American Journal of Molecular Biology, 2016, 6, 12-24
Published Online January 2016 in SciRes. http://www.scirp.org/journal/ajmb
http://dx.doi.org/10.4236/ajmb.2016.61002

Genetic Diversity of Maize Accessions (*Zea mays* L.) Cultivated from Benin Using Microsatellites Markers

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Received 8 October 2015; accepted 2 January 2016; published 5 January 2016

Abstract

Maize (*Zea mays* L.) is the major cereal cultivated in Benin and it is important to know its genetic diversity to improve the yield. The genetic markers of important traits are evaluated in order to improve the maize inbred lines. The aim of this study was to evaluate the genetic diversity of Benin's maize accessions by SSR marker. Thus, one hundred eighty seven maize accessions from three areas (South, Center and North) were analyzed using three SSR markers. A total of 227 polymorphic bands were produced and showed high genetic diversity (Shannon index = 0.51). The polymorphic information content (PIC) values for the SSR loci ranged from 0.58 to 0.81, with an average of 0.71. Genetic distance-based UPGMA dendrogram showed a genetic differentiation between accessions and they were grouped into four clusters in each area. This work provides necessary information that can be used not only to improve the maize production and conservation but also to better manage genetic species resources in Benin.

Keywords

SSR, PCR, Molecular Characterization, *Zea mays* L., Benin

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How to cite this paper: Salami, H.A., Sika, K.C., Padonou, W., Aly, D., Yallou, C., Adjanohoun, A., Kotchoni, S. and Baba-Moussa, L. (2016) Genetic Diversity of Maize Accessions (*Zea mays* L.) Cultivated from Benin Using Microsatellites Markers. *American Journal of Molecular Biology*, **6**, 12-24. http://dx.doi.org/10.4236/ajmb.2016.61002

1. Introduction

Maize is the major cereal growing in the humid tropics and sub-Saharans Africa climate [1]. It is a changeable cereal classified third in world cereal production after wheat and rice [2]. Maize represents an actual source of consumption and income for millions of people in several countries [3]. This cereal belongs to the Andropogoneae tribe, Panicoideae subfamily and Poaceae family [4]. Five species are included in the genus Zea and largely has 2n = 20 chromosomes (except Zea perennis, 2n = 40) [5].

In Benin, maize occupies about 82% of total cereals cultivated area and represents about 84% of national cereal production. Thus, this cereal appears as essential product in Benin [6] and is characterized by large range of varieties (improved and local) managed by producers themselves [7]. Despite its enormous potential, Benin's agriculture is struggling to ensure sustainable food security due to constraints such as low yield [7]. So, researches that contribute to improve of the maize production yields are necessary to lift this constraint. One of the main contributions will be the development of improved varieties, among local maize resources, that meet the expectations of producers. Indeed, the local resources have a very significant phenotypic variability and genetic diversity and constitute an essential component of food security, as they provide the raw material used by breeders to improve the quality and productivity of maize. It is therefore necessary to know the genetic characteristics maize usually grown in Benin. For such characterization, the use of molecular markers provided an opportunity to analyze large-scale of maize populations [8] like previously used to study the structure of plants genetic variation [9] [10].

Various molecular genetic markers such as Restriction Fragment Length Polymorphism (RFLP) [11], Amplified Fragment Length Polymorphism (AFLP) [12], Inter Simple Sequence Repeats (ISSR) [13] and Simple sequence repeats (SSR) [14] were reported to be used in the molecular characterization of various plant genetic resources. Meanwhile, the SSR loci are reported to be highly polymorphic for the basic number of repeat units between species and especially among individuals within species and populations [15]. Widely used in the construction of the genetic map of the human genome, the SSR markers were used in the mapping of the plant genome [16]. Thus, in many plants, microsatellites are known to be more effective in genetic characterization and better indicated for structuring of genetic diversity studies [17] [18]. So, considering their interest in genetics, they are known to be neutral markers, co-dominant, extremely polymorphic and distributed throughout the genome [19]. Then, the aim of this study was to analyze with SSRs markers, the genetic polymorphism that may exist between different corn accessions collected in Benin.

2. Material and Methods

2.1. Plant Materials

Two hundred thirty three accessions of maize from seven agroecological zone in Benin were used in this study (**Figure 1** and **Table 1**). This collection includes the improved cultivars and local cultivars acquired from National Agriculture Research Institute of Benin (INRAB) [20]. In this study, among the two hundred thirty three accessions collected, one hundred eighty seven accessions have germinated.

2.2. DNA Extraction

Maize accessions were grown in the greenhouse. The single plant (3 weeks old) was taken from each accession and stored at −80˚C. Single-plant samples were ground to powder in liquid nitrogen using a mortar and pestle. A total genomic DNA was extracted as described previously [21].

2.3. SSR Analysis

Three SSR primers of maize (**Table 2**) were selected from previous studies [22]. The total volume of PCR mixture was 20 µl containing 10 µl of master mix [AccuStart II PCR ToughMix (2×)], 2.5 µl template DNA, 1 µl of each primer (Forward and reverse) and 5.5 µl water.

The PCR reaction was performed in a thermal cycler (BIO-RAD; T100TM) using an initial 94˚C denaturing step for 3 min followed by 34 cycles of [denaturation at 94˚C for 30 s, annealing for 30 s at the primer's annealing temperature, extension at 72˚C for 1 min 20 s] and a final extension at 72˚C for 5 min.

2.4. Data Analysis

The presence (1) or absence (0) of a PCR amplified SSR markers band were coded. The data base was then regis-

Table 1. Identification numbers, Site of collection, agroecological zone of maize accessions collected in Benin.

