African Journal of Biotechnology Vol. 11(72), pp. 13627-13632, 6 September, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.198
ISSN 1684–5315 ©2012 Academic Journals

*Full Length Research Paper*

# Microsatellite based investigation of genetic diversity in 24 synthetic wheat cultivars

**Muhammad Shakeel[1] and Sadiq Azam[2]***

[1]Department of Biotechnology, Bacha Khan University, Palosa Campus Charsadda, Pakistan.
[2]Centre for Biotechnology and Microbiology, University of Peshawar, Khyber Pakhtunkhwa, Pakistan.

Accepted 23 May, 2012

**Common bread wheat has very low genetic diversity. Plant breeders find it difficult to get appropriate germplasm which can be used for bread wheat improvement. Wild plant species is one of the potential sources for conferring resistant genes which can be exploited for bread wheat improvement. The challenge is to utilize these wild relatives into existing food crops without losing the genetic diversity. This study aimed to monitor genetic variability in the synthetic hexaploid wheats using microsatellites molecular markers. The polymorphism revealed by microsatellites was not very prevalent; however more Simple Sequence Repeat (SSR) need to be used to select diverse synthetic lines which can be crossed with common bread wheat to enhance the crop yield and resistance against various stresses.**

**Key words:** Microsatellite, germplasms, hexaploids, polymerase chain reaction, wheat.

## INTRODUCTION

Plant breeders exploit conventional breeding methods to improve the quality and quantity of food crops and meet the food requirement for increasing population. They can hardly find suitable germplasm which can confer the desired traits for crop improvement. One of the potential sources is the wild plant species that poses a close or distant genetic relationship to food crops. It is difficult to transfer the desired traits from the wild species into domesticated food crops without losing the genetic diversity. In tribe *Triticeae*, these goals are addressed through interspecific and intergeneric hybridization methodologies. Simultaneously, the conventional and molecular diagnostics have been evolved to the level that alien introgression detection no longer remains too complex a process. For speeding up alien introgression and production/maintenance of genetic stock programs, the use of polyhaploidy, through sexual hybridization of bread wheat, has emerged as a stable technique. The above areas form a package that impinges upon exploitation of alien genetic diversity for wheat improvement. Production outputs stand strongly associated with the evolutionary relationships present among the *triticeae* species in their various gene pools (Mujeeb-Kazi, 1998). Today's bread wheat has much reduced polymorphism as compared to its parents, and this polymorphism was reduced upon domestication.

Synthetic hexaploids (SH) wheats are used for the improvement of bread wheat because the conventional wheat germplasm possesses a very low level of DNA polymorphism, hence narrow genetic diversity. SH wheats are thus used to enhance diversity and facilitate molecular applications. SH wheats confer resistance against leaf rust (Kerber and Dyck, 1979; Cox et al., 1992), stripe rust (Ma and Hughes, 1995), karnal bunt (Villareal et al., 1994a), *Septoria tritici* (May and Lagudah, 1992), hessian fly (Gill et al., 1986), green bug (Lage et al., 2003), *Helminthosporium sativum* blotch (Mujeeb-Kazi et al., 1989) and various other biotic and abiotic stresses.

### Microsatellites or simple sequence repeats (SSRs) markers

A class of sequences known as microsatellites can be

*Corresponding author. E-mail: azam_bbt@yahoo.com. Tel: +92-332-9279970.

**Abbreviations: SSR,** Simple sequence repeat; **SH,** synthetic hexaploids.

observed in the genomes of all eukaryotes. In plants, these markers have been used successfully as they are informative and reproducible. Due to multiallelic nature, these markers can be used in evolutionary studies and also studying the genetic variability of bread wheat. It is testing to develop microsatellites in bread wheat due to its large genome size and presence of repetitive DNA sequences. SSR markers are codominant and chromosome specific, which are distributed along the chromosomes. Microsatellites have played a role in tagging resistance genes, marker-assisted selection and verifying the genetic stability of gene bank accessions. Despite its usefulness, the development of microsatellite is expensive and time consuming.

The aim of this project was to identify genetic diversity in the selected synthetic hexaploids and exploit it in the improvement of bread wheat which has very low level of polymorphism.

