

CHROMOSOME LOCATION AND SSR MARKERS OF A STRIPE RUST RESISTANCE GENE FROM WHEAT (*TRITICUM AESTIVUM*) LINE N9738

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Abstract

The objective of present study here was to determine the inheritance and chromosomal location of the resistance gene in N9738. The phenotypic data of F₂ plants, from the crosses N9738/Huixianhong and N9738/Abbondanza, respectively, implied that the stripe rust resistance in N9738 was controlled by a single dominant gene, temporarily designated *YrN9738*. Monosomic (Nullisomic) analysis revealed *YrN9738* is on wheat chromosome 1B. Seventy-eight SSR markers on chromosome 1B were screened on the parents and bulks. Four SSR markers (*Xgpw7422*, *Xgpw5195*, *Xgpw7812*, and *Xcfd65*) were used to construct a linkage map for *YrN9738* and *Xcfd65* was closely linked to *YrN9738* with genetic distance of 3.5 cM. These linked markers were located on chromosome 1BL with Chinese Spring nullisomic-tetrasomics and ditelosomics of homoeologous group 1. Their presence in the deletion stocks of chromosome 1B located *Xgpw7422* and *Xgpw7812* in bin 1BL06-0.32-0.47 and *Xgpw5195* in bin 1BL06-0.32. *YrN9738* should be on wheat chromosome 1B near to the centromere and useful in wheat breeding programs.

Introduction

Stripe rust of wheat, caused by *Puccinia striiformis* f. sp. *tritici* (PST), is one of the most devastating diseases in common wheat (*Triticum aestivum* L.) affecting wheat production. It is also one of the most widespread and destructive disease in China. Fifteen countrywide stripe rust epidemics have been recorded since 1950, and losses of 6.0, 3.2, 1.8 and 1.3 million metric tons of wheat occurred during 1950, 1964, 1990 and 2002, respectively (Wan *et al.*, 2004). The appearance of PST race CYR32 in China, current wheat cultivars with resistance gene *Yr9* and the derivatives of Fan 6 have become susceptible, resulting in the wide spread epidemic in 2002 (Wan *et al.*, 2004). Development and use of resistance genes in wheat breeding is the most effective, economic and environmental friendly approach for controlling stripe rust of wheat (Chen, 2005). More than 70 stripe rust resistance genes have been identified in wheat to date (Cheng & Chen 2010). However, because of co-evolution of the host and pathogen, most of wheat cultivars with the race specific genes have become ineffective in a period of time when they were extensively planted due to the emergence of new virulent rust races, resulting in epidemic (Wang *et al.*, 1996; Wu & Niu 2000; Fu *et al.*, 2009; Ali *et al.*, 2010). In recent years, genetics and plant breeders have emphasized to avoid monoculture of a single wheat variety on large scale, to avoid severe epidemics on wide scale through use diversification of resistance genes (Qamar *et al.*, 2008). Continuous searching for new resources of resistance, localization and pyramiding of more resistant genes in cultivars is important to have sustainable resistance to disease in wheat production. Breeding efforts at the International Wheat and Maize Improvement Center (CIMMYT) have resulted in the release of numerous wheat cultivars that contain the linked genes *Lr34/Yr18* (Singh *et al.*, 1992). Zeng *et al.*, had developed a wheat line YW243 with high quality by multi-resistance genes pyramiding, which pyramided *Yr1*, *Yr2*, *Yr9*, *YrX*, *Sr31* and *Lr26* and

used for developing cultivars (Liu *et al.*, 2006). Many contributions had done, although, identifying new sources of resistance genes and multi-resistance genes pyramiding in cultivar are still urgent and important in wheat breeding program for characterizing the population dynamics of stripe rust pathogen (Chen, 2005; Khan *et al.*, 2012; Zeb *et al.*, 2013).

