Classifying Ethiopian Tetraploid Wheat (*Triticum turgidum* L.)
Landraces by Combined Analysis of Molecular & Phenotypic Data

Negash Geleta*, Heinrich Grausgruber 2

1Department of Plant Sciences, Wollega University, Post Box No: 395, Nekemte, Ethiopia
2BOKU- University of Natural Resources and Applied Life Sciences, Department of Applied Plant Sciences and Plant Biotechnology, Institute of Agronomy and Plant Breeding, Vienna, Austria, A-1180

Abstract
The aim of the study was to investigate the extent of the genetic diversity among gene bank accessions of Ethiopian tetraploid wheat (*Triticum turgidum* L.) landraces using microsatellite markers, qualitative and quantitative data. Thirty five accessions of Ethiopian tetraploid wheat (*T. turgidum* L.) landraces were grown in the greenhouse at IFA Tulln, Austria during spring 2009 for DNA extraction. The same accessions were already grown in spring 2008 at BOKU Vienna, Austria for their phenotypical characterisation. DNA was extracted from each approximately one month old plant according to Promega (1998/99) protocol. A total of 10 µl reaction mixture per sample was used for DNA amplification by PCR. The amplified mixture was loaded to PAGE (12%) containing TE buffer (1×) in CBS electrophoresis chambers and run in an electric field for 2 hrs. The fragments were visualized by scanning with Typhoon Trio scanner. Six and ten quantitative and qualitative morphological traits data respectively were used for combined analysis. Genetic variation was significant within and between wheat species and within and between altitudes of collection site. Genetic distances ranged from 0.21 to 0.73 for all accessions while it ranged from 0.44 within *Triticum polonicum* to 0.56 between *T. polonicum* and *T. turgidum*. Genetic distance between regions of collection ranged from 0.47 (+/2200 m) to 0.56 (+/2500 m). Cluster analysis showed that *T. polonicum* accessions were grouped together whereas *T. durum* and *T. turgidum* formed mixed clusters indicating *T. polonicum* as genetically more distinct from the other two species. We suggest combined analysis of molecular and morphological data for a better classification of accessions.

INTRODUCTION

Microsatellites are tandemly repeated short DNA sequences that are favoured as molecular-genetic markers due to their high polymorphism index (Mun et al., 2006). Tandem repeat in DNA is a sequence of two or more contiguous, approximate copies of a pattern of nucleotides and tandem repeats occur in the genomes of both eukaryotic and prokaryotic organisms (Sokol et al., 2006). Microsatellite markers are the best DNA markers so far used for genetic diversity studies and fingerprinting of crop varieties. Microsatellites motifs are conserved in species and their unique behaviour abundance, co-dominance, robustness and easiness for PCR screening make them the best DNA markers for the evaluation of crop genetic diversity. Furthermore microsatellite markers have many advantages for tracing pedigrees because they represent single loci and avoid the problems
associated with multiple banding patterns obtained with other marker systems (Powell et al., 1996). However, developing microsatellite markers for a plant species requires prior knowledge of its genomic sequences, lack of which makes this technology very expensive and time consuming (Yu et al., 2009).

Microsatellite markers have been applied for genetic diversity studies in many crop plants including wheat (Powell et al., 1996; Gupta & Varshney, 2000; Li et al., 2002; Röder et al., 2002; Alamerew et al., 2004; Khlestkina et al., 2004; Hailu et al., 2005; Teklu et al., 2006a; Teklu et al., 2007), rice (Zeng et al., 2004), pearl millet (Kapila et al., 2008), underutilized crop species (Yu et al., 2009). Microsatellite markers were also applied for checking the identity of different commercial crop varieties and it was proved that the markers correctly identified between different varieties, e.g. varieties of olive for oil production (Pasqualone et al., 2007). Diversity studies based on phenotypic traits in Ethiopian wheat species are ample. However, studies based on molecular markers are few (Alamerew et al., 2004; Hailu et al., 2005; Teklu et al., 2006a; Teklu et al., 2007).

