REGULAR ARTICLE

Phenotypic and genotypic characterization of wheat landraces of Pakistan

Rabia Amir[1], Nasir M. Minhas[1], Alvina Gul Kazi[2]*, Sumaira Farrakh[3], Ahmad Ali[4], Hadi Bux[5] and Mujeeb Kazi[6]

[1]*Department of Plant Breeding and Genetics, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan*
[2]*Atta-ur-Rahman School of Applied Biosciences (ASAB), National University of Sciences and Technology (NUST), H-12, Islamabad, Pakistan*
[3]*Department of Biosciences, COMSATS Institute of Information Technology (CIIT), Islamabad Pakistan*
[4]*Center for Plant Sciences and Biodiversity, University of Swat, Swat, Pakistan*
[5]*Institute of Plant Sciences, University of Sindh Jamshoro 76080, Pakistan*
[6]*National Institute of Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan*

Abstract

Wheat landraces represent a large reservoir of genetic variation of various traits. In this study 28 entries from a collection of 40 maintained in AARI, Faisalabad initially collected from northern Pakistan (Khyber Pakhtunkhwa and Baluchistan) were evaluated for their genetic diversity using microsatellite (SSR) primers. The 40 entries comprised of landraces and earlier local cultivars (C numbers) with all possessing a spring growth habit. Major phonological and biotic stress passport data is on record. The morphological examination of these entries showed that those designated as T2, T3 (*Triticum durum*), T7 (*T. sphaerococcum*), T18 (*T. aestivum*) C-217 (C-516xC-591) and C-258 were agronomically elite as to plant habit. SSR primers amplified total 122 bands out of which 83 were polymorphic. The percentage of polymorphism was 68%. XGWM-337 and XGWM-194 were found to be highly polymorphic. T7, T12 (*T. aestivum*) and C-258 were found to be genetically most diverse landraces using the SSR markers. The polymorphism indicator and phenology profile are the basis for selecting from these germplasm for adding diversity to wheat breeding programs nationally.

Key words: *Triticum aestivum*, Landraces, Simple Sequence Repeats, Genetic diversity, Phenology

Introduction

Wheat (*Triticum* spp) is cultivated all over the world and the most important domesticated grass. In Pakistan, wheat is the major staple food of the people where 9.046 million hectares are covered by wheat having an average annual production equivalent to 24.032 million tons as documented by Hussain et al, (2011); which encompasses approximately 34% of the cultivated area of the country. The yield levels annually fluctuate due to outputs from the rainfed area with the 2012 May harvest touching 25mt at 2.6 tons/hectare. With the continuous increase in population, there is an ever-increasing demand for higher yield. A lot of

research is in place on bread wheat to maximize grain production per unit area. Most of the current cultivars in wheat do not exhibit a lot of genetic diversity rendering it vulnerable to various biotic stresses. There is, therefore, a great need to utilize sources of new diversity in breeding.

To tap on new diversity we have over the last 3 to 4 decades seen researchers exploit various Triticeae genera/species/accessions to widen the wheat genetic base via allelic enrichment using intra-specific, interspecific and intergeneric protocols (Mujeeb-Kazi et al., 2008; Mujeeb-Kazi et al., 2013; Ogbonnaya et al., 2013). There is general agreement that members residing in the primary gene pool occupy priority usage position since allelic transfers are based on homology and are swift. Within this framework access to diverse genomic variation is ample which imparts a solid genetic base to develop varieties that possess disease resistance durability options and a conduit to sustainable agriculture. Additive to the diversity framework are resources of the old varieties (pre-

dwarfing types) and landraces extremely under-utilized but of huge significance value.

Since these landraces have developed through both natural and artificial selection (Belay et al., 1995), thus have a broader genetic base and can provide valuable contribution to breeding (Keller et al., 1991; Tesemma et al., 1998). These are proved to be a good source of tolerance to local stresses (Li et al., 1997) hence increase in yield (Tesemma et al., 1998).

