NARDI FUNDULEA, ROMANIA
www.incda-fundulea.ro

ROMANIAN AGRICULTURAL RESEARCH, NO. 32, 2015
Print ISSN 1222-4227; Online ISSN 2067-5720

GENETIC DIVERSITY AND ITS TEMPORAL CHANGES IN IMPROVED BREAD WHEAT CULTIVARS OF MOROCCO

Fatima Henkrar[1,2,3,4], Jamal El-Haddoury[3], Hassan Ouabbou[3], Nasserlehaq Nsarellah[3], Driss Iraqi[2*], Najib Bendaou[4], Sripada Mahabala Udupa[1*]

[1]ICARDA-INRA Cooperative Research Project, International Center for Agricultural Research in the Dry Areas (ICARDA), B.P. 6299, Rabat, Morocco

[2]Institut National de la Recherche Agronomique (INRA), B.P. 415, Rabat, Morocco

[3]Institut National de la Recherche Agronomique (INRA), B.P. 589, Settat, Morocco

[4]Laboratoire de Physiologie et Biotechnologie Végétale, Faculté des Sciences, B.P. 1014, Université Mohammed V - Agdal, Rabat, Morocco

*Corresponding author. E-mail: s.udupa@cgiar.org

ABSTRACT

Genetic diversity in a set of 20 improved bread wheat cultivars released in Morocco since 1980s, along with 8 exotic bread wheat cultivars, were characterized by employing 14 polymorphic microsatellite markers. A total of 59 alleles (Mean=4.21) in Moroccan cultivars and 53 alleles (Mean=3.78) in exotic cultivars were detected. Genetic diversity at 14 microsatellite loci varied from 0 to 0.895 (Mean=0.576) for 20 Moroccan cultivars and from 0.25 to 0.928 (Mean=0.683) for the exotic cultivars. The genetic distance among the cultivars ranged from 0.143 to 1.00. Using the 14 microsatellite markers, all the bread wheat cultivars could be distinguished for reliable identification, characterization, and diversity analysis. Total number of alleles decreased in the cultivars developed during 1990s (8.3%) compared with that of 1980s. The AMOVA results suggest that change in the cultivar genetic diversity among different decadal groups of bread wheat was very small and non-significant, indicating no significant reduction in over all genetic diversity due to breeding in recent times.

Key words: Bread wheat cultivar characterization, temporal genetic diversity, Microsatellites.

INTRODUCTION

Genetic diversity is the basis for genetic improvement. It is defined as the amount of genetic variability which is reflected in differences of DNA sequence, biochemical characteristics, physiological properties, or morphological characters among individuals of a variety or a population (Rao and Hodgkin, 2002). For the genetic characterization, knowledge on germplasm diversity and genetic relationships among breeding materials could be an invaluable aid in crop improvement (Mohammadi and Prasanna, 2003) and cultivar deployment strategies. However, due to the modern plant breeding practices, it has been argued that the genetic diversity in wheat varieties has been reduced (Fu and Somers, 2009). If this argument is correct, it would have serious consequences both for the genetic vulnerability of crops and for their plasticity to respond to changes in the production environments. It is therefore vital for wheat breeding programs to maintain sufficient diversity to allow for the production of new cultivars able to withstand attack from new races and pathovars of continuously evolving pathogenic microorganisms (Tripp, 1996) and also deploy diverse cultivars in the farmer's field to maintain the sufficient diversity to slow down evolution of the new aggressive pathovars.

Microsatellite markers have been proven to be important tools in wheat genetics and germplasm conservation (Huang et al., 2002; Roussel et al., 2004). These markers were used for characterizing (fingerprinting) varieties and studying temporal changes in Canadian hard red spring bread wheat cultivars released from 1845 to 2004 and showed a reduction in alleles for 4 microsatellite loci for the cultivars released

Received 27 October 2014; accepted 27 April 2015. First Online: June, 2015. DII 2067-5720 RAR 2015-188

from 1970 onwards (Fu et al., 2005). However, no such information available for Moroccan bread wheat cultivars.

