        SLEFTLGRPDWHGADHD 377
sll1-fh673
```

图 4 (网络版彩色)水稻基因*SLL1* 与卷叶基因s*ll1-fh673* 的蛋白质序列比对分析

Figure 4 (Color online) Blast of protein sequence between *SLL1*and s*ll1-fh673*

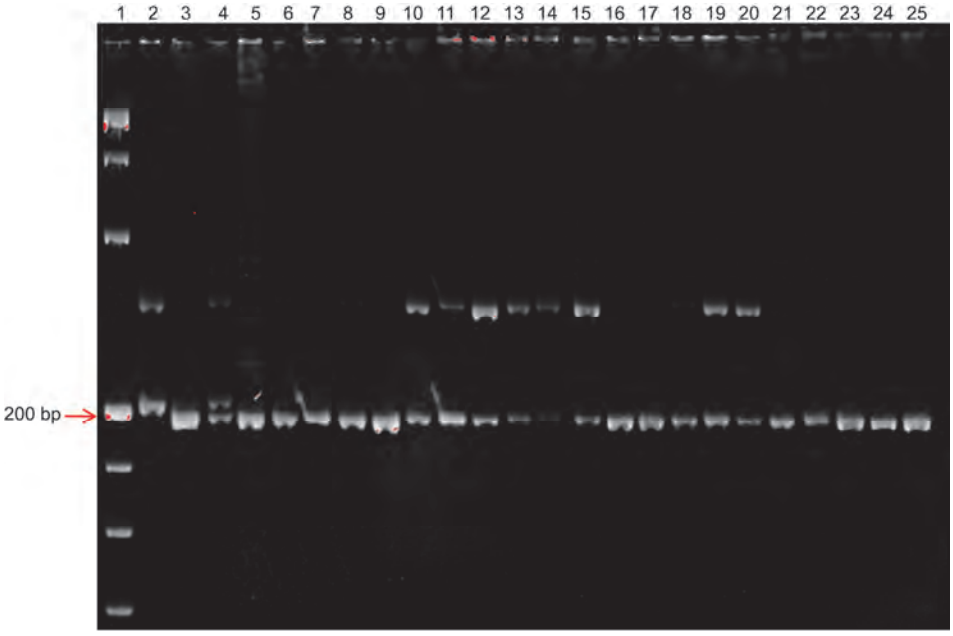


图 5 (网络版彩色)Ri9-30 标记在F_2群体(日本晴/卷叶)的电泳图. 1, 20 bp marker; 2, 平展叶亲本; 3, 卷叶亲本; 4, F_2群体平展叶池; 5, F_2群体卷叶池; 6~25, F_2群体卷叶单株, 箭头, 目的片带的位置

Figure 5 (Color online) The electrophoretogram of Ri9-30 marker in F_2 population of Nipponbare and rolled leaf mutant hybridization. 1, 20-bp marker; 2, flat leaf parent; 3, rolled leaf parent; 4, flat leaf mixed pool of F_2 population; 5, rolled leaf mixed pool of F_2 population; 6–25, rolled leaf individual plant of F_2 population; arrow, the target fragment

高只有野生型的69.9%, 穗形畸形, 穗长变短, 粒形变小, 种子发育不良. 经遗传学分析表明, *sll1-fh673*受1对隐性基因控制, 并将该基因定位于第9染色体InDel标记Ri9-20与Ri9-7之间52 kb的区域. 测序结果表明, 卷叶突变体中的LOC_Os09g23200基因在第1个外显子第447 bp处C替换为A, 447 bp后缺失5个碱基, 导致移码突变, 不能行使正常蛋白功能. 因此, 我们推测卷叶突变体基因*sll1-fh673*是*SLL1*的新等位基因, 丰富了*SLL1*基因的等位资源, 有助于深入研究叶片卷曲的形成机理.

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Summary for "水稻卷叶突变体基因 *shallot like1-Fuhui673* 鉴定、克隆与序列分析"

Identification, cloning and sequence analysis of *shallot like1-Fuhui673* from a rolled leaf mutant in rice

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Leaves are the important organ for photosynthesis in rice and the strong and weak photosynthesis will be directly influenced with their morphologic structure of leaves. Moderate rolling leaf could keep plants upright, improve light transmittance of population, increase efficiency of light utilization and then increase rice yield. We obtained a rolled leaf mutant, named *sll1-fh673* (*shallot like1-Fuhui673*) derived from the restorer line Fuhui673 (*Oryza sativa* L. ssp. *indica*) of hybrid rice, which were treated with 60 Co-γ radiation. The leaves of the *sll1-fh673* rolled inward like a cylinder during the whole growth period. The characteristics of the *sll1-fh673* were distinct from those of the wild-type plants, with shorter on plant height and ear length, malformed spikelets, decreased the grain number per spike, smaller grain shape, worse development of the seed. The result of genetic analysis indicated that the phenotype of rolled leaves of *sll1-fh673* was controlled by a single recessive nuclear gene. Meanwhile, the *sll1-fh673* gene was mapped to a 52-kb region between inDel marker Ri9-20 and Ri9-7 on the Chromosome 9 of rice with Bulked Segregant Analysis and Recessive-class Analysis methods. But there was a cloned rolled leaf gene *SLL1* (LOC_Os09g23200) in the mapped regions, which might be the candidate gene of *sll1-fh673*. The sequencing analysis revealed that there was a single-base substitution from C to A and five-base deletion in the first exon in *sll1-fh673* comparing with the *SLL1* gene, leading to frame-shift mutation after the 148 aa and dysfunction of *sll1* protein. So we speculated that the *sll1-fh673* gene might be allelic to *SLL1* gene. These results will deepen our understanding the mechanisms of the leaf rolling and enriches the allelic resource of the *SLL1* gene. We will predict binding sites of the *sll1-fh673* gene and find interacting protein of down-regulation, and then carry out the molecular mechanism of the leaf rolling, such as electrophoretic mobility shift assay (EMSA), DNA Chip Technology and Yeast One Hybrid System in the future.

rice, rolled leaf, *sll1-fh673*, map-based cloning, sequence analysis

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