## MATERIALS AND METHODS

For molecular analysis, 24 synthetic hexaploid wheats were selected from elite 1 consisting of 95 SH lines (Mujeeb-Kazi, 2003b). These involved six different durum cultivars Altar 84, Doy1, Croc1, D67.2, 68.111 and Ceta). Four *Aegilops tauschii* accessions were associated with each cultivar. The leaves of young seedlings were used for DNA extraction. 4D specific microsatellites were provided by Dr. Manilal CIMMYT. In the growth room at National Agriculture Research Centre (NARC) Islamabad Pakistan, about 7 cm long piece of fresh leaf material were cut from the three weeks-old plants and were placed in 2 ml tubes. Liquid nitrogen was used to freeze the leaves present in the Eppendorf tubes. The leaves were crushed to form powder using a knitting needle. 500 µl DNA extraction buffer (100 mM NaCl, 1% SDS, 100 mM $Na_2EDTA$, 100 mM Tris base, pH: 8.5 by HCl) were added to each tube containing the crushed plant materials/powdered form of leaves. 40 µl phenol:chloroform:isoamyl alcohol (25:24:1) were added to the tubes, mixed and centrifuged at 8000 rpm for 10 min. After centrifugation, the supernatant portion was observed in the upper part of the tube, while the debris accumulated in the bottom of the tube. 40 µl 3 M sodium acetate (pH = 4.8) and 500 µl isopropanol were used to precipitate the DNA. The tubes were centrifuged at 8000 rpm to pellet the DNA and washed with 70% ethanol twice to remove the contaminants. The pellet was dissolved in 50 µl Tris-EDTA (TE) buffer (1 mM EDTA, 10 mM Tris, pH: 8.0) (Weining and Langridge, 1991). DNA was also treated with 50 µg RNase (Promega) to remove the RNA. The quality of DNA was checked after running it on 1.5% agarose gel (Fisher Scientific).

### Polymerase chain reaction

Polymerase chain reaction (PCR) were performed in 25 µl reaction containing 40 to 90 ng genomic DNA template, 0.25 µM of each primer, 200 µM of each dNTP, 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris, and 2.5 units of Go Taq DNA polymerase (Promega). For PCR amplification, denaturation at 94°C for 4 min was used in the initial step. It was followed by 40 cycles, where denaturation at 94°C for 45 s and an extension at 72°C for 1.5 min were used. Annealing temperature was calculated after subtracting five from the melting temperature. 5 min at 72°C were used for the last extension step to complete the extension of all strands. The amplified PCR product was run on 1.5% agarose gel to see the expected size amplicon. Photos were taken using the computer program UVI Photo MW (version 99.03s for Windows; Cambridge, UK). A set of SSR primers specific for the chromosome 4D was used. Three SSRs primers (DMS-34, DMS-40 and DMS-125) were used in this study. This choice is because the SHs of this study are all positive for Na:K discrimination and this trait can be observed on chromosome 4DL and associated with salinity tolerance (Mujeeb-Kazi, 2003b; Shah et al., 1987). Amplification conditions using SSRs were essentially the same as described for random amplification of polymorphic DNAs (RAPDs), except annealing temperature, which were calculated by subtracting five from the melting temperature (Tm).

### Statistical analysis

In this study, all the scorable bands were treated as single locus. They were scored on the basis of presence and absence. Bivariate 1 to 0 data matrix obtained during the study was used for calculating genetic distances using unweighted pair group of arithmetic means as follows (Nei and Li, 1979).

$$GD_{xy} = 1 - d_{xy} / d_x + d_y - d_{xy}$$

Where, $GD_{xy}$ = genetic distance between two genotypes, $d_{xy}$ = total number of common loci in two genotypes, $d_x$ = indicates total number of loci in genotype -1 and $d_y$ = indicates total number of loci in genotype -2. The 1 to 0 bivariate data matrix for each set of wheat lines based on the data of microsatellite primer sets was used to generate dendrogram (Figure 6) using “Popgene32” v. 1.31 (http://www.ualberta.ca./-fyeh/.fyeh).

