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Genetic diversity of Cameroonian bread wheat (*Triticum aestivum* L.) cultivars revealed by microsatellite markers

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The assessment of genetic diversity is a key prerequisite for studying the adaptation of populations to new environmental conditions, and therefore for the selection of new varieties. The present investigation aimed to estimate the levels and genetic structure within bread wheat varieties grown in Cameroon. Thus, genetic diversity was assessed in 17 hexaploid wheat cultivars, using 11 microsatellite markers. Genetic resources were collected in the Northwest, Adamawa and North Regions. All pairs of specific marker loci used gave amplifications with allelic variations of size on all DNA of wheat accessions. A total of 77 alleles were detected among cultivars and the number of alleles per locus ranged from 2 to 13 with an average of 7, comparable to those observed in most previous studies. Gene diversity ranged from 0.46 (Xgdm 125) to 0.90 (Xgwm 177) with an average of 0.88, increasing with the number of alleles, with a correlation coefficient of 0.88 (Adamawa) and 0.76 (Northwest). Microsatellite markers used had an average value of polymorphic information content (PIC) of 0.69, indicating that these markers are highly informative in this study. These markers are valid and will make a contribution to the studies in hexaploid wheat. Moreover, cluster analysis at a genetic similarity of 80% and the principal component analysis, where the first two components explaining 59.86% of variation which structured 17 accessions in 5 main distinct groups. This high diversity revealed among wheat accessions, grown in Cameroon could be used in the breeding programs.

Key words: Genetic diversity, bread wheat (*Triticum aestivum* L.), simple sequence repeats (SSR), Cameroon.

INTRODUCTION

The global demand for wheat yields has been estimated to increase by 50% in 2050, in order to feed the world's

growing population (Grassini et al., 2013; Allen et al., 2017). To meet this demand, wheat production should be increased through agricultural intensification in cropping regions areas. To this end, Rajaram and Hettel (1994) had delimited 12 Mega environments (MEs) for wheat cultivation, where three correspond to several agroecological areas in Cameroon. Among them, the main Cameroonian areas are in the North, North-West and Adamawa Regions.

Wheat production is a means of subsistence for many families in Cameroon. Indeed, the cultivation of wheat has started in Cameroon since 1975, through the Development Society for the Cultivation and Processing of Wheat (SODEBLE). Located in Wassandé (Adamawa's Region), the SODEBLE has grown wheat, converted wheat into flour, marketed and carried trials, in order to improve the production technics. Before its closure, this Company had produced high yielding wheat lines resistant to major fungal diseases (Monthé Biris and Habas, 1980). Twelve of these bread wheat varieties were evaluated for the agronomic traits in the North-West Region (Ayuk-Takem, 1984).

In addition, Ayuk-takem (1984) evaluated the agronomic characteristics of 12 varieties of bread wheat in the Northwest Region to identify high yielding varieties for Bui and other agro-ecological zones in high altitudes in Cameroon. The study showed that the local variety (IRAB-1) had the highest yield (4.1 t/ha), but with a non-significant difference with the varieties Chris Mutageneuse (3.5t/ha) and wheat Blésil 430 (4 t/ha). However, the yields of these three varieties were significantly better compared to all other tested varieties. These varieties had also been subjected to various agronomic tests in 1985/1986. In doing so, certain varieties had not been made available to Cameroonian farmers. Until today, beyond these agronomic evaluations, no studies have ever been carried out on the genetic variability of wheat cultivars grown in Cameroon.

Evaluating the genetic diversity is a prerequisite for studying the adaptation of populations to new environmental conditions and hence for the selection of new varieties. The loss of genetic diversity due to modern breeding practices has been reported in several studies (Fu et al., 2005). Several authors have shown that the narrowness of crop genetic diversity could lead to increased susceptibility to diseases and pests, as well as the inability of plants to respond to different environmental constraints (Gorji and Zolnoori, 2011). Therefore, it is necessary to estimate the level of genetic diversity within existing varieties to serve as a base for strategies development geared in the management and exploitation of genetic resources.