Combined analysis of data from continuous, ordinal and non-ordinal variables were applied for germplam classification e.g. by Franco et al. (1997a), Franco et al. (1998) and Tsivelikas et al. (2009). According to Franco et al. (2001) classifying genotypes into clusters based on DNA fingerprinting and/or agronomic attributes for studying genetic and phenotypic diversity is a common practice. A minimum number of molecular markers combined with morpho-agronomic characters can result in well classified genotypes. Using this strategy Franco et al. (2001) found compact and well-differentiated groups of genotypes for maize, wheat and tomato. In the present study data from microsatellite markers, non-ordinal and continuous traits were combined. Studies based on solely phenotypic traits variations may not be sufficient to characterize genebank accessions. Hence, the objective of this study was to investigate the extent of the genetic diversity among genebank accessions of Ethiopian tetraploid wheat using microsatellite markers, qualitative and quantitative data.

**MATERIALS AND METHODS**

**Plant Material**

Thirty-five accessions of Ethiopian tetraploid wheat (*T. turgidum* L.) landraces (Table 1) were grown in the greenhouse at IFA Tulln, Austria during spring 2009. Ten seeds per accession were planted in order to have enough plants per accession for DNA extraction. The same accessions were already grown in spring 2008 at BOKU Vienna, Austria for their phenotypical characterisation.

**DNA Extraction**

DNA was extracted according to Promega (1998; 1999) protocol. DNA was extracted from each approximately one month old plant. Ten to fifteen centimetres long young leaves were taken and chopped in 2-ml Eppendorf tubes (Eppendorf AG, Hamburg, Germany) and left open to dry for four days in plastic bags containing silica gel. The dried leaves were ground and leaf tissues were lysed by adding 600 µl of nucleic lysing solution to each of the tubes. The tubes were vortexed for 1-3 minutes to wet the cell uniformly and incubated in hot water at 65°C for 15 min. Ribonucleic acids (RNAs) were dissolved by adding 3 µl (4 mg ml⁻¹) RNase solution. Mixing was done by inverting the tubes 2-5 times. The mixture was incubated at 37°C for 15 min and then cooled at room temperature. 200 µl protein precipitation solution was added to each sample and vortexed vigorously for 20 Sec and then centrifuged for 3 min at 16000Xg. The precipitated proteins formed a tight pellet. The supernatant was carefully removed and transferred to another new 1.5 µl micro centrifuge tube containing 600 µl room tempered isopropanol. The solution was gently mixed for each sample by inversion until a thread like mass of DNA strand was visible. Then the mixture was centrifuged at 16000Xg for 2 min at room temperature. The supernatant was carefully decanted for each sample. 600 µl of room tempered ethanol (70%) was added and the tubes were gently inverted several times to wash the DNA and then centrifuged at 16000 × g for 2 min at room temperature. The ethanol was carefully decanted and the tube containing the sample was inverted on clean absorbent paper and the pellet was air dried for 15-20 min. 100 µl TE buffer solution was added to re-hydrate the DNA and incubated at 65°C for 1 hr. For subsequent use of DNA in PCR, it was diluted by 1:50 (v/v) DNA/dH₂O.
Table 1. Accession codes and regions and/or altitudes of collection sites of Ethiopian tetraploid wheat landraces.

| Accession | Region    | Altitude of collection site |
|-----------|-----------|----------------------------|
| T. durum  |           |                            |
| 5325      | Kefa      | 2667                       |
| 5613      | Shewa     | 2400                       |
| 5768      | Shewa     | 2300                       |
| 5888      | Shewa     | 2920                       |
| 5982      | Shewa     | 2930                       |
| 6078      | Arsi      | 2740                       |
| 6137      | Shewa     | 2670                       |
| 6915      | Gojam     | 2030                       |
| 7073      | Arsi      | 2480                       |
| 7472      | Welo      | 2920                       |
| 8317      | Gamu gofa | 2680                       |
| T. polonicum |       |                             |
| 5326      | Kefa      |                            |
| 5655      | Shewa     | 2650                       |
| 5880      | –         |                            |
| 6125      | Shewa     | 2720                       |
| 6370      | –         |                            |
| 7028      | Arsi      | 2880                       |
| 7135      | Shewa     | 2820                       |
| 8085      | –         |                            |
| 8314      | Gamugofa  |                            |
| 204708    | Eritrea   | 2400                       |
| 226637    | –         |                            |
| 241959    | Gojam     | 2125                       |
| 241988    | Welo      | 2845                       |
| 241994    | Tigray    | 2965                       |
| 241996    | Tigray    | 2445                       |
| 241999    | Shewa     | 3030                       |
| 241982-1  | Gonder    | 3080                       |
| 241990-1  | Welo      | 2445                       |
| 241997-1  | Tigray    | 2445                       |