Among the molecular markers, simple sequence repeats (SSR) or microsatellites, are the effective and reliable molecular marker technology, since they are more abundant and display higher levels of polymorphisms. SSR markers have been widely used for tagging stripe rust resistance genes *YrH52* and *Yr15* (Peng *et al.*, 1999, 2000), *Yr26* (Ma *et al.*, 2001), *Yr24* (Zakari *et al.*, 2003), *Yr36* (Uauy *et al.*, 2005), *YrCH42* (Li *et al.*, 2006), *Yr29* (Rosewarne *et al.*, 2006), *YrAlp* (Lin & Chen, 2007), *Yr41* (Luo *et al.*, 2008), *Yr44* (Sui *et al.*, 2009), *Yr43* (Cheng & Chen, 2010), *Yr45* (Li *et al.*, 2011), *Yr46* (Herrera-Foessel *et al.*, 2011) *Yr47* (Bansal *et al.*, 2011), and *Yr48* (Lowe *et al.*, 2011). N9738 (2n=42) is a winter wheat line, developed from the cross N9134/Zhong 4. Zhong 4 was synthesized from (Keqiang/Nanda2419) F₅//*Thinopyrum intermedium* (Sun *et al.*, 1990). N9134 was derived from the cross Abbondanza 5B euploid / *T. dicoccoides* accession As846 (Ji *et al.*, 1999). N9738 is highly resistant to predominant Chinese PST race CYR32 at both the seedling and adult-plant stages. In the present study, the location and the molecular mapping of the resistance gene in N9738 was determined by monosomic (nullisomic) analysis and SSR markers.

Materials and Methods

Plant materials: A total of 167 F₂ plants derived from the 2 crosses N9738/susceptible cv. Huixianhong and N9738/Abbondanza, were used for phenotype test of the stripe rust resistance gene in N9738, respectively. The susceptible common wheat Abbondanza nullisomic (monosomic) series (Xue *et al.*, 1991) were used for locating

the resistance gene. All 21 Abbondanza nullisomic (monosomic) lines as the female parents were crossed to the resistant line N9738. F₁ plants from Abbondanza nullisomic lines/N9738 were directly grown to obtain F₂ seed. Monosomic F₁ plants were confirmed cytologically for Abbondanza monosomic lines and N9738 were grown to obtain F₂ seed. Meiotic chromosome pairing was studied in Acetic acid-Magenta squashes of pollen mother cells (PMC) from anthers fixed in Carnoy I solution. Chinese Spring (CS) and its nullisomic-tetrasomics, and ditelosomics of homoeologous group 1 and wheat chromosome 1B deletion stocks were used for confirmation of the chromosomal location of the markers. The CS nullisomic-tetrasomics, ditelosomics and deletion stocks were kindly provided by National BioResource Project, Japan and the other plant materials were provided by the College of Agronomy, Northwest A & F University.

Resistance evaluation: Seeds were planted in small pots under controlled conditions in a rust-free greenhouse. Seedlings were inoculated with Chinese PST race CYR32 when the first leaf was fully expanded. After inoculation, the seedlings were placed in a dew chamber at 9°C and 100% of relative humidity for 24 h and then transferred to a greenhouse maintained with 14 h light and 10 h dark photoperiod at 12 -17°C. Infection types (IT) were recorded 14-15 days after inoculation when rust was fully developed on the susceptible control Mingxian 169. IT were based on a 0-4 scale (Bariana & McIntosh, 1993), with 0 for no visible uredia, 0⁺ for small chlorotic flecks without sporulation, 1 for chlorosis and necrosis associated with extremely limited uredial development, 2 for chlorosis and necrosis with little intermediate sporulation, 3 for chlorosis with increased uredial development, and 4 for abundant sporulation without chlorosis. The disease assessment standard was further detailed with “+” and “-” and divided into 0, 0⁺, 1, 1⁺, 2, 2⁺, 3, 3⁺ and 4. Rating of the seedling reactions was simplified to 2 classes (resistant and susceptible) as there was a clear distinction between the 2 categories. F₂ plants with IT of 0 to 2⁺ were resistant and those with IT of 3 to 4 were susceptible (Fang *et al.*, 2008). The PST race CYR32 kindly provided by the College of Plant Protection was used for the seedling test.

