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Microsatellite Marker Linked to a Leaf Rust Resistance Gene from *Triticum monococcum* L Transferred to Bread Wheat

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***Triticum monococcum* L, a diploid wheat species closely related to the A genome of cultivated wheats, is highly resistant to leaf rust. A synthetic amphiploid, *T.monococcum – T. durum* was crossed with *T. aestivum cv* WL711, highly susceptible to leaf rust. Leaf rust resistant derivatives were selected among backcross generations with the recurrent parent WL711 and cytologically analysed. Chromosome number of the leaf rust resistant BC_1F_3 progenies varied from 39 to 44. Six leaf rust resistant and susceptible bulks from different BC_1F_3 progenies were analysed using 29 wheat microsatellite(WMS) markers already mapped on A genome of bread wheat and found polymorphic among parents. One *T. monococcum* specific allele of WMS gwm136 locus was found to be closely linked to the leaf rust resistance gene in all the resistant bulks. Differential chromosome number, frequency of univalents and multivalents, however, indicated that the critical *T. monococcum* chromosome might be present in addition to the A genome chromosomes of wheat, substituted either for the B or D genome chromosome of wheat or translocated to chromosome 1A of wheat in one or the other bulks. The association of the *T. monococcum* specific allele of WMS gwm136 locus to leaf rust resistance was further confirmed from bulked segregant analysis in BC_2F_1 generation.**

Key words : microsatellites, wheat, *Triticum monococcum*, leaf rust resistance.

Among the three rust diseases of wheat, leaf rust (syn. brown rust) caused by *Puccinia recondita* Rob ex Desm. f.sp. *tritici* Ericks & Henn, is widely prevalent. In the leaf rust prone areas of the world, successful wheat production has been largely dependent on the development and use of resistant cultivars(1). The wheat cultivars, however, become susceptible to rusts due to their narrow genetic base for resistance and development of new virulences thus making it necessary to search for new sources of resistance against ever evolving pathogens. To-date, 49 leaf rust resistance genes (*Lr*) have been identified in wheat or transferred from related species(2-4). *Triticum monococcum* has been found to be an excellent source for leaf rust resistance(5-8) and a few *Lr* genes have been transferred from *T. monococcum* into *T. aestivum* (8).Tagging of resistance genes with molecular markers not only increases the efficiency of introgression of alien genes into hexaploid wheat (9) but can also facilitate the pyramiding of two or more genes into a cultivar (10). Development of molecular markers for *Lr* resistance genes introgressed from alien species to polyploid wheat have been reported by several workers (11-17). Microsatellites have recently emerged as markers of choice(18). It has been shown that the sequence tagged microsatellite sites (STMS) are capable of detecting more polymorphism than the RFLP markers(19-21). Microsatellites have been successfully used to generate genome specific markers for mapping studies in hexaploid wheat (19,22) and to develop microsatellite maps of wheat (23).

This article deals with the transfer of leaf rust resistance of *T. monococcum* to bread wheat and its tagging with microsatellite markers using bulked segregant analysis.

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Materials and Methods

Plant material — A synthetic amphiploid (*Triticum durum–T. monococcum*) (AABB A^mA^m) developed by Gill *et al* (24) was crossed to a susceptible bread wheat (AABBDD) cultivar WL711(NN) from which Ne_2 gene for hybrid necrosis has been removed to produce F_1. The F_1 was selfed and advanced upto F_3 and backcrossed to generate BC_1 F_3 progenies while selecting for resistance to leaf rust. Resistant BC_1F_3 plants were backcrossed to susceptible recurrent parent WL711 to produce BC_2F_1 population.

Screening for rust resistance — Seedlings of various progenies were inoculated with four individual pathotypes of leaf rust (104B, 104-2, 104-3 and 77-5). The infection types were recorded 14 days after inoculation according to the modified scale of Stakman *et al* (25) where seedlings with infection types 0, 0;, 1 and 2 were classified as resistant and with 3 and 4 infection types as susceptible . At adult plant stage BC_1F_3 progenies alongwith the parents were sprayed with a mixture of urediospores of the leaf rust races. The observation on terminal disease severity and response of individual plants were recorded as percentage of leaf area covered by rust according to modified Cobb's scale as described by Peterson *et al* (26).

