J Biochem Tech (2012) 4(1): 480-484
ISSN: 0974-2328

Genic microsatellite markers for genetic diversity of rust resistant wheat genotypes

Sheikh Arslan Sehgal*, Rana Adnan Tahir, Muhammad Nawaz, Muhammad Younas

Received: 20 March 2013/ Received in revised form: 03 June 2013, Accepted: 04 June 2013, Published online: 18 June 2013,

Abstract

The genetic diversity and molecular characterization of 10 wheat genotypes was investigated using 27 polymorphic microsatellite screened primers including Simple Sequence Repeats (SSR). About twenty one loci were found. Bioinformatics tools were applied for constructing dendogram. Lr-19 gene was present in all ten wheat genotypes and Sr-15 gene is present in nine genotypes except Uqab-2000 that cause resistance against wheat rust. Highest genetic diversity was observed between Shalimar-86 and Pak-81 genotypes and showed the similarity of about 95.34%. Distantly related Uqab-2000 showed minimum genetic diversity and 65.64% dissimilarity with Kohistan-97. Uqab-2000 rust resistant genes may have an insertion or deletion and examined as distantly related to rest of nine genotypes. The current research found that SSR makers identified rust resistant genes in numerous wheat genotypes. Present work also characterized the wheat genotypes at molecular level and found genetic diversity between different wheat genotypes. SSR markers could distinguish and characterize wheat genotypes, more screened primers could be used for saturation of different regions in further research. Bioinformatics also play a vital role in retrieving, analyzing and interpreting the data for further studies.

Keywords: Genetic Diversity, Molecular Characterization, Wheat Rust, SSR markers, Bioinformatics, Lr-19, Microsatellite markers.

Introduction

The common wheat (*Triticum aestivum*) is largely consumed edible of human beings. The common wheat is a domestically cultivated grass and a polyploidy in all over the world. Wheat contains approximately one half of the calories in human's food and can also fulfill the huge part of their nutritional necessities. *Triticum spp* (common wheat) belongs to the *Poaceae* family. This family is one of the most diverse and significant among the families of kingdom *Plantea*. The substantial increase in world's population demands a consistent increase in the production of wheat.

Sheikh Arslan Sehgal*, Rana Adnan Tahir, Muhammad Nawaz

Department of Bioinformatics and Biotechnology, Government College University Faisalabad, Pakistan.

*Tel: 00923217600696
E-mail**:** arslansehgal@yahoo.com

Muhammad Younas

Agricultural Biotechnology Research Institute, AARI. Faisalabad, Pakistan.

The wheat research is very difficult, time consuming, extensive and is used to maximize the production of wheat grains. It can also used to improve and get better yield of grain. However, there is still consideration space for the advancement and improvement in genetics of wheat to conquer the daily problems of increasing requirements of world population. Genetic manipulation and genetic diversity is a good path for increasing yield of wheat. Therefore, it is required to study and estimate different mode of inheritance and genetic variation in different parameters of plants to start the productive wheat breeding programs.

The sluggish yields of wheat in developing countries and Pakistan are due to insufficient diversity in genetic resources used in reproduction programs and breeding new cultivars. A large number of different alleles have been mutated and lost through selection and breeding, so that different problems have to face in modern system of agriculture for the improvement of wheat (Allard 1996, Hoisington et al. 1999).

Molecular and microsatellite markers can show comprehensive divergence in genetic resources. Genetic diversity can directly measured by markers. Microsatellite markers are simple sequence repeats (SSR) of 1-6 nucleotides. Markers profusely scatter in whole genome. SSR markers show the privileged level of polymorphism as compared to other genetic markers. The additional advantages of SSR markers are their co-dominant inheritance and potential for automation when different types of molecular markers are compared with them. These features associated with the easement of detection and also cover all the 21 wheat chromosomes with SSR markers. SSR markers have been also used in the collection of seed bank to improve the wheat germplasm (Borner et al. 2000; Huang et al. 2002) and to characterize the genetic diversity at molecular level in wild relatives (Hammer et al. 2000). Twenty different directly and indirectly related wheat genotypes were also examined for rust resistant genes and found molecular characterization (Sehgal et al. 2012). Bioinformatics is an up growing field of advance studies that efficiently covers all areas of life. Now-a-days, bioinformatics tools are used in all research fields worldwide (Tahir et al. 2012).