In this context, the use of molecular markers to assess genetic diversity is necessary because, unlike phenotypic markers, they are independent from environmental effects (Reza et al., 2015). Several markers, independently or in combination with others, were efficiently used for wheat genetic diversity analyses, including morphological traits (Sonmezoglu et al., 2012). Randomly amplified polymorphic DNAs (RAPDs) (Mukhtar et al., 2002), amplified fragment length polymorphisms (AFLPs) (Reza et al., 2015), restriction fragment length polymorphism (RFLPs) (Bohn et al., 1999) and diversity array technology (DArT) markers have recently been developed and used for genetic diversity assessment and mapping (Ryan et al., 2009), as well as Single nucleotide polymorphisms (SNPs) (Froese and Carter, 2016).

On the other hand, the use of simple sequence repeats (SSRs) markers combines with many desirable marker properties such as abundance, high levels of polymorphism (unlike RFLP), very good reproducibility (compared to RAPD), and co-dominance (contrary to the AFLP for which codominance is not exploitable), but also an even coverage of the genome and the specificity of amplification. In wheat, SSRs markers have been used successfully in a wide range of applications such as genotype identification (Prasad et al., 2000), diversity studies (Akfirat and Uncuoglu, 2013) and genetic mapping. This present study aimed to assess the level of genetic diversity of bread wheat accessions grown in Cameroon.

MATERIALS AND METHODS

Plant material and genomic DNA extraction

The plant material consists of 17 cultivars of bread wheat (*Triticum aestivum* L.) collected in six villages located in two Regions of Cameroon (Table 1). Among them, 11 accessions were collected in five villages of Northwest and 6 were collected from one village (Wassande) of Adamawa region. The cultivars of the North West are mainly local seeds, whereas those of Adamawa were originally given by the SODEBLE and some others were imported from Tchad. In our study, we collected all materials used by farmers in those regions.

An adjusted Doyle and Doyle (1990) protocol was used to extract genomic DNA (gDNA) from seedlings at the two to three leaf stage.

Microsatellite markers and PCR amplification

Eleven wheat microsatellite markers for 11 loci located in the chromosomes 1A, 2A, 2D, 3A, 3B, 4D, 5D, 6B and 7D, were used for genetic diversity analysis. Xgwm and Xwmc markers were obtained, respectively from Röder et al. (1998) and Somers and Isaac (2004; Grain Genes).

PCR reactions were carried out in 14 µl reaction mixtures of KAPA2GTM Fast Multiplex PCR Mix, 6.25 µM of each forward and

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Table 1. Wheat cultivars used and their origins in Cameroon.

S/N	Samples' names	Local name	Village	Region
1	Ngm 2	Ngm 2	Wassande	Adamawa
2	Fuanb2	Fuanb2	Fuanentui	Northwest
3	Babankit	Babankit	Smal Babanki	Northwest
4	Alexander wonder	Alexander wonder	Boyo	Northwest
5	Fuanb1	Fuanb1	Fuanentui	Northwest
6	Sonalika	Sonalika	Wassande	Adamawa
7	Fuanb3	-	Fuanentui	Northwest
8	Fuanb4	-	Fuanentui	Northwest
9	HGW	Hard wheat	Abongphen	Northwest
10	BBT2		Abongphen	Northwest
11	WASSANDE 2	WASSANDE 2	Wassande	Adamawa
12	Vrack	Vrack	Bambui	Northwest
13	Ngderem4	-	Wassande	Adamawa
14	Ngderem1	Ngderem1	Wassande	Adamawa
15	Ngderem3	-	Wassande	Adamawa
16	IRAT 10	IRAT 10	Bambui	Northwest
17	RIBA	RIBA	Boyo	Northwest

reverse primer, 1 µl gDNA and dH_2O. The PCR cycling conditions was set at 94°C for 3 min of denaturation, followed by 45 cycles of 1 min at 94°C, 1 min at the annealing temperature (Ta), 2 min at 72°C and then 72°C for 10 min for extension.