There is always required a solid foundation about the level of genetic diversity available in crop germplasm for a good breeding program. The variation in agronomic, morphological and physiological traits show inaccurate genetic diversity due to environmental factor and polygenic traits. In addition, field evaluations are always tedious and labor-intensive.

The present study has addressed the phenotypic and genotypic characterization of 28 wheat landraces selected on seed abundance from a collection of 40 entries maintained in AARI, Faisalabad that were collected from the northern Pakistan areas of Khyber Pakhtunkhwa and Baluchistan and passport data generated in 2004-2005. The genotypic characterization will contribute in the parental selection in national breeding programs assisting the release of wheat varieties with better agronomic traits.

Material and Methods

This study was conducted on selected wheat landraces of Pakistan (Table 1).

Phenotypic variation

All landraces were grown in the field of National Agricultural Research Center (NARC), Islamabad. The data was taken for three plants from each accession and arithmetic means were calculated. The data was recorded for Pubescence, Plant height, Awn color, Physiological maturity, grain weight, number of grains per spike and spike length.

Genotypic analysis

Genomic DNA of 28 wheat land races of wheat was extracted following the method of Weining and Langridge (1991). DNA quantification was done through spectrophotometer.

SSR analysis

A total of 56 SSR primer pairs were utilized for DNA amplification using Polymerase Chain Reaction (PCR). Each PCR reaction consisted of 25-µl reaction mixture (11.3 µl d3 water, 2.5 µl 10X buffer, 2 µl $MgCl_2$, 2 µl dNTPs, 0.2 µl Taq polymerase, 1 µl of each primer of a primer pair, 5

µl DNA). The samples were incubated at 94°C for 4 min before 45 cycles (94°C for 1 min, primer annealing at 58-60°C for 1 min, extension at 72°C for 1 min). The final extension was done at 72°C for 10 min. The electrophoresis was done on 3% agarose gels with 7 µl of ethidium bromide at 80 V for 1 h to observe under UV transilluminator (Roder et al., 1998).

Table 1. Pedigree list of selected Landraces of Pakistan.

S. No.	Parentage/pedigree Sample detail	Data Information
1	T_1 (*Triticum durum*; 2n=4x=28)	AARI-T_1
2	T_2 (*T. durum*; 2n=4x=28)	AARI-T_2
3	T_3 (*T. durum*; 2n=4x=28)	AARI-T_3
4	T_7 (*T. sphaerococcum*)	AARI-T_7
5	T_8 (*T. aestivum*; 2n=6x=42*)	AARI-T_8
6	T_9 (*T. aestivum*; 2n=6x=42*)	AARI-T_9
7	T_{12} (*T. aestivum*; 2n=6x=42*)	AARI-T_{12}
8	T_{14} (*T. aestivum*; 2n=6x=42*)	AARI-T_{14}
9	T_{15} (*T. aestivum*; 2n=6x=42*)	AARI-T_{15}
10	T_{16} (*T. aestivum*; 2n=6x=42*)	AARI-T_{16}
11	T_{17} (*T. aestivum*; 2n=6x=42*)	AARI-T_{17}
12	T_{18} (*T. aestivum*; 2n=6x=42*)	AARI-T_{18}
13	T_{20} (*T. aestivum*; 2n=6x=42*)	AARI-T_{20}
14	T_{24} (*T. aestivum*; 2n=6x=42*)	AARI-T_{24}
15	8A (selection) (*T. aestivum*; 2n=6x=42*)	AARI-8A
16	D-9 (Barani selection) (*T. aestivum*; 2n=6x=42*)	AARI-D9
17	C-217 (C-516 × C-591) (*T. aestivum*; 2n=6x=42*)	AARI-C217
18	C-228 (hard federation x 9D) (*T. aestivum*; 2n=6x=42*)	AARI-C228
19	C-245 (*T. aestivum*; 2n=6x=42*)	AARI-C245
20	C-247(*T. aestivum*; 2n=6x=42*)	AARI-C247
21	C-248 (*T. aestivum*; 2n=6x=42*)	AARI-C248
22	C-250 (*T. aestivum*; 2n=6x=42*)	AARI-C250
23	C-256 (*T. aestivum*; 2n=6x=42*)	AARI-C256
24	C-258 (*T. aestivum*; 2n=6x=42*)	AARI-C258
25	C-269 (*T. aestivum*; 2n=6x=42*)	AARI-C269
26	C-271 (C-220 x IP165) (*T. aestivum*; 2n=6x=42*)	AARI-C271
27	C-288 (*T. aestivum*; 2n=6x=42*)	AARI-C288
28	C-518 (T9 x 8A) (*T. aestivum*; 2n=6x=42*)	AARI-C518