DNA extraction and bulk segregant analysis: Genomic DNA was extracted from rust-free leaves of two parents (N9738 and Huixianhong) and F₂ individuals using CTAB protocol (Rogers & Bendich 1985). Resistant and susceptible bulks comprising equal amounts of DNA from the ten most resistant (IT 0; to 1) and ten most susceptible (IT 3⁺ to 4) F₂ plants, respectively, were used for bulk segregate analysis (Michelmore *et al.*, 1991). Based on monosomic (nullisomic) analysis results of resistance gene in N9738, 78 primer pairs of SSR on wheat chromosome 1B were synthesized according to the sequences published in the GrainGenes database (<http://www.wheat.pw.usda.gov>) and screened on the parents and bulks. Primer pairs generating bands specific to will be used to genotype individual F₂ plants for molecular mapping. The PCR reaction was performed in a PTC 200 thermo-cycler. A 20μL reaction mixture

contained of 40-60 ng of template DNA, 2.0μl Mg²⁺-free 10 × PCR buffer, 1.2μl 25 mM MgCl₂, 1.6μl 2.5 mM dNTPs, 0.50 unit of *Taq* DNA polymerase (TaKaRa) and 0.5μl 10μM of each primer synthesized by TaKaRa Biotechnology Co. (Dalian City, China). The PCR conditions were conducted with 5 min of denaturation at 94°C, then amplifications were programmed for 36 cycles, each consisting of 45 sec at 94°C, 45 sec at 55-60°C (varied with the primers), 90 sec at 72°C and followed by a 8 min extension step at 72°C. After PCR amplification, products were mixed with one fifth volume of loading buffer (100mmolL⁻¹ EDTA pH 8.0, 10 mmolL⁻¹ TrisHCl pH 7.5, 5% Ficoll 400; 0.05% bromophenol, 0.05% xylene cyanol) and 10μL were loaded for electrophoresis in vertical, no denaturing 8% polyacrylamide gels in 1 × TBE (90 mmolL⁻¹ Tris borate pH 8.3, 2 mmolL⁻¹ EDTA) at 50mA for 2 to 3 h (Wang *et al.*, 2007). After electrophoresis, the gel was silver-stained for visualization.

Linkage mapping: Chi-squared (χ^2) tests were used to evaluate the goodness of fit of observed and expected segregation ratios for stripe rust reaction and molecular markers. The “chitest” procedure in the Excel data analysis of Microsoft Office 2007 was used to calculate *P* values. The Linkage analysis and the calculation of the map distance were conducted with JionMap4 (Van Ooijen, 2006). The genetic map was drawn with the software Mapdraw V2.1 (Liu & Meng, 2003).

Results

Inheritance of stripe rust resistance in N9738: In seedling tests with CYR32, N9738 was highly resistant (IT 0; to 1), whereas huixianhong was highly susceptible (IT 4). Ninety four F₂ plants from the cross N9738/Huixianhong were involved in phenotype test, including 71 resistant individuals (66 individuals with IT 1-2 and 5 individuals with 2⁺) and 23 susceptible individuals (IT 3⁺-4). Whereas in the cross N9738/Abbondanza, 59 individuals were resistant (57 individuals with IT 1-2 and 2 individuals with 2⁺) and 14 individuals were susceptible (IT 3⁺-4). Resistant and susceptible segregation ratios of 2 F₂ populations were well consistent with 3:1 ratio ($\chi^2=0.014$, *P*=0.91 and $\chi^2=1.32$ and *P*=0.25, respectively), indicating that the stripe rust resistance in N9738 was controlled by a single dominant gene, temporarily designated *YrN9738*.

