



Full Length Article

Assessment of D-genome Based Genetic Diversity in Drought Tolerant Wheat Germplasm

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Abstract

The complex nature of drought stress is one of the key bottlenecks in wheat improvement. Besides conventional breeding approaches for wheat improvement under water deficit conditions use of wild relatives like *Aegilops tauschii* (DD), as a source of rich genetic variation has significantly triggered the process. In this study, we have assessed the D-genome based genetic diversity in drought tolerant germplasm by using SSR markers. A total number of 178 alleles were detected on 49 loci with an average of 3.49 alleles per locus with the highest number being of 10 alleles for marker gwm515-2D. Genetic diversity explained by these SSR markers ranged from 4 to 67% with an average of 56.2% per marker. There were 23 SSR markers, which revealed PIC (Polymorphism Information Content) values of more than 50% while overall the PIC values for all markers ranged from 8 to 63.2% with an average of 48.1%. Clustering of genotypes D41, D43, D44, D50 and D51 within two well-known drought tolerant cultivars viz. Dharwar Dry and Sitta showed the suitability of these genotypes for water deficit areas. In conclusion, the genetic diversity present in the studied germplasm may be used in wheat breeding programs targeted for the drought tolerance especially in arid/semi-arid (rain-fed) environments. © 2015 Friends Science Publishers

Keywords: Wheat; *Aegilops tauschii*; Drought tolerance; Genetic diversity; PIC value

Introduction

Drought is the most significant factor among the abiotic stresses which limits productivity of crop plants including wheat (Cattivelli *et al.*, 2008). The scenario has become worse due to prevailing climatic changes which include global warming, uneven distribution/erratic rainfalls and shortage of water supply for agriculture (Battisti and Naylor, 2009; Bloem *et al.*, 2010). Periodic droughts affect half of the world's bread wheat (*Triticum aestivum* L.) production area (Rajaram, 2001) causing substantial losses in grain production (Aprile *et al.*, 2009).

The complex nature of drought stress is one of the key bottlenecks in wheat improvement. Conventional breeding approaches has little affect because drought tolerance is a quantitative trait and through selection high level of heritability is needed for the improvement of yield under such circumstances (Sohail *et al.*, 2011). So among other approaches for wheat improvement the use of wild relatives as a source of rich genetic variation has significantly triggered the process (Ashraf, 2010). Example of such a

wild relative in wheat is *Aegilops tauschii*, which is homologous to the D-genome of bread wheat. This species carries novel genetic diversity not only for biotic (Mujeeb-Kazi *et al.*, 2007) but also for abiotic stress tolerance (Reynolds *et al.*, 2007; Reynolds and Trethowan, 2007) that has tremendous potential for wheat improvement (Jones *et al.*, 2013). In fact of the three genomes of wheat i.e. A, B and D the latter possesses great homology to the D genome of wheat as compared to the A and B genomes (Dubcovsky and Dvorak, 2007). So to widen the genetic diversity of D-genome the International Maize and Wheat improvement Center (CIMMYT) exploited the core collection of *Ae. tauschii* to resynthesize bread wheat termed as “synthetic hexaploid wheat” (SH) (Mujeeb-Kazi *et al.*, 1996). Although SH do not possess superior good agronomic traits like modern wheat cultivars the genetic variability of the *Ae. tauschii* accessions has attracted most of the wheat breeders searching for allelic diversity for drought tolerance (Sohail *et al.*, 2011). In these synthetic hexaploids, *Ae. tauschii* accessions have shown good adaptation to limited supply of water and perform well in rainfed areas. They are hence the

potential source of genetic material for improving drought tolerance of cultivated wheat (Baalbaki *et al.*, 2006). This is the main reason that a significant number (one-third) of all the advanced bread wheat lines developed by CIMMYT for irrigated and low rainfall areas have SH in their pedigrees (van Ginkel and Ogbonnaya, 2007).