## RESULTS

Microsatellites were helpful in describing the genetic diversity in the selected synthetic lines. Minimum number of loci (band) amplified was one and the maximum number of loci amplified was four for the selected SH wheats.

In some cases, no amplification by PCR was detected for durum parents. Polymorphism was observed in some synthetic lines using three SSR primers. Some diagnostic bands were observed in a few synthetic lines which were not present in their durum parents (indicated by arrow in Figures 1, 3, 4 and 5). Size of scorable bands ranged from 200 to 1500 bp. Overall genetic distance for the lines was 0 to 100% (data not shown).

### Dendrogram for SSR primers

The 1 to 0 bivariate data matrix for the selected synthetic lines was used to generate dendrogram. In general, the dendrogram agreed with the average dissimilarity matrix (data not shown). The synthetic lines were grouped into four (A, B, C and D). Group C was the largest and comprised eight synthetic lines. It was further sub-divided into two subgroups J and K.

Similarly, group B was sub-divided into three subgroups G, H and I. Some synthetic lines were found genetically most diverse among the selected synthetic lines on the dendrogram.

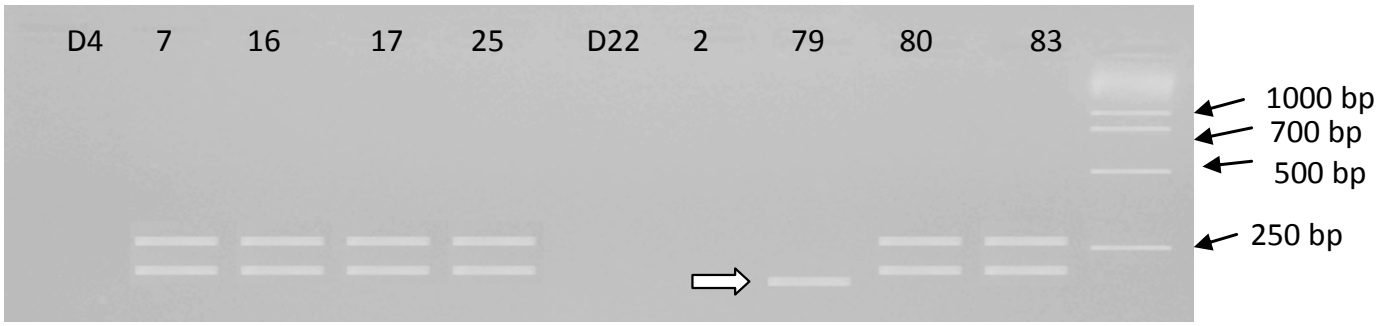


**Figure 1.** PCR profile of durum parent (D4 and D22) and their derivatives (AABBDD; 7, 16, 17, 25, 2, 79, 80 and 83) using SSR primer DMS-34. Arrow indicates SH number 79 which shows PCR amplification at one locus but not at the other locus.


**Figure 2.** PCR profile of durum parent (D1 and D12) and their derivatives (AABBDD; 6, 24, 50, 46, 10, 18, 22 and 56) using SSR primer DMS-34. Molecular sizes (in base pairs) of the bands in 1 Kb DNA ladder are presented on the right side of the figure.

