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

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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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1. Introduction

Maize is the major cereal growing in the humid tropics and sub-Saharan 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-
| No. | ID   | Locality     | Agroecological zone | No. | ID   | Locality     | Agroecological zone | No. | ID   | Locality     | Agroecological zone |
|-----|------|--------------|---------------------|-----|------|--------------|---------------------|-----|------|--------------|---------------------|
| 1   | Zm1  | vidjinan     | Zone VIII           | 36  | Zm36 | Houégledé   | Zone VII            | 71  | Zm71 | Hékpé       | Zone VI             |
| 2   | Zm2  | vidjinan     | Zone VIII           | 37  | Zm37 | Ayahonou    | Zone VII            | 72  | Zm72 | Sénouvhoué  | Zone VI             |
| 3   | Zm3  | vidjinan     | Zone VIII           | 38  | Zm38 | Niaoudi     | Zone VI             | 73  | Zm73 | Sénouvhoué  | Zone VI             |
| 4   | Zm4  | Ayihounzo    | Zone VIII           | 39  | Zm39 | Cové        | Zone VI             | 74  | Zm74 | Sénouvhoué  | Zone VI             |
| 5   | Zm5  | Sémé         | Zone VIII           | 40  | Zm40 | Cové        | Zone VI             | 75  | Zm75 | Sénouvhoué  | Zone VI             |
| 6   | Zm6  | Dossivi      | Zone VIII           | 41  | Zm41 | Avlimé      | Zone VI             | 76  | Zm76 | Agohoué-balimey | Zone VI             |
| 7   | Zm7  | Kodé         | Zone VIII           | 42  | Zm42 | Avlimé      | Zone VI             | 77  | Zm77 | Agohoué     | Zone VI             |
| 8   | Zm8  | Kodé         | Zone VIII           | 43  | Zm43 | Avlimé      | Zone VI             | 78  | Zm78 | Ahohôuya    | Zone VI             |
| 9   | Zm9  | Kodé         | Zone VIII           | 44  | Zm44 | Domado      | Zone VI             | 79  | Zm79 | Ahohôuya    | Zone VI             |
| 10  | Zm10 | Kodé         | Zone VIII           | 45  | Zm45 | Gbédjï      | Zone VI             | 80  | Zm80 | Ahohôuya    | Zone VI             |
| 11  | Zm11 | Kodé         | Zone VIII           | 46  | Zm46 | Gbédjï      | Zone VI             | 81  | Zm81 | Ahohôuya    | Zone VI             |
| 12  | Zm12 | Kakantitché   | Zone VIII           | 47  | Zm47 | Gbédjï      | Zone VI             | 82  | Zm82 | Ahohôuya    | Zone VI             |
| 13  | Zm13 | Kakantitché   | Zone VIII           | 48  | Zm48 | Gbédjï      | Zone VI             | 83  | Zm83 | Ahohôuya    | Zone VI             |
| 14  | Zm14 | Atanka       | Zone V              | 49  | Zm49 | Lohounvodo  | Zone VIII           | 84  | Zm84 | Ahohôuya    | Zone VI             |
| 15  | Zm15 | Atanka       | Zone V              | 50  | Zm50 | Lohounvodo  | Zone VIII           | 85  | Zm85 | Séglahoué  | Zone VI             |
| 16  | Zm16 | Atanka       | Zone V              | 51  | Zm51 | Atikpêta    | Zone VIII           | 86  | Zm86 | Séglahoué  | Zone VI             |
| 17  | Zm17 | Kpankou      | Zone V              | 52  | Zm52 | Djêhadjï    | Zone VIII           | 87  | Zm87 | Séglahoué  | Zone VI             |
| 18  | Zm18 | Kpankou      | Zone V              | 53  | Zm53 | Adjajgbonou | Zone VII            | 88  | Zm88 | Séglahoué  | Zone VI             |
| 19  | Zm19 | Kpankou      | Zone V              | 54  | Zm54 | Adjajgbonou | Zone VII            | 89  | Zm89 | Gbénounkochihoué | Zone VI             |