In Morocco, several bread wheat (*Triticum aestivum*) cultivars had been released since 1980s (Jlibene, 2005 and 2009). However, these cultivars have not yet been characterized using molecular markers for identification to protect cultivar-rights, genetic diversity analysis and estimating genetic relationships. It has been opined that the genetic diversity is being reduced over period of time in wheat cultivars due to breeding and release of improved cultivars. In order to enhance genetic diversity, adaptability and productivity of wheat, recently several exotic lines of bread wheat with desirable traits had been introduced and are being used as parents/donors in the wheat breeding program. Comparing the genetic diversity and estimation of genetic relationships of Moroccan cultivars developed over periods with that of exotic cultivars would be useful for selecting suitable cultivars as parents in crossing program and understanding gain or loss of genetic diversity over periods. However, no such studies had been done on so far for the Moroccan cultivars of bread wheat. Here, we report the use of microsatellite markers for cultivar characterization, genetic diversity analysis, genetic distance estimation and to understand temporal changes in genetic diversity and allele richness in Moroccan bread wheat cultivars developed since 1980s.

MATERIAL AND METHODS

Plant materials

20 bread wheat cultivars released for cultivation in Morocco (Jlibene, 2005 and 2009) were procured from the National Gene Bank of Morocco (Table 1). In addition, 8 potential exotic bread wheat cultivars which are important as a donor in the breeding program were also included for the genetic diversity studies (Yecora Rojo-GPC-B1 [USA], Pavon 76 [CIMMYT], Parula [CIMMYT], Opata [CIMMYT], Dharwar dry [India], Stylet [Australia], Annuello [Australia] and Chinese Spring).

Table 1. Improved bread wheat cultivars of Morocco

Cultivar	Registration year	Pedigree
Saïs	1985	Tob's'/1/NP/2/CC/Inia/3/Cha
Arrehane	1997	L222 introduced from USA
Acsad59	1985	Selection from Arab Center for the Studies of Arid Zones and Dry Lands (ACSAD) nursery
Kanz	1987	Pavon's'/4/Pato (R)/1/Cal/3/7C/2/Bb/Cno
Aguilal	1997	Saïs*2/1/KS-85-14-2
Tilila	1989	Veery's'
Achtar	1988	Hork/1/Ymh/2/Kal/1/Bb
Nasma	1982	Moroccan selection
Khair	1988	Maya/2/LR64/1/LR64/3/TZPP/1/Y54/2/23584
Massira	1992	L2266/1/1406,101/2/Buc's'/3/Vpm/1/Mos 83,11,4,8/2/Nac
Mehdia	1993	Kauz'S'
Rajae	1993	Mor's'/1/Mon's'
Amal	1993	Bow's'/1/Buc's'
Baraka	1988	Vent71/2/Cno67's'/1/SC66/3/Kal/1/Bb (=Pavon)
Jouda	1984	Kal/1/Blue Bird
Saba	1987	Nasma/1/PotamPRL/2*PASTOR
Marchouch	1984	Kal/1/Ciano/2/8156^{2}/3/BT908
Potam	1982	Selection from CIMMYT nursery
Saada	1988	Butte//Arthur/Butte
Salama	2004	Introduced from Europe by SONACOS, Morocco

DNA extraction and microsatellites analysis

The young leaves were harvested from the individual cultivars and DNA was extracted using CTAB (cetyltrimethylammonium bromide) method (Saghai-Maroof et al., 1984) with minor modifications (Udupa et al., 1999). Quality and quantity of the isolated DNA were determined on 1.0% (w/v) agarose gels by comparing bands to known concentrations of lambda DNA.