Molecular techniques have also helped plant breeders and physiologists to select genotypes with improved yield under drought conditions (Chang-Xing *et al.*, 2008). In this regard the advent and development of molecular markers have illustrated a new way of DNA fingerprinting of genotypes and to characterize each genotype in terms of the marker alleles at a number of locations across the genome (Quarrie *et al.*, 2003). Among these molecular markers, microsatellite or simple sequence repeats (SSR's) detect high level of polymorphism. In hexaploid wheat, there are some difficulties to use molecular markers due to the presence of a high proportion of repetitive DNA, large genome size, continuous inbreeding caused by self-pollination and a narrow genetic base (Joshi and Nguyen, 1993) but the high level of polymorphism detected. The ability to analyze by automated systems, high accuracy and repeatability make SSRs suitable for large scale DNA fingerprinting of wheat genotypes (Snape, 1998; Christiansen *et al.*, 2002). These markers are currently used to identify those traits which might be helpful in breeding wheat cultivars under drought conditions. Several quantitative trait loci have been reported for drought in wheat (Kato *et al.*, 2000; Liviero *et al.*, 2002; Quarrie *et al.*, 2003; McCartney *et al.*, 2005; Breseghello and Sorrells, 2007; Kuchel *et al.*, 2007; Maccaferri *et al.*, 2008; von Korff *et al.*, 2008; McIntyre *et al.*, 2010; Pinto *et al.*, 2010). In these studies more of the drought tolerance traits were reportedly located on the A and B genomes of bread wheat. So the present study was designed with the aim to characterize the D-genome of the SH genetic stocks developed in CIMMYT for drought breeding by microsatellite markers and study the contribution of the D genome from *Ae. tauschii*.

Materials and Methods

Plant Material

Fifty seven wheat hexaploid lines from drought tolerant genetic stock based on D-genome developed in CIMMYT along with five well-known drought tolerant spring wheat cultivars i.e. Dharwar Dry, Weebil, Sitta, Nesser and Inquilab were used in this experiment. The seeds of above mentioned plant material were sown in growth chamber in the laboratory of Wheat Wide Crosses, National Agricultural Research Center, Islamabad.

DNA Extraction and PCR Analysis

DNA was isolated from 5 g fresh leaf material of single plants of each genotype according to the protocol described

by Faheem *et al.* (2010). Forty nine SSR markers (Supplementary material, Table 1) distributed over the seven D genome chromosomes were used to characterize the advanced drought tolerant lines and the two drought tolerant cultivars *viz.* Dharwar Dry and Nesser. The PCR reaction was carried out in a final volume of 25 µL. The reaction mixture contained 50–150 ng of total genomic DNA template, 250 nM of each primer, 0.2 mM of each dNTP, 1.5 mM MgCl₂, 10X Taq buffer + KCl and 1 unit of Taq DNA polymerase (Roder *et al.*, 1998). The following PCR profile was used: initial denaturation at 92°C for 3 min followed by 45 cycles of 92°C for 1 min, 50–55–60°C (depending on the annealing temperature of the SSRs) for 1 min, 72°C for 2 min, and a final extension step of 10 min at 72°C (Röder *et al.*, 1998). Thermocycler “Amplitrionyx 6” was used for all amplification reactions. The PCR products were separated on 1.5% agarose/TBE gel. Gels were visualized by ethidium bromide under the UV light chamber and observed using the computer program UVI PhotoMW. To estimate the molecular weights of PCR products 1 Kbp ladder (Fermentas) was used.

Data Analysis

For statistical analysis the presence or absence of PCR amplified products were converted into binary data i.e. 0 and 1 respectively. Genetic distances were calculated using un-weighted the pair group of arithmetic means (Nei and Li, 1979) procedure as: $GD_{xy} = 1 - d_{xy}/d_x + d_y - d_{xy}$. In which GD_{xy} = Genetic distance between two genotypes, d_{xy} = Total number of common loci (bands) in two genotypes, d_x = Total number of loci (bands) in genotype 1, d_y = Total number of loci (bands) in genotype 2. Allelic polymorphism information content (PIC) was calculated according to following formula as described by Botstein *et al.* (1980):