Polymerase Chain Reaction (PCR)
A total of 10 µl reaction mixture per sample was used for DNA amplification by PCR. The 10 µl PCR mixture contained 0.025 µl forward primers (10 µM), 0.25 µl (10 µM) reverse primers, 0.225 µl of fluorescent M-13 labelled tail of 10 µM (HEX or FAM), 5 µl GoTaq® Green master mix (Promega Corporation, Madison, USA) (a, b), and 1.2 µl dH2O. GoTaq® Green master mix (a, b) contains dNTPs (dATP, dGTP, dCTP and dTTP), MgCl2 and reaction buffers at optimal concentrations for efficient amplification of DNA templates by PCR. GoTaq® Green master mix (a, b) (Flanagan et al., 2005) is a premixed ready to use solution containing a non-recombinant modified form of TaqDNA polymerase that lacks 5’→3’ exonuclease activity. It also contains two dyes (blue and yellow) that allow monitoring of progress during electrophoresis. PCR program SSR M13 was used for amplification. The following temperatures and times were used for PCR amplification of genomic DNA: (1) 95°C for 2 min (to heat the lid); (2) 95°C for 45 s to denature the double stranded DNA; (3) 68°C for 45 s to anneal the primers to the single stranded DNA; (4) 72°C for 1 min for TaqDNA polymerase to extend the primers. Steps 2 to 4 were repeated for 7 times; (5) 95°C for 45 s to denature the DNA; (6) 54°C for 45 s to anneal the primers to the single stranded DNA; (6) 72°C for 1 min for TaqDNA polymerase to extend the primer ends and steps 5 to 6 were repeated 30 times; (7) further extension of primers was done at 72°C for 5 min by TaqDNA polymerase; (8) finally the reaction was stopped and cooled at 8°C.

Polyacrylamide Gel Electrophoresis (PAGE) and Scanning
The amplified mixture was loaded to PAGE (12%) containing TE buffer (1×) in CBS electrophoresis chambers (C.B.S. Scientific Co., Del Mar, USA) and run in an electric field for 2 hrs. The fragments were visualized by scanning with Typhoon Trio scanner (GE Healthcare Europe GmbH, Regional Office Austria, Vienna).

Microsatellite Loci
Microsatellite loci were selected based on available information. Out of 30 microsatellite loci only 11 of them gave polymorphic bands that can be scored as either 0 or 1. However, the microsatellite markers Xgwm181 and Xgwm340 are located on the same chromosome arm, i.e. 3BL, very near to each
Table 2. Repeat number, chromosomal location and genetic diversity for SSR markers.

| Primers   | Chromosome | Repeat              | Annealing temperature | Number of alleles | Genetic diversity |
|-----------|------------|---------------------|-----------------------|------------------|------------------|
| Xgwm294  | 2A         | (GA)9TA(GA)15       | 55°C                  | 4                | 0.62             |
| Xgwm495  | 4B         | (GA)20              | 60°C                  | 5                | 0.57             |
| Xgwm340  | 3B         | (GA)26              | 60°C                  | 5                | 0.41             |
| Xgwm160  | 4A         | (GA)21              | 60°C                  | 2                | 0.39             |
| Xgwm135  | 1A         | (GA)20              | 60°C                  | 6                | 0.53             |
| Xgwm397  | 4A         | (CT)21              | 55°C                  | 4                | 0.61             |
| Xgwm626  | 3B         | (CT)5(GT)13         | 50°C                  | 5                | 0.37             |
| Xgwm595  | 5A         | (GA)39imp           | 60°C                  | 5                | 0.44             |
| Xgwm400  | 7B         | (CA)21              | 60°C                  | 4                | 0.60             |
| Xgwm344  | 7B         | (GT)24              | 55°C                  | 2                | 0.09             |
| **Total**|            |                     |                       | **42**           | **4.63**         |
| **Mean** |            |                     |                       | **4.2**          | **0.46**         |

imp, imperfect repeat (Source: Röder et al., 1998)