Fourteen polymorphic microsatellites representing the chromosomes 1A, 4A, 7A, 1B, 3B, 6B and 7B were selected (from the initial screening of over 45 microsatellite markers) for genotyping. Sequence information of the microsatellite primers was available from Ward et al. (2003) (*Xbarc263*), Röder et al. (1998) (*Xgwm*), Somer et al. (2004) (*Xwmc*) and Devos et al. (1995) (*Xpsp2999*). The PCR reactions were performed in total volume of 10 µL, containing 1X PCR buffer (1.5 mM $MgCl_2$), 200 µM of each dNTPs, 10 pmoles of each primer, 0.5 U of *Taq* DNA polymerase and approximately 50 ng of genomic DNA. The amplification reaction was performed in the Eppendorf Master cycler with an initial denaturation for 5 minutes at 94°C, followed by 35 cycles of each cycle with 30 seconds denaturation at 94°C, 30 seconds annealing at 59°C, 45 seconds extension at 72°C. Final extension was carried out at 72°C for 5 minutes followed by cooling at 4°C for infinite period. Amplified products were separated on 6% (w/v) denaturing polyacrylamide gels. The amplified bands were detected by silver staining. Size of each band was estimated simultaneously by means of a 100-bp DNA Ladder.

Data analysis

Alleles amplified by microsatellite primers for each cultivar were scored and genetic diversity (*H*) was calculated (Nei, 1987):

$$n/(n-1)(1-\sum \rho^2)$$

where:

n is the number of samples and ρ is the frequency of an allele. Using PowerMarker software (Ver. 3.0; Liu and Muse, 2005), the microsatellite polymorphism data was used to estimate the shared allele genetic distance (Jin and Chakraborty, 1993) between the different cultivars. A dendrogram was constructed based on the genetic distance by using Neighbor-joining (NJ) method (Saitou and Nei, 1987). The Analysis of Molecular Variance Analysis (AMOVA) was performed using software GenAlEx 6 (Peakall and Smouse, 2006)

RESULTS

Microsatellite polymorphism

Survey with 14 microsatellite loci in 20 Moroccan bread wheat cultivars revealed 59 alleles. The number of alleles per locus ranged from 1 for *Xbarc263* to 8 for *Xgwm577* with an average number of 4.21. The microsatellite markers used in this study also showed different levels of genetic diversity. Genetic diversity for 14 microsatellite loci at 20 Moroccan bread wheat cultivars varied from 0 (*Xbarc263*) to 0.895 (*Xgwm577*) with an average of 0.551. These results reveal that the chosen primers were efficient enough to detect diversity in the bread wheat cultivars of Morocco.

The dendrograms showing genetic relationships among the cultivars are presented (Figure 1). Using all the 14 microsatellite markers, we could able to distinguish all the cultivars of Morocco. The genetic distance (data not shown) was lowest between cultivar Aguilal and Saïs (0.143). The genetic distance was highest (1.00) between the Massira and Stylet, Baraka and Stylet, Dharwar dry and Stylet, followed by other Moroccan cultivars (Jouda, Mehdia, Marchouch, Tillila and Nasma) and Stylet (0.929), and between exotic cultivars (Chinese Spring, Yecora Rojo-Gpc-B1 and Opata) and Stylet, indicating that the choice of exotic cultivars for crossing program to improve bread wheat cultivars was good enough to improve genetic diversity and genetic gains in coming years. At 0.30 shared allele genetic distance all the cultivars formed 7 groups (Figure 1); the first group consists of Kanz,

Amal, Saada and Achtar; the second major group consists of Massira, Arrehane, Rajae, Khair, Tilila, Mehdia, Jouda. Marchouch, Baraka and 4 other exotic cultivars; and the third group consist of Acsad59, Potam, Saïs and Aguilal and 3 other exotic cultivars. Other four groups consist of single cultivars each namely, Stylet (exotic cultivar), Nasma, Saba and Salama.