$$\text{Polymorphism Information Content PIC} = 1 - \sum (P_{ij})^2$$

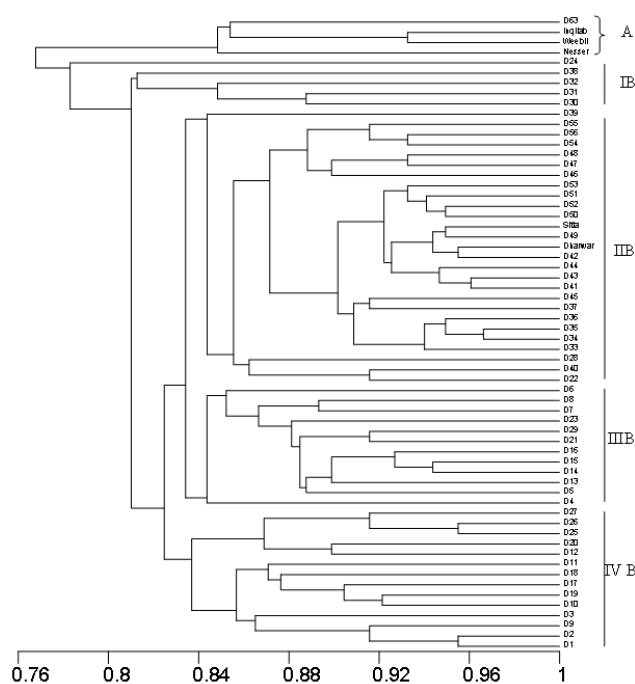
Where P_{ij} is the frequency of the i th pattern revealed by the j th primer summed across all patterns revealed by the primers. The analysis was performed using computer soft wares MVSP V3.1 (Multi variate statistical package) (Kovach, 2005) and Power marker V3.0 (Liu and Muse, 2005).

Results

The data obtained was used to analyze the genetic diversity present in the drought tolerant germplasm based on the D-genome. The summarized results of all the studied SSR's for gene diversity, number of alleles per locus and their respective PIC value are presented in Table 1. Forty nine SSR markers, covering all the seven D-genome chromosomes of hexaploid wheat with 3 to 11 primers per chromosome were used to compare the 62 wheat genotypes. Population genetic analysis showed that a total number of 178 alleles were detected on 49 loci with an average of 3.49

Table 1: Characteristics of the SSR markers and their number of alleles, gene diversity and PIC values calculated for 62 wheat genotypes

Marker	AlleleNo	Gene Diversity	PIC Value	Marker	Allele No	Gene Diversity	PIC Value
gwm337-1D	3.00	0.571	0.480	gwm 3-3D	3.00	0.619	0.546
gwm458-1D	1.00	0.453	0.350	gwm 52-3D	5.00	0.646	0.588
gwm642-1D	3.00	0.556	0.459	gwm 71-3D	2.00	0.508	0.387
gwm106-1D	3.00	0.52	0.508	gwm 484-4D	2.00	0.568	0.474
gwm232-1D	1.00	0.499	0.375	gwm 165-4D	5.00	0.570	0.478
gwm33-1D	6.00	0.571	0.481	gwm 194-4D	4.00	0.649	0.594
gwm249-2D	3.00	0.556	0.460	gwm 639-5D	5.00	0.599	0.521
gwm261-2D	1.00	0.492	0.371	gwm 271- 5D	3.00	0.571	0.480
gwm296-2D	4.00	0.648	0.592	gwm 358-5D	4.00	0.508	0.387
gwm349-2D	3.00	0.582	0.495	gwm 565 - 5D	6.00	0.674	0.632
gwm455-2D	5.00	0.552	0.453	gwm 583-5D	5.00	0.645	0.587
gwm515- 2D	10.00	0.645	0.590	gwm 174- 5D	1.00	0.500	0.375
gwm539-2D	5.00	0.650	0.595	gwm 182-5D	1.00	0.481	0.365
gwm157- 2D	3.00	0.624	0.556	gwm 192-5D	2.00	0.047	0.047
gwm208-2D	2.00	0.554	0.456	gwm 205-5D	5.00	0.569	0.476
gwm30-2D	4.00	0.587	0.502	gwm 212-5D	6.00	0.642	0.583
gwm102-2D	4.00	0.617	0.543	gwm 16-5D	5.00	0.668	0.623
gwm314-3D	3.00	0.543	0.439	gwm 325- 6D	1.00	0.092	0.088
gwm341-3D	3.00	0.622	0.555	gwm 469-6D	1.00	0.494	0.372
gwm383-3D	4.00	0.604	0.529	gwm 55-6D	3.00	0.629	0.563
gwm497-3D	4.00	0.538	0.431	gwm 295-7D	3.00	0.650	0.595
gwm161-3D	3.00	0.537	0.431	gwm 350-7D	6.00	0.523	0.410
gwm183-3D	4.00	0.642	0.583	gwm 428- 7D	3.00	0.571	0.480
gwm2-3D	4.00	0.611	0.539	gwm 635-7D	4.00	0.632	0.567
Mean				gwm 111-7D	7.00	0.652	0.599
					3.49	0.562	0.481

