ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 09, pp.38413-38417, September, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

GENETIC ANALYSIS OF RECOMBINANT INBRED LINES OF WHEAT FOR RESISTANCE TO KARNAL BUNT (*NEOVOSSIA INDICA*) USING ISSR MARKERS

[1]Mamta Gupta, [*,1]Bunty Sharma, [1]Veena Chawla and [2]Neelam R. Yadav

[1]Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar-125 004 (Haryana), India
[2]Department of Molecular Biology and Biotechnology, CCS HAU, Hisar-125 004 (Haryana), India

ARTICLE INFO

Article History:
Received 14th June, 2016
Received in revised form
29th July, 2016
Accepted 25th August, 2016
Published online 30th September, 2016

Key words:

Karnal bunt,
Neovossia indica,
ISSR markers,
Molecular diversity.

ABSTRACT

In the present investigation 94 recombinant inbred lines from wheat cross Aldan (resistant) and WH542 (susceptible), were used. There was significant variation among all the recombinant inbred lines for coefficient of infection of Karnal bunt (*Neovossia indica*). The chi-square analysis indicated that a single dominant gene was responsible for Karnal bunt resistance. Total thirty five inter simple sequence repeat (ISSR) primers were used for amplification of DNA from the two parents. ISSR markers based finger print database were generated using only 25 primers. Out of 25, six primers showed 100% polymorphism. A total of 193 alleles were detected at 25 ISSR loci. The number of alleles per locus ranged from 4-14 with an average of 7.72 alleles per locus. The two parents were genetically diverse (similarity coefficient 0.419). The cluster analysis led to distribution into two groups: group 1 had resistant parent Aldan and group 2 had susceptible parent WH542, resistant bulk and susceptible bulk respectively. Among all the primers used one primer (IS-43) was identified to be associated with Karnal bunt resistance. This showed an amplification profile (650 bp product) characteristic of resistant parent, in corresponding bulk and in individual genotyping of the recombinant inbred lines. This might have an application in marker assisted selection after converting it to SCAR marker.

Citation: Mamta gupta, Bunty Sharma, Veena Chawla and Neelam R. Yadav, 2016. "Genetic analysis of recombinant inbred lines of wheat for resistance to Karnal bunt (*Neovossia indica*) using ISSR markers" *International Journal of Current Research*, 8, (09), 38413-38417.

INTRODUCTION

Wheat is a source of staple food in many countries of the world. The most demanding aspect, which has interferred greatly with the production of wheat, is the management of diseases. Every year about twenty per cent of wheat, that otherwise would have been available for food and feed worldwide, is lost due to diseases either in the field or storage. Karnal bunt is a seed, soil / air borne disease that affects wheat production in the Indian subcontinent (Ykema *et al*., 1996, Haq *et al*., 2002, Kumar *et al*., 2007). To avoid introduction and spread of disease, legal restrictions on seed movement have been enforced by many countries (Babadoost, 2000). In order to maintain the sales of wheat in the foreign market phytosanitary certificates are required indicating that wheat lots are free from karnal bunt. The most-effective and economical method of disease management is through host plant resistance. Developing karnal bunt resistant wheat cultivars with conventional methods is time consuming and labor-intensive. An alternative to direct selection for karnal bunt resistance is the indirect selection using DNA markers linked to genes conferring karnal bunt resistance. Among different types of molecular markers, inter-simple sequence repeat polymorphic DNA (ISSR) (Zietkiewicz *et al*., 1994) have been proposed as a new source of genetic marker that overcomes many of the technical limitations of RFLP (Rafalski *et al*., 1991) and RAPD (Devos and Gale, 1992) .These markers represent an efficient, reproducible and suitable marker system for discriminating among closely related plant systems (Cooke and Lees, 2004) due to higher stringency of amplification in the form of longer primers (approximately 15-25 bases long) at elevated annealing temperatures (36-65ºC).

Corresponding author: Bunty Sharma
Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar-125 004 (Haryana), India.

MATERIALS AND METHODS

Plant Material

Wheat genotypes viz. Aldan, WH542 and their recombinant inbred lines (94) were used in the present investigations.