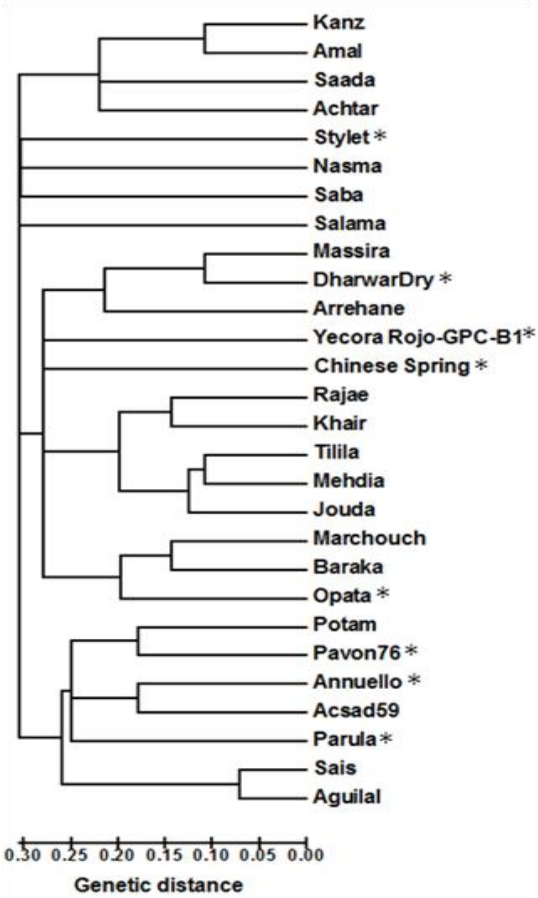

Figure 1. Dendrogram showing relationships among the 20 Moroccan and 8 exotic cultivars (marked with "*") of bread wheat as revealed by Neighbour-joining method based on shared allele genetic distance

Change in genetic diversity due to wheat genetic improvement

In order to understand the temporal genetic diversity among Moroccan wheat cultivars, we grouped the cultivars into decadal groups according to their registration dates, and compared their average number of alleles, unique alleles and genetic diversity of the cultivars developed in each period (Table 2). There were two temporal groups in the bread wheat cultivars (1980s and 1990s). These temporal groups were also compared with the exotic varieties currently used in the breeding programs. Total number of alleles decreased in the cultivars developed during 1990s (8.3%) comparing with that of 1980s. The genetic diversity of the different temporal groups of bread wheat cultivars were also compared (Table 2). The genetic diversity and total alleles of the bread wheat cultivars decreased from 1980s to 1990s, but unique allele increased from 0.38 for 1980s to 0.43 for 1990s.

The genetic distances were calculated for each pair of decadal groups to estimate the extent of their divergence (Table 3). The highest genetic distance (0.383) was found between 1990s and exotic, whereas the lowest (0.263) was observed between the 1980s and 1990s. The dendrogram (Figure 2) resulting from the cluster analysis based on shared allele genetic distance had separated the cultivars of exotic origin, whereas the decadal groups from the 1980s and 1990s grouped together. These exotic cultivars had 12 unique alleles which are not present in the Moroccan cultivars.

Table 3. Genetic distance (shared allele) among the cultivars registers during 1980s, 1990s and the exotic bread wheat cultivar

Groups	Genetic distance		
	1980s	1990s	Exotic cultivars
1980s	0.000	0.263	0.367
1990s		0.000	0.383
Exotic cultivars			0.000

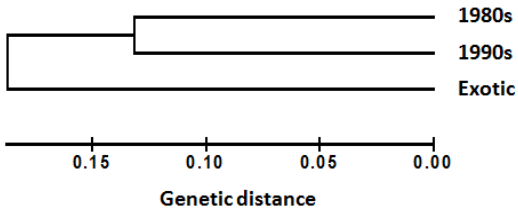

Figure 2. Dendrogram showing relationships among the 2 decadal groups and the exotic cultivars of bread wheat as revealed by Neighbor-joining method based on shared allele genetic distance

The AMOVA showed that the proportion of variance among cultivars within decadal groups was highest (98%), whereas among decadal groups accounted for only 2%. These results suggest that change in the cultivar genetic diversity among different decadal groups of bread wheat was very small and non-significant.