Molecular and Phenotypic Data

Data from the 10 microsatellite markers were recorded in a binary way (0 or 1). Zero means no allele for the locus while 1 means there is an allele. In total 42 alleles were present. Quantitative data of six morphological traits, i.e. days to heading, spike density, awn length, thousand kernel weight, yellow pigment content and protein content which were used for the combined analysis. Furthermore ten qualitative traits included beak shape, beak length, glume colour, awn color, glume hairiness, seed color, seed size, seed shape, vitreousness and seed plumpness were used.

Statistical Analysis

Gene diversity among accessions for microsatellite markers was calculated according to Nei (1973):

$$Gene\ \text{diversity} = 1 - \sum_{i,j} P_{ij}^2$$

where $P_{ij}$ is the frequency of the $j^{th}$ allele for the $i^{th}$ locus summed across all alleles of the locus. The gene diversity coefficient is also referred to as the allelic polymorphic information content according to Anderson et al. (1993). Data from SSR marker, qualitative and quantitative traits were combined and analysed modified after Franco et al. (1997a). Regions with only a few number of accessions were pooled together and four groups were formed, i.e. Northern (Eritrea, Tigray, Welo, Gonder, Gojam), Central (Shewa) and Southern (Arsi, Kefa, Gamu Gofa) Ethiopia. Accessions with no available information of their original collection site were pooled together in one group. Similarly, altitudes of collection sites were classified as ≤2200 m, ≤2500 m, ≤2800 m, >2800 m and genotypes with no available information. Genetic distances between accessions, within and between species, within and between regions, and within and between altitudes were computed using Gower’s distance (Gower, 1971). Using the dissimilarity distances between accessions a GLM analysis of variance was run for species, regions and altitudes to check significances between these effects and in order to obtain means and standard errors. Hierarchical cluster analysis was performed for all genotypes using the dissimilarity matrix of Gower’s distance and the Ward fusion method. All analyses were carried out using SAS Vers. 9.1 software (SAS Institute, Cary, USA).

RESULTS

The used microsatellite markers revealed a total of 42 alleles. The number of alleles per locus ranged from two for Xgwm160 and Xgwm344 to six for Xgwm135. Genetic diversity ranged from 0.09 (Xgwm344) to 0.62 (Xgwm294) (Table 2). Based on combined data Gower’s dissimilarity ranged from 0.21 between ID 5585 and ID 241997-1 (T. turgidum) to 0.73 between ID 241982-2 and ID 209774 (T. turgidum and T. polonicum, respectively).
Analysis of variance of the Gower dissimilarity matrix showed that the difference within and between species and altitudes were significant (P<0.0001), whereas the differences within and between regions were not significant (P>0.05) (Table 3). Mean dissimilarities within and between species, regions and altitudes are presented in Tables 4, 5 and 6, respectively. At species level the dissimilarity ranged from 0.44 (within T. polonicum) to 0.56 (between T. polonicum and T. turgidum). On the other hand, within species variability was higher for T. durum and T. turgidum genotypes. Within region dissimilarity ranged from 0.51 for Central Ethiopia to 0.53 for accessions of unknown origin while between regions dissimilarity ranged from 0.51 between Central and Southern Ethiopia to 0.54 between accessions of unknown origin and Northern and/or Southern Ethiopia. Generally, accessions of unknown origin had higher within and between regions dissimilarities. The most probable reason is that these accessions have been collected in different regions of Ethiopia.

Table 3. ANOVA for species, region & altitude.

| Source of variation | DF | Mean Square | Pr>F       |
|---------------------|----|-------------|------------|
| Species             | 5  | 0.045       | <0.0001    |
| Region              | 9  | 0.008       | 0.2590     |
| Altitude            | 14 | 0.017       | <0.0004    |

For altitude, within altitude dissimilarity ranged from 0.47 (≤2200 m) to 0.56 (≤2500 m) while between altitudes dissimilarity ranged from 0.49 between ≤2200 m and accessions of unknown altitude and between ≤2800 m and >2800 m to 0.55 between ≤2200 m and ≤2500 m. Clustering of genotypes using Gower’s dissimilarity matrix grouped the 35 genotypes into 6 subgroups (Figure 1). The most remarkable result of the dendrogram is that almost all T. polonicum accessions are grouped together, indicating the indigenous evolution of this tetraploid wheat species. T. durum and T. turgidum accessions were randomly mixed together throughout all clusters.