Evaluation of karnal bunt resistance

The screening of both the parental genotypes and their recombinant inbred lines against *N. indica* under artificial epiphytotic conditions was carried out in the net-house and field. These were inoculated with the sporidial suspension of concentration 10,000 sporidia per ml. After maturity, the inoculated ear heads were harvested. Grains were removed carefully by hand and were separated into different grades. The percentage of infected grains and the coefficient of infection were worked out as described by Aujla *et al*. (1989).

DNA isolation and Polymerase chain reaction (PCR) amplification

DNA was isolated from leaf tissues using modified CTAB procedure (Saghai-Maroof *et al*., 1984). DNA amplification were carried out in 20 µl reaction mixture, each containing 25 ng of template DNA, 2 µM inter-simple sequence repeat primers, 250 µM each of dNTPs, 2 µl of 10X buffer, 1 unit *Taq* polymerase and 1.5 mM $MgCl_2$. PCR amplification were performed on a PTC-100 Thermal Cycler (MJ Research, PTC-0150) under the following conditions: Initial denaturetion at 94^0C for 4 min, followed by 45 cycles of denaturing at 94^0C for 1 min, annealing at 50^0C / 55^0C / 60^0C for 1 min, extension at 72^0C for 1 min and final extension at 72^0C for 15 min.

Similarity coefficient

The frequency of ISSR polymorphism was calculated based on presence or absence of common bands (Ghosh *et al.,* 1997). The binary data was used to compute pair-wise similarity coefficient (Jaccard, 1908) and the similarity matrix thus obtained was subjected to cluster analysis using the UPGMA (unweighted pair group method with arithmetic average) algorithm on NTSYS-PC (numerical taxonomy and multivariate analysis system program).

RESULTS AND DISCUSSION

The numbers of infected grains with Karnal bunt were recorded. Percentage of infected grains and coefficients of infection were calculated. The data were transformed using angular transformations and analysed following Completely Randomized Design. There was significant variation among the recombinant inbred lines (Table 1). The range of percentage of infection (PI) on the RILs was 0-65.46 and coefficient of infection (CI) was 0-41.70. RILs with coefficient of infection less than 5% were taken as resistant and those with more than 5% coefficient of infection were taken as susceptible. Sixty four recombinant inbred lines were found to be resistant and thirty were susceptible. Chi-square test for goodness of fit was carried out. The data suggested a good fit (χ^2=2.39) for 3:1 ratio. This indicated that a single dominant gene was responsible for karnal bunt resistance.

Bulk segregant analysis

The bulk segregant analysis was used to identify ISSR markers linked to Karnal bunt resistance. A total of 10 resistant and 10 susceptible RILs were selected from 94 RILs of cross Aldan x WH542 based on their coefficient of infection (CI). Two bulks were prepared to obtain molecular marker linked to the Karnal bunt resistance. The list of 20 selected RILs, their mean CI and codes used are given in Table 2.

ISSR markers based polymorphism

ISSR markers based fingerprint database was generated for both the parents, karnal bunt resistant and susceptible bulks and has been shown in Fig. 1-4. Data recorded for different alleles for each primer was scored as 1 if the band was present and scored as 0 if the band was absent. A total of 193 alleles were detected at 25 ISSR loci. The number of alleles per locus ranged from 4 (ISSR 878) to 14 (ISSR 835) with an average of 7.72 alleles per locus. The overall size of PCR products amplified ranged from 200-3000 bp. Out of 25 markers, 6 markers (ISSR 814, 841, 844, 852, 818 and IS-43) showed 100% polymorphism (Table 3). The ISSR allelic database for both bulks and the parental wheat genotypes were used for generating similarity matrix data and UPGMA tree cluster analysis. Similarity coefficients of both the parents and bulks are presented in Table 4.