Monosomic(nullisomic) analysis of *YrN9738* : F₂ populations from crosses of 10 Abbondanza monosomic lines, 11 nullisomic lines susceptible to stripe rust and line N9738 to were tested with PST race CYR32. All of the F₂ populations, except the crosses involving chromosome 1B, segregated in a ratio of 3 resistant: 1 susceptible, confirming that the resistance in N9738 was controlled by a dominant monogenic gene (Table 1). The segregation of the cross Abbondanza-nullisomic-1B/N9738 deviated significantly (*p*<0.001) from the expected ratio of 3:1, indicating that *YrN9738* is located on chromosome 1B (Table 1).

Table 1. Segregation for seedling reaction to PST race CYR32 in F₂ progenies from crosses of euploid 'Abbondanza' and wheat line N9738.

Chromosome involved	Number of the resistant plants	Number of the susceptible plants	χ^2	P
1AM	60	18	0.15	0.70
1BN	79	4	18.03**	< 0.001
1DN	80	29	0.15	0.70
2AN	80	22	0.64	0.42
2BM	65	16	1.19	0.28
2DN	70	21	0.18	0.67
3AM	84	27	0.03	0.86
3BM	52	16	0.08	0.78
3DN	80	27	0.003	0.96
4AN	56	13	1.40	0.24
4BM	51	15	0.18	0.67
4DN	79	23	0.33	0.57
5AM	49	13	0.54	0.46
5BM	51	20	0.38	0.54
5DM	63	26	0.84	0.36
6AN	84	30	0.11	0.74
6BN	69	22	0.03	0.86
6DN	62	22	0.06	0.81
7AM	63	22	0.04	0.84
7BN	66	17	0.90	0.34
7DM	48	18	0.18	0.67

** Segregation ratio is distinctly deviated from 3:1.

N: Abbondanza nullisomic line M Abbondanza monosomic line

Bulked segregant analysis and linkage analysis: Of the 78 SSR markers on wheat chromosome 1B, *Xgpw7422*, *Xgpw5195*, *Xgpw7812*, and *Xcfd65* showed reproducible polymorphic bands in bulked segregant analysis (Fig. 1) The 94 F₂ individuals were genotyped with 4 markers. Results indicated that marker *Xcfd65* was dominant and the other 3 markers were co-dominant (Fig. 2). Four markers segregated in 3:1 ratios for presence and absence of special amplified fragment (Table 2), indicating that these markers were single-locus markers. Four SSR markers were used to construct a linkage map for *YrN9738* and *YrN9738* was linked to the marker *Xcfd65* at 3.5 cM (Fig. 3).

Chromosome bin assignment: The marker *Xcfd65* was located in bin 1BL06-0.32 nearby the centromere already (<http://wheat.pw.usda.gov/ggpages/SSRclub/GeneticPhysical/>). All four linked markers were placed on chromosome 1BL using CS aneuploid stocks of the homologous group 1 (Fig. 4). Further amplification with chromosome 1B deletion stocks indicated that *Xgpw7422* and *Xgpw7812* were located in bin 1BL06-0.32-0.47 and *Xgpw5195* was placed in bin 1BL06-0.32 (Fig. 5). The results indicated that *YrN9738* was located on wheat chromosome 1B. Because no markers on the side of *YrN9738* next to the centromere were found out, it is not certain that whether *YrN9738* was on the long arm of chromosome 1B or not.

Discussion

The conventional monosomic analysis provides a basis for assigning resistance genes to specific chromosomes (McIntosh *et al.*, 1996). In recent years, molecular markers