Cytogenetic studies — Spikes from resistant plants of BC_1F_3 plants were fixed at pre-booting stage in Carnoy's solution II (6:3:1, ethanol : chloroform : acetic acid). The fixative was replaced after 48h with 70% ethanol and samples were stored at 4°C until observation. Anthers were squashed in 2% acetocarmine stain and the meiocytes at diplotene through late metaphase stages were used for recording chromosome pairing.

DNA extraction — Total genomic DNA was extracted from 3 to 4 week old seedlings from the parents and from progeny of each of the BC_1 F_3 plants found to be resistant or susceptible. DNA was extracted using CTAB method according to the protocols of Hoisington *et al* (27). Aliquots of DNA (20 µg) from resistant and susceptible plants were pooled to make separate resistant and susceptible bulks(28).

Microsatellite analysis — A total of 59 microsatellite markers (WMS) mapped on A-genome of wheat (23) were used for polymorphism survey between the parents and those markers which were polymorphic between parents were tested against resistant and susceptible bulks. PCR reactions were carried out in 25µl volume containing 10mM Tris-HCl pH8.3, 50mM KCl, 1.5mM $MgCl_2$, 0.005 per cent Tween 20, 100 µM of each of the dNTPs, 0.4µM of each primer, 50ng of genomic DNA and 1 unit of *Taq* DNA polymerase. Each sample was overlaid with 20 µl of light mineral oil. PCR amplification was performed in MJ Research (PTC-100) Inc. thermal cycler programmed for 30 cycles of 1 min (4 min for the first cycle) at 94°C, 1 min at 50-60°C and 2 min at 72°C, followed by a final extension step of 15 min at 72°C. Amplified DNA fragments were resolved by electrophoresis in 3% high resolution Metaphor gel (FMC, Rockland, NY). Gels were visualized by staining with ethidium bromide using UVP Gel Documentation System.

Results and Discussion

Disease reaction studies — Although *T. monococcum* was found to be susceptible at seedling stage to some of the leaf rust races tested, it showed immune reaction at adult plant stage whereas the recurrent wheat cultivar WL711 was highly susceptible (5) (Table 1). Out of 449 BC_1 F_3 progenies screened, a total of 62 lines were found to be resistant to leaf rust at adult plant stage under similar epiphytotic conditions. Some of the resistant BC_1 F_3 plants were backcrossed to recurrent parent WL 711 (NN) to produce BC_2 F_1. All the plants of BC_2 F_1 were uniformly resistant indicating that the leaf rust resistance was dominant. The BC_2 F_2 grown at IARI, Regional Station, Wellington, The Nilgiri's also maintained leaf rust resistance against diverse pathogen population in the region.

Cytogenetic studies — The somatic chromosome number (2n) of resistant BC_1 F_3 plants ranged from 39 to 44 (Table 2). Large number of plants showed 2n=42 followed by 2n=41 chromosomes. All BC_1 F_3 plants with 2n = 42 showed some frequency of univalents and multivalents except two plants where only bivalents were observed. However, BC_2F_1 plants with 2n=42,43 also showed univalents (Table 3).

Table 1. Leaf rust reaction of the parents at seedling and adult plant stages

Parent/cultivar	Seedling reaction (Pathotype)				Adult plant reaction*
	104B	104-2	104-3	77-5	
T. monococcum	3		2^+	1^-	0
T. durum cv PBW 114	3^+	0	3^+	2^-	5S-20S
T. aestivum cv WL 711 (NN)	4^-	4	3^+	4	80S-100S
Agra Local	4	4	4	4	80S-100S

* Against a mixture of the above pathotypes

Table 2. Chromosome pairing data of some of the leaf rust resistant plants from BC_1F_3 population of crosses of synthetic amphidiploid (*T.durum -T.monococcum*) x *T. aestivum cv* WL 711(NN)