The PCR products were electrophoresed on 6% non-denaturing polyacrylamide gels containing 1xTBE (Tris Borate EDTA). The amplified band sizes for each SSR locus were determined on the basis of their migration relative to the 50 bp marker.

Data analysis

The molecular diversity within all accessions was estimated for each SSR locus, using the Power Marker 3.25 software (Liu and Muse, 2005). To measure the informative character of the SSR markers, the PIC for each marker was calculated using the formula of Nei (1973):

$$PIC = 1-\Sigma_{i=1}^{k} P_i^2$$

Where, k is the total number of alleles detected per locus and Pi the frequency of the allele i in all 17 accessions.

Genetic similarity (GS; Dice, 1945) was calculated as:

$$GS = 2N_{ij}/(N_i + N_j)$$

Where, N_{ij} is the number of fragment common to individual i and j, and (Ni + Nj) is the total number of fragment in both individuals.

Genetic distance (GD) among group pairs was calculated following Nei and Li (1979),

$$(GD_{xy}) = 1-(2N_{xy}/N_x + N_y)$$

The dendrogram was constructed using the method based on the genetic distance (SAHN method, UPGMA algorithm) of the 17 accessions and using the software Statistica 12. To calculate allele frequency (A_{xy}) from one of the variation to another in each locus, the formula of Khlestkina et al. (2004) was used:

$$A_{xy} = \Sigma|P_{xi} - P_{yi}|N_{xy}$$

Where, P_{xi} and P_{yi} are the frequencies of the i^{th} allele in regions X and Y, respectively, and N_{xy} is the total number of alleles for the two groups X and Y. The allelic frequency variation was calculated separately for each of the 11 loci and then for all of them as an average. All fragments were used to generate GS matrix for Principal Component Analysis (Sneath and Sokal, 1973).

RESULTS

Characteristics of markers and genetic diversity

All pairs of primers specific for SSR locus used resulted in a positive amplification with allelic variations in size on all DNA of wheat accessions. A total of 77 microsatellite alleles were detected. The number of alleles per locus varied from 2 (Xgwm 125 and Xgwm 331) to 13 (Xwmc 177), with an average of 7 alleles per locus. Genetic diversity for microsatellite loci ranged from 0.46 (Xgdm 125) to 0.90 (Xgwm 177) with an average of 0.88. The polymorphism information Content (PIC) varied from 0.25 (Xwmc 331) to 0.89 (Xwmc 177), with an average of 0.69 (Table 2).

The results indicated a significant correlation ($P < 0.01$) between gene diversity and number of alleles across wheat accessions in both Regions (Figure 1). The correlation coefficient between these two variables over the 11 loci were 0.88 (Adamawa) and 0.76 (Northwest).

Genetic relationship and diversity among different geographical regions

Genetic distance value (GD) indicates that some

Table 2. Description of SSR Markers.