**DISCUSSION**

In the present study of combined analysis of molecular marker and quantitative and qualitative phenotypic data variation within and between tetraploid species of Ethiopian origin was evident. Due to the larger number of T. durum and T. turgidum genotypes variation within these two species were higher than within T. polonicum. Genetic dissimilarity within T. polonicum was lower than within the other two species. The lower variation within T. polonicum genotypes is most probably due to the fewer number of investigated genotypes and the narrower, more indigenous evolution of this species. Therefore, dissimilarity between T. polonicum and the other two species is significantly higher than within dissimilarity. The higher variation within T. durum and T. turgidum and the random mixing of these species in the clusters following cluster analysis of Gower’s dissimilarity matrix is not astonishing.

Table 4. Genetic dissimilarity within and between wheat species.

| Species                          | Mean | Standard Error |
|----------------------------------|------|----------------|
| T. durum                         | 0.51 | 0.010          |
| T. polonicum                     | 0.44 | 0.024          |
| T. turgidum                      | 0.52 | 0.006          |
| T. durum vs T. polonicum         | 0.53 | 0.010          |
| T. durum vs T. turgidum          | 0.52 | 0.005          |
| T. polonicum vs T. turgidum      | 0.56 | 0.008          |

Table 5. Genetic dissimilarity within and between regions.

| Region                 | Mean | Standard Error |
|------------------------|------|----------------|
| Northern Ethiopia      | 0.52 | 0.012          |
| Central Ethiopia       | 0.51 | 0.011          |
| Southern Ethiopia      | 0.52 | 0.017          |
| Unknown origin         | 0.53 | 0.017          |
| Northern vs Central    | 0.52 | 0.008          |
| Northern vs Southern   | 0.53 | 0.009          |
| Northern vs unknown origin | 0.54 | 0.009          |
| Central vs Southern    | 0.51 | 0.009          |
| Central vs unknown origin | 0.53 | 0.009          |
| Southern vs unknown origin | 0.54 | 0.011          |
### Table 6. Genetic dissimilarity within and between altitudinal classes.

| Altitude (m)                  | Mean  | Standard Error |
|-------------------------------|-------|----------------|
| ≤2200                         | 0.47  | 0.045          |
| ≤2500                         | 0.56  | 0.015          |
| ≤2800                         | 0.49  | 0.020          |
| >2800                         | 0.51  | 0.013          |
| Unknown altitude              | 0.53  | 0.013          |
| ≤2200 vs ≤2500                | 0.55  | 0.016          |
| ≤2200 vs ≤2800                | 0.52  | 0.018          |
| ≤2200 vs >2800                | 0.51  | 0.015          |
| ≤2200 vs unknown altitude     | 0.49  | 0.015          |
| ≤2500 vs ≤2800                | 0.53  | 0.011          |
| ≤2500 vs >2800                | 0.53  | 0.009          |
| ≤2500 vs unknown altitude     | 0.55  | 0.009          |
| ≤2800 vs >2800                | 0.49  | 0.011          |
| ≤2800 vs unknown altitude     | 0.53  | 0.011          |
| >2800 vs unknown altitude     | 0.53  | 0.009          |

**Figure 1.** Cluster analysis for 35 genotypes of tetraploid wheats using Gower’s distance dissimilarity matrix.
considering the different developments in wheat taxonomy. Dorofeev et al. (1979) clearly differentiated between T. durum and T. durum at species level, whereas MacKey (1988) classified durum wheat as a variety of subspecies turgidum of species turgidum, i.e., T. turgidum subsp. turgidum convar. durum, van Slageren (1994) followed this idea at the subspecies level, i.e. T. turgidum subsp. durum, and Kimber & Sears (1987) classified all tetraploid wheats with a BA genome as T. turgidum (for a Triticum comparative classification table see http://www.k-state.edu/wgrc/Taxonomy/comptri.html).