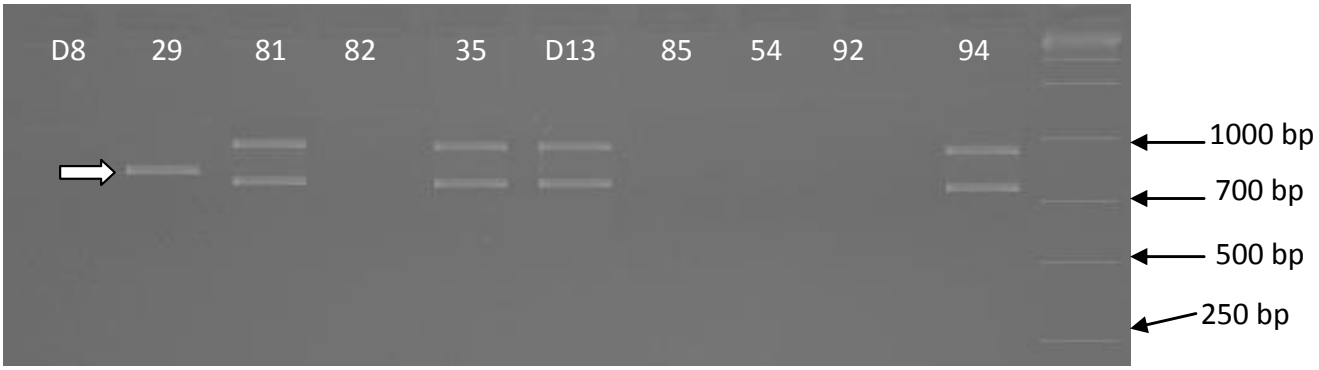


**Figure 3.** PCR profile of durum parent (D8 and D13) and their derivatives (AABBDD; 29, 81, 82, 35, 85, 54, 92 and 94) using SSR primer DMS-34. Arrow indicate diagnostic band present in SH number 29 but absent in durum parent.

## DISCUSSION

Microsatellites have widely been used in studying genetic diversity in bread wheat (Naghavi et al., 2008; Pestsova et al., 2000) and have shown promising results. In plants, these markers have been reported to be highly informative, reproducible and locus specific. Microsatellites have shown significantly larger diversity in hexaploid bread wheat as compared to other marker system. In wheat, it is difficult and expensive to develop these markers due to its large genome size. These markers have been used for the identification of resistance genes, genetic stability of wheat cultivars and marker-assisted selection. Moreover they require low level of genomic DNA (Huang et al., 2002). Microsatellites have been shown to be an easily

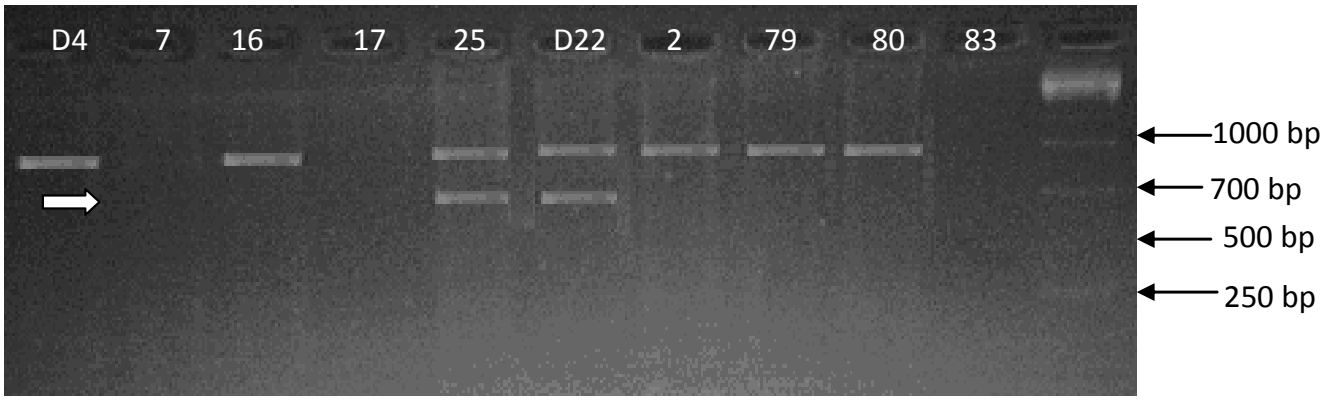


**Figure 4.** PCR profile of durum parent (D4 and D22) and their derivatives (AABBDD; 7, 16, 17, 25, 2, 79, 80 and 83) using SSR primer DMS-40. Arrow indicates band which is present in durum parent but absent from most of SH except number 25.

