J. Agric. Sci. Mansoura Univ., 31 (10): 6275 - 6282, 2006

MOLECULAR DIVERSITY OF FOUR EGYPTIAN WHEAT CULTIVARS REVEALED BY MICROSATELLITES.

El-Shihy, O.M.[1]; A. M. Ghallab[1]; H. M. Youssef Ibrahim[1] and M. S. Röder[2]

1- Plant Physiology Dept., Faculty of Agric., Cairo University, Egypt

2- Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), 06466 Gatersleben, Germany

ABSTRACT

The objective of this study was to evaluate the genetic diversity of the most important Egyptian wheat cultivars grown in 2005 by using microsatellites (SSR) as molecular markers. The four varieties of wheat (Triticum aestivum L.) tested were Giza 168, Sids 1, Gemmeza 7 and Gemmeza 9) including Chinese Spring as standard. The 24 microsatellites used revealed a total of 93 alleles. The fragment size ranged from 75 bp in GWM3 to 285 bp in GWM931. The average number of alleles was 3.9 ranging from 2-9 alleles per locus. The average allele number was different for each cultivar. With 66 alleles the number was highest in Giza 168, followed by 33 alleles in Sids 1, then Gemmiza 9 with 32 alleles and the lowest allele number was found in Gimmeza 7 (30 alleles). The polymorphism information content (PIC value) reflecting the gene diversity of the 24 microsatellite loci ranged from 0.48 to 0.82 with an average of 0.66. The genetic similarity level ranged from 0.22 for Chinese Spring with the Egyptian cultivars to 0.58 for Gimmeza 7 and Gimmeza 9. The correlation coefficient between PIC and number of alleles over 24 microsatellites loci was 0.62.

INTRODUCTION

Wheat is an important crop occupying around 16% of the arable lands of the world, with increasing global demand and associated shortages of production in many countries.

Wheat is one of the oldest and most important of the cereal crops in Egypt. Although wheat production per unit area in Egypt has significantly increased during the past years, wheat production supplies only 40% of its annual domestic demand. The reasons for the lacking ability of Egypt to produce sufficient wheat for domestic consumption are that the total cultivated area represents less than one quarter of the total area; and that Egypt has one of the highest rates of wheat consumption per capita of any country in the world (200 kg per capita, compared with a world average of less than 60 to 75 kg per capita). The population growth rate (2.1% annually) increases higher than the increase of wheat production and little efforts are made for improving salt tolerance in wheat crops, e.g. only two genotypes (Sakha 8 and Sakha 93) among Egyptian wheat genotypes are tolerate to salinity. There exists a competition for cultivated lands to grow wheat, forage and cotton crops. Most importantly, Egypt still is one of the largest countries that import wheat. Wheat imports in 2004/05 (July/June) were about 6.5 million tons, with a cost of about 986 million US $ annually (FAO; http://www.fao.org/). Therefore, the Egyptian Government needs to make a great effort to increase wheat productivity. Extending wheat growing outside

the Nile Valley is the first effort toward overcoming the described problems. However, most of the area outside the Nile Valley suffers from salinity or depends on water sources that are affected by salinity, therefore, increasing salt tolerance for wheat genotypes is one of the cheapest methods to spread growing wheat in these areas.

The power of molecular markers as powerful tools to evaluate the genetic diversity of germplasm is increasingly recognized (Melchinger *et al.*, 1991 and Melchinger *et al.*, 1994). Such markers have been used to trace the geographic origins of accessions by comparing genetic fingerprints of diverse material (Salamini *et al.*, 2002; Beak et al 2003) and to classify germplasm resources (Alamerew *et al.*, 2004).

Microsaellites are, compared to other marker types, abundant and possess a high polymorphism information content (PIC) and are often multiallelic (Röder *et al.*, 1995; Gupta *et al.*, 1996). A limited number of microsatellite markers are often sufficient to detect differences even in very closely related wheat genotypes (Plaschke *et al.*, 1995). Furthermore, a large number of wheat microsatellite markers has been developed, which are widely used in genomic mapping populations and evolutionary studies, as well as for fingerprinting and pedigree analyses (Röder *et al.*, 1998; Röder *et al.*, 2004).