Source: Dr. Aziz-ur-Rehman, AARI, Faisalabad (2004-2005).

Statistical analysis

The presence and absence of bands was scored as 1 and 0 respectively for cluster analysis of 28 genotypes using the Pop Gen software version 1.32 (Yeh et al., 2000) to calculate genetic diversity and similarity among genotypes.

Results

Phenotypic observations

The morphological data of 28 wheat landraces of Pakistan is summarized in Table 2.

SSR analysis

For the SSR analysis, total 15 chromosome specific primers were used. These primers amplified a total of 122 scorable bands ranging from 50bp to 500bp. Out of 122 scorable bands, 83 were polymorphic. SSR markers detected 68% of polymorphism among the landraces (Figure 1). The highest number of allele was detected by XGWM-609 whereas XGWM 337 and XGWM-194 detected the highest level of polymorphism. The coefficient of similarities ranged from 40% to 95%. The lowest genetic similarity was found between T7 (*T. sphaerococcum*) with C-288 and T15 (*T. aestivum*) with C-288. The dendrogram analysis showed three main clusters. Cluster I included three genotypes with the genetic distance ranging from 0.08 (T1 and T2) to 0.32 (C-217). A total of 32 genotypes were included in cluster II. The genetic distance ranged from 0.05 (C-269, T18, T20) to 0.44 (T7). The cluster III included two genotypes. The genetic distance ranged from 0.64 (C-258) to 0.89 (T12).

Table 2. Phenotypic evaluation of 28 wheat landraces.

S. No.	Genotype	GC	PUB	FLOW	HT	AWN	P.MA	GWT	Nodes/ Spike	GR/Spike	Spikelng
1	T1	A1	-ive	130	106	B	145	32	12	47	8
2	T2	A1	-ive	130	112	W	144	31	12	36	8.7
3	T3	A1	-ive	129	108	LB	142	30	12	25	10.2
4	T7	A2	-ive	129	94	-	137	21	10	42	7.3
5	T8	A2	-ive	128	106	DB	137	30	8	34	8.2
6	T9	A2	-ive	127	104	DB	137	24	8	49	9.3
7	T12	A2	-ive	126	103	DB	145	25	7	79	9.6
8	T14	R2	-ive	123	99	LB	144	24	10	58	11
9	T15	R2	-ive	122	98	LB	145	26	10	34	11
10	T16	A2	-ive	128	104	LB	133	23	8	51	9.7
11	T17	A2	-ive	121	104	-	137	20	11	40	12.3
12	T18	A2	-ive	122	111	-	137	23	9	47	9.4
13	T20	A2	-ive	127	100	-	137	34	10	70	11.8
14	T24	A2	-ive	126	99	-	137	28	9	52	8.5
15	8A	A2	-ive	121	90	DB	144	29	11	31	8.8
16	D-9	A1	-ive	122	105	LB	140	29	9	47	10.2
17	C-217	A2	-ive	125	106	B	140	36	8	48	8.7
18	C-228	A2	-ive	119	111	B	144	31	9	45	9.8
19	C-245	A1	-ive	120	108	LB	144	30	9	45	8.7
20	C-247	A2	-ive	119	114	DB	144	30	10	55	8
21	C-248	A2	-ive	126	104	B	144	27	11	40	9.8
22	C-250	A2	-ive	128	102	LB	145	30	8	26	12
23	C-256	A2	-ive	124	100	LB	133	21	8	43	9.3
24	C-258	A2	-ive	125	85	-	133	37	7	29	8.8
25	C-269	A3	-ive	124	94	-	137	32	10	51	10
26	C-271	A2	-ive	119	113	B	137	41	10	55	10.4
27	C-288	A1	-ive	126	101	LB	137	33	10	46	7.5
28	C-518	A2	-ive	119	103	B	140	28	8	43	7.7