The current research was conducted to estimate the genetic diversity of ten different wheat genotypes by using microsatellite markers. This study addressed the genetic characterization of ten different genotypes of wheat and showed the utilization of SSR markers to determine the genetic diversity and molecular characterization among wheat genotypes. The phylogenetic relationships, genetic diversity and molecular characteristics concluded in current study will facilitate in breeding programs for the selection of parents and

to grow high-docile wheat varieties. Bioinformatics tools also help researchers to work efficiently. Scientist can grow rust resistant genes in wheat by using already identified rust resistant genes.

Materials and Methods

Plant materials/wheat germplasm

Seeds of 10 genotypes (Chenab-2000, Uqab-2000, Kohistan-97, Rotas-90, Punjab-06, MH-97, Shakhar-95, Chakwal-86, Pak-81, Shalimar-86) were collected from the Agriculture Biotechnology Research Institute (ABRI) Faisalabad. Study was done in the Department of Bioinformatics and Biotechnology Government College University Faisalabad (GCUF) and Agriculture Biotechnology Research Institute (ABRI) Faisalabad. The seedlings of ten wheat genotypes were raised in clay pots in GCUF. Normal and required conditions were provided for better growth. Young and vigorously growing fresh sample of leaves from seedlings were obtained from 21 days old seedling for the extraction of DNA. Healthy portion of fresh leaf of the rudder were cut apart with sterilized scissors and washed in solution of ethanol and distilled water. Tissue papers were used to dry the leaves for the removal of spores of microorganism and any other foreign DNA. The collected leaf samples were crammed in polythene bags for avoiding damages and stored at -80˚ C freezer.

DNA extraction and SSR analysis

The genomic DNA of wheat genotypes were extracted from young leaves according to the methodology described by Khan et al. (Khan et al. 2004). Spectrophotometer was utilized to check the quality and concentration of extracted genomic DNA material for the amplification of PCR. Twenty seven screened pairs of primers were utilized for the analysis of rust in ten selected genotypes of wheat. The conditions of PCR amplification were maintained as described by Roder (Roder et al.1998).

Each PCR product was carried out in about 25 µl volume of reaction containing double distilled deionizer H_2O, $MgCl_2$, 10x buffer, dNTPs, taq polymerase and both primer pairs according to the primers profile. The amplification of PCR product was done by the incubation of DNA samples for 5-6 minutes at 94˚ C. 45 cycles of PCR product was done that comprised on 94 ˚ C for 60 seconds.

Microsatellite primers were annealed for 60 seconds at 58-60˚ C and the extension for 60 seconds at 72˚C. The final PCR product extension was carried out for 8-10 minutes at 68-72˚ C (Table 1). The amplified product of PCR were electrophorized on 5 % of agrose gel containing 8 µl ethidium bromide, at 90 volts for 60 minutes and UV transluminator was used for observation of gel. Bands appeared on agrose gel were examined and absence and presence of bands on gel were scored as 0 and 1 respectively. A excel sheet was prepared and names of used primers for gene detection were written in columns and selected genotype names in rows. 0 and 1 numeric digits were used for scoring the sheet. The 0 in the field of row and column show the absence of that gene or amplification of that gene in specific genotype and 1 represents the presence of gene in the genotype. NTSys pc software version 2.2 was used for cluster analysis of ten wheat genotypes to determine genetic diversity and similarity among genotypes. This software compares the results of the genotypes and then constructs the dendogram.

Table 1: Table of utilized SSR markers for wheat characterization at molecular level