Crop diversity studies using molecular markers have been conducted in different cereals such as barley (Hordeum vulgare L.; Macaulay *et al.*, 2001; Matus and Hayes, 2002; Koebner *et al.*, 2003), rice (*Oryza sativa* L.), Ishii and McCouch, 2001), maize (*Zea mays* L.); Mumm and Dudley, 1994 and Lu and Bernardo, 2001) and in wheat (*Triticum spp.*), Donini *et al.*, 2000; Prasad *et al.*, 2000; Röder *et al.*, 2002; Huang *et al.*, 2002; Eujail *et al.*, 2002). Microsatellites have a high potential for genome analyses of self-pollinating crops because of their specific properties and their high degree of polymorphism (Plaschke *et al.*, 1995; Röder *et al.*, 1995).

This study was conducted to evaluate the genetic diversity of the most important Egyptian wheat cultivars grown in 2005 by using microsatellites (SSR) as molecular markers.

MATERIALS AND METHODS

Plant materials

Four varieties of spring wheat (*Triticum aestivum* L.) were used in this study (Giza 168, Sids 1, Gemmeza 7 and Gemmeza 9). They were obtained from the Wheat Department, Field Crops Institute, Agricultural Research Centre in Giza, Egypt. The variety Chinese spring was obtained from the Gene Bank, Gatersleben, Germany, and used as refernce. Seeds from all cultivars were planted in the green house of the Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany.

DNA extraction

Total genomic DNA was extracted from pooled leaves of five one-week old plants. The young seedling leaves of each cultivar were harvested and frozen in liquid nitrogen 15 mints. Approximately 2-4 g of leaf material were ground in 2 ml tubes in "Retsch-Schwingmuehle MM 300" 2x 30 sec

and a frequency of 25/s. The extraction was performed according to the protocol of Doyle & Doyle 1990. Polymerase Chain Reaction amplifications were performed as described by Röder *et al.*, (1998). The substrate subjected to PCR contained 50-100 ng template DNA, 250 nM cy5-labelled forward primer, 250 nM unlabelled reverse primer, 0.2 mM dNTPs, 2.5 µl PCR buffer (10 x), 1.5 mM $MgCl_2$ and 1U Taq DNA polymerase in a total volume of 25 µl.

Fragment detection was performed with an Automated Laser Fluorescence (ALFexpess) sequencer (Amersham Biosciences Europe GmbH, Freiburg, Germany) as described by Röder *et al.*, (1998) and fragment sizes were calculated using the compuer programme fragment Anayser 1.02 (Amersham Biosciences) by comparison with internal size standards. The cultivar Chinese spring was used as a reference to standardize different gel runs. In the case of weak or lacking fragment products, PCR amplifications were repeated to exclude failed PCR reaction as the cause of null alleles.

Microsatellite markers (SSR)

A total of 24 Gatersleben Wheat Microsatellites (GWM) were used for analysis. The GWM markers used were previously described by Röder et al. (1998).

Data analysis

The presence and absence of alleles was scored as binary data matrix. The gene diversity also called polymorphism information content (PIC) was computed according to Nei (1973) as:

$$PIC = 1 - \acute{O}P^2_{ij}$$

Where P_{ij} is the allele frequency of the j th allele for the i th marker summed over the number of alleles. Anderson *et al.*, (1993) suggested that gene diversity is the same as the Polymorphism Information Content (PIC).

Genetic similarity was calculated according to Dice (1945). Cluster analysis was performed using the UPGMA method.

Results:

Microsatellite diversity

The 24 wheat microsatellite markers used reveled a total of 93 alleles. The fragment size ranged from 75 bp in GWM3 located on chromosome 3D to 285 bp in GWM931 on chromosome 5D. The average number of alleles per locus was 3.9 and the largest number of alleles was 9 detected on locus GWM1002. The lowest number of alleles (2 alleles) was found at GWM 291 and GWM674 (Table1). Average of allele numbers was different for each cultivar. The allele number was highest (66 alleles) in the variety Giza 168 followed by 33 alleles in Sids 1, then Gemmiza 9 with 32 alleles and the lowest was Gimmeza 7 with 30 alleles.

