Genome-based Identification of Simple Sequence Repeats Markers in *Tilletia indica* and Virulence Analysis of Monosporidial Population

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Abstract

Tilletia indica is a floret infecting quarantined fungal pathogen of wheat causing Karnal bunt of wheat. The population of *Tilletia indica* is highly genetically diversed and it is difficult to breed durable and resistant cultivars of wheat. Simple sequence repeats (SSRs) are extensively used to uncover the population structures in fungal pathogens. In present study, a total of 5,772 simple sequence repeat loci were identified in the *T. indica* genome. *In silico* analysis, forty microsatellite markers were used to genotype of 20 *Tilletia indica* isolates from north-western plain zone of India to analyze population structure. SSRs were found in 863 scaffolds. Among SSRs, tri-nucleotide was most abundant (42 %), followed by di-nucleotide (28 %), mono-nucleotide (23 %), tetra-nucleotide (3 %), hexa-nucleotide (3 %) and penta-nucleotide (1 %). 130 alleles were amplified in 40 SSR markers among the 20 *T. indica*. The polymorphic information value content (PIC) values ranged from 0.20 to 0.81 with an average of 0.51. Maximum PIC (0.81) was obtained for TiSSR34 marker. 18 SSRs were highly informative (PIC ≥ 0.5), 15 SSRs were moderately informative (0.5 >PIC <0.25) and remaining 7 SSRs were less informative marker (PIC<0.25). In cluster analysis, *T. indica* isolates did not cluster to region wise of isolates. Further, the monosporidial (haploid) population of *Tilletia indica* was also developed. Ten highly polymorphic SSR markers were amplified in 60 monosporidial lines (ms) of *T. indica*. Differently amplified monosporidial lines were chosen for monosporidial lines compatibility assay. 27 monosporidial crosses of *T. indica* were produced Karnal bunt disease reaction on susceptible host (WH542). KB7MS1 x KB18MS5 with 36 % coefficient of infection and KB7MS1 x KB16MS1 (coefficient of infection of 24.17 %) was found most virulent. These haploid monosporidial lines may have different mating types. These ms lines were found in distinct clusters. The newly developed SSR markers will be useful for genetic and population studies.

INTRODUCTION

Karnal bunt disease is a major constraint for exporting wheat. The disease is endemic to north-western plain zone of India. It was first reported by Manoranjan Mitra in 1931. It is also reported in few other countries *viz*. Afghanistan, Pakistan, Nepal, Mexico and in some parts of the United States, Iraq, Iran, Lebanon, Syria, Sweden and Turkey, United States, and South Africa. It has major bio security concern for exporting countries. Import of wheat is restricted in 77 countries due this disease. The present study aimed to identify the simple sequence repeats in the whole genome of *T. indica* and developed 40 polymorphic SSR markers in population of *T. indica*.

MATERIALS AND METHODS

Whole genome sequence of *T. indica* RAKB_UP_1 isolate (*GenBank* accession numbers MBSW00000000) was used to identify SSR markers and the genome size is 33.7 Mb. The simple sequence repeats were identified through Microsatellite identification tool (MISA). 20 *T. indica* isolates were isolated and maintained. Fungal DNA was isolated from mycelia of *T. indica* isolates using CTAB method. 40 tri-nucleotides were assessed in 20 isolates of *T. indica*. Polymorphism among isolates was calculated as polymorphism information content (PIC). Developed of 60 monosporidial lines of *T. indica*. Monosporidial lines compatibility assay was performed. Coefficient of infection was calculated.