GC: Grain quality (A-Amber color; R-Red color; 1-Bold grain; 2-Medium grain; 3-Small, shrivelled grain); PUB: Pubescence; FLOW: Days to flowering; HT: Plant height at maturity; AWN: Awn color; P. MA: Days to physiological maturity; GWT: 1000 grain weight; NODES/SPIKE: Number of nodes per spike; GR/SPIKE: Number of grains per spike; SPIKELNG: Spike length.
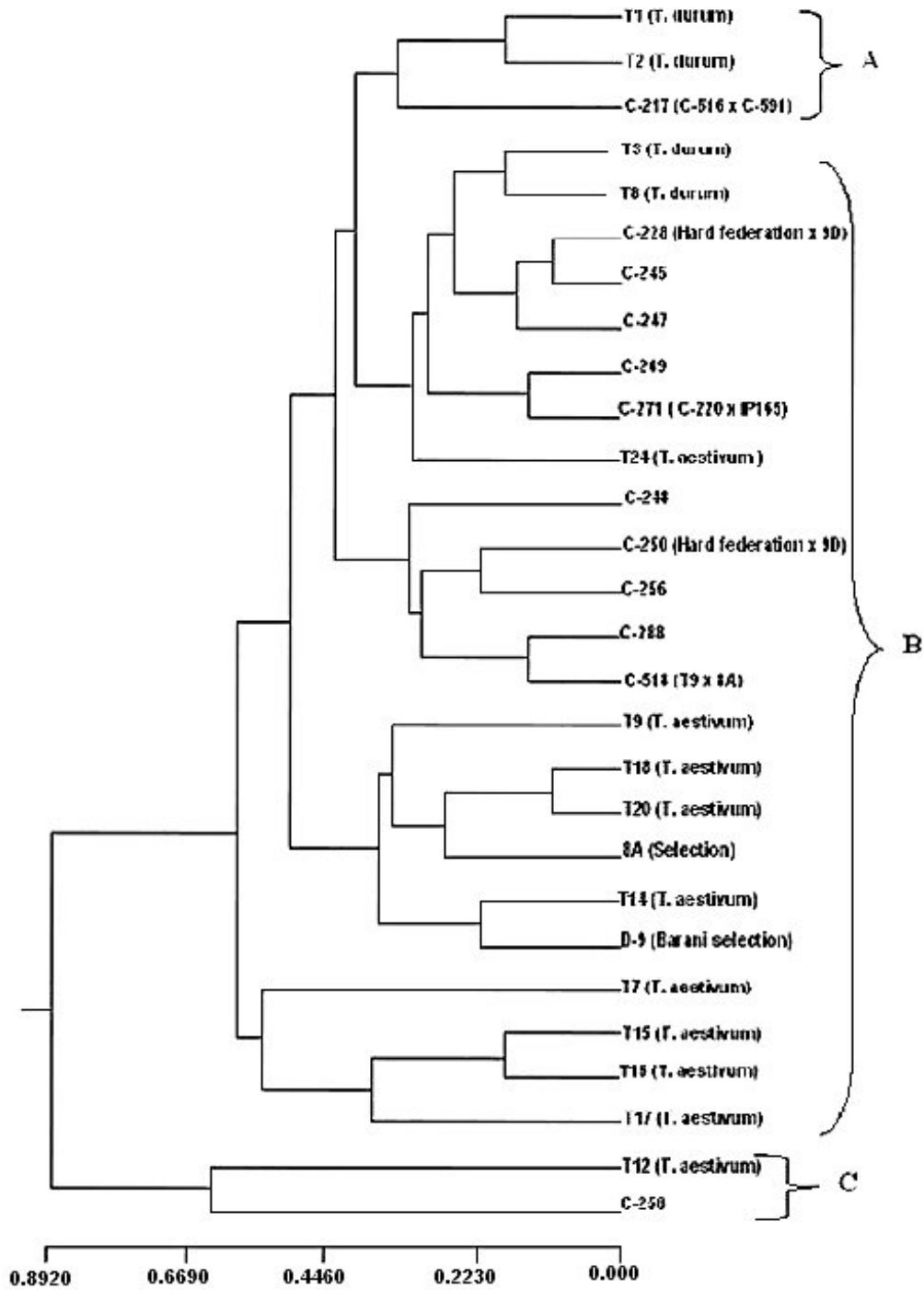
Figure 1. Cluster analysis of 28 wheat landraces using SSR primers.