Locus	Chromosome position	Primers sequences	Repeat	Bases expected	Annual temperature	Alleles frequency	Number of alleles	Gene diversity	PIC
Xwmc 11	1A, 3A	5' TTGTGATCCTGGTTGTGTTGTGA 3' 5' CACCCAGCCGTTATATATGTTGA 3'	CT	177	61	0.29	8	0.83	0.81
Xwmc 59	1A. 6A	5' TCATTCGTTGCAGATACACCAC 3' 5' TCAATGCCCTTGTTTCTGACCT 3'	(CA)19	197	58	0.18	10	0.89	0.87
Xwmc 177	2A	5' AGGGCTCTCTTTAATTCTTGCT 3' 5' GGTCTATCGTAATCCACCTGTA 3'	(CA)21	184	52	0.18	13	0.90	0.89
Xgwm 190	5D	5' GTGCTTGCTGAGCTATGAGTC 3' 5' GTGCCACGTGGTACCTTTG 3'	(CT)22	201-253	55	0.18	9	0.87	0.86
Xgwm 437	7D	5' GATCAAGACTTTTGTATCTCTC 3' 5' GATGTCCAACAGTTAGCTTA 3'	(CT)24	109-111	47	0.18	10	0.88	0.87
Xgwm 539	2D	5' CTGCTCTAAGATTCATGCAACC 3' 5' GAGGCTTGTGCCCTCTGTAG 3'	(GA)27	143-157	60	0.24	8	0.83	0.81
Xdgm 125	4D	5' GCAGGCGTGTTACTCCAAGT 3' 5' CCGAGGTGGATAGGAGGAAA 3'	-	-	60	0.65	2	0.46	0.35
Xwmc 331	4D	5' CCTGTTGCATACTTGACCTTTTT 3' 5' GGAGTTCAATCTTTCATCACCAT 3'	-	128	61	0.82	2	0.29	0.25
Barc 133	3B	5' AGCGCTCGAAAAGTCAG 3' 5' GGCAGGTCCAACTCCAG 3'	(CT)24	-	-	0.65	4	0.52	0.47
Xgwm 133	6B	5' ATCTAAACAAGACGGCGGTG 3' 5' ATCTGTGACAACCGGTGAGA 3'	(CT)39	-	-	0.35	4	0.72	0.67
Xgwm 644	6B	5' GTGGGTCAAGGCCAAGG 3' 5' AGGAGTAGCGTGAGGGGC 3'	(GA)20	-	-	0.29	7	0.79	0.76
Mean	-	-	-	-	-	0.36	7	0.72	0.69

Xgwm and Xwmc markers were obtained respectively from Röder et al. (1998) and Somers and Isaac (2004; Grain Genes); PIC, Polymorphism information content.

accessions are closely related. The GD over accessions in all regions ranged from 0.18 (between Wassande2 and NGDEREM3) to 1 with a mean of 0.8 (80%). So, at 80% of genetic divergence, the 17 wheat cultivars studied were structured into 5 main groups (A, B, C, D and E) in the dendrogram based on the UPGMA analysis using SSR data (Figure 2). Group A included 4 cultivars (Alexander wonder, Riba, Vrack and FUANB3).

Very close to 80% of genetic dissimilarity, the group B could be divided into two subgroups: subgroup B1 contained 6 accessions (BABANKIT, FUANB1, FUANB4, FUANB2, BBT2 and HGW) while subgroup B2 included only one cultivar (IRAT 10). It is noteworthy that the two varieties FUANB1 and FUANB4 are identical. Group C contained 4 cultivars (Ngderem1, Ngderem3, WASSANDE 2 and NGM2) while Groups D and E each contained 1 cultivar, respectively (Ngderem4 and SONALIKA).

Furthermore, the principal component analysis (PCA) for the six-collection village split the accessions into five clearly distinct groups. The first two principal components had Eigen values of 6.36 and 3.81. The PCA grouped the 17 wheat accessions into various components with the first two explaining 59.86 and 37.44% of the total variation. Accessions from each village were approximal clustered together (Figure 3). So, 80% of the genetic material from the same geographical village could be clustered in specific groups.

