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Short Communication

Genetic diversity and structure found in samples of Eritrean bread wheat

Zeratsion Abera Desta[1]*, Jihad Orabi[2], Ahmed Jahoor[3] and Gunter Backes[4]

[1]*Department of Plant Breeding, Swedish University of Agricultural Sciences, Sundsvagen 14, Box 101, Alnarp, SE 23053, Sweden,* [2]*Department of Agriculture and Ecology, Faculty of Life Sciences, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg, Copenhagen, Denmark,* [3]*Nordic Seed, Højbygårdvej 14, 4960 Holeby, Denmark and* [4]*Department of Organic Plant Breeding and Agrobiodiversity, Faculty of Organic Agricultural Sciences, Kassel University, Nordbahnhofstr. 1a, 37213 Witzenhausen, Germany*

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Abstract

Genetic diversity and structure plays a key role in the selection of parents for crosses in plant breeding programmes. The aim of the present study was to analyse the genetic diversity and structure of Eritrean bread wheat accessions. We analysed 284 wheat accessions from Eritrea using 30 simple sequence repeat markers. A total of 539 alleles were detected. The allele number per locus ranged from 2 to 21, with a mean allele number of 9.2. The average genetic diversity index was 0.66, with values ranging from 0.01 to 0.89. Comparing the three genomes of wheat, the B genome had the highest genetic diversity (0.66) and the D genome the lowest diversity (0.61). A STRUCTURE analysis based on the Bayesian model-based cluster analysis followed by a graphical representation of the distances by non-parametric multidimensional scaling revealed a distinct partition of the Eritrean wheat accessions into two major groups. This is the first report of the genetic diversity and structure of Eritrean bread wheat.

Keywords: Bayesian structure; genetic distance; genetic diversity; microsatellites; population structure; *Triticum aestivum*

Experimental

Most diversity studies on wheat from the Horn of Africa have dealt with tetraploid wheat (Teklu and Hammer, 2006; Hailu *et al.*, 2010; Takenaka *et al.*, 2010), but studies on the analyses of diversity of bread wheat (Alamerew *et al.*, 2004) are extremely rare. A rapid loss of genetic resources has been reported in Ethiopia by Teklu and Hammer (2006), mainly due to an increasing adoption of higher performing varieties coming from abroad. This phenomenon has not yet been investigated in Eritrea and, therefore, analysis of genetic diversity within and between populations of Eritrean wheat is of paramount importance.

Plant material

Accessions of 284 bread wheat (*Triticum aestivum*) landraces were collected directly from Eritrean farmers' fields (Supplementary Fig. S1, available online only at http://journals.cambridge.org).

* Corresponding author. E-mail: zeraygreat@gmail.com

DNA isolation and genotyping

DNA of the Eritrean wheat accessions was extracted using the Cetyl Trimetheyl Ammonium Bromide (CTAB) method according to Saghai-Maroof *et al.* (1984). All samples were genotyped using 30 microsatellite primers distributed over the whole wheat genome (Supplementary Table S1, available online).

PCR amplification was performed in Thermo-Fast 96-well plates with a final reaction volume of 10 µl containing 50 ng template DNA. The amplification reaction was carried out with a GeneAmp® PCR System 2700 thermal cycler (Applied Biosystems, Foster City, CA, USA). The resulting fragments were detected on an ABI 3130xl DNA Analyser (Applied Biosystems) and analysed using GeneMarker software version 1.85 (Softgenetics, State College, PA, USA).

Statistical analysis

The modified Roger's distance (MRD) was calculated using the Microsoft Excel (Microsoft, Redmond, WA, USA) Visual Basic for Applications program according to the formula of Wright (1978):

$$\mathrm{MRD} = \sqrt{\frac{1}{2m}\sum_{i=1}^{m}\sum_{k=1}^{a_i}(p_{ij} - q_{ij})^2},$$

where p_{ij} and q_{ij} are the allele frequencies of the jth allele at the ith marker, a_i is the number of alleles at the ith marker and m is the number of loci or simple sequence repeats (SSRs).

Gene diversity (GD) was calculated according to the formula of Nei (1973):

$$\mathrm{GD} = 1 - \sum_{i=1}^{n}(p_{ij})^2,$$

where p_{ij} is the frequency of the jth allele for the ith locus summed across all the alleles for the SSR locus. The polymorphic information content of individual markers was calculated with the formula of Yasuda (1988):

$$\mathrm{PIC} = 1 - \sum(p_{ij})^2 - (\sum(p_{ij})^2)^2 + \sum((p_{ij})^2)^2.$$

A total of 539 alleles were obtained (Table 1). The average gene diversity of the Eritrean wheat accessions was 0.66, with values ranging from 0.01 to 0.89 (Supplementary Table S2 available online). The average allele number per locus ranged from 2 to 21, with a mean value of 9.2. Among the three genomes of wheat, the highest gene diversity was found for the B genome (0.69) compared with the A genome (0.67) and D genome (0.61).

