Molecular Identification of a Newwheat-*Thinopyrumintermedium* Cryptictranslocation Line for Resistance to Powdery Mildew

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***Abstract*—Powdery mildewis one of the destructive diseases of wheat in many regions of the world. Wheat-*Thinopyrumintermedium* derived line CH5382 conferring novel powdery mildew resistance was characterized using molecular and cytogenetic methods. The conventional GISH analysis probed by *Th. Intermedium* cannot detect the alien fragmentof CH5382. Two PCR-based Landmark Unique Gene markers (TNAC1102 and TNAC1567), which were assigned on wheat chromosome 2L and 5S, respectively, canamplfy unique bands of CH5382, and the bands were traced to wheat-*Th. intermedium* partial amphiploiddonor TAI7044 and *Th. intermedium*. The result suggested that CH5382 is a new wheat-*Thinopyrumintermedium* cryptic alien translocation line with powdery mildew resistance.**

***Index Terms*—Cryptic alientranslocation, landmark unique gene markers, powdery mildew, *Thinopyrumintermedium*.**

I. Introduction

Powdery mildew, caused by Blumeriagramin is f. sp. Tritici (Bgt) is a destructive disease of wheat (Triticumaestivum L.) grows in regions with temperate and maritime climates. The most effectiveapproach of controlling thedisease is the developmentand utilization of resistantvarieties. Over 70 alleles have been identifiedfor resistance to wheat powdery mildew [1]. Most of these resistance genes are rapidly overcome by virulent races of the pathogen.The recent study showed that powdery mildew resistance genePm21 is one of the most effective in both Europeand China, but extensive useof Pm21 may render it usceptible to new pathogen races [2]. Therefore it is of importance to search for new sources of resistance from the tertiary gene pool to reduce the wheat yield loss caused byepidemics of powdery mildew.

Thinopyrumintermedium(2*n*=6*x*=42, genome JJsS), the tertiary gene pool ofwheat,conferring many valuable traits, has been widely used in wheat geneticimprovement. It is reported that variousadditionlines, substitutionlinesand translocation lines involving chromosomes of Th. intermedium with powdery mildew resistance have been bred[3],[4]. Many introgressionscontained large aliensegment, despite carrying the desired genes, since the large introduced chromosome segments were unable to adequately compensate for the wheatgenes they replace, or carry undesirable traits. Thus, the introgressedlines with minimum length of alien chromosome segments but retaining the useful geneswasimportant for using *Th. Intermedium* chromatin.

CH5382 is a stable wheat-*Th.intermedium* derived line carrying novel genes for resistance to powdery mildew. The purpose of the present study is to prove the resistance gene of CH5382 derived fromTh. *intermediumby* employing molecular and cytological methodologies.It will be beneficial to accelerate the applications of CH5382 to wheat breeding programs.

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II. Materials and Methods

A. Materials

CH5382 was BC2F7 derived lines developed by back crossing wheat-Th. *intermedium* partial amphiploid "TAI7044" as resistance donor with susceptible wheat cultivars.TAI7044, Jing411 and Jimai26werebredor provided by Shanxi Academy of Agricultural Sciences.Chinese Spring (CS) was maintained at the Triticeae Research Institute, Sichuan Agricultural University, China, Th. *intermedium*(PI440125) was provided by National Plant Germplasm System (NPGS), USA.

B. Evaluation of Powdery Mildew Resistance and GISH Analysis

The resistance survey of seedlings of *Th.intermedium*, resistance donor TAI7044, CH5382 and two wheat cultivars, was evaluated in the greenhouse using Bgt isolates E09.

For GISH,Th. *Intermedium* genomic DNA was labeled with digoxigenin-11-dUTP by nick translation according to the manufacturer's instruction (Roche Diagnostics, Indianapolis, USA).Sheared genomic DNA of CS (ABD, 2*n*=42) was used as blocking DNA.