No.	ID	Locality	Agroecological zone	No.	ID	Locality	Agroecological zone	No.	ID	Locality	Agroecological zone
1	Zm1	vidjinan	Zone VIII	**36**	Zm36	Houèglè	Zone VII	**71**	Zm71	Hèkpè	Zone VI
2	Zm2	vidjinan	Zone VIII	**37**	Zm37	Ayahonou	Zone VII	**72**	Zm72	Sènouhoué	Zone VI
3	Zm3	vidjinan	Zone VIII	**38**	Zm38	Niaouli	Zone VI	**73**	Zm73	Sènouhoué	Zone VI
4	Zm4	Ayihounzo	Zone VIII	**39**	Zm39	Covè	Zone VI	**74**	Zm74	Sènouhoué	Zone VI
5	Zm5	Sèmé	Zone VIII	**40**	Zm40	Covè	Zone VI	**75**	Zm75	Sènouhoué	Zone VI
6	Zm6	Dossivi	Zone VIII	**41**	Zm41	Avlimè	Zone VI	**76**	Zm76	Agohoué-balimey	Zone VI
7	Zm7	Kodé	Zone VIII	**42**	Zm42	Avlimè	Zone VI	**77**	Zm77	Agohoué	Zone VI
8	Zm8	Kodé	Zone VIII	**43**	Zm43	Avlimè	Zone VI	**78**	Zm78	Ahogbéya	Zone VI
9	Zm9	Kodé	Zone VIII	**44**	Zm44	Domado	Zone VI	**79**	Zm79	Ahogbéya	Zone VI
10	Zm10	Kodé	Zone VIII	**45**	Zm45	Gbédji	Zone VI	**80**	Zm80	Ahogbéya	Zone VI
11	Zm11	Kodé	Zone VIII	**46**	Zm46	Gbédji	Zone VI	**81**	Zm81	Ahogbéya	Zone VI
12	Zm12	Kakanitchoé	Zone VIII	**47**	Zm47	Gbédji	Zone VI	**82**	Zm82	Ahogbéya	Zone VI
13	Zm13	Kakanitchoé	Zone VIII	**48**	Zm48	Gbédji	Zone VI	**83**	Zm83	Ahogbéya	Zone VI
14	Zm14	Atanka	Zone V	**49**	Zm49	Lohounvodo	Zone VIII	**84**	Zm84	Ahogbéya	Zone VI
15	Zm15	Atanka	Zone V	**50**	Zm50	Lohounvodo	Zone VIII	**85**	Zm85	Sèglahoué	Zone VI
16	Zm16	Atanka	Zone V	**51**	Zm51	Atikpéta	Zone VIII	**86**	Zm86	Sèglahoué	Zone VI
17	Zm17	Kpankou	Zone V	**52**	Zm52	Djéhadji	Zone VIII	**87**	Zm87	Sèglahoué	Zone VI
18	Zm18	Kpankou	Zone V	**53**	Zm53	Adjaïgbonou	Zone VII	**88**	Zm88	Sèglahoué	Zone VI
19	Zm19	Kpankou	Zone V	**54**	Zm54	Adjaïgbonou	Zone VII	**89**	Zm89	Gbénounkochihoué	Zone VI
20	Zm20	Vloko	Zone V	**55**	Zm55	Adjaïgbonou	Zone VII	**90**	Zm90	Gbénounkochihoué	Zone VI
21	Zm21	Issaba	Zone VII	**56**	Zm56	Adjaïgbonou	Zone VII	**91**	Zm91	Gbénounkochihoué	Zone VI
22	Zm22	Issaba	Zone VII	**57**	Zm57	Adjaïgbonou	Zone VII	**92**	Zm92	Massi	Zone VII
23	Zm23	Ayogo	Zone VI	**58**	Zm58	Adjaïgbonou	Zone VII	**93**	Zm93	Massi	Zone VII
24	Zm24	Sédjè	Zone VI	**59**	Zm59	Adjaïgbonou	Zone VII	**94**	Zm94	Atoungon	Zone VII
25	Zm25	Houezeto	Zone VI	**60**	Zm60	Gnamamé	Zone VII	**95**	Zm95	Atoungon	Zone VII
26	Zm26	Sédjè	Zone VI	**61**	Zm61	Gnamamé	Zone VII	**96**	Zm96	Hlanhonou	Zone VII
27	Zm27	Glégbodji I	Zone VI	**62**	Zm62	Gnamamé	Zone VII	**97**	Zm97	Hlanhonou	Zone VII
28	Zm28	Glégbodji I	Zone VI	**63**	Zm63	Gnamamé	Zone VII	**98**	Zm98	Hlanhonou	Zone VII
29	Zm29	Glégbodji I	Zone VI	**64**	Zm64	Banigbé	Zone VII	**99**	Zm99	Kotokpa	Zone VII
30	Zm30	Dohinhonko	Zone VI	**65**	Zm65	Banigbé	Zone VII	**100**	Zm100	Koussoukpa	Zone VII
31	Zm31	Dohinhonko	Zone VI	**66**	Zm66	Banigbé	Zone VII	**101**	Zm101	Koussoupka	Zone VII
32	Zm32	Dohinhonko	Zone VI	**67**	Zm67	Atchouhoué	Zone VI	**102**	Zm102	Agoïta	Zone VII
33	Zm33	Agonmey	Zone VII	**68**	Zm68	Atchouhoué	Zone VI	**103**	Zm103	Dohouimey	Zone V
34	Zm34	Agonmey	Zone VII	**69**	Zm69	Atchouhoué	Zone VI	**104**	Zm104	Honhoun	Zone V
35	Zm35	Agonmey	Zone VII	**70**	Zm70	Atchouhoué	Zone VI	**105**	Zm105	Honhoun	Zone V
106	Zm106	Lantêdié	Zone V	**143**	Zm143	Kpari	Zone V	**180**	Zm180	Biro	Zone III
107	Zm107	Lantêdié	Zone V	**144**	Zm144	Boue	Zone V	**181**	Zm181	Yambérou	Zone II
108	Zm108	Lantêdié	Zone V	**145**	Zm145	Boue	Zone V	**182**	Zm182	Yambérou	Zone II