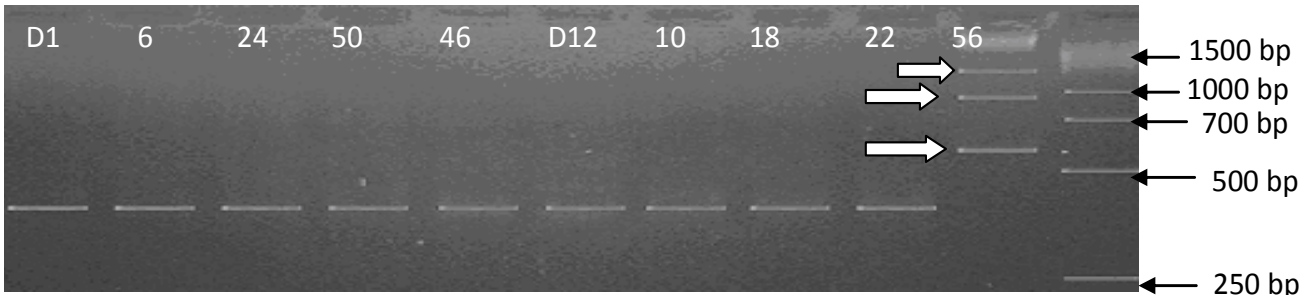


**Figure 5.** PCR profile of durum parent (D1 and D12) and their derivatives (AABBDD; 6, 24, 50, 46, 10, 18, 22 and 56) using SSR primer DMS-125. Arrows indicate diagnostic bands which are absent in durum parents but present in SH number 56.

applicable, highly informative (Plaschke et al., 1995), robust and reproducible. They are helpful in studies comparing the closely related genotypes (Guadagnuolo et al., 2001). Genetic diversity of wheat cultivars helps in identifying different parental combinations that are needed for promoting selection (Franco et al., 2001). In conventional wheat breeding, breeders make crosses between different varieties. These crosses have few limitations and most of the improvement is based on genetic recombination and selection. Also, utilization of landraces for introducing genetic diversity has been successful in wheat breeding. For further improvement of the present cultivars for resistance against biotic and abiotic stresses, alternative genetic resources possessing diversity are required. These valuable genetic resources for wheat improvement are found among different species in the *Triticeae* tribe and reside within the three gene pools of this family.

Total genomic DNA from synthetic lines # 2, 18, 22, 56, 82, 85, 54 and 92 did not amplify using DMS-34 and hence were not included in the analysis. No band was detected in the durum parents D4, D1, D8, D12 and D22. It is inferred that the primer was 4D specific which targeted D genome only, not A and B genome. Since the durum cultivars used are genomically AABB and the donor diploids are DD with different accessions, therefore genetic diversity indicates that using the primer set DMS-34 polymorphism was not prevalent. Polymorphism is essential when molecular mapping is involved. From the detail of the Figures 1, 2, 3, 4 and 5, the conclusion drawn is that genetic diversity among the selected synthetic lines was not impressive. This suggests that more SSRs need to be utilized in order to detect genetic diversity. Some diagnostic bands which were observed in SH lines but could not observe in their durum parents suggest that these bands are donated by the D genome of *A. tauschii*. On the other hand, the bands that are present in durum parents but not in some SH lines suggests that these bands are either missing or replaced by the D genome of *A. tauschii.* The reason for using a subset of 24 SH lines was that all these lines have shown salinity tolerance in the earlier studies (Gorham, 1990; Colmer et al., 2006).

## CONCLUSION AND RECOMMENDATIONS

Microsatellites are suited in studying the genetic diversity in synthetic hexaploid wheat as compared to RAPDs and other markers, as the selected SH wheat of this study are