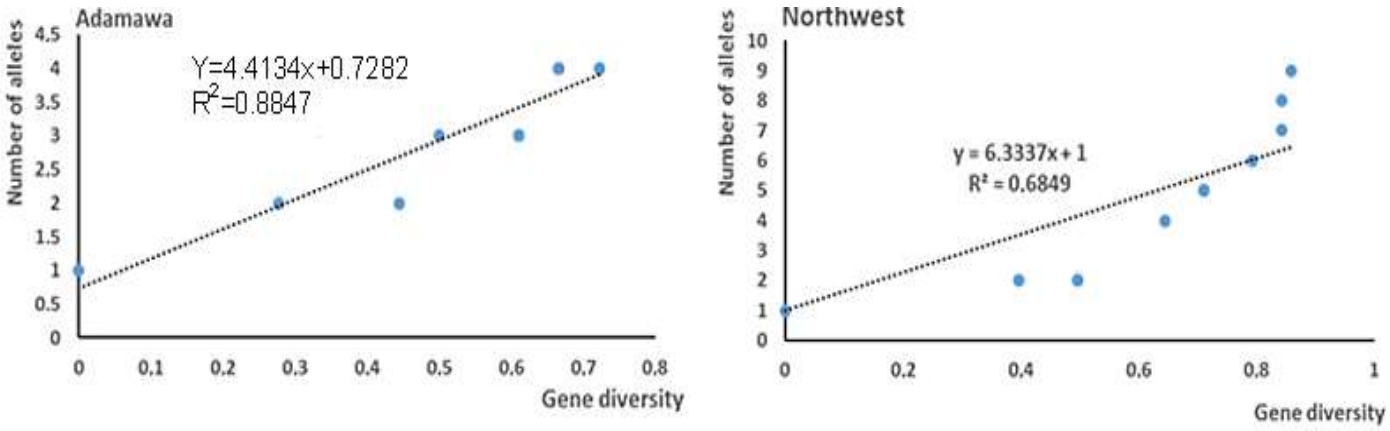

Figure 1. Correlation between gene diversity and the number of alleles over 11 microsatellite loci in hexaploid wheat.

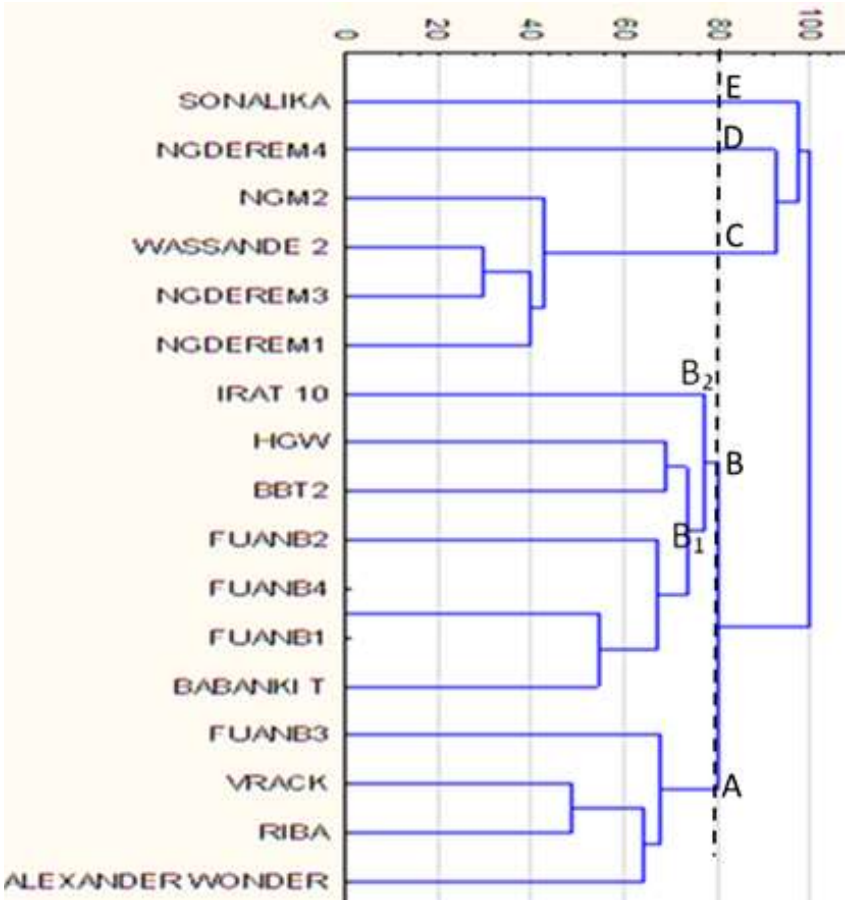

Figure 2. Grouping according to the dissimilarities between 17 accessions of hexaploid wheat on the basis of the SSR profiles of 11 loci.