The present data was enough to depict variation within and between species. Using 22 SSR markers Alamerew et al. (2004) studied genetic diversity among Ethiopian wheat accessions (T. aestivum, T. aethiopicum and T. durum) and found that all T. aestivum accessions grouped together while T. durum and T. aethiopicum accessions were not grouped into distinct clusters. Using only molecular markers data may not group accessions into the respective species/subspecies level. Combining molecular with phenotypic data might be more promising. Another study by Hailu et al. (2005) using 8 ISSR marker showed that genetic distances were higher between T. turgidum and T. dicoccum but lower between T. turgidum and T. durum and clustering of genotypes did not completely group according to their region of origin or species level. Using 29 microsatellite markers, Teklu et al. (2006a) studied genetic diversity among Ethiopian tetraploid wheat landraces and found a lower genetic distance between T. turgidum and T. durum compared to T. turgidum and T. dicoccum or T. durum and T. dicoccum.

Although within region and between regions dissimilarities were not significant accessions of unknown origin were responsible for higher dissimilarities. The most probable reason for this observation is that these accessions were collected in different regions. From our results we conclude that accessions of the Ethiopian genebank with no available information about their collection site are the most variable group and, therefore, can be valuable sources for crop improvement programmes despite the fact that more or less no passport data about their origin is available. Based on 29 SSR marker Teklu et al. (2006b) found highest within region genetic diversity for Shewa (Central) and Gonder (Northern Ethiopia), however, the authors did not find significant correlations between genetic distances and geographic distances. Hailu et al. (2005) on the other hand found lower values for between region diversity. However, the latter authors used only a few ISSR markers for their genetic diversity study. With regard to altitude, in the present study within altitude diversity was highest for ≤2500 m while between altitudes diversity was highest between ≤2200 m and ≤2500 m. This is in agreement with Teklu et al. (2006b).

Molecular tools alone may not be sufficient to group wheat species and/or genotypes efficiently. To establish good groupings according to species/subspecies level, pedigree background etc. a large number of molecular data would be needed. For instance Zhang et al. (2002) determined the minimum number of SSR markers to completely classify common wheat varieties into parental breeding lines and large-scale breeding varieties. The authors suggested 350 to 400 alleles to be enough to cluster varieties into their respective groups. This high number of needed markers/alleles would be too costly for screening of stored genebank accessions for e.g. the development of core sets. On the other hand, Tsivelikas et al. (2009) studied the genetic diversity among squash accessions using RAPD data and morpho-physiological data and they found that the best groupings were obtained when molecular data were combined with morpho-physiological data. Vollmann et al. (2005) used both phenotypic and RAPD markers data to analyse genetic diversity in Camelina sativa accessions and they found that the genetic diversity between the two different clustering approaches. In our study, the combined approach better grouped together T. polonicum genotypes than molecular or morphological data alone would have done. The uses and preferences of different statistical tools for analysis of genetic diversity in crop plants were reviewed by Mohammadi & Prasanna (2003). The authors stated that each data set (morphological, biochemical or molecular) has its own strengths and constraints and there is no single or simple strategy to address effectively various complex issues related to choice of distance measure(s), clustering methods, determination of optimal number of clusters or analysis of individual, and combined data sets by means of various statistical tools. Crossa & Franco (2004) recommended a two stage sequential clustering strategy using all variables, continuous and categorical, to produce more homogenous groups of individuals than other clustering.
strategies. Franco et al. (1997b) applied Normix after Ward method for classifying genebank accessions of maize and obtained a good estimation of optimum group number and formation of more compact and separated groups than using only the Ward method. Gutiérrez et al. (2003) compared racial classification by visual observation and numerical taxonomy for the classification of maize landraces. The authors found that numerical taxonomy using the Ward-MLM (modified location model) strategy generated more homogenous clusters than the initial racial method.