Table 1. Analysis of variance for coefficient of infection with *N. indica*

Source of variation	D.F.	M.S.	F-Cal
Treatments	95	183.19**	120.43
Error	192	1.52	

** significant 1% level

Table 2. List of twenty RILs, their coefficient of infection (CI) and code used for bulk segregant analysis (I to X genotypes represent resistant bulk while XI to XX susceptible bulk)

RIL No.	C.I.	Code	RIL No.	C.I.	Code
1	0.00	I	6	6.03	XI
3	0.14	II	19	10.57	XII
4	0.00	III	28	16.31	XIII
17	0.00	IV	43	7.35	XIV
41	0.00	V	50	8.01	XV
70	0.09	VI	55	13.74	XVI
75	0.17	VII	56	5.86	XVII
79	0.00	VIII	61	23.16	XVIII
81	0.55	IX	65	12.83	XIX
86	0.29	X	85	10.27	XX

Table 3. Allelic diversity among parents Aldan and WH 542 as assessed by 25 ISSR markers

Number of primers	25
Number of alleles	193
Range of alleles	4-14
Average number of alleles	7.72
Number of markers showing 100% polymorphism	6

Table 4. Similarity matrix data among parents, resistant and susceptible bulks obtained using the allelic diversity at 25 ISSR loci

	R.	R.B.	S.	S.B.
R.	1.000			
R.B.	0.383	1.000		
S.	0.419	0.673	1.000	
S.B.	0.419	0.683	0.730	1.000

R: resistant parent; R.B: resistant bulk; S: susceptible parent; S.B.: susceptible bulk.

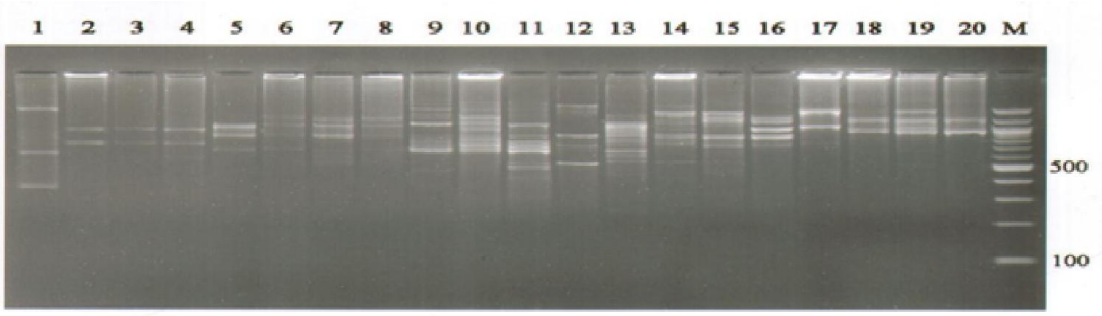

Fig. : Electrophoretic pattern of amplified products in parent Aldan (Lanes 1,5,9,13,17), resistant bulk (Lanes 2,6,10,14,18), WH542 (Lanes 3,7,11,15,19) and susceptible bulk (4,8,12,16,20) using five primers: 814 (1-4), 822 (Lanes 5-8), 835 (Lanes9-12), 836 (Lanes13-16) and 844 (Lanes17-20), Lane M:100 bp DNA Ladder.

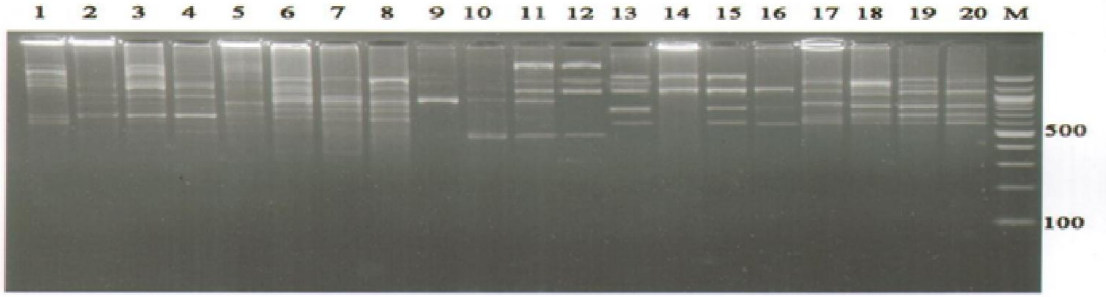

Fig. : Electrophoretic pattern of amplified products in parent Aldan (Lanes 1,5,9,13,17), resistant bulk (Lanes 2,6,10,14,18), WH542 (Lanes 3,7,11,15,19) and susceptible bulk (4,8,12,16,20) using five primers: 845 (Lanes 1-4) , 849 (Lanes 5-8) ,852 (Lanes 9-12) , 854 (Lanes 13-16) and 856 (Lanes 17-20) , Lane M :100 bp DNA Ladder.