Sl. No.	Primer name	Cycles	Incubating	Comprising	Annealing	Extension	Final Extension	Base pair
01	Sr-2, Xgwm-533	44	94°C at 4.30 min	94°C at 60 sec	60°C at 6 sec	72°C at 120 sec	72°C at 10 min	120 bp
02	Lr-19	32	94°C at 4.45 min	94°C at 90 sec	55°C at 120 sec	72°C at 90 sec	72°C at 5 min	130 bp
03	Xgwm-37, Lr-19	48	94°C at 4.30 min	94°C at 60 sec	50°C at 60 sec	72°C at 120sec	72°C at 7 min	130 bp
04	STS-9-10, Yr-5	48	94°C at 4.30 min	94°C at 30 sec	45°C at 30 sec	72°C at 45 sec	72°C at 7 min	139 bp
05	STS-7-8, Yr-5	48	94°C at 4.45 min	94°C at 30 sec	45°C at 30 sec	72°C at 45 sec	72°C at 7 min	139 bp
06	GDM-35, Lr-39	32	94°C at 4.15 min	94°C at 30 sec	55°C at 30 sec	72°C at 30 sec	72°C at 5 min	145 bp
07	Sr-36, WMC-477	45	94°C at 10 min	94°C at 60 sec	58°C at 60 sec	72°C at 120 sec	72°C at 10 min	155bp
08	STS-638, Sr-15, Xgwm-11	45	94°C at 4.45 min	94°C at 60 sec	55°C at 60 sec	72°C at 120 sec	72°C at 10 min	202 bp
09	Lr-25	35	94°C at 3.30 min	94°C at 60 sec	60°C at 60 sec	72°C at 120 sec	72°C at 10 min	210 bp
10	XWMC-44, Yr-46, Sr-29	35	94°C at 9.45 min	94°C at 60 sec	61°C at 60 sec	72°C at 120 sec	72°C at 10 min	242 bp
11	Xgwm-210	46	94°C at 4.45 min	94°C at 60 sec	60°C at 60 sec	72°C at 120 sec	72°C at 20 min	245 bp
12	Xgwm-130	45	94°C at 4.45 min	94°C at 60 sec	55°C at 60 sec	72°C at 120 sec	72°C at 10 min	245 bp
13	Lr-10	35	94°C at 3.15 min	94°C at 45 sec	57°C at 45 sec	72°C at 30 sec	72°C at 3 min	310 bp
14	Xgwm-295	46	94°C at 5 min	94°C at 60 sec	60°C at 60 sec	72°C at 120 sec	72°C at 10 min	310 bp
15	Lr-20, Sr-15, STS-638	45	94°C at 5 min	94°C at 60 sec	55°C at 60 sec	72°C at 60 sec	72°C at 10 min	355 bp
16	SCS-464, Lr-28	40	94°C at 2 min	94°C at 60 sec	60°C at 60 sec	72°C at 60 sec	72°C at 7 min	378 bp
17	Lr-28, Xgwm-319	45	94°C at 5 min	94°C at 60 sec	60°C at 60 sec	72°C at 120 sec	72°C at 10 min	378 bp
18	SCS-253, Lr-19	35	95°C at 2 min	94°C at 60 sec	60°C at 60 sec	72°C at 60 sec	72°C at 7 min	736 bp

Results

Lr-19 translocation originally produce by Sharma and Knott (Sharma and Knott 1966) when they transform leaf rust resistance genes 7el1 chromosome of Thinopyrum ponticum a long arm of chromosome 7D of common wheat . Huerta-Espino and Singh (Heurta-Espino and Singh 1994) reported first virulence in Puccinia Triticinan to Lr-19 and it is an effective source of leaf rust resistance worldwide.

The Lr-19 gene has a cut-of-point and middle long arm of 7D chromosome is dislocated and find that the distal half of 7D was

replaced by Thinopyrum Chromatinv (Knott 1980). During meiosis, Thinopyrum segment of 7DL does not pair with homologous wheat segment, complicating attempts to study linkage relationship or to recombine its genes (Kim et al. 1993; Marais and Marais 1990).

Lr-19 gene is a leaf rust resistant gene. Lr-19 gene is present in all the examined ten wheat genotypes and cause resistant against rust. Sr-15 is a stripe rust resistant gene and present in nine genotypes except Uqab-2000. Twenty seven microsatellites screened and polymorphic primer pairs were used to evaluate the extent of molecular characterization and genetic diversity among ten different genotypes of wheat. These twenty seven microsatellite primers were indicated 21 polymorphic loci. All the ten wheat genotypes showed resistant against rust. These ten wheat genotypes were closely and distinctly related. High level of polymorphism was observed by SSR markers ranging from 8.32% to 94.19%. A dendogram (Fig 3) was constructed to study the genetic relationship between ten wheat genotypes. Cluster analysis of ten wheat genotypes showed two groups. Cluster I showed nine wheat genotypes and cluster II indicated remaining one genotype. Shalimar-86 and Pak-81 were closely related each other and show approximately 95.34% similarities. Genotypes of cluster I are more closely related to each other. A minimum similarity was observed between Uqab-2000 and Kohistan-97 which showed that they are 65.64% dissimilar.

The agarose gel result shows the presence of Lr-19 gene in all the wheat genotypes (Fig 1). The bands amplified at 736 base pair. The presence of Sr-15 gene observed in the agarose gel result and bands amplified at 202 base pair (Fig. 2). Both the genes are efficiently effective for rust in wheat.