Discussion

The landraces that have been cultivated around the world are especially important as a genetic resource because they have evolved adaptations to various environmental conditions. These landraces have acquired this adaptation to diverse natural conditions due to natural selection and farmers' selection. They provide a good source of genetic diversity to various global breeding programs and are considered the most important genetic resource. Landraces have attracted the scientific community

due to their genetic variability in well-adapted backgrounds (Fathi et al., 2011). The study of genetic diversity provides important information about their breeding potential. Also, heterogeneity of wheat landraces is too complicated to analyze systematically (Nevo and Payne, 1987). For transgressive segregation, genetically diverse parents are mandatory (Joshi et al., 2004). Although, thousands of landraces are preserved in various seed banks of the world, the majority of these are insufficiently evaluated for exploitation in wheat breeding. The biochemical and molecular characterization have become a prerequisite for the modern seed industry. The evaluation of genetic diversity using molecular markers is an important tool in the effective management of genetic resources (Virk et al., 1995; Ford-Lloyd et al., 1997). SSR or microsatellites provide an efficient, rapid and reliable method for quality control in seed certification programs to identify the sources of seed contamination in order to maintain pure germplasm collection. SSRs are reproducible, co-dominant in inheritance hence multiallelic and comparatively abundant due to extensive genomic coverage (Plaschke et al., 1995; Fu et al., 2005; Gupta and Varshney, 2000; Achtar et al., 2012).

This study was focused on the evaluation of genetic diversity of 28 genotypes of landraces using 15 SSR primers. The SSR primers generated a total of 122 alleles. The primer pair XGWM608 amplified maximum 8 bands whereas the minimum of 2 bands were amplified by the primer pair XGWM550. The highest level of polymorphism was detected by XGWM-337 and XGWM-194. This study confirmed the ability of microsatellite loci to reveal allelic diversity as already reported (Ravi et al., 2003; Ram et al., 2007). In this study the genotypes T2 (*T. durum*), T3 (*T. durum*), T4 (*T. sphaeorococcum*), T18 (*T. aestivum*), C.217 (C-516 x C-519) and C-258 were the most diverse genotypes. These genotypes showed very good morphological characters as well.

Current observations exhibit a wide array of phonological and molecular polymorphism diversity. Both these variables provide selective sieves to target entries in pre-breeding programs. Height variations are indices of selections for irrigated and rainfed agriculture. Yield components (grains/spike, 1000 kernel weight) are integrated crucially with yield maximization targets and days to physiological maturity vital to combat climate change. We see variability for all aspects in the germplasm studied that gives us optimism to harness this novel diversity in a volatile manner.

Further it is fortuitous that another 700 landraces have been reported from the NARC genebank that will add further value to their exploitation after their stringent phenotyping together with genotyping assays based on GoldenGate (Chao et al., 2011), BeadExpress (Trebbi et al., 2011), Infinium platforms (Cavanagh et al., 2013) and 90K iSelect (Akhunov et al., 2013), or direct sequencing of populations through genotype-by-sequencing (GBS) approach (Davey et al., 2011; Poland et al., 2012).