RESULTS

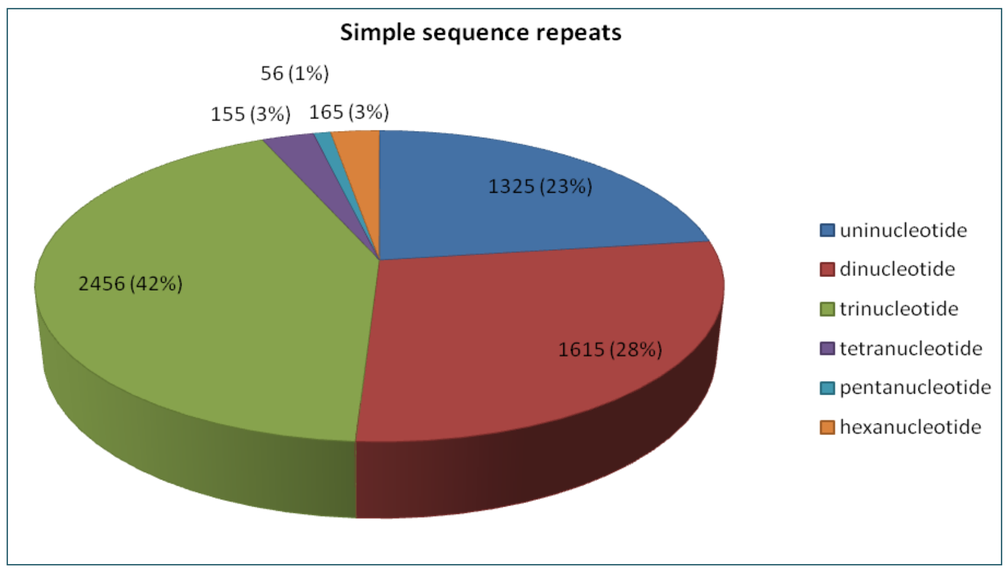

Fig. 1 Identified SSR markers in the genome of *T. indica*

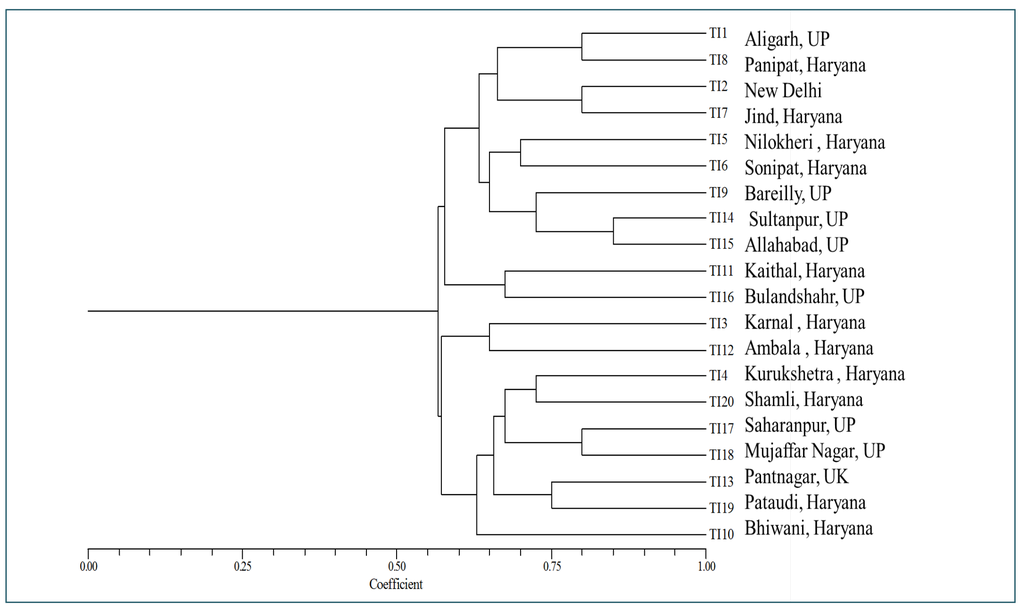

Fig.2 Relationship of 20 *Tilletia indica* isolates based on genotypes of 40 SSR markers

Table. 1 **SSR analysis of 60 *T. indica* MS lines for 10 selected markers**

SSR Markers	Allele size range (bp)	Number of alleles	PIC value
TiSSR 1	220-1200	5	0.65
TiSSR 2	250-490	4	0.69
TiSSR 3	280-650	3	0.59
TiSSR 5	250-410	4	0.65
TiSSR 9	280-800	6	0.79
TiSSR14	280	1	n/a
TiSSR19	240-300	2	0.18
TiSSR25	300-810	3	0.58
TiSSR 34	310-800	6	0.78
TiSSR 40	300	1	n/a