C. Molecular Marker Analysis

Total genomic DNA was extracted from young leaves according to the SDS protocol [5]. A total of 125 pairs of the PCR-based landmark unique gene (PLUG) primers

were synthesized according to Ishikawa *et al.* [6]. The PCR procedures are: an initial 5 minute enaturation at 95℃, followed by 32 cycles of 95℃ for 45 seconds, 57℃ for 45 seconds, 72℃ for 2 minutes, and a final incubation at 72℃ for10 minutes before cooling to 4℃. The PCR was performed in an Icyclerthermalcycler (Bio-Rad, Hercules, USA). An 8μL aliquot of the amplification product was analyzed by electrophoresis on a 1.5% agarose gel in 40 mMTris-acetate. To obtain high levels of polymorphism, an 8 μLaliquot of the product was digested 2 hours with TaqI (65℃), HaeIII (37℃), orHpaII (37℃), respectively. Digested fragments were fractionated by electrophoresis on 2% agarosegel in Tris-Acetate-EDTA buffer.

III. Results

A. Agronomic Traits and Resistance Observation of CH5382

CH5382 derived from *Th.intermedium*shows some phenotypic differences compared to its wheat parents, such ashigh number of tillers, semi-dwarfism, awnedness and high resistance to powdery mildew, leaf rust and yellow rust. The survey of powdery mildew resistance Demonstrated that CH5382, resistance donor TAI7044 and the *Th. intermedium*wereimmune to *Bgtisolates* E09, whereas the wheat parents, Jing411 and Jimai26 were susceptible. The resultconfirmed that powdery mildew resistance gene has been transferred from*Th.intermedium*toCH5382.

TABLE 1 HOMOLOGOUS GROUP RELATIONSHIP OF THE PLUG MARKERS DERIVED FROM WHEAT ESTS

Primer	Homologous relationship	Primer	Homologous relationship
TNAC1010	1S	TNAC1457	4AL,4BS,4DS
TNAC1019	1AS,1BL,1DL	TNAC1463	4AL,4BS,4DS
TNAC1021	1L	TNAC1468	4AL,4BS,4DS
TNAC1026	1L	TNAC1510	4AL,4BS,4DS
TNAC1038	1L	TNAC1485	5S
TNAC1063	1AL,1BS,1DS	TNAC1497	5S
TNAC1085	1L	TNAC1503	5S
TNAC1088	1L	TNAC1588	5S
TNAC1102	2S	TNAC1535	5L
TNAC1176	2S	TNAC1540	5L
TNAC1183	2S	TNAC1541	5L
TNAC1233	2S	TNAC1545	5L
TNAC1142	2L	TNAC1567	5L
TNAC1118	2L	TNAC1577	5L
TNAC1125	2L	TNAC1605	5L
TNAC1132	2L	TNAC1610	5L
TNAC1137	2L	TNAC1613	5L
TNAC1139	2L	TNAC1616	5L
TNAC1140	2L	TNAC1559	5L
TNAC1195	2L	TNAC1614	5L
TNAC1199	2L	TNAC1528	5L
TNAC1200	2L	TNAC1554	5L
TNAC1248	3S	TNAC1864	5L
TNAC1380	3L	TNAC1677	6S
TNAC1383	3L	TNAC1678	6S
TNAC1386	3L	TNAC1679	6S
TNAC1627	3S	TNAC1683	6S
TNAC1644	3S	TNAC1674	6S
TNAC1648	3S	TNAC1685	6S
TNAC1291	3S	TNAC1752	6S
TNAC1294	3S	TNAC1702	6L
TNAC1296	3S	TNAC1726	6L
TNAC1300	3S	TNAC1740	6L
TNAC1301	3S	TNAC1741	6L
TNAC1314	3S	TNAC1743	6L
TNAC1326	3S	TNAC1748	6L
TNAC1252	3L	TNAC1763	6L
TNAC1263	3L	TNAC1776	7AS,4AL,7DS
TNAC1267	3L	TNAC1781	7S
TNAC1273	3L	TNAC1782	7S
TNAC1277	3L	TNAC1787	7S
TNAC1278	3L	TNAC1926	7S
TNAC1280	3L	TNAC1805	7S
TNAC1283	3L	TNAC1806	7S
TNAC1286	3L	TNAC1917	7S
TNAC1335	3L	TNAC1929	7S
TNAC1356	3L	TNAC1943	7S
TNAC1626	3A	TNAC1803	7L
TNAC1364	3L	TNAC1811	7L
TNAC1367	3L	TNAC1812	7L
TNAC1373	3L	TNAC1815	7L
TNAC1377	3L	TNAC1822	7L
TNAC1378	3L	TNAC1826	7L
TNAC1656	4AL,4BS,4DS	TNAC1829	7L
TNAC1663	4AL,4BS,4DS	TNAC1834	7L
TNAC1391	5AL,4BL,4DL	TNAC1845	7L
TNAC1398	4L	TNAC1867	7L
TNAC1403	4L	TNAC1956	7L
TNAC1408	4AS, 4BL, 4DL	TNAC1957	7L
TNAC1412	4AS,4BL,4DL	TNAC1825	7L
TNAC1421	4L		