Population structure was analysed with a Bayesian model-based clustering approach using 'STRUCTURE' v.2.3 software (Pritchard *et al.*, 2009). The number of groups (K) was set from 1 to 12 having 20 replicates of runs with a 'burn-in' period of 10,000 iterations and 10,000 Markov chain Monte Carlo (MCMC) iterations (Supplementary Fig. S1, available online) to determine the optimal number of groups according to Evanno *et al.* (2005). Finally, ten replicates with an optimal number of groups were run at a 'burn-in' period and MCMC iterations of 100,000.

Based on the MRD, a non-parametrical multidimensional scaling (npMDS) was used to visualize the similarity between the two Eritrean groups inferred by the STRUCTURE analysis. For this purpose, the R language and environment software (v.2.11.1; R Development

Table 1. Different genetic diversity indices split up into the different genomes (A, B and D genomes) for the modified Roger's distances (MRD), the total number of allele observations, the average number of alleles, gene diversity and polymorphic information content (PIC)

	Distances[a]	Total number of allele observations			Number of alleles			Gene diversity[b]			PIC		
Genomic markers[c]	Er-1–Er-2	Er-1[d]	Er-2[d]	Total	Er-1	Er-2	Total	Er-1	Er-2	Total	Er-1	Er-2	Total
All genomes	0.49	343.23	196.00	539.23	7.90	5.97	9.20	0.60	0.47	0.66	0.57	0.44	0.63
A	0.47	346.73	194.91	541.64	7.18	6.64	8.64	0.55	0.55	0.67	0.52	0.52	0.63
B	0.49	341.23	197.54	538.77	9.5	6.23	10.69	0.65	0.47	0.69	0.62	0.44	0.66
D	0.52	343.23	196.00	539.23	6.00	4.43	7.29	0.57	0.34	0.61	0.52	0.30	0.58

[a] Genetic distance was calculated based on the MRD formula (Wright, 1978): $MRD = \sqrt{\frac{1}{2m}\sum_{i=1}^{m}\sum_{k=1}^{a_i}(p_{ij} - q_{ij})^2}$. [b] Gene diversity was according to the formula of Nei (1973): $GD = 1 - \sum_{i=1}^{n}(p_{ij})^2$. [c] All genomes indicate the markers employed in the A, B and D genomes [d] 'Er-1' and 'Er-2' represent the Eritrean wheat accessions of groups 1 and 2, respectively, inferred by the STRUCTURE analysis.