PAU plant no. (1998-99)	2n Chromosome No.	Chromosome pairing				
		Univalents	Bivalents		Trivalents	Quadrivalents
			Rods	Rings		
1424-8	39	3.07 (± 1.70)	5.73 (±1.62)	11.8 (±2.36)	0.2 (±0.41)	0.07 (±0.25)
1425-1	40	1.50 (± 0.93)	5.37 (±1.19)	13.87 (±1.24)	0.00	0.00
1425-4	41	1.35 (± 0.81)	1.20 (±1.19)	17.9 (±1.71)	0.15 (±0.36)	0.25 (±0.55)
1425-6*	41	0.33 (± 0.59)	1.83 (±1.24)	16.7 (±1.52)	0.78 (±0.42)	0.28 (±0.46)
1425-8	41	1.25 (± 1.032)	2.5 (±1.93)	16.37 (±2.18)	0.20 (±0.41)	0.33 (±0.48)
1425-11	40	1.00 (± 1.25)	4.76 (±1.46)	14.31 (±1.68)	0.71 (±0.38)	0.10 (±0.31)
1426-1*	42	1.45 (± 0.69)	3.2 (±2.09)	15.95 (±2.01)	0.60 (±0.59)	0.10 (±0.31)
1426-2	44	0.08 (± 0.29)	3.25 (±1.86)	17.75 (±2.63)	0.083 (±0.29)	0.5 (±0.52)
1427-1	40	1.79 (± 1.06)	2.12 (±1.48)	16.04 (±1.78)	0.58 (±0.65)	0.04 (±0.20)
1427-2	40	0.12 (± 0.50)	2.12 (±1.50)	17.5 (±1.46)	0.00	0.18 (±0.40)
1427-4	41	1.75 (± 1.28)	2.08 (±1.44)	17.08 (±1.44)	0.08 (±0.28)	0.17 (±0.38)
1428-3	41	1.40 (± 0.98)	3.87 (±2.11)	15.34 (±2.09)	0.16 (±0.46)	0.18 (±0.38)
1428-4	41	1.75 (± 1.13)	3.75 (±1.71)	15.58 (±1.83)	0.08 (±0.028)	0.08 (±0.028)
1428-7	39	1.56 (± 0.89)	3.00 (±1.83)	15.38 (±2.33)	0.06 (±0.25)	0.06 (±0.25)
1443-3	43	0.88 (± 0.69)	1.94 (±1.19)	18.47 (±1.41)	0.23 (±0.43)	0.12 (±0.33)
1443-5	43	2.64 (± 1.16)	3.28 (±1.73)	15.64 (±2.07)	0.50 (±0.57)	0.25 (±0.51)
1446-10	42	0.125 (±0.50)	1.81 (±1.47)	19.12 (±1.40)	0.00	0.00
1483-2	42	1.18 (±1.67)	2.94 (±1.19)	17.10 (±2.30)	0.00	0.18 (±0.39)
1483-4	42	0.00	0.00	21.00	0.00	0.00

Contd....