**Fig. 1:** SSR marker based cluster formation of 62 wheat genotypes

alleles per locus. The number of alleles per locus ranged from 1 for gwm182-5D, gwm232-1D, gwm261-2D, gwm325-6D, gwm458-1D, gwm469-6D, gwm174-5D to 10 for gwm515-2D (Table 1). However many of the SSR primers had more than 3 alleles indicating wide genetic

variability among the genotypes. The size of amplification products ranged from 50 bp to 1000 bp.

The genetic diversity explained by SSR makers in the studied germplasm ranged from 4% to 67% with an average of 56.2% per marker (Table 1). Maximum gene diversity

(67.4%) was revealed by gwm565-5D, while gwm16-5D also showed higher diversity of 66.8% (Table 1). SSR markers which detected the least gene diversity were gwm192-5D and gwm325-6D for which the gene diversity values were 4% and 9% respectively. Twenty SSR markers detected more than 60% gene diversity, which depicted that a lot of the genetic variation found in the germplasm was due to the presence of different alleles on the D-genome. Polymorphism information content value is one of the most reliable tools used scientifically to measure genetic diversity by any molecular marker and to distinguish one individual from another. The loci polymorphism can be categorized into three main categories viz. high, medium and low depending upon the value of PIC 0.5, $0.5 > \text{PIC} > 0.25$ and $\text{PIC} < 0.25$. PIC values calculated for the studied SSR markers ranged from 8% to 63.2% for marker gwm192-5D and gwm565-5D respectively with an average of 48.1% per marker (Table 1). It is noteworthy that the same markers i.e. gwm192-5D and gwm565-5D also exhibited the minimum and maximum gene diversity respectively, thus, proving that the PIC values actually reflected the presence of gene diversity. The results show that there were 23 SSR markers, which revealed P values of more than 50%. Among these markers gwm515-2D, gwm296-2D, gwm194-4D, gwm295-7D, gwm539-2D, gwm111-7D, gwm16-5D and gwm565-5D were very significant due to their high PIC values (59%, 59.2%, 59.4%, 59.5%, 59.5%, 59.9%, 62.3% and 63.2%) respectively (Table 1).

A similarity matrix based on simple matching algorithm as described by Nei and Lei, (1979) was generated for the 49 SSR primers by using software MVSP. The value of the similarity co-efficient of these genotypes ranged from 0.103 (10.30%) to 0.875 (87.50%). Minimum similarity of 10.30% was shown by D5 with D40 while genotypes D34 and D35 were 87.50% related to one another which was computed to be the maximum similarity. Cluster analysis based on UPGMA grouped the 62 genotypes into two main clusters named as A and B (Fig. 1). Cluster A had only four genotypes i.e. Nesser, Weebil, Inquilab and D63. Clustering of these cultivars in one group might be due to the similarity of D-genome with one another and with the D63. Separation of these cultivars from rest of the genotypes shows that the D-genome of these cultivars was different from that of genotypes in which different accessions of *Ae. tauschii* has been used as diploid progenitors. Genotype D24 was the most diverse in the germplasm due to its unique clustering pattern. The remaining genotypes along with cultivar Dharwar Dry and Sitta were clustered in cluster B. To understand the relationship among the genotypes cluster B was further divided into four groups. Four genotypes D38, D32, D31 and D30 were in group-I (Fig. 1) among which D30 and D31 were almost similar. Among these, group II was the most significant group of cluster B. It contained two well known drought tolerant cultivars i.e. Dharwar Dry and Sitta and twenty four drought tolerant genotypes. Among these genotypes D42 was found more similar to Dharwar