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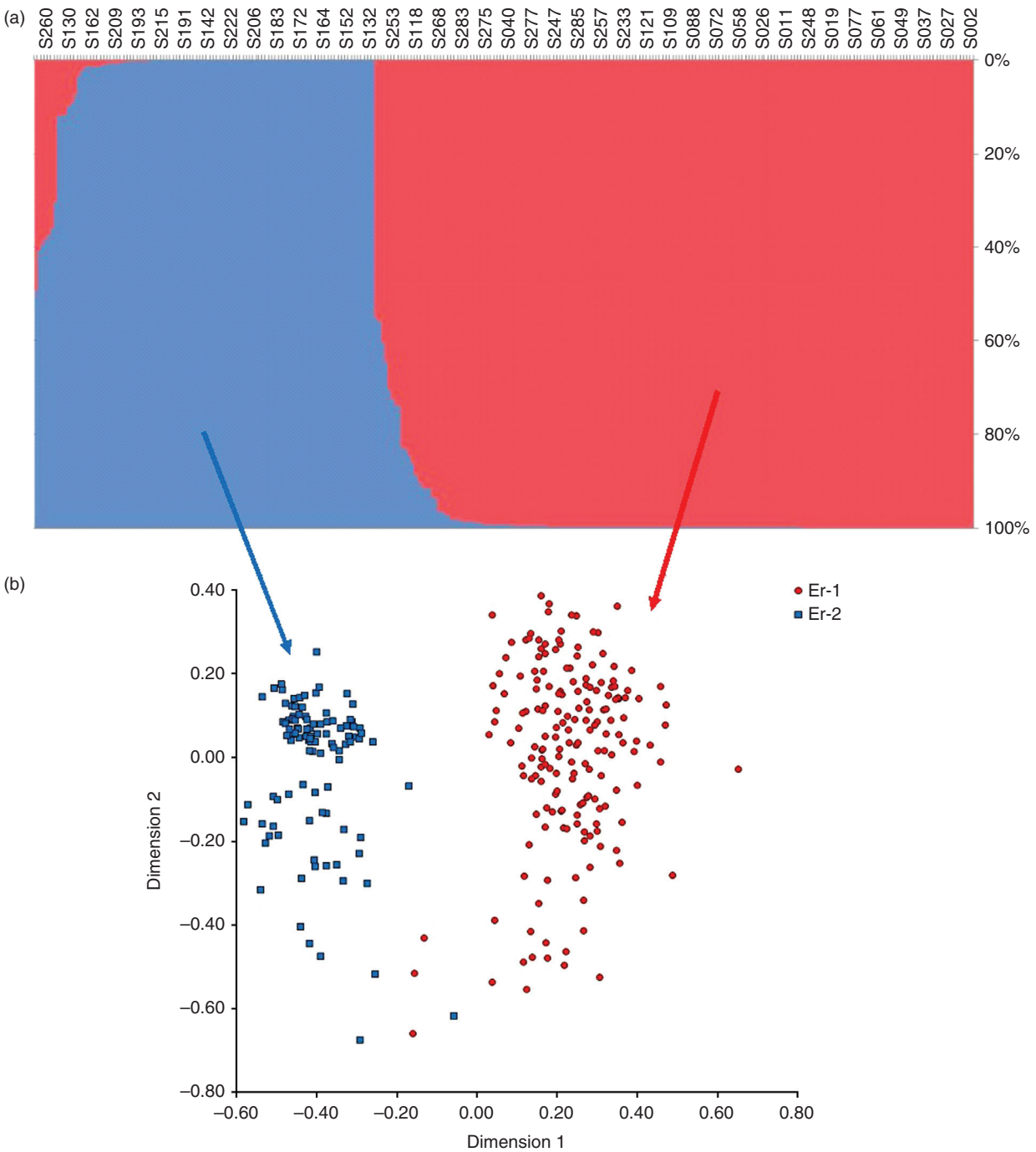

Fig. 1. (colour online) (a) Stacked bar diagram of the *q* values of the analysed accessions was generated by the program STRUCTURE (only the name of every tenth accession is shown) for the two Eritrean groups: red bars, Er-1 group; blue bars, Er-2 group. (b) Results of the non-parametric multidimensional scaling (npMDS) with two dimensions. The distance in the 2D space approximates the genetic distance between the accessions. Er-1 refers to Eritrean group 1 indicated by circles and red colour, while Er-2 refers to Eritrean group 2 represented by squares and blue colour. The MDS showed a stress statistic of 18.23%. The arrows indicate the corresponding groups inferred by the STRUCTURE analysis (a) to the accessions in the npMDS graph (b).

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Core Team, 2011) including the 'Modern Applied Statistics with S-plus' package was employed (Venables and Ripley, 2002).

The npMDS analysis separated the 284 Eritrean bread wheat accessions into two major groups, which was in agreement with assignments generated by the STRUCTURE analysis (Fig. 1). The two major groups of Eritrean bread wheat consisted of 181 accessions, of which 103 accessions were related to Er-1 and Er-2, respectively (Supplementary Fig. S1 available online only at http://journals.cambridge.org). The genetic distance between the two groups of Eritrean accessions (Er-1 and Er-2) was 0.49 (Table 1).

Discussion

The clear separation of the Eritrean accessions into two groups (Fig. 1) was rather surprising, as the genetic distance between the Er-1 and Er-2 groups was 0.49 (Table 1). The interpretation of the finding of these two different groups in Eritrean accessions is difficult based on the available data only. As this is the first report on Eritrean bread wheat, no other published data can be consulted to help in the interpretation. Location was not in agreement for the separation of these two groups. It is a usual cropping system in Eritrea to plant mixed varieties as a means of increasing yield stability and risk minimizing strategy (Woldeamlak *et al.*, 2008). One possibility could be that the farmer's conscious selection (for different purposes such as baking quality, disease and pest resistant) might lead to two separate groups within Eritrean wheat landraces. Brown (2000) described how genetic structure could be formed by selection and population fragmentation, and, again, these can be maintained by selection, isolation and the absence of hybridization. Another possibility could be that germplasm was exchanged among farmers from adjacent countries. Therefore, one of the two groups (perhaps the improved bread wheat cultivar or landrace) might be brought from a neighbouring country such as Ethiopia.

The Eritrean wheat gene pool presents an important genetic resource, as it shows a high genetic diversity compared with the small area where it is grown. The detection of two clearly separated sub-populations in the first report on Eritrean bread wheat makes it clear that conservation efforts (both *in situ* and *ex situ*) have to deal with both groups.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S1479262113000324

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