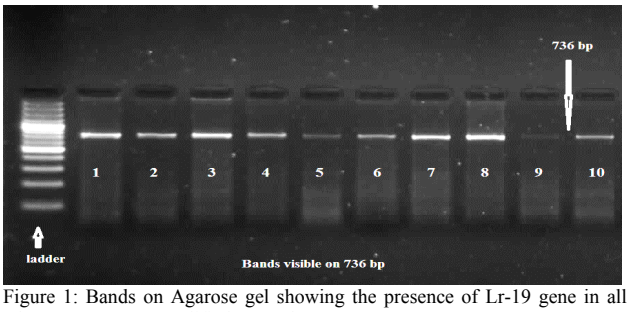

Figure 1: Bands on Agarose gel showing the presence of Lr-19 gene in all wheat genotypes (Amplified at 736 bp).

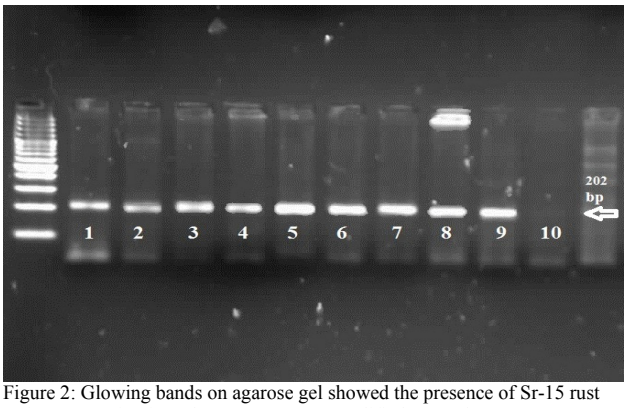

Figure 2: Glowing bands on agarose gel showed the presence of Sr-15 rust resistant gene in nine wheat genotypes (Amplified at 202 bp)

Discussion

The wheat cultivars are of different types and become susceptible to different types of rust because it has narrow genetic bases for resistance. The evolution rates of pathogens are very fast and rapid. So, it is necessary to find out new and better sources for resistance.

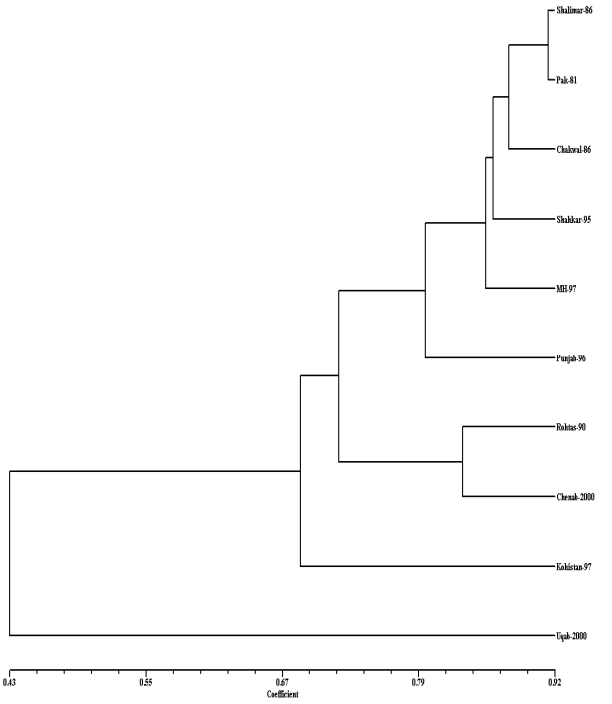

Figure 3: Dendogram of ten wheat genotype

The genetic resistance is important to control many phytopathogenic epidemics. The wheat production and yield of wheat has been dependent on the use and development of rust resistance genotypes having well characterized and diverse genes. It is suggested that diversity, variation and different combinations of genes give long lasting and better resistance for rust diseases than given by any individual genes (Dyck and Sambroski 1982).

The molecular markers have prospective to detect the genetic diversity and characterization at molecular level (Virk et al. 1995, Ford-lloyd et al. 1997; Song et al. 2003). A variety of SSR molecular markers are often selected as preferred molecular markers for application in breeding and diversity of significance. SSR markers have extensive genome coverage, relative abundance, co-dominant inheritance and multi-allelic nature (Gupta and Varshei 2000). Microsatellite markers or SSR markers are becoming the best choice markers due to high level of polymorphism and higher reliability (Fu et al. 2005; Plaschke et al. 1995).