Table 2. Temporal changes in number of alleles, unique alleles and genetic diversity in bread wheat cultivars of Morocco and their comparison to the exotic cultivars

		Moroccan cultivars of 1980s				Moroccan cultivars of 1990s				Moroccan cultivars of 1980s and 1990s			Exotic cultivars			
Locus	Location	Sample size *(n)*	Number of alleles	Number of unique alleles	Genetic diversity *(H)*	Sample size *(n)*	Number of alleles	Number of unique alleles	Genetic diversity *(H)*	Sample size (n)	Number of unique alleles	Genetic diversity *(H)*	Sample size *(n)*	Number of alleles	Number of unique alleles	Genetic diversity *(H)*
Xgwm33	1A	13	4	1	0.526	7	3	1	0.524	20	5	0.505	8	3	0	0.464
Xgwm389	3B	13	3	0	0.602	7	3	0	0.524	20	4	0.553	8	4	1	0.642
Xgwm146	7B	13	4	0	0.602	7	4	1	0.809	20	5	0.679	8	5	1	0.857
Xgwm397	4A	13	2	0	0.282	7	1	0	0	20	2	0.189	8	3	1	0.607
Xgwm136	1A	13	5	1	0.756	7	5	0	0.905	20	6	0.795	8	5	1	0.893
Xbarc263	1A	13	1	0	0	7	1	0	0	20	1	0	8	4	3	0.75
Xgwm130	7A	13	4	1	0.718	7	3	1	0.762	20	5	0.721	8	3	0	0.714
Xwmc89	4A	13	3	0	0.667	7	3	0	0.667	20	3	0.653	8	3	0	0.714
Xwmc420	4A	13	2	0	0.513	7	1	0	0	20	2	0.395	8	2	0	0.25
Xgwm193	6B	13	2	0	0.154	7	2	1	0.286	20	3	0.195	8	3	1	0.464
Xgwm273	1B	13	3	0	0.564	7	2	0	0.571	20	3	0.542	8	4	1	0.643
Xpsp2999	1A	13	5	1	0.756	7	5	0	0.908	20	7	0.805	8	4	0	0.821
Xwmc24	1A	13	4	1	0.769	7	4	1	0.809	20	5	0.789	8	4	1	0.821
Xgwm577	7B	13	6	0	0.885	7	7	1	1	20	8	0.895	8	6	2	0.928
Total			48	5			44	6			59			53	12	
Mean			3.42	0.35	0.557		3.14	0.42	0.554		4.21	0.551		3.78	0.85	0.683
Standard deviation (±)			1.39	0,49	0.252		1.74	0.51	0.357		2.01	0.269		1.05	0.86	0.190

DISCUSSION

Our study identified a set of 14 microsatellites markers for identification, characterization and diversity analysis of 28 bread wheat cultivars. The DNA profiles obtained in the study can be used as 'DNA fingerprints' for reliable identification and more importantly for cultivar-right-protection of the Moroccan cultivars. The microsatellite analysis generated information on cultivars relatedness, which is very useful for the breeding program for identification of suitable cultivars to be used as parents in the crossing programs. The close genetic relationships observed between some of the cultivars have also an agreement with their pedigree information. For instance, the close genetic relationship of bread wheat cultivars Aguilal and Sais observed by microsatellite analysis can be explained to the fact that Saïs in one of the parent of Aguilal (Jlibene, 2005 and 2009). This study is the first report on genetic characterization of improved bread wheat cultivars of Morocco.

In general, average number of alleles detected in this study in Moroccan bread wheat cultivars (4.21) was higher than that of bread wheat cultivars of Egypt (3.2; Salem et al., 2008) and Iran (3.26; Gorji et al., 2011), but less than the cultivars from Libya (4.5; Amer et al., 2001), Europe (6.2; Plaschke et al., 1995) and Canada (8.6; Fu et al., 2005).