Local genotypes provide a great source of alleles as multi locus combinations are suitable for different environment of each country (Allard, 1996). When diverse lines are utilized in breeding programs, there is more chance of transgressive segregation due to reshuffling of alleles by recombination resulting in high yielding genotypes (Sofalian et al., 2008). In addition, this high genetic variation can be used for effective gene tagging and genome mapping to pyramid genes for high yield, disease and insect resistance (Talebi et al., 2010).

Conclusion

The genetic structure of wheat land races is an evolutionary approach for survival and performance where combined effects of natural and human selection have orchestrated various suitable combinations of traits. This genetic diversity acts as a buffer against the outbreak of epidemics by delaying the formulation of new pathotypes. The development of new wheat varieties from land races is a practical strategy to improve the performance of crop in the farmers' field. These land races are the source of success for global breeding programs. This global effort to assemble and characterize these genetic resources is paramount for the world's fight against hunger.

References

Achtar, S., M. Y. Moualla, A. Kalhout, M. S. Röder and N. M. Ali. 2012. Assessment of Genetic Diversity among Syrian durum (*Triticum* ssp. durum) and Bread Wheat (*Triticum aestivum* L.) using SSR markers. Russ. J. Gen. 46:1320–1326.

Akhunov, E., S. Sehgal, H. Liang, S. Wang and A. Akhunova. 2013. Comparative analysis of synthetic genes in grass genomes reveals accelerated rates of gene structure and coding sequence evolution in polyploid wheat. Plant Physiol. 161:252-265.

Allard, R. W. 1996. Genetic basis of evolution of adaptedness in plants. Euphytica 92:1–11.

Aziz-ur-Rehman, AARI, Faisalabad (2004-2005, Personal communication.

Belay, G., T. Tesemma, E. Bechere and D. Mitiku. 1995. Natural and human selection for purple-grain tetraploid wheats in the ethiopian highlands. Gen. Res. Crop Evo. 42:387–391.

Cavanagh, C., S. Chao, S. Wang, B. E. Huang and S. Stephen. 2013. Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. Proc. Natl. Acad. Sci., USA (IN PRESS).

Chao, S., J. Dubcovsky, J. Dvorak, M. C. Luo and S. P. Baenziger. 2010. Population- and genome-specific patterns of linkage disequilibrium and SNP variation in spring and winter wheat (*Triticum aestivum* L.). BMC Genom. 11:727.

Davey, J. W., P. A. Hohenlohe, P. D. Etter, J. Q. Boone and J. M. Catchen. 2011. Genome wide genetic marker discovery and genotyping using next-generation sequencing. Nat. Rev. Genet. 12:499-510.

Fathi, R., T. Reza and F. Farzad. 2011. Characterization of Iranian landrace wheat accessions by inter simple sequence repeat (ISSR) markers. J. Appl. Environ. Biol. Sci. 1(10):432-436.

Ford-Lloyd, B. V., M. T. Jackson, H. J. Newbury. 1997. Molecular markers and the management of genetic resources in seed genebanks: A case study of rice. In: J. A.Callow (Ed.) pp. 103-118. CAB International, Wallingford Biotechnology and plant genetic resources - conservation and use, Ford-Lloyd BV and Newbury HJ.

Fu, Y. B., G. W. Peterson, K. W. Richards and D. Somers. 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, P. K. and R. K. Varshnei. 2000. The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. Euphytica 113:163-185.

Hussain, M., N. Ahmad, F. Mohammad , M. A. Khan and S. Ajmal. 2011. Wheat breeding for high yield potential and durable resistance against yellow rust. Pak. J. Phytopathol. 23:56-61.

Joshi, B. K., A. Mudwari, M. R. Bhattaand and G. O. Ferrara. 2004. Genetic diversity in Nepalese wheat cultivars based on agromorphological traits and coefficients of parentage. Nep. Agri. Res. J. 5:7-17.