In wheat, many microsatellite markers have been mapped for wheat genome and now also commercially available (Roder et al. 1998). Microsatellite markers are functional and becoming famous for various applications in breeding of wheat due to their easy handling and high level of polymorphism (Bryan et al. 1997; Lelley et al. 2000, Roder et al. 1995; Roy et al. 1999). SSR markers are utilizes to evaluate the molecular characterization of wheat hexaploid landraces in associated with their geographical origin (Al-khanjari et al. 2007). Sehgal et al. (2012) also suggested the wheat rust resistant genes for better production and yield of wheat and discussed twenty wheat genotypes. The current work examined the effective wheat genotypes that cause resistance against rust. The selected genotypes show resistance in short period of time.

In this study, one of the polymerase chain reaction based system (SSR) has been used and compared for studying the genetic

diversity and molecular characterization between ten different genotypes of wheat. The SSR system is different in principle type and amount of polymorphism detected.

The current study showed the efficacy of microsatellite markers in revealing assessment of molecular characteristics and genetic variability among ten different wheat genotypes, wherein 27 microsatellite screened polymorphic markers were used. SSR markers loci generated by 27 primers pairs were used to study the molecular characterization and genetic diversity among ten wheat genotypes. This work also showed that a maximum number of bands were generated by SCS-253, STS-638 and xgwm-11 primer pairs.

All the ten wheat genotypes were showed rust resistant. These ten wheat genotypes were closely and distinctly related. High level of polymorphism was shown by SSR markers ranging from 8.32% to 94.19%. Bioinformatics is applied on extracted lab data and drew a dendogram. Dendogram was constructed to investigate the genetic relationship between ten wheat genotypes. Cluster analysis of ten wheat genotypes showed two groups. Cluster I showed nine wheat genotypes and cluster II indicated remaining one genotype. Shalimar-86 and Pak-81 were closely related each other and showed 95.34% similarity. Genotypes of cluster I are more closely related to each other. A minimum similarity was observed between Uqab-2000 with other nine genotypes. Lr-19 is a wheat rust resistant gene and present in all ten genotypes. Sr-15 gene is present in nine wheat genotypes except Uqab-2000. These gene causes resistance against rust diseases. Different mutations occur in Uqab-2000 leads to the isolation of this genotype from others.

Conclusion

It is concluded that more polymorphic wheat microsatellites could be used for efficient screening of the germplasm by saturating more regions of the wheat genome. Genome of Uqab-2000 may have occurred any insertion or deletion and presents distantly related among rest of the genotypes. The selected genotypes show effective and consistency against rust. Lr-19 and Sr-15 genes explained high level of rust resistance in wheat genotypes. Dendogram represented phylogenetic relationship between wheat genotypes. High level of divergence occurs in the genome of Shalimar-86 and Pak-81. There are still lots of work remaining on controlling the effects of pathogens and rust disease. This struggle will lead to the improvement of the yield grains and solving the food problems of developing countries. How to improve wheat rust resistant genes and insert resistant genes in non-resistant genotypes is a burning question.

Acknowledgements

Sheikh Arslan Sehgal acknowledges Sheikh Farhan Ashraf and Babar Ashraf Sheikh for their kind help and support in studying and preparing this manuscript. The author is grateful to his Lab fellow and friend Sajjad Ahmad Larra for research helps and acknowledges Agriculture Biotechnology Research Institute AARI Faisalabad, Pakistan for giving wheat genotypes and support throughout the research.

References

Allard RW (1996) Genetic basis of the evolution of adaptednes in plants. Euphytica 92: 1-11.

Al-khanjari S, Hammer K, Buerkert A, Roder MS (2007) Molecular diversity of Omani wheat revealed by microsatellites: II. Hexaploid landraces. Genet. Resour. Crop Evol. 54: 1407-1417.

Borner A, Chebotar S, Korzun V (2000) Molecular characterization of the genetic integrity of wheat (*Triticum aestivum* L.) germplasm after long-term maintenance. Theor. Appl. Genet. 100: 494-497

Bryan GJ, Collins AJ, Stephensons P, Orry A (1997) Isolation and characterization of microsatellites from hexaploid bread wheat. Theor. Appl. Genet. 94: 557-563.

