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P Strelchenko

K Street

O Mitrofanova

Helen Hill
Southern Cross University

Robert J. Henry
Southern Cross University

See next page for additional authors

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Comparative assessment of wheat landraces from AWCC, ICARDA and VIR germplasm collections based on the analysis of SSR markers

Strelchenko P[1], Street K[2], Mitrofanova O[1], Hill H[3], Henry R[3], Mackay M[4]

[1]All-Russian Research Institute of Plant Industry (VIR), St. Petersburg, Russia, [2]Genetic Resources Unit, International Centre for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria, [3]Centre for Plant Conservation Genetics, Southern Cross University, Lismore, Australia, [4]Australian Winter Cereals Collection (AWCC), Tamworth, Australia

INTRODUCTION

Wheat landraces are potentially a useful germplasm resource of genetic variability for traits of interest such as stress resistance and tolerance and grain quality characteristics. For thousands of years wheat landraces have evolved under the influence of natural and artificial selection as performed by many generations of farmers. The differences in soil and climatic conditions between areas of wheat cultivation and heterogeneity of environments within these areas have led to the development of a large number of landraces adapted to local environments and which are characterized by yield stability in conditions of traditionally low input agricultural systems. Accurate assessment of landrace material is necessary to detect its genetic differentiation and facilitate efficient involvement in future breeding programs. The use of morphological and agronomical traits have been used to classify landraces, however, until recently little information about their genetic relationships was available.

The last century, only a small proportion of the landraces gene pool was utilized in wheat breeding programs. The objectives of this work were to (i) determine SSR based genetic diversity among bread wheat landraces collected in different agro- ecological zones and stored in the three gene banks, (ii) to evaluate the usefulness of SSR markers as a tool to improve the management of wheat genetic resources.

MATERIALS AND METHODS

About 16,000 bread wheat landraces from three significant gene banks were amalgamated into a virtual collection. Each of the landraces was characterized by collection site coordinates (latitude and longitude). Climatic and edaphic attributes were estimated for each collection site. In total 976 bread wheat landraces maintained in AWCC (187 accessions), ICARDA (338) and VIR (451) originated from 675 collection sites. These landraces belong to different agro ecological zones (according to FAO classification) were collected from 48 countries covering regions from Europe, Caucasus, Central Asia, and Africa were compared on the structure of 13 SSR loci mapped on different wheat chromosomes. In this study the set of landraces consisted of a random subset of 512 accessions and a subset of 479 landraces collected in dry areas.

Crude DNA was extracted from individual seedlings using one genotype per each accession. Thirteen microsatellite primer pairs, detecting 13 polymorphic loci localized on different chromosomes were selected mainly from the work of Baulforier et al. (2007). PCR amplification was performed according to Röder et al. (1998). PCR was performed using the Applied Biosystems 9700 thermocycler and cycling conditions were an initial 5-min denaturation at 95ºC followed by 34 cycles, each consisting of 30 s denaturation at 95ºC, 30 s annealing at temperatures from 45ºC to 60ºC according to primer specificity and 30 s extension at 72ºC and a final 5-min extension at 72ºC. Fragment analysis was carried out on Applied Biosystems 3730 automated sequencer and fragment sizes were calculated using ABI PRISM GeneMapper software v. 3.0. Amplified SSR fragments of different sizes were scored as different alleles and for subsequent statistical analyses the data was coded in a binary form such as the presence or absence (1 and 0) of an allele. For each microsatellite locus and for the whole set of accessions, the total number of alleles observed and the number of rare alleles were recorded.

The genetic similarity matrix was calculated using Nei's coefficient (Nei & Li, 1979). This matrix was subjected to cluster analysis according to Ward's minimum variance method (Ward, 1963). In Ward's method, the distance between two clusters is the analysis of variance (ANOVA) sum of squares between the two clusters summed over cluster members. Calculations were performed using the software package STATISTICA 6.

RESULTS AND DISCUSSION

The numbers of detected alleles for loci *Xgwm46* (chromosome 7 B), *Xgwm149* (4 B), *Xgwm186* (5 A), *Xgwm190* (5 D), *Xgwm257* (2 B), *Xgwm261* (2 D), *Xgwm285* (3 B), *Xgwm341* (3 D), *Xgwm413* (1 B), *Xgwm437* (7 D), *Xgwm469* (6 D), *Xgwm610* (4 A), *Xgwm626* (6 B) were 26, 13, 32, 18, 5, 22, 26, 27, 20, 26, 19, 20, and 15 respectively. The average number of alleles per locus was 20.7 and allele frequency ranged from 0.001 to 0.543, with an average frequency of 0.048. From a total of 269 alleles detected, 191 (71.0%)

were characterized by a frequency of less than 0.05 and considered as rare alleles and 40 alleles (14.9%) were identified as unique. For each locus, between two to four alleles were detected and had a frequency of more than 0.10, whilst only for two alleles (*Xgwm*261_{176} and *Xgwm*626_{104} on chromosomes 2D and 6B respectively) the frequency was more than 0.50.