Analysis of gene diversity

The PIC value reflecting the gene diversity of the 24 microsatellite loci ranged from 0.48 at locus GWM 291 and GWM 674 to 0.82 at locus GWM 1016 with an average of 0.66 (Table1).

Table 1: SSR markers and their chromosomal locations, Fragment size, polymorphism information content (PIC) and allele number in four Egyptian wheat cultivars.

SSR markers	Chromosomal locations	Fragment size range	PIC	Allele no
gwm 3	3D	75-110	0.78	6
gwm234	5B	202 258	0.64	4
gwm294	2A	89-118	0.77	5
gwm408	5B	Nul, 167, 182	0.56	3
gwm497	1A, 2A, 3D	156-159	0.64	3
gwm18	1B	Null,196,189	0.64	3
gwm389	3B	114-131	0.61	3
gwm619	2B	148-164	0.67	4
gwm162	3A	208-215	0.72	4
gwm148	2B	159-166	0.72	4
gwm113	4B	Null, 147-156	0.72	4
gwm1016	5B, 6B	111 155	0.82	6
gwm1002	7D	null, 140-185	0.78	9
gwm940	6B, 4B, 2B	149 165	0.61	4
gwm777	5B	106-115	0.72	4
gwm291	5A	null, 153	0.48	2
gwm37	2D	161 189	0.68	4
gwm161	3D	151 156	0.56	3
gwm674	3A	163 165	0.48	2
gwm540	5B	124 132	0.64	3
gwm931	5D	null,270 284	0.72	4
gwm980	3B	null, 152 155	0.64	3
gwm1078	1B	139 141	0.64	3
gwm1184	7B	139 143	0.64	3
			0.661667	3.875

The correlation coefficient between Polymorphism Information Content (PIC) and number of alleles over 24 microsatellites loci was 0.62. (Figure 1).

Cluster analysis

The genetic similarity values between the cultivars were used to produce a dendogramme. The analysis was derived from a UPGMA cluster analysis which helps to explain the relationship between wheat cultivars. The genetic similarity level ranged from 0.22 for Chinese Spring with the Egyptian cultivars and 0.58 for Gimmeza 7 and Gimmeza 9.

Cluster analysis allowed to discriminate two groups. The first cluster consisted only of cultivars Gimmeza 7 and Gimmeza 9, while the second cluster comprised the other two cultivars Sids 1 and Giza 168 (Figure 2).

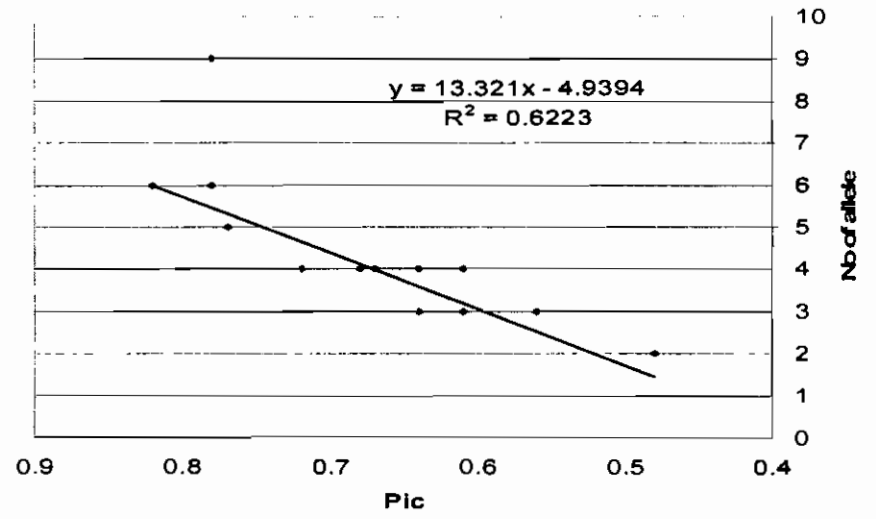

Figure 1. Correlation between gene diversity and number of alleles over 24 microsatellites loci in 4 Egyptian wheat cultivars.