Table 2 Coefficient of infection of monosporidial crosses of *T. indica* on wheat host

Sl. No.	Crosses of ms lines of *T. indica*	% COI
1	TI1 Ms1 X TI7 Ms1	18.00
2	TI 1 Ms 1 X TI 16 Ms 1	5.80
3	TI 1 Ms 1 X TI 18 Ms 5	66.10
4	TI2 Ms 1 X TI 7 Ms 1	3.03
5	TI2 Ms 1 X TI 9 Ms 3	5.41
6	TI 2 Ms 1 X TI 10 Ms 7	5.56
7	TI 2 Ms 1 X TI 11 Ms 1	6.56
8	TI 2 Ms 1 X TI 16 Ms 1	51.69
9	TI 2 Ms 1 X TI 18 Ms 5	40.00
10	TI 2 Ms 1 X TI 20 Ms 7	14.29
11	TI 7 Ms 1 X TI 10 Ms 7	11.24
12	TI 7 Ms 1 X TI 13 Ms 4	4.71
13	TI 7 Ms 1 X TI 16 Ms 1	96.67
14	TI 7 Ms 1 X TI 18 Ms 5	144.00
15	TI 7 Ms 1 X TI 20 Ms 7	3.00
16	TI 7 Ms 9 X TI 9 Ms 3	5.80
17	TI 7 Ms 9 X TI 10 Ms 7	2.86
18	TI 7 Ms 9 X TI 16 Ms 1	9.09
19	TI 9 Ms 3 X TI 10 Ms 7	4.00
20	TI 9 Ms 3 X TI 16 Ms 1	4.17
21	TI 9 Ms 3 X TI 18 Ms 5	3.03
22	TI 10 Ms 7 X TI 13 Ms 4	26.00
23	TI 10 Ms 7 X TI 16 Ms 1	77.78
24	TI 10 Ms 7 X TI 20 Ms 7	10.67
25	TI 13 Ms 4 X TI 18 Ms 5	48.57
26	TI 16 Ms 1 X TI 18 Ms 5	8.42
27	TI 18 Ms 5 X TI 20 Ms 7	32.00

A total of 5,772 unique SSR were identified in the genome of *T. indica* using MISA script. SSRs were found in 863 scaffolds. Among SSRs, tri-nucleotide was most abundant (42 %), followed by di-nucleotide (28 %), mono-nucleotide (23 %), tetra-nucleotide (3 %), hexa-nucleotide (3 %) and penta-nucleotide (1 %) (Fig.1). The size of SSR motif varied from 132bp $(CTAACC)_{22}$ to 10bp $(T)_{10}$. Overall, 130 alleles were amplified among the 20 *T. indica* isolates with a range of 2-6 alleles/marker. Amplified product was in the range of 200-1000bp. The highest number of alleles was 6 in TiSSR1 and TiSSR34 loci. 18 marker were highly informative, 15 moderately informative and remaining 7 markers were less informative. Cluster analysis did not group the *Ti* isolates according to their isolation states (Fig. 2). Sixty monosporadial lines were established from *Ti* culture. Ten highly polymorphic SSR markers were amplified in 60 monosporidial lines (ms) of *T. indica* (Table 1). Differently amplified monosporidial lines were chosen for monosporidial lines compatibility assay. 27 monosporidial crosses of *T. indica* were produced Karnal bunt disease reaction on susceptible host (WH542). KB7MS1 x KB18MS5 with 36 % coefficient of infection and KB7MS1 x KB16MS1 (coefficient of infection of 24.17 %) was found most virulent (Table 2).

CONCLUSIONS

40 SSR markers were identified in the genome of *T. indica*. 18 SSRs markers were highly informative, 15 SSRs were moderately informative. In cluster analysis, *T. indica* isolates did not cluster to region wise of isolates. Further, the ms lines were developed for SSR analysis. The ms lines were found in distinct clusters. These haploid monosporidial lines may have different mating types. The newly developed SSR markers will be useful for genetic and population studies of *T. indica*.

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