Continued

109	Zm109	Lantêdié	Zone V	**146**	Zm146	Boue	Zone V	**183**	Zm183	Yambérou	Zone II
110	Zm110	Fonkpodji	Zone V	**147**	Zm147	Boue	Zone V	**184**	Zm184	Kpako soro kpika	Zone II
111	Zm111	Fonkpodji	Zone V	**148**	Zm148	Gounin	Zone III	**185**	Zm185	Kpako soro kpika	Zone II
112	Zm112	Agoua	Zone V	**149**	Zm149	Gounin	Zone III	**186**	Zm186	Kpako soro kpika	Zone II
113	Zm113	Boobè	Zone V	**150**	Zm150	Gounin	Zone III	**187**	Zm187	Yaroubosso	Zone II
114	Zm114	Boobè	Zone V	**151**	Zm151	Gounin	Zone III	**188**	Zm188	Yaroubosso	Zone II
115	Zm115	Agoua	Zone V	**152**	Zm152	Gounin	Zone III	**189**	Zm189	Yaroubosso	Zone II
116	Zm116	Pira	Zone V	**153**	Zm153	Gounin	Zone III	**190**	Zm190	Ounet	Zone II
117	Zm117	Aguélé	Zone V	**154**	Zm154	Bounyérou	Zone III	**191**	Zm191	Ounet	Zone II
118	Zm118	Aguélé	Zone V	**155**	Zm155	Bounyérou	Zone III	**192**	Zm192	Ounet	Zone II
119	Zm119	Azongnihogon	Zone V	**156**	Zm156	Bounyérou	Zone III	**193**	Zm193	Ounet	Zone II
120	Zm120	Azongnihogon	Zone V	**157**	Zm157	Bounyérou	Zone III	**194**	Zm194	Gomparou	Zone II
121	Zm121	Azongnihogon	Zone V	**158**	Zm158	Bounyérou	Zone III	**195**	Zm195	Gomparou	Zone II
122	Zm122	Ayéladjou	Zone V	**159**	Zm159	Banhounkpo	Zone III	**196**	Zm196	Zougoupantrossi	Zone II
123	Zm123	Ayéladjou	Zone V	**160**	Zm160	Banhounkpo	Zone III	**197**	Zm197	Badou	Zone II
124	Zm124	Koutoukou	Zone V	**161**	Zm161	Banhounkpo	Zone III	**198**	Zm198	Badou	Zone II
125	Zm125	Pounga	Zone V	**162**	Zm162	Banhounkpo	Zone III	**199**	Zm199	Badou	Zone II
126	Zm126	Atchakpa	Zone V	**163**	Zm163	Banhounkpo	Zone III	**200**	Zm200	Badou	Zone II
127	Zm127	Atchakpa	Zone V	**164**	Zm164	Sakarou	Zone III	**201**	Zm201	Badou	Zone II
128	Zm128	Atchakpa	Zone V	**165**	Zm165	Sakarou	Zone III	**202**	Zm202	Badou	Zone II
129	Zm129	Atchakpa	Zone V	**166**	Zm166	Sakarou	Zone III	**203**	Zm203	Bagou	Zone II
130	Zm130	Atchakpa	Zone V	**167**	Zm167	Sakarou	Zone III	**204**	Zm204	Warra	Zone II
131	Zm131	Gbanlin	Zone V	**168**	Zm168	Sakarou	Zone III	**205**	Zm205	Warra	Zone II
132	Zm132	Gbanlin	Zone V	**169**	Zm169	Ponaga	Zone III	**206**	Zm206	Partago	Zone IV
133	Zm133	Yaoui	Zone V	**170**	Zm170	Ponaga	Zone III	**207**	Zm207	Partago	Zone IV
134	Zm134	Yaoui	Zone V	**171**	Zm171	Ponaga	Zone III	**208**	Zm208	Partago	Zone IV
135	Zm135	Kassehlo	Zone V	**172**	Zm172	Ponaga	Zone III	**209**	Zm209	Partago	Zone IV
136	Zm136	Kpassatona	Zone V	**173**	Zm173	Ganrou	Zone III	**210**	Zm210	Monmongou	Zone IV
137	Zm137	Kpassatona	Zone V	**174**	Zm174	Ganrou	Zone III	**211**	Zm211	Sérou	Zone IV
138	Zm138	Kpassatona	Zone V	**175**	Zm175	Ganrou	Zone III	**212**	Zm212	Angara	Zone IV
139	Zm139	Kpassatona	Zone V	**176**	Zm176	Kassakpéré	Zone III	**213**	Zm213	Angara	Zone IV
140	Zm140	Kpassatona	Zone V	**177**	Zm177	Kassakpéré	Zone III	**214**	Zm214	Firou	Zone II
141	Zm141	Kpari	Zone V	**178**	Zm178	Kassakpéré	Zone III	**215**	Zm215	Firou	Zone II
142	Zm142	Kpari	Zone V	**179**	Zm179	Biro	Zone III	**216**	Zm216	Firou	Zone II
217	Zm217	Kaobagou	Zone II	**223**	Zm223	Yédékanhoun	Zone IV	**229**	Zm229	Holli	Zone IV
218	Zm218	Kaobagou	Zone II	**224**	Zm224	Yédékanhoun	Zone IV	**230**	Zm230	Holli	Zone IV
219	Zm219	Djoléni	Zone II	**225**	Zm225	Boliféri	Zone IV	**231**	Zm231	Kotari	Zone IV
220	Zm220	Pikéré	Zone II	**226**	Zm226	Boliféri	Zone IV	**232**	Zm232	Kotari	Zone IV
221	Zm221	Komgourou	Zone II	**227**	Zm227	Boliféri	Zone IV	**233**	Zm233	Kotari	Zone IV
222	Zm222	Yédékanhoun	Zone IV	**228**	Zm228	Holli	Zone IV				