Dyck PL and Sambroski DJ (1982) The inheritance of resistance to puccinia in a group of common wheat cultivars. Can. J. Genet. Cytol. 24: 2733-2783.

Ford-lloyd BV, Jackson M.T, Newbury HJ (1997) Molecular Markers and the Management of Genetic Resources in Seed Genebanks: A Case Study of Rice. In: Biotechnology and Plant Genetic Resources - Conservation and Use (Callow JA, Ford-Lloyd BV and Newbury HJ, eds.). CAB International (Centro de Biociência Agrícola International), Wallingford, 103-118.

Fu YB, Peterson GW, Richards KW, Somers D (2005) Allelic reduction and genetic shift in the Canadian hard red spring wheat germplasm released from 1845 to 2004. Theor Appl. Genet. 110: 1505-1516.

Gupta PK and Varshei RK (2000) The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. Euphytica, 113: 163-185.

Hammer K, Filatenko AA, Korzun V (2000) Microsatellite markers - a new tool for distinguishing diploid wheat species. Genet. Resour. Crop Evol. 47: 497-505.

Hoisington D, Khairallah M, Reeves T, Ribaut JM (1999) Plant genetic resources: what can they contribute toward increased crop productivity? Proc. Natl. Acad. Sci. U. S. A. 96: 5937-5943.

Huang Q, Borner A, Roder A, Ganal W (2002) Assessing genetic diversity of wheat (*Triticum aestivum* L.) germplasm using microsatellite markers. Theor. Appl. Genet. 105: 699-707.

Heurta-Espino J and Singh RP (1994) First report of virulence to wheat with leaf rust resistant gene Lr-19 in Mexcico. Plant disease 78:640.

Knott DR (1980) Mutation of a gene for yellow pigment link to Lr - 19 in wheat. Canadian journal of genetics and cytology 22:651-654

Kim NS, Amstrong K, Knott DR (1993) Molecular detection of Lophopyrum chromatin in wheat Lophopyrum recombinants and their use in the physical mapping of chromosome 7D. Theoretical and applied genetics 85561-567

Khan IA, Awan FS, Ahmad A, Khan A (2004) A modified mini-prep method for economical and rapid extraction of genomic DNA in plants. Plant Mol. Biol. Rep. 22: 89a-89e.

Lelley T, Stachel M, Grausgruber H, Vollmann J (2000) Analysis of relationships between Aegilops tauschii and the D genome of wheat utilizing microsatellites. Genome, 43: 661-668.

Marais GF, Marais AS (1990) The assignment of a Thinopyrum Disticum (thunb) love derived translocation to the long arm of wheat chromosome 7D using endopeptidase polymorphism. Theoretical and applied genetics 79:182-186

Plaschke J, Ganal WM, Roder MS (1995) Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor. Appl. Genet. 91: 1001-1007.

Roder MS, Plaschke J, Koing SU, Borner A (1995) Abundance, variability and chromosomal location of microsatellites in wheat. Mol. Gen. Genet. 246: 327-333.

Roder MS, Korzun V, Wendehake V, Plaschke J (1998) A microsatellite map of wheat. Genetics 149: 2007-2023.

Roy JK, Prasad M, Varshney RK, Balyan HS (1999) Identification of a microsatellite on chromosomes 6B and a STS on 7D of bread wheat showing an association with preharvest sprouting tolerance. Theor. Appl. Genet. 99: 336-340.

Sehgal SA, Tahir RA, Nawaz M (2012) Molecular Characterization of Wheat Genotypes Using SSR Markers. Int J Bioautomation, 16:119-128.

Sharma D, Knott DR (1966) The transfer of leaf rust resistance from Agropyron to Triticum by irradiation. Can. J. Genet. Cytol. 8: 137-143.

Song ZP, Xu X, Wang B, Chen JK (2003) Genetic diversity in the northernmost Oryza rufipogon populations estimated by SSR markers. Theor. Appl. Genet. 107: 1492-1499.

Tahir RA, Sehgal SA, Ijaz A (2012) in silico comparative modeling of papa1 and papa2 proteins involved in mycobacterium tuberculosis sulfolipid-1 biosynthesis pathway. Int. J. Bioautomation, 16: 155-164.

Virk PS, Ford-lloyd BV, Jackson MT, Newbury HJ (1995) Use of RAPD for the study of diversity within plant germplasm collections. Heredity, 74 (Pt 2): 170-179.