Accessions were then analyzed separately according to their region of origin (Adamawa and Northwest). A comparison of the genetic diversity of wheat accessions was done between two germplasm pools. The mean of gene diversity, number of alleles per locus, total number of alleles and the number of accessions carrying rare alleles were higher in Northwest, compared to those in Adamawa Region (Table 3). These results suggest that

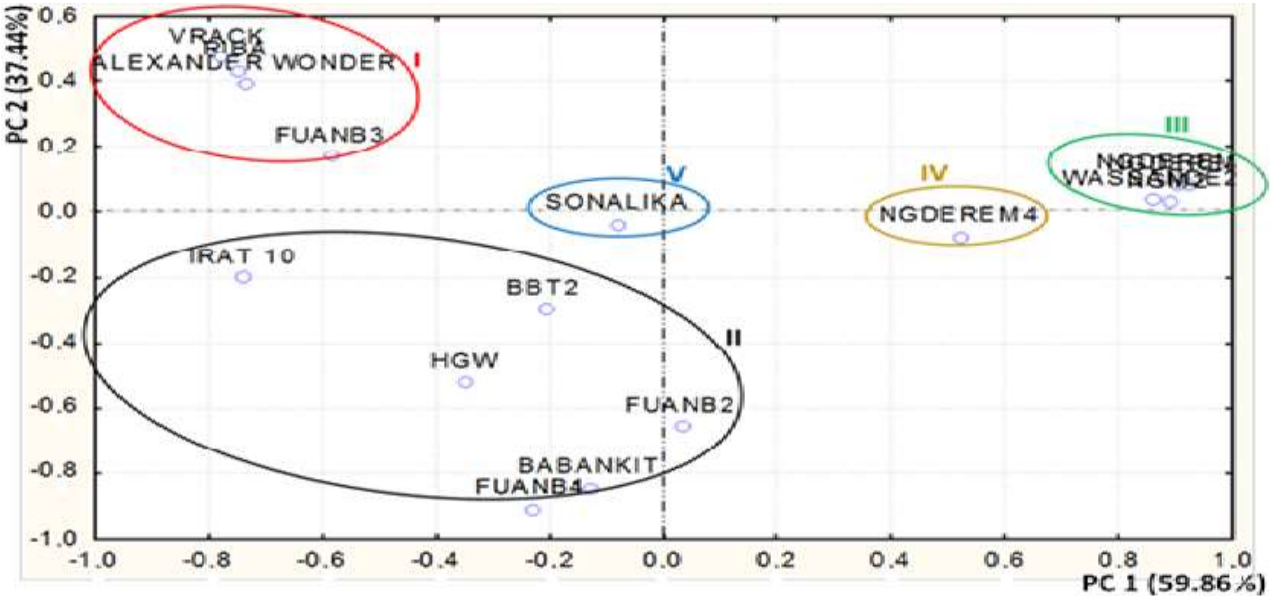

Figure 3. Principal component analysis of 17 hexaploid wheat accessions from 6 villages in Cameroon. The grouping is based on Dice's similarity coefficients.

Table 3. Analysis of geographical regions.

Item	Northwest	Adamawa
Number of accessions	n = 11	n= 6
Total number of alleles	51	34
Average number of alleles per marker	4.64	3.09
Number of rare alleles	5	6
Mean of PIC-values	0.57	0.48
Mean of Gene diversity	0.62	0.54

the Northwest area exhibited greater genetic diversity than Adamawa region, even after taking into account the effect of collection size.

DISCUSSION

Diversity of SSR markers

In the present study, 11 microsatellite markers revealing 77 alleles allowed to discriminate 17 cultivars of hexaploid wheat collected in Cameroon. The number of alleles per locus ranged from 2 to 13 with an average of 7. Röder et al. (2002) detected an average of 10.5 alleles per locus from 502 recent European wheat varieties, using 19 microsatellite markers. Khaled et al. (2015) used 17 SSR markers to assess genetic diversity of 33 genotypes of hexaploid wheat from Egypt and detected an average of 5.59 alleles per locus.