Zm: Zea mays; Zone II: Cotton zone of Northern Benin; Zone III: Food area south Borgou; Zone IV: Area west Atacora; Zone V: Cotton zone of central Benin; Zone VI: Land area bar; Zone VII: Suction zone; Zone VIII: Fishery Zone.

Table 2. Characteristics of SSR primers used in this study.

Markers Name	Bin[1]	Motif	Sequence (5'-3')
Umc1222	1.01	(AG) 20	For: CTCAGAACAGAAGCCATCAAAAGC Rev: CGTCTTCGTGAGAGACATCCTGT
Umc 1335	1.06	(AG) 24.	For: ATGGCATGCATGTGTTTGTTTTAC Rev: ACAGACGTCGCTAATTCCTGAAAG
Umc 1327	8.01	(GCC) 4	For: AGGGTTTTGCTCTTGGAATCTCTC Rev: GAGGAAGGAGGAGGTCGTATCGT

[1]: Position in the chromosome; For: Forward; Rev: Reverse.

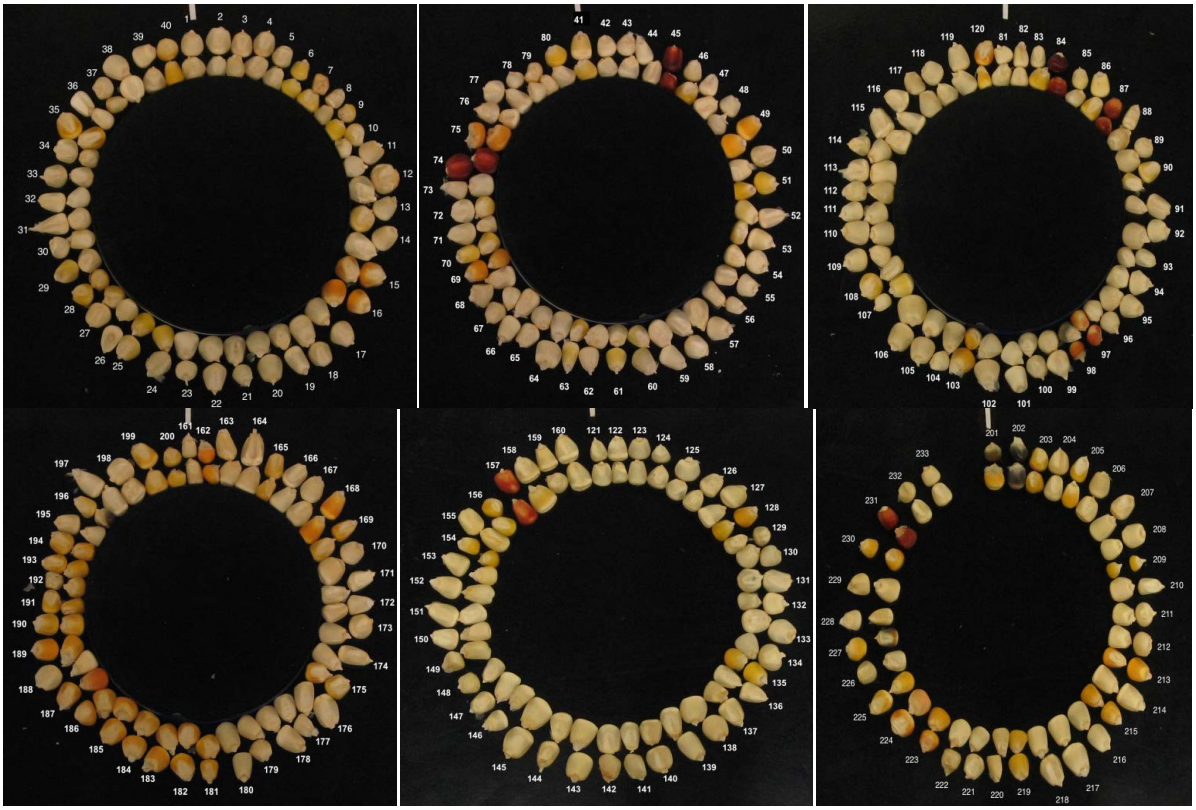

Figure 1. Picture showing the phenotypical diversity of maize accession collected in Benin.

tered in an MS Excel spreadsheet in order to generate the analysis matrix. Genetic diversity parameters such as Polymorphism Information Content (PIC) as previously describe by Anderson *et al.* [23]; polymorphism rate (P), number of alleles (Na), expected heterozygosity (He) and Shannon's phenetic index (H) were estimated according to the method used by Adoukonou-Sagbadja *et al.* [9].

Cluster analysis by Un-weighted Pair Group Method using Arithmetic Averages (UPGMA) and principal coordinate analysis (PCoA) were performed to identify genetic variation patterns among the maize genotypes using DarWin and NTSYSpc (2.2) data bases software respectively.