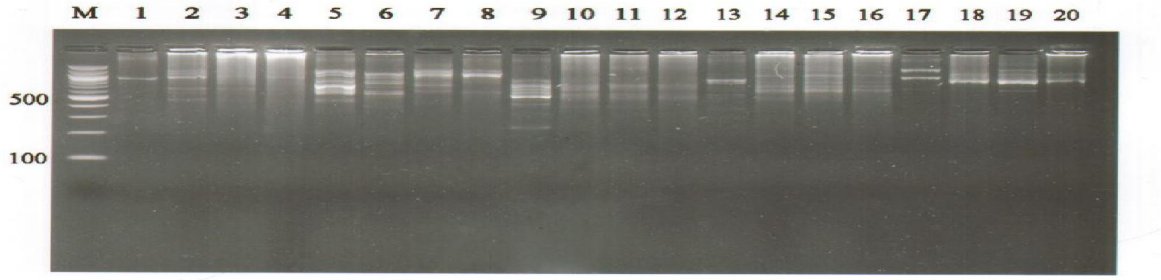

Fig. : Electrophoretic pattern of amplified products in parent Aldan (Lanes 1,5,9,13,17), resistant bulk (Lanes 2,6,10,14,18), WH542 (Lanes 3,7,11,15,19) and susceptible bulk(4,8,12,16,20) using five primers: 858 (Lanes 1-4) , 873 (Lanes 5 -8) , 809 (Lanes 9-12) , 818 (Lanes 13-16) and 841 (Lanes 17-20), Lane M:100 bp DNA Ladder.

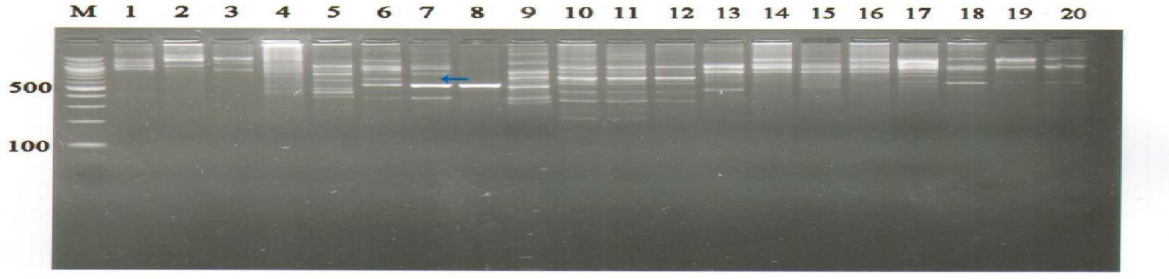

Fig. : Electrophoretic pattern of amplified products in parent Aldan (Lanes 1,5,9,13,17), resistant bulk (Lanes 2,6,10,14,18), WH542 (Lanes 3,7,11,15,19) and susceptible bulk(4,8,12,16,20) using five primers: 860 (Lanes 1-4) , IS- 43 (Lanes 5-8) ,IS-55 (Lanes 9-12) , IS-101 (Lanes 13-16) and 893 (Lanes 17-20), Lane M:100 bp DNA Ladder.

Fig.1-4.

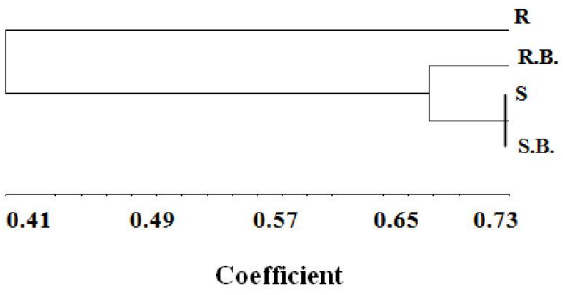

Fig. 5. Dendrogram of parents (R Aldan while S WH542) and both the bulks

The similarity coefficient between the two parental genotypes was 0.419, between the resistant bulk and resistant parent (Aldan) was 0.383 and between the resistant bulk and susceptible parent (WH542) was 0.673. The similarity coefficient between the susceptible bulk and resistant parent, was 0.419 and between the susceptible bulk and susceptible parent was 0.730. The cluster analysis lead to distribution of both the bulk and parental genotypes into two groups. Group 1 had Aldan and group 2 had WH542, resistant bulk and susceptible bulk respectively (Fig.5).