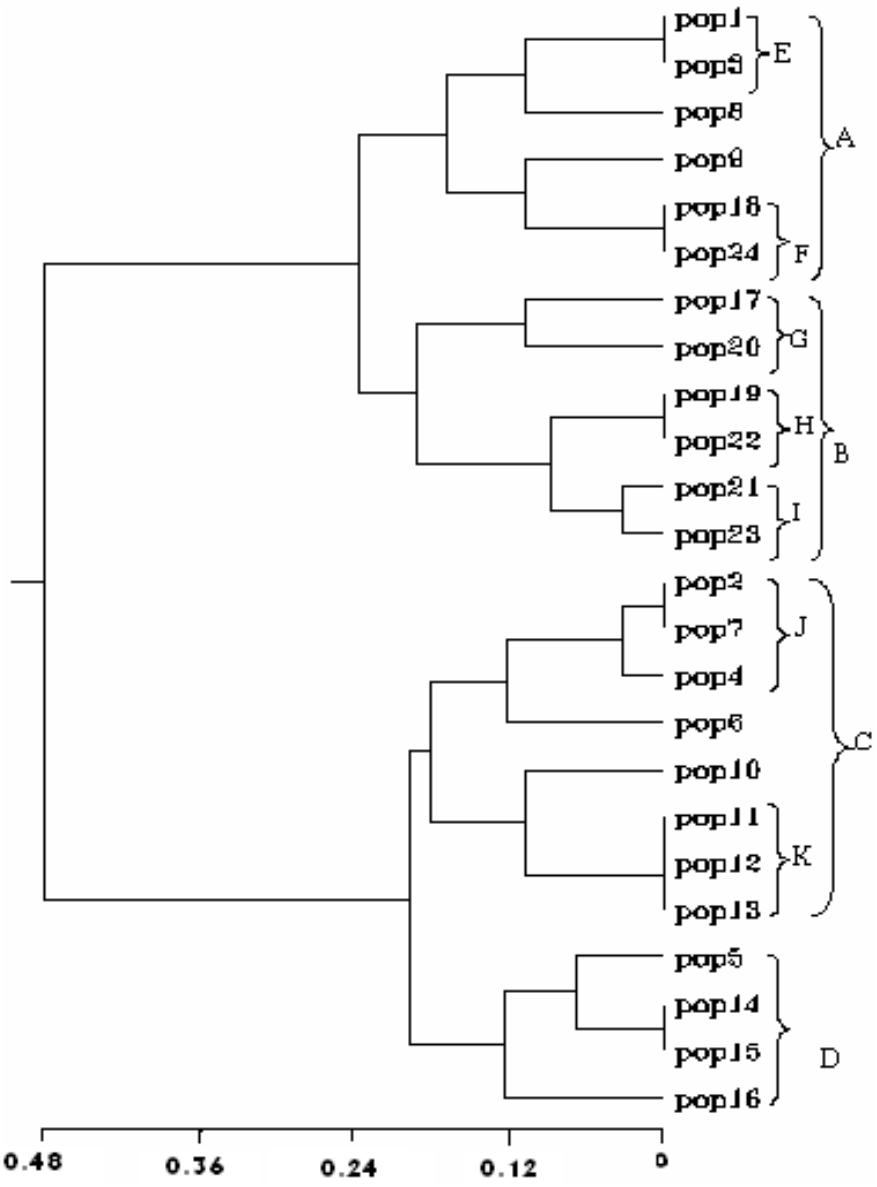


**Figure 6.** Dendrogram of 24 synthetic lines (elite 1) constructed using data of three SSR primer set. Pop 1, elite 1 # 7; Pop 2, elite 1 #16; Pop 3, elite 1 # 17; Pop 4, elite 1 # 25; Pop 5, elite 1 # 2; Pop 6, elite 1 # 79; Pop 7, elite 1 # 80; Pop 8, elite 1 # 83; Pop 9, elite 1 # 6; Pop 10, elite 1 # 24; Pop 11, elite 1 # 50; Pop 12, elite 1 # 46; Pop 13, elite 1 # 10; Pop 14, elite 1 # 18; Pop 15, elite 1 # 22; Pop 16, elite 1 # 56; Pop 17, elite 1 # 29; Pop 18, elite 1 # 81; Pop 19, elite 1 # 82; Pop 20, elite 1 # 35; Pop 21, elite 1 # 85; Pop 22, elite 1 # 54; Pop 23, elite 1 # 92 and Pop 24, elite 1 # 94.

all positive for K:Na discrimination and this characteristics can be observed on chromosome 4DL which is associated with salinity tolerance (Mujeeb-Kazi, 2003b; Shah et al., 1987). The microsatellites used showed polymorphism in some SH lines but a large set of these markers should be employed to get better knowledge about genetic diversity and their subsequent use in bread wheat improvement. As most of the genes responsible for high yield and resistance against various biotic and abiotic stresses are derived from the D genome of *Aegilops tauschii,* it would be sensible to focus on 4D specific microsatellites markers to get useful information about genetic variability in SH lines which may provide strong basis for bread wheat improvement.