Contd. Table 2

1483-5	42	0.00	1.07 (±1.16)	19.73 (±1.53)	0.13 (±0.52)	0.00
1484-1	43	2.92 (±1.85)	3.15 (±2.44)	14.69 (±1.75)	1.46 (±0.87)	0.00
1484-2	43	2.45 (±1.75)	4.18 (±3.60)	14.72 (±2.69)	0.90 (±0.70)	0.00
1485-4*	41	1.94 (±1.29)	2.76 (±1.78)	15.88 (±1.96)	0.35 (±0.49)	0.176 (±0.39)
1486-1	43	1.43 (±0.79)	1.00	18.14 (±0.90)	1.28 (±0.48)	0.00
1486-2	42	0.85 (±0.38)	0.85 (±1.21)	18.57 (±1.27)	0.57 (±0.53)	0.00
1486-5	42	2.22 (±0.37)	4.89 (±1.17)	15.00 (±1.11)	0.00	0.00
1487-1	42	0.17 (±0.57)	2.13 (±1.25)	18.78 (±1.20)	0.00	0.00
1487-2	43	0.50 (±1.00)	3.25 (±0.95)	17.5 (±1.29)	0.00	0.25 (±0.5)
1489-3	43	1.54 (±1.37)	3.63 (±1.96)	14.81 (±1.78)	1.27 (±0.90)	0.18 (±0.40)
1489-4*	42	0.00	0.57 (±0.74)	20.43 (±0.74)	0.00	0.00
1490-1	41	1.03 (±0.44)	0.73 (±0.91)	19.23 (±1.14)	0.00	0.00
1490-3	41	3.54 (±1.33)	4.46 (±2.33)	13.00 (±2.48)	1.00 (±0.81)	0.00
1490-6	42	2.00	4.00	16.00	0.00	0.00
1490-7	41	2.72 (±1.42)	3.09 (±1.81)	14.90 (±2.07)	0.63 (±0.67)	0.09 (±0.30)
1490-8*	42	0.09 (±0.41)	2.26 (±1.29)	18.70 (±1.29)	0.00	0.00

* Progenies used for bulk segregant analysis.

Table 3. Leaf rust reaction and chromosome pairing data of leaf rust resistant BC_2F_1 progenies of cross of synthetic amphidiploid (*T. durum* –*T. monococcum*) with *T. aestivum cv* WL 711(NN)

PAU plant no. (1999-2000)	2n Chromosome No.	Chromosome pairing				
		Univalents	Bivalents		Trivalents	Quadrivalents
			Rods	Rings		
617-4	42	2.48 (±1.24)	3.44 (±1.49)	13.10 (±1.89)	2.00 (±0.52)	0.69 (±0.25)
621-2	43	1.26 (±0.96)	2.53 (±1.69)	18.1 (±1.62)	0.13 (±0.34)	0.03 (±0.18)

Normal diploid pairing without multivalents in resistant plants with 2n=42 suggests that leaf rust resistance from *T. monococcum* has been transferred to wheat or the critical *T. monococcum* chromosome has been substituted for the corresponding homoeologous chromosomes of the A genome of wheat.

The presence of univalents and multivalents in a number of aneuploid resistant plants with 2n=43 or 44 suggest that critical *T. monococcum* chromosome(s) may be present as monosomic, double monosomic or disomic addition. However, in leaf rust resistant aneuploid plants with 2n=39 to 41, the critical *T. monococcum* chromosome may be present or the resistance might have been transferred to *T. aestivum* but the full complement of the D genome might not have been recovered.

Presence of multivalents in some leaf rust resistant plants with 2n=42 suggest that the critical *T. monococcum* chromosome is present in addition to 14 A genome chromosomes of bread wheat. The critical *T. monococcum* chromosomes carrying genes for leaf rust resistance might have been substituted for one of the B or D genome homoeologues.

Bulked segregant analysis — The bulked segregant analysis (28,29) was used to identify microsatellite markers linked to the rust resistance gene(s) transferred from *T. monococcum* to *T. aestivum.* A total of 29 microsatellite markers out of 59 WMS markers mapped on the A genome of wheat were found to be polymorphic between parents. Leaf rust resistant bulks and susceptible bulks were prepared from lines which segregated for resistance and susceptible reactions. Number of plants used for bulking varied from 6-10 in different bulks. Bulked genomic-DNA was used as template for PCR amplification. A total of 6 resistant and 6 susceptible bulks were tested against the 29 polymorphic WMS markers. Out of 29 microsatellite markers only one marker (gwm136) mapped on 1AS of bread wheat, generated polymorphic alleles between all the resistant and susceptible bulks. The WMS gwm136 locus had a low molecular weight allele in *T. monococcum* amplifying a fragment of approximately 270 base pairs (Fig. 1a). However, this primer pair generated a high molecular weight allele in. *T. aestivum cv* WL 711 (NN) (Fig. 1a). As expected synthetic amphiploid showed both the alleles. The high molecular weight allele of *T. aestivum* was present in both the resistant and susceptible bulks whereas the low molecular weight allele of *T. monococcum* was present only in resistant bulks and was absent in susceptible bulks (Fig. 1a). This indicated that the low molecular