Specific ISSR marker showing association with karnal bunt resistance

One ISSR marker (IS-43) was detected which could possibly be linked to karnal bunt resistance. This specific marker IS-43 gave specific band only in resistant parent and resistant bulk. Individual RILs used for making the resistant and susceptible bulk were tested using this primer. The specific band was present in individuals RILs making the resistant bulk and was absent in RILs used for making susceptible bulk. Rest of the RILs from the cross Aldan x WH542 were also tested using above primer. In this population this specific band was present in 46 resistant lines tested. Twenty susceptible recombinant inbred lines (excluding those in susceptible bulk) were also tested for presence of this band using IS-43. This band was absent in all of these susceptible lines. The goodness of fit for the ratio of 3:1 for resistant and susceptible lines indicated that resistance was under the control of single dominant gene. Identification of recombinant inbred lines with lower and higher coefficient of infection than Aldan and WH542 parents suggested that WH542, probably, has some minor genes for Karnal bunt resistance. The inheritance of Karnal bunt resistance have also been reported to be controlled by 2 recessive genes by Singh *et al*., (1993) and Fuentes Davila *et al*. (1995). Singh *et al*., (1996) reported that resistance was controlled by few major genes along with some minor genes. Presence of minor genes in genotypes WH542 was also reported by Kumar *et al*., (2007) and Kaur *et al*., (2011). They observed that the skewed distribution of Karnal bunt resistance was towards the resistant parent and suggested the segregation of multiple genes with dominant or complementary gene action in wheat line HD29. Sharma *et al*., (2005) studied the genetics of karnal bunt resistance in population derived from crosses of four resistant stocks (HD29, W485, ALDAN 'S'/IAS58, H567.71/3*PAR) and a highly susceptible cultivar WH542. The screening was performed under optimal conditions for disease development with a mixture of isolates from North Western Plains of India using syringe method of inoculation. Genetic analysis revealed that HD 29, W485 and ALDAN 'S'/IAS 58 each carried two resistance genes whereas 3 genes were indicated in H 567.71/3*PAR. Similar results were also observed by Bala *et al*. (2011) in near isogenic lines (NILs) developed using six donor stocks viz., ALDAN ' S' / IAS 58, CMH 77.308, H567.7I/3*PAR, HD29, HP1531 and W485 in the background of susceptible cultivar WH542.

Genetic relationship among both the parents, resistant and susceptible bulks

Problem arises in finding useful genes for a particular trait when numerous genes are associated with the expression of that trait. The smaller the contribution of individual gene, the more difficult is to detect it. However, still it is important for breeding crops, all the major genes associated with a trait should be detected. Several new types of markers are now available for gene mapping, ISSR markers are certainly the best for most of the purposes (Fernandez *et al*., 2002). Parveen *et al*., (2013) studied, genetic relationships among ten isolates of *Tilletia indica* collected from different locations of India and 15 monosporidial lines raised from these isolates. Then these isolates were investigated by using 34 RAPD and 28 ISSR primers. In this study, both marker systems were similar except for the percentage polymorphism which was found to be greater using ISSR, thus indicating the greater effectiveness of ISSR primers for estimating genetic diversity of *Tilletia indica*.

A total of 25 ISSR marker were tested on two bulks (resistant and susceptible) along with parents in the present study. With 24 of the 25 primers, no apparent association between the markers and Karnal bunt resistance was observed. The remaining one primer, IS-43 was found to be associated with the Karnal bunt resistance as it exhibited amplification profile characteristic of resistant parent and in the corresponding bulk in bulk segregant analysis.

REFERENCES

Aujla, S.S., Sharma, I. and Singh B.B. 1989. Rating scale for identifying wheat varieties resistant to *Neovossia indica* (Mitra) Mundkur. *Ind Phytopath*. 42(1): 161-162.