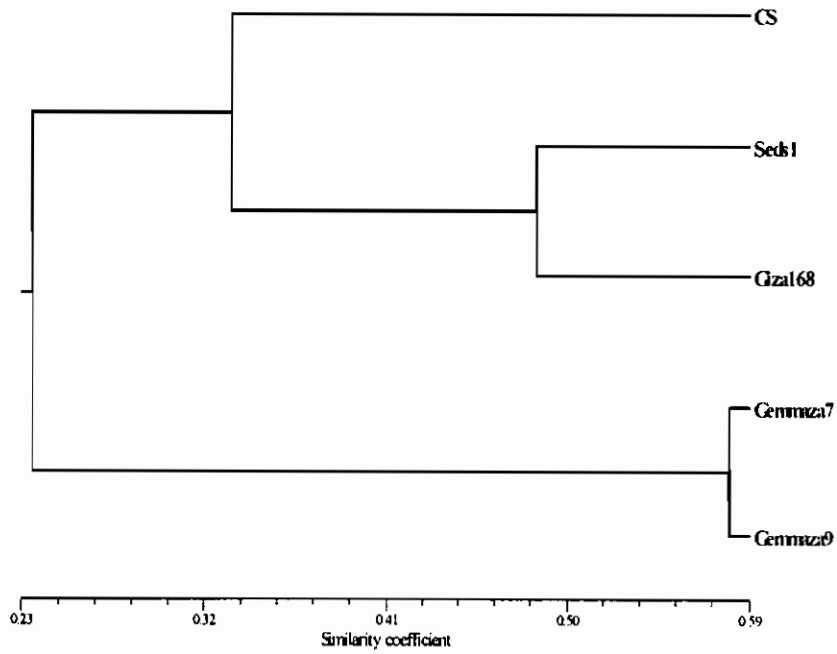

Figure 2: Dendrogramme of 4 Egyptian wheat cultivars clustered according to UPGMA using Dice's similarity coefficients.

DISCUSSION

In this study 24 microsatellites revealing 93 alleles from 5 wheat cultivars were sufficient to discriminate the germplasm for these cultivars. The average number of alleles was 3.9 and genome B was more polymorphic than A. These results were similar to previous studies on wheat (Figliuolo and Perrino, 2004) they noted that 15 markers produced 63 bands with an average of 7.7 alleles. Moreover, Teklu *et al.* (2005) reported a higher number of alleles per locus for *T. durum* than for *T turgidum*. He noted that 29 microsatellite markers revealed 320 and 271 alleles in *T. durum* and *T. turgidum*, respectively, with average number of alleles per locus of 11.0 in *T. durum* and 9.3 in *T. turgidum*. On the other hand, Bertin *et al.*, (2001) found an average number of 5.2 alleles per locus in spelt wheat. While, Eujail *et al.*, (2001) detected an average of 5.5 alleles per locus with 64 genotypes and Ben Amer et al. (2001) found an average off 4.5 alleles per locus from 24 wheat microsatellite markers with 15 Libyan wheat genotypes. The results also were confirmed by Khlestkina *et al.*, (2004) reported an average PIC-value of 0.7 in 54 Siberian wheat plants. Huang *et al.*, (2002) reported a gene diversity of 0.77 in 998 European wheat varieties. While Prasad *et al.*, (2000) found a PIC-value of 0.71 in 55 wheat genotypes.

The cluster study discriminated the investigated four Egyptian wheat cultivars. Similar results were found by Khlestkina (2004) for old and modern Siberian spring wheat varities. Huang (2002) found that not all accessions originating from the same region clustered in the same group, indicating that the genetic diversity of *T. aestivum* is not completely related to geographic distribution. In contrast, Alamerew *et al*, (2004) found that all of the used accession in his study could be separated, clustering in two large groups.

Acknowledgements

The authors grateful to the professional and personal support from InWent Organization also to Dr. Röder and her group staff of the Gene and Genome Mapping, IPK Gatersleben.