CONCLUSION

The present data was enough to depict variation within and between species. Combining molecular with phenotypic data might be more promising. Although within region and between regions dissimilarities were not significant, accessions of unknown origin were responsible for higher dissimilarities. The most probable reason for this observation is that these accessions were collected in different regions. From our results we conclude that accessions of the Ethiopian genebank with no available information about their collection sites are the most variable group and, therefore, can be valuable sources for crop improvement programmes despite the fact that more or less no passport data about their origin is available. From the results of the present study the combined use of molecular markers and phenotypic data is suggested as a promising way for the characterization of genebank accessions.

Acknowledgement

The authors are grateful to Prof. Tamas Lelley, IFA Tulln, Austria, for providing the laboratory facilities for analysis of the molecular markers. This work was part of the PhD study for first author and financed by Austrian Agency for International Cooperation in Education and Research.

REFERENCES

Alamerew S., Chebotar, S., Huang, X., Röder, M., Börner, A. (2004). Genetic diversity in Ethiopian hexaploid and tetraploid wheat germplasm assessed by microsatellite markers. Genetic Resources and Crop Evolution 51: 559-567.

Anderson, J.A., Churchill, G.A., Antrique, J.E., Tanksley, S.D., Sorrels, M.E. (1993). Optimising parental selection for genetic linkage maps. Genome 36: 181-188.

Crossa, J., Franco, J. (2004). Statistical methods for classifying genotypes. Euphyla 137: 19-37.

Dorofeev, V.F., Filatenko, A.A., Migushova, E.F., Udaczin, R.A., Jakubziner, M.M. (1979). Wheat, Vol. 1. In: Dorofeev, V.F., Korovina, O.N. (Eds.), Flora of cultivated plants. Kolos, Leningrad, Russia (in Russian).

Flanagan, L., Wheeler, S., Koeff, M., Knoche, K. (2005). GoTaq<sup>®</sup> Green Master Mix: from amplification to analysis. Promega Notes 91 (9/05): 13-16. [Available online: http://www.promega.com/pnotes/91/12972_13/euro/12972_euro.pdf; verified 12 October 2009]

Franco, J., Crossa, J., Villaseñor, J., Taba, S., Eberhart, S.A. (1997a). Classifying Mexican maize accessions using hierarchical and density search methods. Crop Science 37: 972-980.

Franco, J., Crossa, J., Diaz, J., Taba, S., Villaseñor, J., Eberhart, S.A. (1997b). A sequential clustering strategy for classifying gene bank accessions. Crop Science 37: 1656-1662.

Franco, J., Crossa, J., Villaseñor, J., Taba, S., Eberhart, S.A. (1998). Classifying genetic resources by categorical and continuous variables. Crop Science 38: 1688-1696.

Franco, J., Crossa, J., Ribbhat, J.M., Betran, J., Warburton, M.L., Khairallah, M. (2001). A method for combining molecular markers and phenotypic attributes for classifying plant genotypes. Theoretical and Applied Genetics 103: 944-952.

Gower, J.C. (1971). A general coefficient of similarity and some of its properties. Biometrics 27: 857-874.

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

Gutiérrez, L., Franco, J., Crossa, J., Abadie, T. (2003). Comparing a preliminary racial classification with numerical classification of the maize landraces of Uruguay. Crop Science 43: 718-727.

Hailu, F., Merker, A., Belay, G., Johansson, E. (2005) Molecular diversity and phylogenetic relationships of tetraploid wheat species as revealed by intersimple sequence repeats (ISSR) from Ethiopia. Journal of Genetics and Breeding 59: 329-338.

Kapila, R.K., Yadav, R.S., Plaha, P., Rai, K.N., Yadav, O.P., Hash, C.T., Howarth, C.J. (2008). Genetic diversity among pearl millet maintainers using microsatellite markers. Plant Breeding 127: 33-37.

Khlestkina, E.K., Röder, M.S., Efremova, T.T., Börner, A., Shumny, V.K. (2004). The genetic diversity of old and modern Siberian varieties of spring wheat determined by microsatellite markers. Plant Breeding 123: 122-127.
Kimber, G., Sears, E.R. (1987). Evolution in the genus *Triticum* and the origin of cultivated wheat. In: Heyne, E.G. (Ed.), Wheat and Wheat Improvement, 2nd Ed., American Society of Agronomy, Madison, WI. pp 154-164.