have played a significant role in tagging stripe rust resistance (*Yr*) genes in wheat (McIntosh *et al.*, 2007, 2009). In this study, chromosome location of a resistant gene in wheat line N9738 was confirmed by both monosomic (nullisomic) analysis and SSR markers. The marker *Xcfd65* was closed linked to *YrN9738* with a distance of 3.5cM. According to the linkage map, it implied that *YrN9738* was near the centromere of chromosome 1B. That might be why we could not find out more polymorphic markers flanked to *YrN9738* on the side next to the centromere. Among the resistance genes identified on 1B, *Yr24*, *Yr26*, *YrH52*, and *YrCH42* have been located on chromosome 1BS near the centromere (Ma *et al.*, 2001; Smith *et al.*, 2002; Li *et al.*, 2006; Lin & Chen, 2007). Work has been reported that *Yr24*, *Yr26* and *YrCH42* represent the same locus and *YrCH42* was flanked by markers *Xbarc187* and *Xgwm498* with genetic distances of 2.2cM and 1.5cM (Li *et al.*, 2006). *Xgwm498* was located in deletion bin 1BL-0.32. (<http://wheat.pw.usda.gov/ggpages/SSRclub/GeneticPhysical/>). *YrH52* was flanked by marker *Xgwm273* and *Xgwm413* and mapping on 1BS. *Xgwm413* was located in deletion bin 1BS10-0.50. *YrCH42* and *YrH52* were located on 1BS near the centromere. Based on our linkage map, *Yr9738* is probably allelic to *YrCH42* or *YrH52*. Postulation and allelism tests would be necessary to understand the relationships between *YrN9738* and *Yr24*, *Yr26*, *YrH52*, *YrCH42*. N9738 is an improved wheat line with better agronomic traits that can potentially be used as a breeding parent. The four linked markers will be diagnostic markers for *YrN9738* in marker-assisted selection.

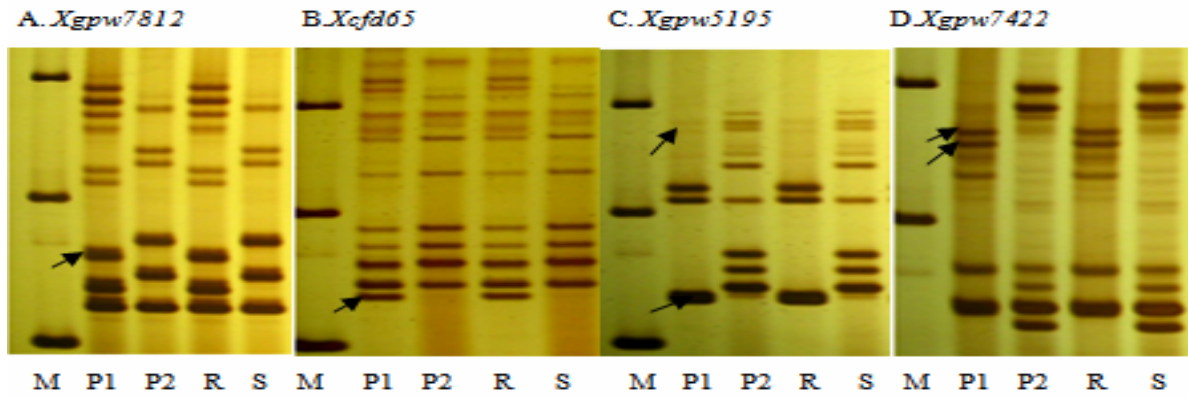


Fig. 1. Gel scan showing markers *Xgpw7812*(A), *Xcfd65*(B), *Xgpw5195*(C) and *Xgpw7422*(D) amplification products in parents, resistant bulk and susceptible bulk derived from the cross N9738 and Huixianhong. Arrow shows the special amplified fragment with yielded markers linked to *YrN9738*. M DNA ladder DL2000, P1 N9738, P2 Huixianhong, R resistant bulk, S susceptible bulk.