3. Results

3.1. Classification of the Maize Accessions According to Germination Time

Figure 2 shows the germination percentage of maize accessions according to the number of day after seeding. Analyze of this figure shows that the percentage of germination varied not only according the number of day after seeding but also according to the zone. Thus, in south (**Figure 2(a)**) the accessions can be grouped in two clusters. The accessions of cluster I (63%) have a middle germination time (three or four days after seeding).

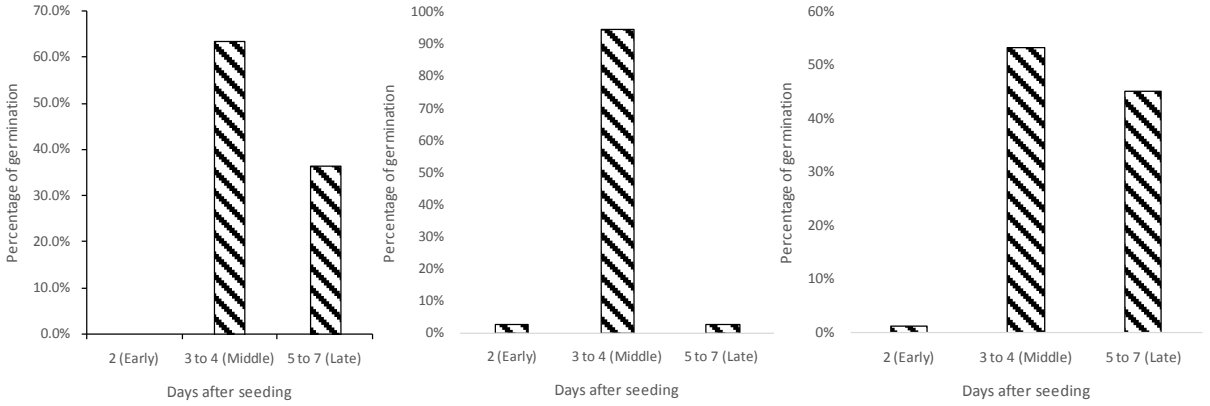

Figure 2. Percentage of germination according to the day after seeding of maize accessions. (a) Southern; (b) Center; (c) Northern.

The cluster II, regroup 37% of accessions, have a late germination time (≥ five days after seeding).

As for maize accessions of center, they can be classified in three clusters according to the germination time (**Figure 2(b)**). The cluster I contain the accessions of maize that have early germination time (two days after seeding). This cluster regroups 3% of the whole accessions. The cluster II contain 93% of accessions and was characterized by a middle germination time (three to four day after seeding). The cluster III maize accessions (4%) have a late germination time (≥ five days after seeding).

Figure 2(c) shows the classification of north maize accessions in three clusters. The first cluster contains the accessions that have early germination time (two days after seeding). The accessions of cluster II have a middle germination time (three to four day after seeding) and the cluster III was characterized by a late germination time (≥ five days after seeding).

3.2. SSR Polymorphism

The SSR markers selected to analyze the genetic diversity of the maize accessions displayed different characteristic profiles. Thus, different numbers of polymorphic bands, percentage of polymorphism, Polymorphism Information Content (PIC), and expected heterozygosis have been generated using the SSR markers (**Table 3**). All microsatellite markers used were found to be polymorphic, in other words a loci polymorphic rate of 100% was observed and the number of bands generated by each marker varied from 58 to 102 (76 a mean value). The level of polymorphism ranged from 25.33% to 44.54%. The discriminating power of each primer pair, estimated by the value of the PIC varied between 0.58 and 0.81 with an average rate of 0.71% for all SSRs analyzed.

3.3. Genetic Differentiation

Among the 227 distinct scored bands (~2 bands/accessions); 41% (n = 92) were recorded for south accessions, 23% (n = 52) for Center's accessions and 36% (n = 83) for the North accessions (**Table 4**). There were no specific bands belonging to accessions of the same production area. The South and North's accessions showed a high polymorphism and the number of accessions per zone had no effect on the percentage of polymorphism. To end, the Shannon index varied between 0.49 and 0.53 with an average of 0.52 for all accessions.

3.4. Genetic Relationship and Cluster Analyses

Genetic relationships among maize cultivars were determined by the Unweighted Pair Group Method with Arithmetic mean (UPGMA) using the Nei distances [24]. This method showed a dendrograms profiles of the maize accession respectively from Southern, Central and Northern Benin. The analysis of dendrograms showed the heterogeneity between local and improved accession in each area (**Table 5** and **Figures 3-5**).

The first dendogram shows the threshold of 14% similarity, the southern cultivars were grouped into four clusters (**Figure 3**). The clusters I and II were composed of as many individuals and contain both local and improve cultivars collected from South Benin areas. Cultivars of cluster I and II have a large height of plant and

Table 3. Number of scored polymorphic bands, percentage of polymorphism, estimated PIC, and expected heterozygosis (He) of three SSR markers.

Loci	Number of scored polymorphic	Polymorphism %	PIC	He
Zm1	69	30.13	0.75	0,46
Zm2	102	44.54	0.58	0.50
Zm3	58	25.33	0.81	0.43

Zm: *Zea mays*; He: Heterozygosis expected, PIC: Polymorphism Information Content.

Table 4. Genetic diversity of cultivars based on maize growing in Benin.

Area	Number of cultivars	Number of Loci amplified	Polymorphism (%)	Shannon index
Southern	74	92	45.53	0.53
Center	38	52	22.91	0.49
North	75	83	36.56	0.53
Total	187	227	100	

Table 5. Result showing the characteristic of the dendrogram cluster of different area of Benin.