Dry and D49 also had close similarity with Sitta. This may suggest that the D-genome of these genotypes share some common alleles like that of Dharwar Dry and Sitta which might be involved in combating drought stress in these genotypes. Clustering of Dharwar Dry and Sitta in this group also suggested that some genetic differences were found in the D-genomes of these cultivars as compared to Nesser, Weebil and Inquilab. Similarity index among the genotypes with that of Dharwar Dry and Sitta showed that genotypes D41, D43, D44, D50, D51 and D52 were genetically similar and thus were clustered in a tight linkage in group II (Fig. 1). Fourteen genotypes D4, D5, D6, D7, D8, D13, D14, D15, D16, D21, D23 and D29 clustered in the same group designated as group III of cluster B. The dendrogram showed that among these genotypes D14 and D15 are about 95% similar based upon the D-genomic constitution. The remaining 14 genotypes in group IV of cluster B among which was D1 and D2 had very close genetic proximity due to the involvement of same *Ae. tauschii* accession in their SH parentage.

Discussion

Of the three genomes (A, B and D) of hexaploid wheat, the D-genome donated by *Ae. tauschii* has the least diversity (Dubcovsky and Dvorak, 2007) there by acts as a potential barrier for its improvement. The availability of few genes for developing drought tolerant wheat cultivars has compelled the wheat breeders to use the genetic diversity of wild species to achieve their goals (Sohail *et al.*, 2011). In this regard *Ae. tauschii* appeared to be the most desirable species as many desirable genes for biotic and abiotic resistance were found its accessions (Mujeeb-Kazi and Hettell 1995; Zaharieva *et al.*, 2001). Using this D-genome diversity International center for maize and wheat improvement (CIMMYT) resynthesized the hexaploid wheat by crossing elite durum lines with *Ae. tauschii* accessions (Mujeeb-Kazi 2003; Trethowan and Mujeeb-Kazi, 2008). These synthetic hexaploid wheats (SHW's) have thus been used widely to incorporate drought tolerant traits into modern wheat cultivar due to their good performance under water deficit conditions. In this study we assessed the diversity of D-genome from *Ae. tauschii* by SSR markers in the background of the SH wheat. The genetic diversity explained by SSR makers in the studied germplasm ranged from 4% to 67% with an average of 56.2% per marker which is promising. Similarly, the number of alleles also varied from 4 to 10 with an average value of 3.49 alleles per locus. However there were about 23 primers which have more alleles than average value. This suggests the presence of more than two alleles on the D-genome for the markers studied. Sohail *et al.* (2011) reported wider genetic diversity for drought in SH wheats as compared to the corresponding accessions of *Ae. tauschii* and concluded that the expression of traits in wild species may vary in expression in SH wheats derived from them.

PIC value which is considered as one of the most reliable parameters to detect diversity assessed by any marker ranged from 8% to 63.2% for 49 SSR primers. Hao *et al.* (2006) also suggested that to evaluate the genetic diversity in any germplasm the PIC value and number of alleles per locus must be considered. Huang (2002) and Hai *et al.* (2007) proved that there is positive correlation between the number of alleles per locus and PIC value. Bousba *et al.* (2012) also used the same strategy to evaluate the genetic diversity for drought tolerance in durum wheat and suggested the suitability of SSR makers and PIC value for characterizing the germplasm. The value of the similarity co-efficient of these genotypes ranged from 0.103 (10.30%) to 0.875 (87.50%). Das *et al.* (2007) also reported that the similarity coefficient among drought tolerant synthetics and conventional wheat ranged from 0.16 to 0.79. Clustering of D41, D43, D44, D50, D51 and D52 with Dharwar Dry and Sitta suggested the suitability of these genotypes for arid and semi-arid areas which are mostly affected by drought globally. Dharwar Dry is an outstanding cultivar selected in India where it performed exceptionally well in water stressed regions. The pedigree of this cultivar is unknown and some scientists believe that it may be derived from CIMMYT germplasm (Kirigwi *et al.*, 2007). Similarly Sitta is also one of the high yielding cultivar for drought affected areas developed in CIMMYT and has close proximity to the above mentioned genotypes that includes Dharwar Dry. This proximity can be explained that there might be some genes or quantitative trait loci associated with drought tolerance located on the D-genome. On other hand clustering of three other drought tolerant cultivars i.e. Weebil, Nesser and Inquilab separately in one cluster showed that their D-genomes were different from the remaining genotypes offering useful potent resources for allelic diversity.