Keller, L., J. E. Schmid and E. R. Keller. 1991. Are cereal landraces a source for breeding? Landwirtschaft Schweiz 4:197–202.

Li, S., F. Sun, B. Guo, L. Liu and C. Pang. 1997. Evaluation of abiotic stress resistance in Hebei winter wheat genetic resources. Wheat Inform. Serv. 85:1–6.

Mujeeb-Kazi, A., A. Gul, M. Farooq, S. Rizwan and I. Ahmad. 2008. Rebirth of synthetic hexaploids with global implications for wheat improvement. Aust. J. Agric. Res. 59:391-398.

Mujeeb-Kazi, A., A. G. Kazi, I. Dundas, A. Rasheed, P. Chen, M. Kishi, D. Bonnett, R. R. C. Wang, H. Bux and S. Farrukh. 2013. Genetic diversity for wheat improvement as a conduit to food security. In: Donald Sparks Eds. Advances in Agronomy (IN PRESS).

Nevo, E. and P. I. Payne. 1987. Wheat storage proteins: diversity of HMW glutenin subunits in wild emmer from Israel. Theor. Appl. Gen. 74:827-836.

Ogbonnaya, F., A. Mujeeb-Kazi, A. G. Kazi, E. L. Lagudah, S. S. Xu and D. Bonnett. 2013. Synthetic hexaploid in wheat improvement. In: Jules Janick (Ed.). pp. 35-122. Plant Breeding Reviews. John Wiley & Sons Inc.

Plaschke, J., M. W. Ganal and M. S. Röder. 1995. Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor. Appl. Genet. 91:1001-1007.

Poland, J. A., P. J. Brown, M. E. Sorrells and J. L. Jannink. 2012. Development of high density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. PLoS One 7:e32253.

Ram, S. G., V. Thiruvengadam and K. K. Vinod. 2007. Genetic diversity among cultivars, landraces and wild relatives of rice as revealed by microsatellite markers. J. Appl. Genet. 48:337-345.

Ravi, M., S. Geethanjali, F. Sameeyafarheen and M. Maheswaran. 2003. Molecular marker

based genetic diversity analysis in rice (*Oryza sativa* L.) using RAPD and SSR markers. Euphy. 133:243-252.

Roder, M. S., V. Korzun, K. Wendehake and J. Plaschke. 1998. A microsatellite map of wheat. Genet. 149:2007-2023.

Sofalian, M., N. Chaparzadeh, A. Javanmard and M. S. Hejazi. 2008. Study the genetic diversity of wheat landraces from northwest of Iran based on ISSR molecular markers. Int. J. Agri. and Biol. 10:466-468.

Talebi, R., A. Haghnazari and I. Tabatabai. 2010. Assessment of genetic variation within international collection of *Brassica rapa* genotypes using inter simple sequence repeat DNA markers. Biharean Biologist. 4(2):145-151.

Tesemma, T., S. Tsegaye, G. Belay, E. Bechere and D. Mitiku. 1998 Stability of performance of tetraploid wheat landraces in the Ethiopian highland. Euphy. 102:301–308.

Trebbi, D., M. Maccaferri, P. de Heer, A. Sorensen and S. Giuliani. 2011. High-throughput SNP discovery and genotyping in durum wheat (*Triticum durum* Desf.). Theor. Appl. Genet. 123:555-569.

Virk, P. S., B. V. Ford-Lloyd, M. T. Jackson and H. J. Newbury. 1995. Use of RAPD for the study of diversity within plant germplasm collections. Hered. 74:170-179.

Weining, S. and P. Langridge. 1991. Identification and mapping in cereals based on the polymerase chain reaction in Brassica. Theor. Appl. Genet. 82:632-727.

Yeh, F. C., R. C. Yang and T. B. J. Boyle. 2000. PopGene32, microsoft windows-based freeware for population genetic analysis. Version 1.32. Molecular biology and biotechnology centre, University of Alberta, Edmonton.