Based on the analysis of 13 microsatellite loci, 937 unique genotypes were identified from 976 landraces. At the same time, it was revealed that 21 groups each had between two to nine identical genotypes. Some of the landraces which formed the groups were taken mainly from the same gene bank and predominantly were collected in the same site or neighbor sites. Such landraces might be considered as doublets. Quantitatively allele diversity was somewhat similar among the genotypes from each of the three gene banks. In total, 194, 210 and 236 alleles were revealed in the landraces from AWCC, ICARDA, and VIR respectively. Among these alleles the number of specific alleles detected in these gene banks amounted to 10, 19, and 32. On average Nei's coefficients for all possible pairs of genotypes was 0.18. In the cluster analysis a complex pattern of genetic relationships between the landraces has been shown.

All of the analyzed genotypes have been divided into six groups with genetic linkage about 10 (Fig 1). Group 1 was the most distant and included 38 genotypes from Tunis and 21 genotypes from Algeria. All of these from Group 1 were chosen from ICARDA and were very similar between each other. Based on the analysis of their introduction history it was shown that these genotypes are a set of lines selected from a few landraces received from the Bari Germplasm Institute, Italy. This group of genotypes had very low levels of SSR loci polymorphism and also based on morphological traits this material has confirmed genetic uniformity. Genotypes originating from different regions were combined in each of the groups 2-6. However, genotypes from Central Asia were mainly included into groups 2-4, while genotypes from Europe and Africa – into groups 5-6. The majority of genotypes from the Caucasus clustered into groups 2 and 3. All identified groups were differentiated based on the frequency of many alleles. Such distinctions for 11 alleles have been demonstrated on Fig. 1. Interestingly, for the *Xgwm* 257 locus on chromosome 2B, 3 alleles out of the 5 alleles identified showed the most significant differences.

We estimated distribution of 41 very rare SSR alleles, which were represented only in 4 -7 genotypes. These alleles were revealed in landraces from different countries and had a different level of genetic similarity. More frequently very rare alleles were represented in landraces from Central Asia, especially from Tajikistan, and more seldom in landraces from other regions. The presence of two very rare alleles was detected in 24 genotypes and approximately half of these originated from Pakistan and Tajikistan. Based on this data it may be proposed that such alleles have mainly arisen independently in different regions or that these genotypes were more widely distributed in former times, but have since remained in only a few sites. Analysis of the genotype distribution originating from one collection site among six clusters has demonstrated genotypes from each of 18 collection sites included in anyone cluster, while the genotypes from 70 sites were detected in different clusters. Despite there being 26 specific alleles identified among the D-subset of landraces, accessions of this subset were included in all clusters.

The application of modern technologies, which allow the study of DNA polymorphisms in a large set of landraces, makes it possible to identify the structure of genetic diversity in wheat collections from different gene banks and help preserve the maximum amount of wheat variability. The identification of germplasm collection structure is useful for strategy establishment in germplasm management and will increase the efficiency of selecting the initial material for wheat breeding.

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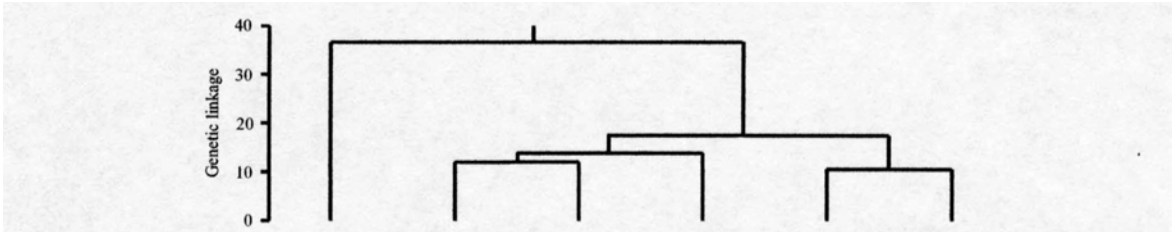

Allele	Chr.	1 (66)	2 (206)	3 (148)	4 (164)	5 (175)	6 (217)	Total (976)
257_{192}	2B	0.92	0.07	0.83	0.09	0.08	0.20	0.28
257_{194}	2B	0.03	0.81	0.10	0.20	0.22	0.40	0.35
257_{196}	2B	0.05	0.13	0.05	0.72	0.69	0.41	0.37
261_{176}	2D	0.00	0.37	0.64	0.86	0.62	0.39	0.52
149_{153}	4B	0.97	0.10	0.37	0.05	0.37	0.76	0.39
149_{163}	4B	0.00	0.16	0.25	0.47	0.41	0.14	0.25
149_{165}	4B	0.00	0.64	0.23	0.31	0.09	0.04	0.25
610_{169}	4A	0.02	0.52	0.47	0.49	0.26	0.27	0.37
626_{104}	6B	0.98	0.60	0.61	0.24	0.85	0.28	0.54
626_{120}	6B	0.00	0.12	0.07	0.55	0.04	0.03	0.14
626_{133}	6B	0.02	0.17	0.21	0.05	0.03	0.57	0.21

Fig. 1. Genetic relationship between six groups of wheat landraces based on analysis of microsatellites and differences in the frequency of some alleles in the group.