According to the registration periods, we detected a decrease in allelic richness in the period 1990s compared with that of the period 1980s for Moroccan bread wheat cultivars. The genetic distance estimates also clearly showed that the cultivars of decadal group 1980s were closely related compared to the

genetic distance estimates between recent decadal group 1990s and exotic cultivars, indicating there is an increase in genetic relatedness between the decadal groups, thereby decreases in genetic diversity. Increased genetic distance between exotic cultivars and the decadal groups indicated good choice of the exotic cultivars deployed currently for genetic improvement in Morocco. These findings clearly demonstrate various natures of the breeding impacts on Moroccan bread wheat cultivars, not only reducing the allelic richness and also changing the genetic relatedness in the released cultivars.

Although there was a decrease in genetic diversity in the recent decadal group compared to 1980s, AMOVA analysis showed that the proportion of variance among cultivars within decadal groups was highest (98%) and a very little non-significant variability among the decadal groups (2%; P>0.06), justifying that substantial genetic variability still exists among the cultivars of Morocco. The observation that no substantial change of diversity was due to the plant breeding in UK (Donini et al., 2000), supports our observation. The fact that most of the cultivars released in recent times in Morocco are derived from ICARDA or CIMMYT international wheat nurseries (Jlibene, 2005 and 2009), resulted in maintaining substantial genetic diversity among the cultivars, thereby there is no immediate genetic threat to current bread wheat breeding in Morocco. However, there is a need to improve further wheat productivity, diversity, quality and abiotic and biotic tolerance to adapt to climate change and emerging pathogens/pests which are posing real problems in Morocco. Exotic wheat cultivars can bring in unique alleles which are not present in the Moroccan cultivars, when used as parents in the breeding program for broadening the genetic base and improve genetic gain and enhance adaptation in the cultivars as a part of long-term breeding efforts.

Acknowledgements

Authors are grateful to the ICARDA/ Morocco Collaborative Grants Program for the support.

CONCLUSIONS

The present study confirmed the usefulness of microsatellite markers as a powerful tool for bread wheat cultivar identification, to protect cultivar-rights, and for characterization and diversity analysis. A decrease in allelic richness at microsatellite loci in the period 1990s comparing with that of the period 1980s for Moroccan bread wheat cultivars, has clearly indicated the need to deploy the exotic cultivars in the breeding program for making crosses and to enhance the genetic base of new cultivars to be developed in future for adaptation to changing environments, abiotic and biotic stresses.

REFERENCES

Amer, I.M.B., Borner, A., Röder, M.S., 2001. *Detection of genetic diversity in Lybian wheat genotypes using wheat microsatellite markers*. Genetic Resources and Crop Evolution, 48: 579-585.

Devos, K.M., Bryan, G.J., Collins, A.J., Stephenson, P., Gale, M.D., 1995. *Application of two microsatellite sequences in wheat storage proteins as molecular markers.* Theoretical and Applied Genetics, 90**:** 247-252.

Donini, P., Law, J.R., Koebner, R.M.D., Reeves, J.C., Cooke, R.J., 2000. *Temporal trends in the diversity of UK wheat*. Theoretical and Applied Genetics, 100: 912-917.

Fu, Y.B., Peterson, G.W., Richards, K.W., Somers, D., De Pauw, R.M., Clarke, J.M., 2005. *Allelic reduction and genetic shift in the Canadian hard red spring wheat germplasm released from 1845 to 2004*. Theoretical and Applied Genetics, 110: 1505-1516.

Fu, Y.B., Somers, D.J., 2009. *Genome-Wide Reduction of Genetic Diversity in Wheat Breeding.* Crop Science, 49**:** 161-168.