REFERENCES

Alamerew, S.; Chebotar, S.; Huang, X.Q.; Röder, M.S. and Borner, A. (2004). Genetic diversity in Ethiopian hexaploid and tetraploid wheat germplasm assessed by microsatellite markers. Gen.Res. Crop. Evol. 51, 559-567.

Anderson, J.A.; Churchill, G.A.; Autrique, J.E.; Sorrells, M.E. and Tanksley, S.D. (1993). Optimizing parental selection for genetic linkage maps. Genome 36, 181-186.

Baek, H.J.; Beharav, A. and Nevo, E. (2003). Ecological genomic diversity o microsatellites in wild barley Hordeum spontaneum, populations in Jordan. Theor. Appl. Gen. 106, 397-410.

Ben Amer, I.M.; A. Börner and M.S. Röder (2001) Detection of genetic diversity in Libyan wheat genotypes using wheat microsatellite markers. Genetic Resources and Crop Evolution 48, 579-585.

Bertin , P.; Gregoire, D.; Massart, S. and de Froidmont, D. (2001). Genetic diversity among European cultivated spelt revealed by microsatellites. Theor. Appl. Gen. 102, 148-156.

Dice, L.R. (1945). Measures of the amount of ecological association between species. Ecology 26, 297-302.

Donini, P.; Law, J.R.; Koebner, R.M.D.; Reeves, J.C. and Cooke, R.J. (2000). Temporal trends in the diversity of UK wheat. Theor. Appl. Gen. 100, 912-917.

Doyle, J.J. and Doyle, J.L. (1990) Isolation of plant DNA from fresh tissue. Focus 12, 13-15.

Eujail, M.E.; Sorrells, M.; Baum, M. ; Wolters, W. and Powell, W. (2002). Isolation of EST-derived microsatellite markers for genotzping the A and B genomes of wheat. Theor. Appl. Gen. 10, 399-407.

Eujail, M.E.; Sorrells, M.; Baum, M.; Wolters, W. and Powell, W. (2001). Assessment of genotypic variation among cultivated durum wheat based on EST-SSRs and genomic SSRs. Euphytica 119, 39.43.

Figliuolo, G. and Perrino, P., (2004). Genetic diversity and intra-specific phylogeny of *Triticum turgidum* L. subsp. dicoccon (Schrank) Thell. Revealed by RFLPs and SSRs. Gen. Res. Crop Evol., 51, 519-527.

Gupta, P.K.; Balyan, H.S. and Sharma, B.(1996). Microsatellites in Plant: new class of molecular markers, Curr. Sci. 70, 45-54

Huang, X.Q.; Börner, A., Röder, M.S. and Ganal, M.W. (2002) Assessing genetic diversity of wheat (*Triticum aestivum* L.) germplasm using microsatellite markers. Theor. Appl. Genet. 105, 699-707.

Ishii, T.; Xu, Y. and McCouch, S.R. (2001). Nuclear- and chloroplast – microsatellite variation in A-genome species of rice. Genome 44(4), 658-666.

Khlestkina, E.K.; Than M.H.M.; Pestsova, E.G.; Röder, M.S.; Malyshev,S.V; Korzun, V. and A. Börner (2004) Mapping of new 99 new microsatellite loci in rye (*Secale cereale* L.) including 39 expressed sequence tags. Theor. Appl. Genet. 109, 725-732.

Koebner, R.; Donini, P.; Reeves, J.; Cooke, R. and Law, J. (2003). Temporal flux in the morphological and molecular diversity of UK barley, Theor. Appl. Gen. 106, 550-558.

Lu, H. and Bernardo, R. (2001). Molecular marker diversity among current and historical maize inbreds. Theor. Appl. Gen. 103, 613-617.

Macaulay, M.; Ramsay, L.; Powell, W. and Waugh, R. (2001). A representative, highly informative ' genotyping set' of barely SSRs. Theor. Appl. Gen. 102, 801-809.

Matus, I.A. and Hayes, P.M. (2002). Genetic diversity in three groups of barely germplasm assessed by simple sequence repeats. Genome 45 (6), 1095-1106.