Area of Benin	Clusters	Characteristic of cluster
South-Benin	Cluster 1	Large height plant, good husk cover, late flowering and large height cob insertion.
	Cluster 2	Large height plant, good husk cover and late flowering
	Cluster 3	Medium husk cover and average height plant.
	Cluster 4	Small height plant, bad husk cover, middle germination time and early flowering
Center-Benin	Cluster 1 (A and B)	Small height, bad husk cover, early germination time and early flowering
	Cluster 2 (C and D)	Medium height and medium husk cover.
North-Benin	Cluster 1	Large height plant, good husk cover and late flowering.
	Cluster 2	Medium height and bad husk cover.
	Cluster 3	Small height, bad husk cover, late germination time and early flowering.
	Cluster 4	Medium height, bad husk cover and late flowering.

have a good husk cover with late flowering only that the plants cluster I were larger. Cluster III consisting of fourteen accessions have a mediumhusk cover and have an average height of plant. Cluster IV composed of twenty-seven accessions were small height, have a bad husk cover, and have a middle germination time but unlike cluster I and II plants early flowering (**Table 5**).

The second dendogram shows the threshold of 20% similarity, the cultivars collected from center Benin were grouped into two clusters and each cluster into two sub-clusters (**Figure 4**). Cluster I with its two sub-clusters (A and B) is consists of eighteen accessions and different from the cluster II to the threshold of 15%. This group consists of cultivars from all villages of Central corn production area. The sub-cluster A composed of ten accessions are morphologically different from those of the sub-cluster B. The plants of this cluster were small height and have a bad husk cover, early flowering and early germination time. The cluster II as consisting of two sub-clusters (C and D) is composed of 21 accessions. The plants of this cluster are medium height and have a medium husk cover (**Table 5**).

The CAH analysis based on the Euclidean distance computed using the UPGMA method clustered the north accessions into four clusters at the similarity threshold of 0.75 (**Figure 5**). The cluster I different of other clusters to 0.60 thresholds is composed of 19 local and improved collected from North. Plants of this group were large height and have a good husk cover but late flowering. Twenty accessions composed the cluster II. This

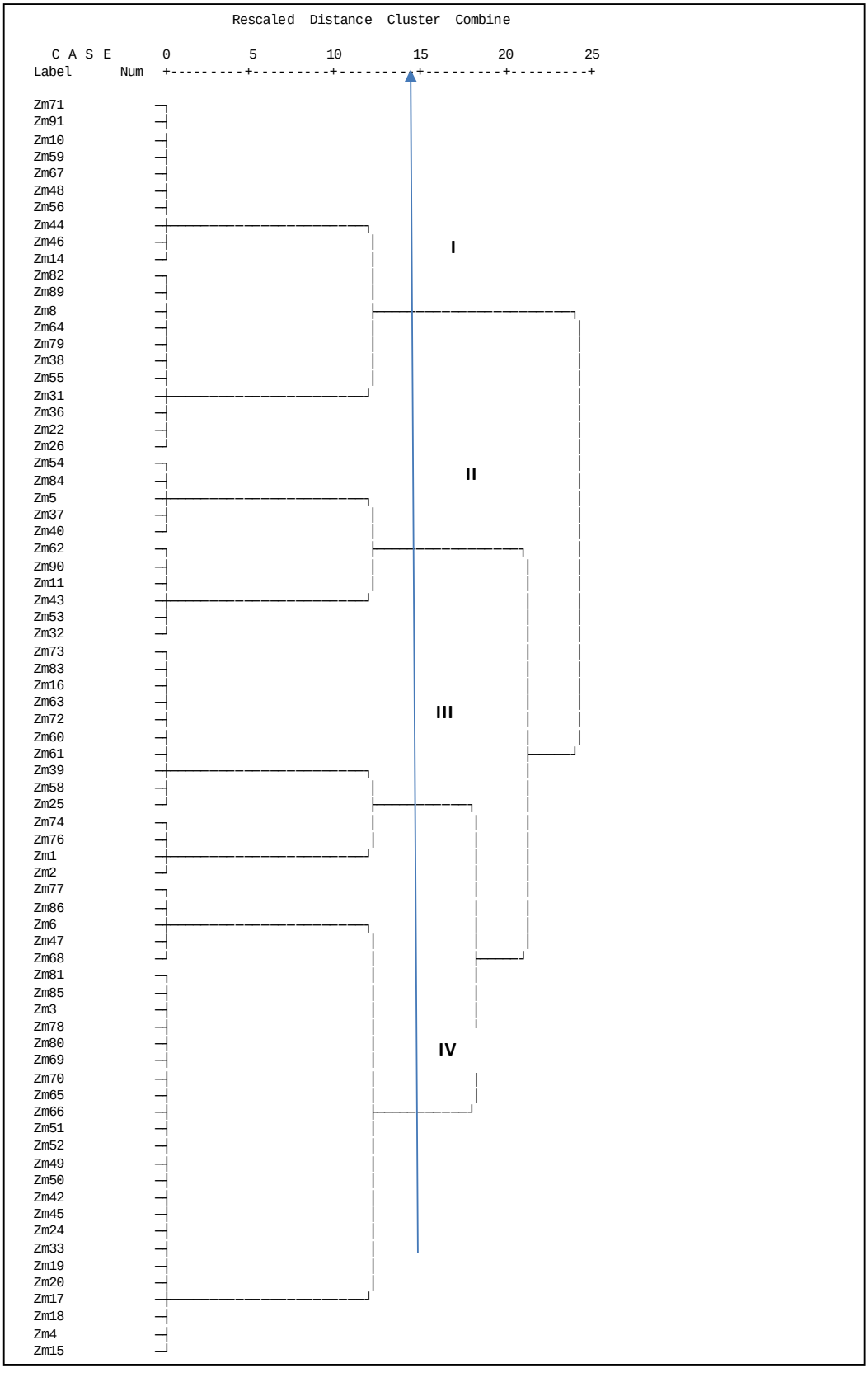

Figure 3. Dendrogram showing the genetic relationships between cultivars maize of South by UPGMA analysis.