The results of present study have elucidated that the genetic diversity present in the studied germplasm can be used in wheat breeding programs targeted for the drought tolerance especially in arid and semi-arid areas.

References

- Aprile, A., A.M. Mastrangelo, A.M. De Leonardi, G. Galiba, E. Roncaglia, F. Ferrari, L. De Bellis, L. Turchi, G. Giuliano and L. Cattivelli, 2009. Transcriptional profiling in response to terminal drought stress reveals differential responses along the wheat genome. *BMC Genomics*, 10: 279
- Ashraf, M., 2010. Inducing drought tolerance in plants: Recent advances. *Biotechnol. Adv.*, 28: 169–183
- Baalbaki, R., N. Hajj-Hassan and R. Zurayk, 2006. Species from semiarid areas of Lebanon: Variation in quantitative attributes under water stress. *Crop Sci.*, 46: 799–806
- Battisti, D.S. and R.L. Naylor, 2009. Historical warnings of future food insecurity with unprecedented seasonal heat. *Science*, 323: 240–244
- Bloem, M.W., R.D. Semba and K. Kraemer, 2010. Castel gandolfo workshop: An introduction to the impact of climate change, the economic crisis, and the increase in the food prices on malnutrition. *J. Nutr.*, 140: 132S–135S
- Botstein, D., R.L. White, M. Skolnick and R.W. Davis, 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Amer. J. Hum. Genet.*, 32: 314–331
- Bousba, R., M. Baum, A. Djekoune, S. Labadidi and A. Djighly, 2012. Screening for drought tolerance using molecular markers and phenotypic diversity in durum wheat genotypes. *World Appl. Sci. J.*, 16: 1219–1226
- Bresegghello, F. and M.E. Sorrells, 2007. Qtl analysis of kernel size and shape in two hexaploid wheat mapping populations. *Field Crops Res.*, 101: 172–179
- Cattivelli, L., F. Rizza, F.W. Badeck, E. Mazzucotelli, A.M. Mastrangelo, E. Francia, C. Mare, A. Tondelli and A.M. Stanca, 2008. Drought tolerance improvement in crop plants: An integrated view from breeding to genomics. *Field Crops Res.*, 105: 1–14
- Chang-Xing, Z.G. Ling-Yu, C.A. Jaleel, S. Hong-Bo, Y. Hong-Bing, 2008. Prospectives for applying molecular and genetic methodology to improve wheat cultivars in drought environments. *Cpmptes rendus. Biologies*, 331: 579–586
- Christiansen, M.J., S.B. Andersen and R. Ortiz, 2002. Diversity changes in an intensively bred wheat germplasm during the 20th century. *Mol. Breed.*, 9: 1–11
- Das, M., G. Bai and A. Mujeeb-Kazi, 2007. Genetic diversity in conventional and synthetic wheats with drought and salinity tolerance based on aflp. *Can. J. Plant Sci.*, 87: 691–702