Li, Y.C., Röder, M.S., Fahima, T., Kirzhner, V.M., Beils, A., Korol, A.B., Nevo, E. (2002). Climatic effects on microsatellite diversity in wild emmer wheat (*Triticum dicocoides*) at the Yehudiyya microsite, Israel. *Heredity* 89: 127-132.

MacKey, J. (1988). A plant breeder's perspective on taxonomy of cultivated plants. *Biologisches Zentralblatt* 107: 369-379.

Mohammadi, S.A., Prasanna, B.M. (2003). Analysis of genetic diversity in crop plants – salient statistical tools and considerations. *Crop Science* 43: 1235-1248.

Mun, J.H., Kim, D.J., Choi, H.K., Gish, J., Debelle, F., Mudge, J., Denny, R., Endre, G., Saurat, O., Dudez, A.M., Kiss, G.B., Roe, B., Young, N.D., Cook, D.R. (2006). Distribution of microsatellites in the genome of *Medicago truncatula*: a resource of genetic markers that integrate genetic and physical maps. *Genetics* 172: 2541-2555.

Neil, M. (1973). Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences USA* 70: 3321-3323.

Pasqualone, A., Montemurro, C., Summo, C., Sabetta, W., Caponio, F., Bianco, A. (2007). Effectiveness of microsatellite DNA markers in checking the identity of protected designation of origin extra virgin olive oil. *Journal of Agricultural and Food Chemistry* 55: 3857-3862.

Powell, W., Marchray, G.C., Provan, J. (1996). Polymorphism revealed by simple sequence repeats. *Trends in Plant Science* 1: 215-222.

Promega (1998/99). Technical manual: *Wizard®* Genomic DNA Purification Kit.

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

Röder, M.S., Wendehake, K., Korzun, V., Bredemeijer, G., Laborie, D., Bertrand, L., Isaac, P., Rendell, S., Jackson, J., Cooke, R.J., Vosman, B., Ganal, M.W. (2002) Construction and analysis of a microsatellite-based database of European wheat varieties. *Theoretical and Applied Genetics* 106: 67-73.

Sokol, D., Benson, G., Tojeira, J. (2006). Tandem repeats over the edit distance. *Bioinformatics* 23: 30-35.

Teklu, Y., Hammer, K., Huang, X.Q., Röder, M.S. (2006b). Regional patterns of microsatellite diversity in Ethiopian tetraploid wheat accessions. *Plant Breeding* 125: 125-130.

Teklu, Y., Hammer, K., Röder, M.S. (2007) Simple sequence repeats marker polymorphism in emmer wheat (*Triticum dicoccum* Schrank): analysis of genetic diversity and differentiation. *Genetic Resources and Crop Evolution* 54: 543-554.

Tsivelikas, A.L., Koutita, O., Anastasiadou, A., Skaracas, G.N., Traka-Mavrona, E., Koutriska-Sotiriou, M. (2009). Description and analysis of genetic diversity among squash accessions. *Brazilian Archives of Biology and Technology* 52: 271-283.

van Slageren, M.W. (1994). Wild wheats: a monograph of *Aegilops* L. and *Amblyopyrum* (Jaub. & Spach) Eig (*Poaceae*). *Wageningen Agricultural University Papers*.

Vollmann, J., Grausgruber, H., Stift, G., Dryzhyruk, V., Lelley, T. (2005) Genetic diversity in camellina germplasm as revealed by seed quality characteristics and RAPD polymorphism. *Plant Breeding* 124: 446-453.

Yu, J.W., Dixit, A., Ma, K.H., Chung, J.W., Park, Y.J. (2009). A study on relative abundance, composition and length variation of microsatellites in 18 underutilized crop species. *Genet. Genetic Resources and Crop Evolution* 56: 237-246.

Zeng, L., Kwon, T.R., Liu, X., Wilson, C., Grieve, C.M., Gregorio, G.B. (2004). Genetic diversity analysis by microsatellite markers among rice (*Oryza sativa* L.) genotypes with different adaptations to saline soils. *Plant Science* 166: 1275-1285.

Zhang, X.Y., Li, C.W., Wang, L.F., Wang, H.M. (2002). *Z. Zentralblatt für Agronomie, Pflanzenzüchtung und Pflanzenbau* 53: 1115-1126.