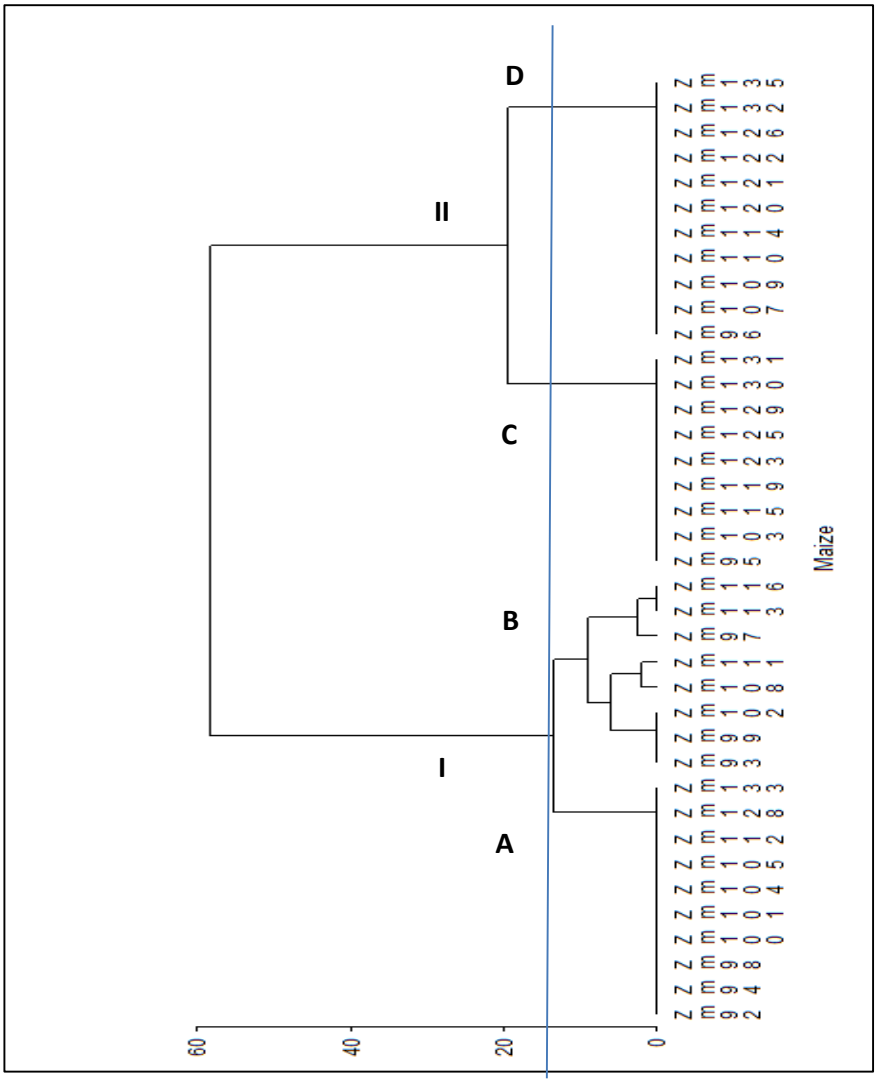

Figure 4. Dendrogram showing the genetic relationships between cultivars maize of Center by UPGMA analysis.

group shows a heterogeneous as I cluster and explained an eco-genetic relationship. The plants of this cluster are medium height and have bad husk cover. The cluster III is composed of 18 accessions and different from other clusters and the threshold of 0.90. The plants of this group were small height, have bad husk cover and have late germination time but early flowering. The cluster IV is different to the cluster III at the threshold of 0.10 and composed of 18 accessions. These plants are substantially similar to those of cluster III except the fact that these last cluster have average height and flowering (**Table 5**).

4. Discussion

4.1. Polymorphism Analysis

In this study, all microsatellite markers used were polymorphic and a high discriminatory power (0.71 average) that allowed discrimination of maize accessions from Benin by each marker. The high level of the PIC values showed that the fragments generated in this study were very informative. Al-Badeiry *et al.* [25] reported that the PIC demonstrates the informativeness of the SSR loci and their potential to detect differences among the varieties based on their genetic relationships. The efficiency of the molecular marker technique depends on the level of polymorphism and discriminatory power among the set of accessions [26]. The result obtained in this study