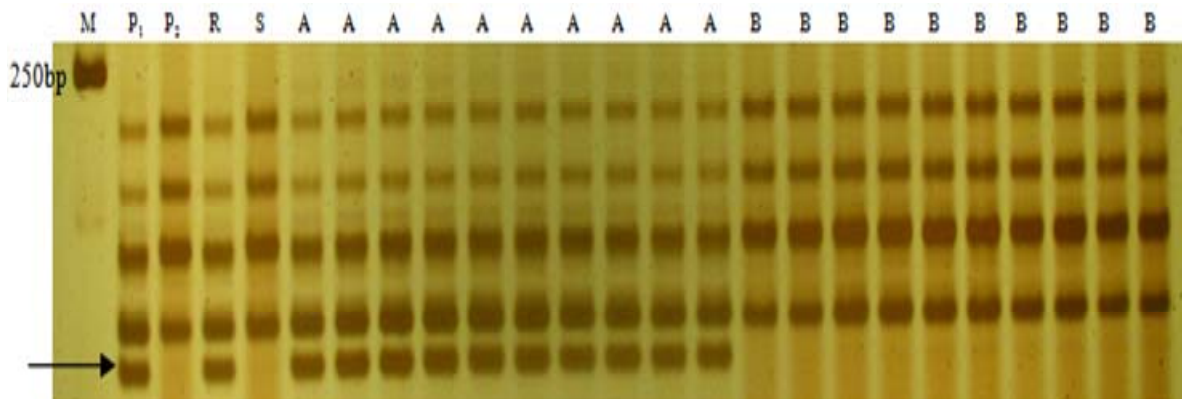


Fig. 2. Gel scan showing marker *Xcfd65* amplification products in parents, resistant bulk, and susceptible bulk and F₂ plants derived from the cross N9738 and Huixianhong. Arrow shows the special amplified fragment with *Xcfd65* linked to *YrN9738*. M DNA ladder DL2000, P₁ N9738, P₂ Huixianhong, R resistant bulk, S susceptible bulk, A resistant plants with homozygous bands, B susceptible plants.

Table 2. Segregation of four SSR markers in the 94 F₂ individuals derived from the cross N9738 / Huixianhong.

Marker	Resistant plants		Susceptible plants		χ^2	P
	With ^a	Without ^b	With ^a	Without ^b		
<i>Xgpw7422</i>	70	1	5	18	1.15	0.28
<i>Xgpw5159</i>	71	0	6	17	2.40	0.12
<i>Xgpw7812</i>	71	0	5	18	1.72	0.19
<i>Xcfd65</i>	71	0	3	20	0.70	0.40

^a = Numbers of the individuals with the specific bands of N9738.

^b = Numbers of the individuals without the specific bands of N9738 and only with the specific bands of Huixianhong

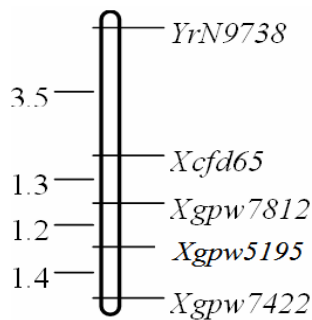


Fig. 3. A linkage map for *YrN9738* and four SSR markers on chromosome 1B. Locus names are indicated on the right side of the map. Kosambi map distances (cM) are shown on the left side.

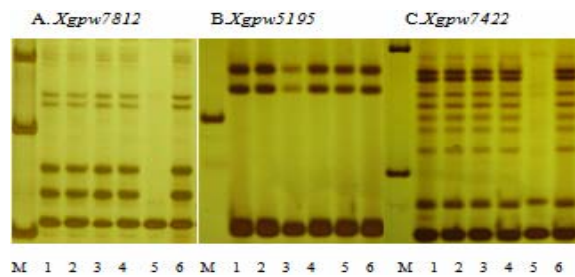


Fig. 5. Gel scan showing markers *Xgpw7812* (A), *Xgpw5195* (B) and *Xgpw7422*(C) amplification products in Chinese Spring (CS) 1B chromosome deletion lines. M: 100bp Ladder; CS: Chinese Spring; the lanes, 1 1BS-01 (FL=0.35), 2 1BS-09 (FL=0.84), 3 1BS-10 (FL=0.5), 4 1BL-01 (FL=0.47), 5 1BL-06 (FL=0.32), 6 1BL-10 (FL=0.89), FL: fraction length.