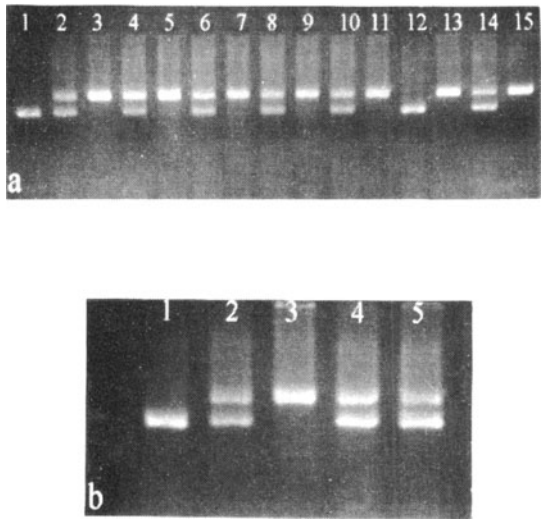

Fig.1. PCR amplification pattern of genomic DNA obtained using WMS gwm136 primer in parents and leaf rust resistant and susceptible bulks from derivatives of synthetic amphiploid (*T. durum-T. monococcum*)xWL711(NN). **(a) BC_1F_3 derivatives:** Lane 1, *T. monococcum;* lane 2, Synthetic amphiploid; lane 3, *T. aestivum cv* WL711(NN); lane 4, 1425-6 (R); lane 5, 1425-7 (S); lane 6, 1426-1 (R); lane 7, 1426-5 (S); lane 8, 1442-6 (R); lane 9, 1442-2 (S); lane10, 1485-4 (R); lane 11, 1485-1 (S); lane 12, 1489-4 (R); lane 13, 1489-10 (S); lane14, 1490-8 (R), and lane15, 1490-10 (S). **(b) BC_2F_1 derivatives:** Lane1, *T. monococcum;* lane 2, Synthetic amphiploid; lane 3, *T. aestivum cv* WL711 (NN); lane 4, 617 (R), and lane 5, 621 (R). (Where, R= Resistant and S= Susceptible).

weight allele specific to *T. monococcum* was co-segregating and linked with the rust resistance gene(s) derived from *T. monococcum*. The co-segregating low molecular weight allele of WMS gwm136 locus, mapped on the short arm of chromosome 1A (23) suggested that the leaf rust resistance gene closely linked to it is present on 1AS of *T. monococcum*. This is in agreement with the earlier report by Hussien *et al* (8) that chromosomes 1A, 5A and 6A of *T. monococcum* carry leaf rust resistance genes.

The presence of both the parental alleles (donor and recipient) in most of the resistant bulks shows that the marker alleles are in either heterozygous state or critical chromosome or a segment of *T. monococcum* carrying the marker allele and leaf rust resistance could be present in addition to 14 A genome chromosomes. However, the presence of only *T. monococcum* specific allele in one leaf rust resistant bulk 1489-4(R) indicates that the resistance might have been transferred from 1AS of *T. monococcum* to 1A of wheat.

The microsatellite markers mapped on the long arm of 1A, however, did not co-segregate with rust resistance indicating the possibility of translocation of 1AS carrying rust resistance genes to the B or D genome chromosomes of bread wheat. The exact chromosomal location of the resistance genes, however, can be identified by monosomic analysis.

To confirm the cosegregation of the *T. monococcum* specific allele of WMS gwm 136 locus with the rust resistance gene of *T. monococcum*, two DNA bulks from two different resistant lines of BC_2F_1s were tested against the above WMS marker. In both the resistant bulks, the *T. monococcum* specific allele of WMS gwm136 locus was present confirming the association of the WMS marker and the leaf rust resistance gene of *T. monococcum* (Fig.1b).

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