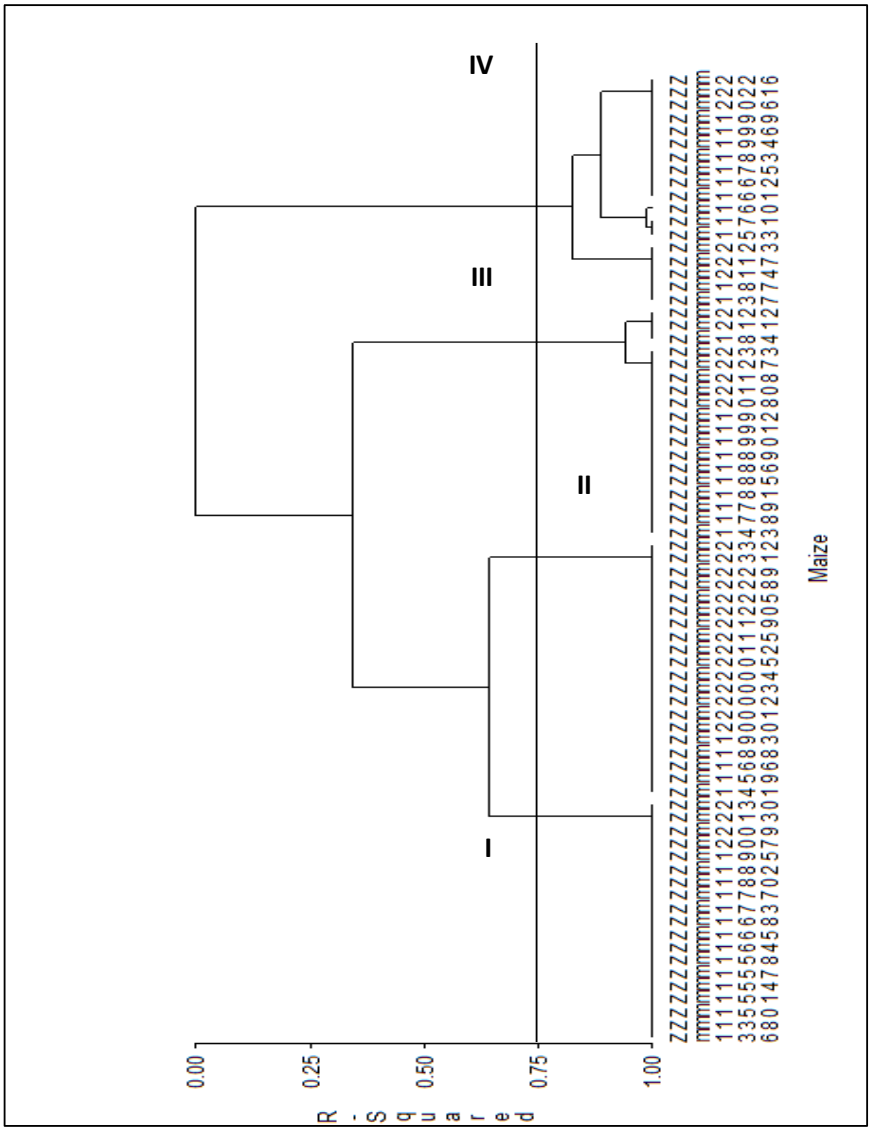

Figure 5. Dendrogram showing the genetic relationships between cultivars maize of North by UPGMA analysis.

were superior to that obtained by Shehata *et al.* [27] who obtained the PIC value of 0.57 on maize inbred lines in Saudi Arabia and also superior to the 0.44 funded by Al-Badeiry *et al.* [25] but similar to 0.69 obtained by Elçi and Hançer [28] on maize accession in Turkey. Considering the heterozygoty, the mean of 0.46 obtained in this study was lower than the one (0.54 and 0.55) obtained in previous studies [25] [29] on maize accession. However, our found is higher than those of Yao *et al.* [30] and Aci *et al.* [22], where they observed respectively an average value of 0.39 and 0.396.

4.2. Genetic Diversity of Maize Accession

In this study 227 distinct scored bands were recorded for Benin accessions. The Shannon index (0.52) obtained in this study seems high and may suggest a higher genetic diversity and differentiation of maize accession in Benin. These results were in agreement with the 0.54 Shannon index reported on sorghum using the microsatellites markers [31].

The higher diversity of maize accessions obtained in this study can be explained by the fact that during the collection of maize accession, several accessions (improve and local accession) were collected. In the different

agro-ecological zone, the farmers used to keep the accessions based not only in their culture but also in the nutritional characteristics. So because of their technological and organoleptic qualities found to be very different from local ecotypes, improved maize varieties developed by research are reported to be very few adopted and therefore little cultivated by peasants [32]. In addition, the cross-pollination between different varieties of maize from neighboring fields is also supplementary factors that increase genetic diversity. High levels of genetic diversity in maize are caused by active transposable elements, meiotic recombination following out crossing, new introgressions from exotic germplasm of this highly traded crop species, genetic drift following new introductions, and natural and artificial selection by farmers as the crop adapts to new environments [33].

To more understand the genetic diversity of maize accessions analyzed, the genotypic data obtained for three SSR markers were used to generate three UPGMA dendrograms depending on the area. Considering the dendrogram, a great similarity is observed between plants of the same group. However, the grouping of accessions in different cluster, reflects the genetic history, agronomic and eco-geographical affinity between the different accessions. The dendrograms revealed four different groups both in the north and in the south of Benin against two groups recorded among the center accessions. The highest diversity observed in the South and North can be explained by the fact that those areas are reported to be a large corn producing areas in Benin, incontrast with the Center area known to be producer of groundnuts and cassava [20].

Indeed, northern accessions were discriminated by the germinal parameters, plant and ear height, and early ears maturity while in the Center apart of the earliness and plant height the husk cover and sensitivity streak were considered. The discrimination of the accessions characteristics in the South is based on the germination time and female flowering [34]. This diversity of discriminative parameters depending on the area can not only be due to the difference of soil type but also to the climate. [35] asserted that farmers' choice of which maize genotype to grow is influenced by the major vegetation/climatic conditions found in Ghana. The traditional management of genetic resource of maize held by farmers participating in this great diversity of maize accessions.

5. Conclusion

In the present study, the SSR markers revealed the genetic relationships and diversity of maize accession in Benin. This study provides useful information that can be used in a breeding program for genetic improvement and characterization of new varieties. In addition, the results of this study are relevant for developing management the maize genetic resources. Further research on the sequencing of different maize gene is necessary to confirm the genetic diversity.

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