- Dubcovsky, J. and J. Dvorak, 2007. Genome plasticity a key factor in the success of polyploid wheat under domestication. *Science*, 316: 1862–1866
- Faheem, M., T. Mahmood, A.G. Kazi, H.A. Ayaz and A. Mujeeb-Kazi, 2010. Phenological and molecular characterization of D-genome synthetic hexaploid wheats targeted for drought tolerance. *Annu. Wheat Newsletter*, 56: 179–183
- Hai, L., C. Wagner and W. Friedt, 2007. Quantitative structure analysis of genetic diversity among spring bread wheats (*Triticum aestivum* L.) from different geographical regions. *Genetica*, 130: 213–225
- Hao, C., X. Zhang, L. Wang, Y. Dong, X. Shang and J. Jia, 2006. Genetic diversity and core collection evaluations in common wheat germplasm from the northwestern spring wheat region in china. *Mol. Breed.*, 17: 69–77
- Huang, X., A. Börner, M. Röder and M. Ganai, 2002. Assessing genetic diversity of wheat (*Triticum aestivum* L.) germplasm using microsatellite markers. *Theor. Appl. Genet.*, 105: 699–707
- Jones, H., N. Gosman, R. Horsnell, G. Rose, L. Everest, A. Bentley, S. Tha, C. Uauy, A. Kowalski, D. Novoselovic, R. Simek, B. Kobiljski, A. Kondic-Spika, L. Brbaklic, O. Mitrofanova, Y. Chesnokov, D. Bonnett and A. Greenland, 2013. Strategy for exploiting exotic germplasm using genetic, morphological, and environmental diversity: The *Aegilops tauschii* Coss. Example. *Theor. Appl. Genet.*, 126: 1793–1808
- Joshi, C.P. and H.T. Nguyen, 1993. Application of the random amplified polymorphic DNA technique for the detection of polymorphism among wild and cultivated tetraploid wheats. *Genome*, 36: 602–609
- Kato, K., H. Miura and S. Sawada, 2000. Mapping qtls controlling grain yield and its components on chromosome 5A of wheat. *Theor. Appl. Genet.*, 101: 1114–1121
- Kirigwi, F., M. Van Ginkel, G. Brown-Guedira, B. Gill, G. Paulsen and A. Fritz, 2007. Markers associated with a QTL for grain yield in wheat under drought. *Mol. Breed.*, 20: 401–413
- Kovach, W.L., 2005. *MVSP-A MultiVariate Statistical Package for Windows*, Ver. 3.1. Kovach Computing Services, Pentraeth, Wales, U.K
- Kuchel, H., K.J. Williams, P. Langridge, H.A. Eagles and S.P. Jefferies, 2007. Genetic dissection of grain yield in bread wheat. I. QTL analysis. *Theor. Appl. Genet.*, 115: 1029–1041
- Liu, K. and S.V. Muse, 2005. Power marker: Integrated analysis environment for genetic marker data. *Bioinformatics*, 21: 2128–2129
- Liviero, L., E. Maestri, M. Gulli, E. Nevo and N. Marmioli, 2002. Ecogeographic adaptation and genetic variation in wild barley, application of molecular markers targeted to environmentally regulated genes. *Genet. Resour. Crop Evol.*, 49: 133–144