B. Cytological Study of CH5382

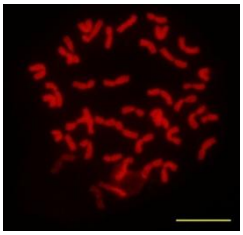

Fig. 1.Analysis of Th.intermediumchromosomes or segments inthe CH5382 by GISH using Th. intermediumgenomic DNA as a probe.

GISH analysis using *Th. intermedium* genomic DNA as probe was conducted to determine the *Th.intermedium*fragmentinCH5382. However, no signal was observed in mitotic metaphase of CH5382 (Fig. 1). The result indicates that alien fragment was small and unable to be detected by GISH analysis.

C. Molecularcharacterizationofalien Translocation Line

To understand the presence of *Th.intermedium* alien fragment to powdery mildewin CH5382, a total of 125 pairs of PLUGprimersevenly distributed throughout the seven homologous groups of wheat chromosomes were applied to amplify the DNA of CS and CH5382. We found that 7 primer pairs gave polymorphic bands between them. The markersTNAC1102 and TNAC1567, came from wheat homologous group-2 short arm and group-5 long arms, respectively, generated additional bands inresistance donor TAI7044and CH5382 compared to CS (Fig. 2). The results suggested that the *Th.intermedium* alien fragments in CH5382was possibly belonged to homoeologous group 2 and 5.Therefore, the molecular evidence may confirm that CH5382 contains small fragments introgression of *Th.intermedium* chromatin.

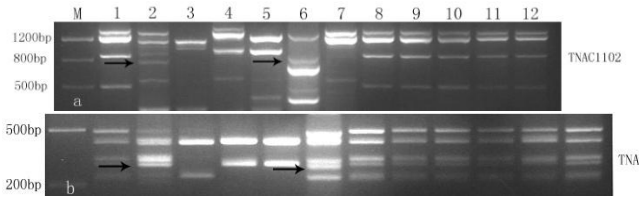

Fig. 2.PCR patterns from PLUG primersTNAC1102 (a) and TNAC1567(b).Lanes 1 to 6 were Chinese Spring, TAI7044, Pseudoroegneriaspicata, Thinopyrumelongatum, Thinopyrumponticmand CH5382, Lanes 7-12 representcommon wheatjintai170,jinmai66, jinchun15, jingshuang16, mingxian169 and chuanmai45. The arrows indicate the Th. intermedium specific bands

IV. DISCUSSION

The application of GISH provides a powerful method for the characterization of wheat-alien translocations and has been applied successfully on identification of intergeneric hybrids. However, some recent studies had demonstrated that some traits of interest were transferred to recipient genotypes without detectable cytological or genetic changes [7], [8]. The absent of GISH signals were observed in CH5382using *Th.intermedium* genomic DNA as probe. It indicates that the introgressed *Th. Intermedium* segments were relatively small and cytologically undetectable.

The PLUG primers are designed based on rice syntenic region, and presumably amplify fragments corresponding to the similar linkage group(s) of wheat relatives [9]. They play important roles in detecting alien chromatin in wheat backgrounds. In this study, total of 125 PLUG markers are developed to screen and identify *Th.intermedium* alien fragments in CH5382. The results demonstrate that the presence of *Th.intermedium* alien fragment to powdery mildew in CH5382. It will be interesting to study the genetics of powdery mildew resistance gene(s) in CH5382, and further application to wheat breeding for resistance.

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