Babadoost, M. 2000. Comments on the zero-tolerance of quarantine of karnal bunt of wheat. *Plant Dis*. 84(7): 711-712.

Bala, R., Sharma, I. and Bains, N.S. 2011. Genetic analysis of Karnal bunt (*Tilletia indica Mitra*) in near isogenic lines of bread wheat (*Triticum aestivum* L.). *J. Wheat Res.* 3(1): 59-62.

Cooke, D.E.L. and Lees, A.K. 2004. Markers, old and new, for examining *Phytophthora infestans* diversity. *Plant Pathology.* 53: 692-704.

Devos, K.M. and Gale, M.D. 1992. The use of random amplified polymorphic DNA markers in wheat. *Theor. Appl. Genet*. 84: 567-572.

Fernandez, M.E., Figueiras, A.M. and Benito, C. 2002. The use of ISSR and RAPD markers for detecting DNA polymorphism, genotypes identification and genetic diversity origin. *Theor. Appl. Genet.,* 104: 845-851.

Fuentes-Davila, G., Rajaram, S. and Singh, G. 1995. Inheritance of resistance to karnal bunt (*Tilletia indica* Mitra) in bread wheat (*Triticum aestivum* L.). *Plant Breed*, 114(3): 250-252.

Ghosh, S., Karanjawala, Z.E. and Houser, E.R. 1997. Methods for precise sizing automated inning of alleles and reduction or error rates in large scale genotyping using fluorescently labeled dinucleotide markers. *Genome Res*., 7: 165-178.

Haq, U.E., Rehman – Rattu, A.E and Ji.M. 2002. Prevalence of karnal bunt of wheat in NWFP (Pakistan). *Int. J. Agri. Biol.,* 4(1): 150-152.

Jaccard, P. 1908. Nauvelles, recherches sur la distribution florale. *Bull. SOC. Vaudoise Sci. Nat.* 44: 223-270.

Kaur, A., Joshi, M. and Sharma, I. 2011. Genetics of Karnal bunt resistance in a new set of bread wheat lines. *Crop Imp.* 38(1): 38-44.

Kumar, M., Luthra , O.P., Yadav. N.R., Chaudhary, L., Saini, N., Kumar, R., Sharma, I. and Chawla, V. 2007. Identification of microsatellite markers on chromosomes of bread wheat showing an association with karnal bunt resistance. *African J. Biotech*. 6(15): 1617-1622.

Parveen, S., Saharan, M.S., Verma. A. and Sharma., I. 2013. Comparative analysis of RAPD and ISSR marker assays for detecting genetic polymorphism in *Tilletia indica. Eur. J. Experi. Bio*. 3(1):380-387.

Rafalski, J.K., Tingey, S.V. and Williams, J.G.K. 1991. RAPD markers- a new technology for genetic mapping and plant breeding. *Ag. Biotech. News Inf*. 3:645-648.

Saghai-Maroof, M.A., Soliman, K.M.M., Jorgensen, R.A. and Allard, R.W. 1984. Ribosomal DNA spacer length polymorphism in barley. Mandelion inheritance, Chromosomal location and population dynamics. *Proc. Natl. Acad. Sci. (USA)*. 81: 8014-8019.

Sharma I., Bains, N.S., Singh, K. and Nanda, G.S. 2005. Additive genes at nine loci govern Karnal bunt resistance in a set of common wheat cultivars. *Euphytica*. 142: 301-307.

Singh, D.V., Srivastava, K.D., Aggarwal, R., Bahadur P and Tandon, S. 1993. Source of resistance to Karnal bunt (*Neovassia indica*) of wheat. (*Triticum* species). *Indian J. Agric. Sci.* 63(7): 454-455.

Singh, J., Aujla, S.S. and Sharma, I. 1996. Karnal bunt resistance in wheat genetic studies. *Plant Dis. Res*. 11(1): 35-42.

Ykema, R.E., Floyd, J.P. and Palm, M.G. 1996. First report of karnal bunt of wheat in United States. *Plant Dis*. 80(10): 12-14.

Zietkiewicz, E., Rafalski. A. and Labuda, D. 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Geno*. 20: 176-183.

****