Mumm, R.H. and Dudley, J.W. (1994). A classifications of 148 U.S. maize inbreds. I. cluster analysis based on RFLPs. Crop Sci. 34, 842-851.

Nei, M. (1973) Analysis of gene diversity in subdivided populations. Proc. Natl.Acad. Sci. USA 70, 3321-3323.

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

Prasad, M.; Varshney, R.K.; Roy, J.K.; Balayan, H.S. and Gupta, P.K. (2000). The use of microsatellites for detecting DNA polymorphism, genotype identification and genetic diversity in wheat. Theor. Appl. Gen. 100, 584-592.

Röder, M.S.; Plaschke, J.; König, S.U.; Börner, A.; Sorrells, M.E.; Tanksley, S.D. and Ganal, M.W. (1995). Abundance, variability and chromosomal location of microsatellites in wheat . Mol. Gen. 246, 327-333.

Röder, M.S.; Korzun, V.; Wendehake, K.; Plaschke, J.; Tixier, M.H.; Leroy, P. and M.W. Ganal (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. and M.W. Ganal (2002). Construction and analyis of a microsatellite-based database of European wheat varieties. Theor. Appl. Genet. 106, 67-73.

Röder, M.S.; Huang, X.Q. and M.W. Ganal (2004). Wheat microsatellites in plant breeding-potential and implications. In: Molecular Markers in Plant Breeding. Springer-Verlag Heidelberg, eds. Loerz H, Wenzel G, pp. 255-266.

Teklu, Y.; K. Hammer; X.Q. Huang and M.S. Röder (2005). Analysis of Microsatellite Diversity in Ethiopian Tetraploid Wheat Landraces. Genetic Resources and Crop Evolution, 53, (6). 1115-1126.

التنوع في الجزيئات الوراثية لأربعة اصناف قمح مصرية باستخدام المعلمات الوراثية احادية الجزيئية

اسامه محمد الشيحى[١]، عبد الرحمن مرسى غلاب[١]، حلمى محمد يوسف ابراهيم[١] و ماريون رودر[٢]

١- قسم فسيولوجيا النبات - كلية الزراعه - جامعة القاهره

٢-معهد ابحاث النبات - جاترسليبين - المانيا

تهدف هذه الدراسة الى تقييم التنوع الوراثي في اهم اصناف القمح المصرية المتداولة عام ٢٠٠٥ باستخدام المعلمات االوراثية احادية الجزيئية حيث اختبرت اربعة اصناف من القمح المصري هي جيزة ١٦٨ ، سدس ١ ، جميزة ٧ و جميزة ٩ مقارنة بصنف القمح الربيعي الصيني ككنترول. استخدم ٢٤ من المعلمات الوراثية احادية الجزيئية وكنتيجة لذلك تم اظهار ٩٣ اليلا. وتراوح حجم الجزيئات الوراثية بين ٧٥ bp في GWM 3 الى ٢٨٥ bp في GWM 931 وكان متوسط عدد الاليلات ٣,٩ وتراوح بين ٢ الى ٩ اليلا لكل موقع. اختلف متوسط عدد الاليلات لكل صنف حيث اعطى اعلى قيمة له (٦٦ اليلا) في صنف جيزة ١٦٨ يتبعه صنف سدس ١ (بـ ٣٣ اليلا) ثم صنف جميزة ٩ (٣٢ اليلا) وكان اقل عدد من الاليلات في صنف جميزة ٧ (٣٠ اليلا) . ووجد ان الاختلافات الوراثية التي تعكس التنوع الجيلي من ٢٤ من المعلمات الوراثية احادية الجزيئية المستخدمة تراوح بين ٠,٤٨ الى ٠,٨٢ بمتوسط ٠,٦٦ وتراوح مستوى التماثل الوراثي من ٠,٢٢ لصنف القمح الربيعي الصيني مع الاصناف المصرية المستخدمة الى ٠,٥٨ لصنفي جميزة ٧ و جميزة ٩ ويوجد ارتباط قوي بين التنوع الجيلي وعدد الاليلات حيث كان معامل الارتباط ٠,٦٢.