Gorji, A.H., Zolnoori, Z**.,** 2011. *Genetic diversity in hexaploïde wheat genotypes using microsatellites markers.* Asian Journal of Biotechnology**,** 3**:** 368-377.

Huang, X.Q., Boerner, A., Roeder, M.S., Ganal, M.W., 2002. *Assessing genetic diversity of wheat (Triticum aestivum L.) germplasm using microsatellite markers*. Theoretical and Applied Genetics, 105: 699-707.

Jin, L., Chakraborty, R., 1993. *Estimation of genetic distance and coefficient of gene diversity from single-probe multilocus DNA fingerprinting Data*. Molecular Biology and Evolution, 11**:** 120-127.

Jlibène, M**.,** 2005. *L'amélioration génétique du blé tendre au Maroc*. In: Abbad-Andaloussi, F. and Chahbar, A. (eds), La création variétale à l'INRA,

méthodologie, acquis et perspectives, Institut National de la Recherche Agronomique, Rabat, Morocco: 42-43.

Jlibène, M., 2009. *Amélioration génétique du blé tendre au Maroc à l'aube du 21ème siècle*. Edition INRA, Rabat, Maroc: 33.

Liu, K., Muse, S.V., 2005. *PowerMarker: An integrated analysis environment for genetic marker analysis.* Bioinformatics, 21: 2128-2129.

Mohammadi, S.A., Prasanna, B.M., 2003. *Analysis of Genetic Diversity in Crop Plants-Salient Statistical Tools and Considerations*. Crop Science, 43**:** 1235-1248.

Nei, M., 1987. *Molecular evolutionary genetics*. Columbia University Press, New York.

Peakall, R., Smouse, P.E., 2006. *GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research.* Molecular Ecology Notes, 6: 288-295.

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

Rao, R.V., Hodgkin, T., 2002. *Genetic diversity and conservation and utilization of plant genetic resources*. Plant Cell Tissue and Organ Culture, 68: 1-19.

Röder, M.S., Korzun, V., Gill, B.S., Wendehake, K., Pleaschke, J., Tixier, M., Leroy, P., Ganal, M.W., 1998. *A microsatellite map of wheat*. Genetics, 149: 2007-2023.

Roussel, V., Koenig, J., Bechert, M., Balfourier, F., 2004. *Molecular diversity in French bread wheat accessions related to temporal trends and breeding programmes*. Theoretical and Applied Genetics, 108: 920-930.

Saghai-Maroof, M.A., Soliman, K.M., Jorgensen, R.A., Allard, R.W., 1984. *Ribosomal DNA spacer length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics.* Proceedings of the National Academy of Sciences USA, 81: 8014-8018.

Saitou, N., Nei, M., 1987. *The neighbor-joining method: a new method for reconstructing phylogenetic trees.* Molecular Biology and Evolution, 4: 406-425.

Salem, K.F.M., El-Zanaty, A.M., Esmail, R.M., 2008. *Assessing wheat (Triticum aestivum L.) genetic diversity using morphological characters and microsatellite markers.* World Journal of Agricultural Science, 4: 538-544.

Somers, D.J., Isaac, P., Edwards, K., 2004. *A high-density microsatellite consensus map for bread wheat (Triticum aestivum L.).* Theoretical and Applied Genetics 109: 1105-1114.

Tripp, R., 1996. *Biodiversity and modern crop varieties: Sharpening the debate*. Agric. Hum. Values, 13:48-63.

Udupa, S.M., Robertson, L.D., Weigand, F., Baum, M., Kahl, G., 1999. *Allelic Variation at (TAA)n Microsatellite Loci in a World Collection of Chickpea (Cicer arietinum L.) Germplasm.* Molecular and General Genetics, 261: 354-363.

Ward, R., Cregan, P., Song, Q., Shi, J., Gill, B., Singh, S., 2003. *544 BARC SSRs from Rick Ward.* http://wheat.pw.usda.gov/ggpages/whatsnew/2003.html