- Maccaferri, M., M.C. Sanguineti, S. Corneti, J.L.A. Ortega, M.B. Salem, J. Bort, E. DeAmbrogio, L.F.G. del Moral, A. Demontis and A. El-Ahmed, 2008. Quantitative trait loci for grain yield and adaptation of durum wheat (*Triticum durum*) across a wide range of water availability. *Genetics*, 178: 489–511
- McCartney, C., D. Somers, D. Humphreys, O. Lukow, N. Ames, J. Noll, S. Cloutier and B. McCallum, 2005. Mapping quantitative trait loci controlling agronomic traits in the spring wheat cross rl4452x'ac domain'. *Genome*, 48: 870–883
- McIntyre, C.L., K.L. Mathews, A. Rattey, S.C. Chapman, J. Drenth, M. Ghaderi, M. Reynolds and R. Shorter, 2010. Molecular detection of genomic regions associated with grain yield and yield-related components in an elite bread wheat cross evaluated under irrigated and rainfed conditions. *Theor. Appl. Genet.*, 120: 527–541
- Mujeeb-Kazi, A., 2003. New genetic stocks for durum and bread wheat improvement. In: *Tenth International Wheat Genetics Symposium*, pp: 772–774. Paestum, Italy
- Mujeeb-Kazi, A. and G.P. Hettel, 1995. *Utilization of Wild Grass Biodiversity in Wheat Improvement*. CIMMYT Research report Number 2, pp 1–140
- Mujeeb-Kazi, A., A. Gul, I. Ahmad, M. Farooq, S. Rizwan, H. Bux, S. Iftikhar, S. Asad and R. Delgado, 2007. *Aegilops tauschii*, as a spot blotch (*Cochliobolus sativus*) resistance source for bread wheat improvement. *Pak. J. Bot.*, 39: 1207–1216
- Mujeeb-Kazi, A., V. Rosas and S. Roldan, 1996. Conservation of the genetic variation of *Triticum tauschii* (Coss.) Schmalh. (*Aegilops squarrosa* Auct. Non l.) in synthetic hexaploid wheats (*T. turgidum* 1 S. Lat. x *T. tauschii*; 2n= 6x= 42, AABBDD) and its potential utilization for wheat improvement. *Genet. Resour. Crop Evol.*, 43: 129–134
- Nei, M. and W.H. Li, 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. National Acad. Sci.*, 76: 5269–5273
- Pinto, R.S., M.P. Reynolds, K.L. Mathews, C.L. McIntyre, J.J. Olivares-Villegas and S.C. Chapman, 2010. Heat and drought adaptive qtl in a wheat population designed to minimize confounding agronomic effects. *Theor. Appl. Genet.*, 121: 1001–1021
- Quarrie, S., D. Dodig, S. Pekic, J. Kirby and B. Kobijlski, 2003. Prospects for marker-assisted selection of improved drought responses in wheat. In: (Proceedings of the European Workshop on Environmental Stress and Sustainable Agriculture, Varna, Bulgaria) *Bul. J. Plant Physiol.*, Special Issue: 83–95
- Rajaram, S., 2001. Prospects and promise of wheat breeding in the 21st century. *Euphytica*, 119: 3–15
- Reynolds, M., F. Dreccer and R. Trethowan, 2007. Drought-adaptive traits derived from wheat wild relatives and landraces. *J. Exp. Bot.*, 58: 177–186
- Reynolds, M. and R. Trethowan, 2007. Physiological interventions in breeding for adaptation to abiotic stress. *Frontis*, 21: 127–144
- Röder, M.S., V. Korzun, K. Wendehake, J. Plaschke, M.H. Tixier, P. Leroy and M.W. Ganal, 1998. A microsatellite map of wheat. *Genetics*, 149: 2007–2023
- Snape, J.W., 1998. Golden calves or white elephants? Biotechnologies for wheat improvement. *Euphytica*, 100: 207–217
- Sohail, Q., T. Inoue, H. Tanaka, A.E. Eltayeb, Y. Matsuoka and H. Tsujimoto, 2011. Applicability of *Aegilops tauschii* drought tolerance traits to breeding of hexaploid wheat. *Breed. Sci.*, 61: 347–357
- Trethowan, R.M. and A. Mujeeb-Kazi, 2008. Novel germplasm resources for improving environmental stress tolerance of hexaploid wheat all rights reserved. *Crop Sci.*, 48: 1255–1265
- van Ginkel, M. and F. Ogbonnaya, 2007. Novel genetic diversity from synthetic wheats in breeding cultivars for changing production conditions. *Field Crops Res.*, 104: 86–94
- Von Korff, M., S. Grando, A. Del Greco, D. This, M. Baum and S. Ceccarelli, 2008. Quantitative trait loci associated with adaptation to mediterranean dryland conditions in barley. *Theor. Appl. Genet.*, 117: 653–669
- Zaharieva, M., P. Monneveux, M. Henry, R. Rivoal, J. Valkoun and M. Nachit, 2001. Evaluation of a collection of wild wheat relative *Aegilops geniculata* roth and identification of potential sources for useful traits. *Euphytica*, 119: 33–38

(Received 13 August 2014; Accepted 02 December 2014)