## REFERENCES

Colmer TD, Flowers TJ, Munns R (2006). Use of wild relatives to improve salt tolerance in wheat. J. Exp. Bot. 57(5):1059-1078.
Cox D, Condeelis J, Wessels D, Soll D, Kern H, Knecht DA (1992). J.

Targeted disruption of the ABP-120 gene leads to cells with altered motility. J. Cell Biol. 116:943-955.
Franco J, Crossa J, Ribaut JM, Betran J, Warburton ML, Khairallah M (2001). A method for combining molecular markers and phenotypic attributes for classifying plant genotypes. Theor. Appl. Genet. 103: 944-952.
Gill BS, Gnocchi M, Perenzin, Heun M (1986). Chromosome banding methods, standard chromosome band nomenclature, and applications in cytogenetic analysis. In: Heyne, EG (Ed.) Wheat and wheat Improvement, 2nd ed., Agronomy Monograph No. 13. American Society of Agronomy, Madison, WI, pp. 243-254.
Gorham J (1990). Salt Tolerance in the Triticeae: K/Na Discrimination in Synthetic Hexaploid Wheats. J. Exp. Bot. 41(5):623-627.
Guadagnuolo R, Savova BD, Felber F (2001). Gene flow from wheat (*Triticum aestivum* L.) to jointed goatgrass (*Aegilops cylindrica* Host.), as revealed by RAPD and microsatellite markers. Theor. Appl. Genet. 103:1-8.
Huang XQA, Borner MS, Roder, Ganal MW (2002). Assessing genetic diversity of Wheat (*Triticum aestivum L*.) germplasm using microsattelite markers. Theor. Appl. Genet. 105:699-707.
Kerber ER, Dyck EL (1979). Resistance to stem and leaf rust of wheat in Ae. squarrosa and transfer of a gene for stem rust resistance to hexaploid wheat. Proc. 5th. Intern. Wheat Genet. Symp., New Delhi, India. pp. 358-364.
Lage J, Skovmand B, Anderon SB (2003). Expression and suppression of resistance to greenbug in synthetic hexaploid wheats derived from *Triticum dicoccum* x *Aegilops tauschii* crosses. J. Econ. Entomol. 96:202-206.
Ma H, Hughes GR (1995). Genetic control and chromosomal location of *Triticum timopheevi* derived resistance to *Septoria nodorum* blotch in durum wheat. Genome 38:332-338.
May CE, Lagudah ES (1992). Inheritance in hexaploid wheat of septoria tritici blotch resistance and other characteristics derived from *Triticum tauschii* Aus. J. Agric. Res. 43:433-442.
Mujeeb KA (1998). Evolutionary relationships and gene transfer in the *Triticeae*. In A. A. Jaradat, ed. Proc. 3rd Int. *Triticeae* Symp., ICARDA, Aleppo, Syria, pp. 59-65. Science Publishers.
Mujeeb KA (2003b). Wheat improvement facilitated by novel genetic diversity and *in vitro* technology: a review. Plant Tissue Cult. 13(2):179-210.
Mujeeb KA, Roldan S, Suh DY, Ter KN, Farooq S (1989). Production and cytogenetics of *Triticum aestivum* L. hybrids with some rhizomatous species. Theor. Appl. Genet. 77:162-168.
Naghavi MR, Aghaei MJ, Taleei AR, Hassani ME (2008). Genetic diversity of the D-genome in *T. aestivum* and *Aegilops* species using SSR markers. Genet. Resour. Crop Evol. 56(4):499-506.
Nei N, Li W (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Nat. Acad. Sci. 76:5269-5273.
Pestsova E, Ganal GMW, Roder MS (2000). Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome 43:689-697.
Plaschke J, Ganal MW, Roder MS (1995). Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor. Appl. Genet. 91:1001-1007.
Shah S, Gorham J, Forster B, Wyn JRG (1987). Salt tolerance in the *Triticeae*: the contribution of the D genome to cation selectivity in hexaploid wheat. J. Exp. Bot. 38:254-269.
Villareal RL, Mujeeb KA, Fuentes DG, Rajaram S, Toro ED (1994a). Resistance to Karnal bunt in synthetic hexaploid wheats derived from *Triticum turgidum* × *T. tauschii*. Plant Breed 112:63-69.