The average number of alleles per locus in the study is thus comparable to those observed in previous studies. In addition, the microsatellite markers we used had an average PIC value of 0.69, which means that these markers are highly informative in our study. Indeed, Botstein et al. (1980) reported that a PIC value higher than 0.5 is considered to be a sign of a very informative marker, while 0.5> PIC> 0.25 corresponding to an informative marker. In previous studies, Röder et al. (2002) found an average PIC value of 0.67 in 500 genotypes. The choice of these SSR loci is therefore relevant for our study.

Genetic relationship between wheat cultivars

Cluster analysis discriminated all cultivars of Cameroonian hexaploid wheat into five main groups. Most cultivars clustered according to their geographical location. Indeed, all accessions from groups B and C are cultivated in the Northwest and Adamawa Regions,

respectively. Similarly, the 4 varieties of group A were collected in the Northwest Region. Moreover, the variety NGDEREM4 of group D comes from Adamawa and the SONALIKA variety of group E was introduced in Cameroon since 1975 through the SODEBLE Company, from Mexico. Huang et al. (2002) reported that the genetic diversity of hexaploid wheat was not completely related to geographic distribution. They also reported that, these results might be explained by the fact that similar genetic variation occurred independently in the different geographic regions or that artificial transfer of accessions from one region to others resulted in a false determination of the geographic origin.

Similar results were obtained by Khaled et al. (2015) in hexaploid wheat genotypes grown in Egypt. On the other hand, Al-Khanjari et al. (2007) found that all local varieties of wheat from the same geographical area clustered in the same group. In our case, we can hypothesize that the genetic proximity of the cultivars based on their geographical origin results from a local selection and diversification, coupled with weak or non-existent exchanges of seeds between regions, inducing a geographical structuration and a form of isolation by distance.

The overall gene diversity increased with the number of alleles at a given locus. We found significant correlation between gene diversity and the number of alleles in Adamawa ($r = 0.88$, $P < 0.01$) and Northwest ($r = 0.76$, $P < 0.01$). Therefore, the number of alleles could be used for the assessment of genetic diversity in hexaploid wheat. Similar results were found by Huang et al. (2002) in a set of 24 microsatellite markers used to characterize 998 accessions of hexaploid wheat germplasm. Consequently, these authors reported that the characterization of a reliable correlation coefficient needs a large sample size. The results in the present study disagrees with those reported by Prasad et al. (2000) who indicated that the polymorphism information content value was not correlated with the number of alleles in 55 wheat accessions. According to Huang et al. (2002), the number of alleles was also correlated with the repeat number of microsatellite DNA and its relative distance from the centromere. It has been suggested that the three mechanisms for creating a new allele at SSR loci are slippage replication (Tachida and Iizuka, 1992), unequal crossing-over and genetic recombination (Harding et al., 1992). The value of genetic distance (GD) indicated that some accessions were closely related. Averages of GD over accessions in all regions were ranged from 0.18 (between Wassande2 and NGDEREM3) to 1 with a mean of 0.8 (80%). The high GD coefficient values indicate the presence of high gene diversity in the accessions.

The mean of gene diversity was relatively higher in Northwest (0.62) compared to Adamawa (0.54). These results suggested that the Northwest exhibited greater genetic diversity than Adamawa region. The Northwest was the presumed center of origin of hexaploid wheat in Cameroon and Adamawa was the sites were the SODEBLE was established. The results obtained in our study provided new information on the relationships between the Cameroonian bread wheat cultivars.

The set of the used microsatellite markers showed a high level of polymorphism and sufficient information to discriminate the cultivars of hexaploid wheat grown in Cameroon. Generally, our study provides a first description about the molecular genetic diversity of Cameroonian wheat varieties. The results are consistent with expectations and provide a first base for further investigations. The important level of the genetic diversity reported in the present study should be taken into account in developing wheat breeding programs in agro-ecological zones of Cameroon. Morphological and phenotypic studies will also be required to couple our results of molecular analyzes.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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