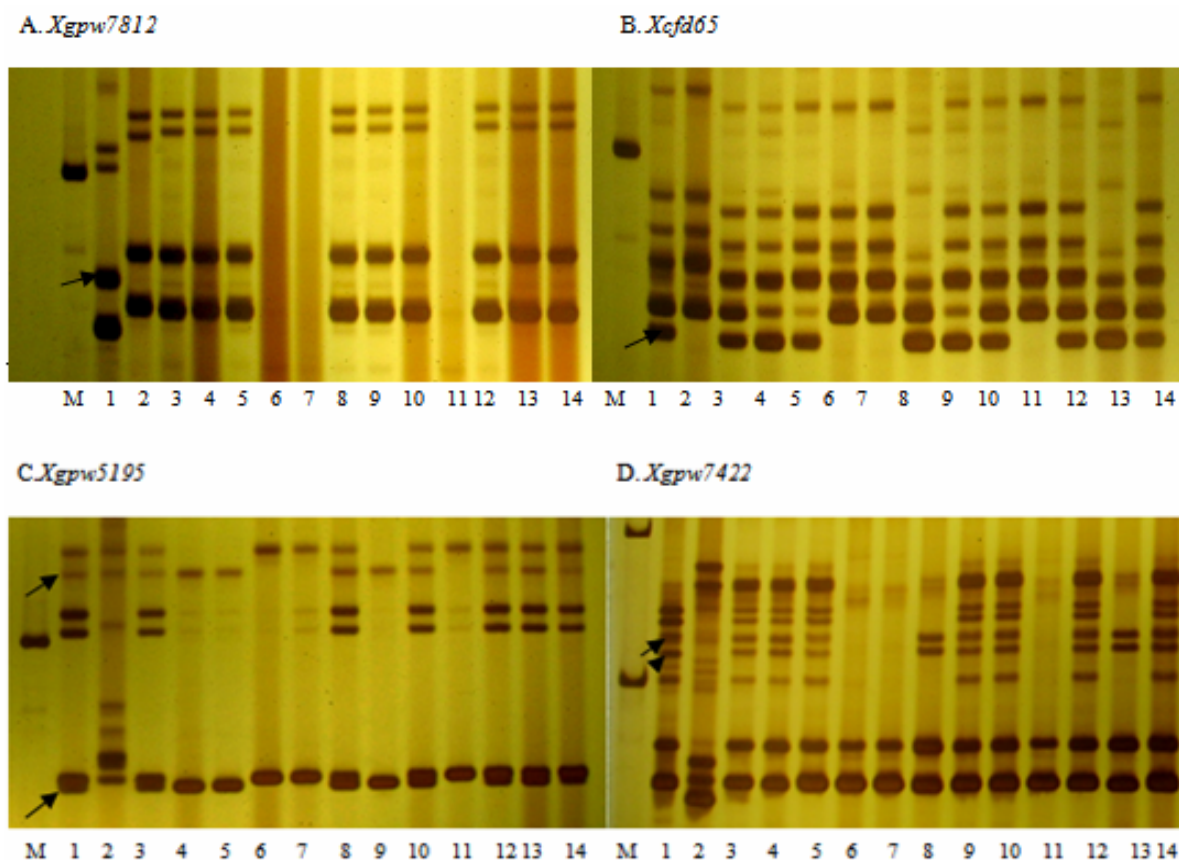


Fig. 4. Gel scan showing markers *Xgpw7812* (A), *Xcfd65* (B), *Xgpw5195*(C) and *Xgpw7422*(D) amplification products in CS aneuploid stocks of the homologous group 1. Arrow shows the special amplified fragment with yielded markers linked to *YrN9738*. M DNA ladder DL2000, the lanes, 1 N9738, 2 Huixianhong, 3 CS, 4 CSN1AT1B, 5 CSN1AT1D, 6 CSN1BT1A, 7 CSN1BT1D, 8 CSN1DT1B, 9 CSDt1AS, 10 CSDt1AL, 11 CSDt1BS, 12 CSDt1BL, 13 CSDt1DS and 14 CSDt1DL.

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