| 20  | Zm20 | Vloko        | Zone V              | 55  | Zm55 | Adjajgbonou | Zone VII            | 90  | Zm90 | Gbénounkochihoué | Zone VI             |
| 21  | Zm21 | Issaba       | Zone VII            | 56  | Zm56 | Adjajgbonou | Zone VII            | 91  | Zm91 | Gbénounkochihoué | Zone VI             |
| 22  | Zm22 | Issaba       | Zone VII            | 57  | Zm57 | Adjajgbonou | Zone VII            | 92  | Zm92 | Massi       | Zone VII             |
| 23  | Zm23 | Ayogo        | Zone VI             | 58  | Zm58 | Adjajgbonou | Zone VII            | 93  | Zm93 | Massi       | Zone VII             |
| 24  | Zm24 | Sédjé        | Zone VI             | 59  | Zm59 | Adjajgbonou | Zone VII            | 94  | Zm94 | Atoungon    | Zone VII             |
| 25  | Zm25 | Houezeto     | Zone VI             | 60  | Zm60 | Gnammâmè    | Zone VII            | 95  | Zm95 | Atoungon    | Zone VII             |
| 26  | Zm26 | Sédjé        | Zone VI             | 61  | Zm61 | Gnammâmè    | Zone VII            | 96  | Zm96 | Hlanhonou   | Zone VII             |
| 27  | Zm27 | Glégbondji I | Zone VI             | 62  | Zm62 | Gnammâmè    | Zone VII            | 97  | Zm97 | Hlanhonou   | Zone VII             |
| 28  | Zm28 | Glégbondji I | Zone VI             | 63  | Zm63 | Gnammâmè    | Zone VII            | 98  | Zm98 | Hlanhonou   | Zone VII             |
| 29  | Zm29 | Glégbondji I | Zone VI             | 64  | Zm64 | Baniighbé  | Zone VII            | 99  | Zm99 | Kotoroka    | Zone VII             |
| 30  | Zm30 | Dohinonko    | Zone VI             | 65  | Zm65 | Baniighbé  | Zone VII            | 100 | Zm100 | Kousoukpa   | Zone VII             |
| 31  | Zm31 | Dohinonko    | Zone VI             | 66  | Zm66 | Baniighbé  | Zone VII            | 101 | Zm101 | Kousoukpa   | Zone VII             |
| 32  | Zm32 | Dohinonko    | Zone VI             | 67  | Zm67 | Atchouhoué  | Zone VI             | 102 | Zm102 | Agoïta      | Zone VII             |
| 33  | Zm33 | Agonnmy      | Zone VII            | 68  | Zm68 | Atchouhoué  | Zone VI             | 103 | Zm103 | Dohouimey   | Zone V              |
| 34  | Zm34 | Agonnmy      | Zone VII            | 69  | Zm69 | Atchouhoué  | Zone VI             | 104 | Zm104 | Honhouen    | Zone V              |
| 35  | Zm35 | Agonnmy      | Zone VII            | 70  | Zm70 | Atchouhoué  | Zone VI             | 105 | Zm105 | Honhouen    | Zone V              |
| 106 | Zm106| Lantédéi     | Zone V              | 143 | Zm143| Kpari       | Zone V              | 180 | Zm180 | Biro        | Zone III            |
| 107 | Zm107| Lantédéi     | Zone V              | 144 | Zm144| Boua        | Zone V              | 181 | Zm181 | Yamhêrou    | Zone II             |
| 108 | Zm108| Lantédéi     | Zone V              | 145 | Zm145| Boua        | Zone V              | 182 | Zm182 | Yamhêrou    | Zone II             |
| Zm   | Zone    | Location                          |
|------|---------|-----------------------------------|
| Zm109 | 109     | Lantêdié Zone V                   |
| Zm110 | 110     | Booue Zone V                      |
| Zm111 | 111     | Yambéréou Zone II                 |
| Zm112 | 112     | Agoua Zone V                      |
| Zm113 | 113     | Boobè Zone V                      |
| Zm114 | 114     | Zm114 Boobè Zone V                |
| Zm115 | 115     | Agoua Zone V                      |
| Zm116 | 116     | Pira Zone V                       |
| Zm117 | 117     | Aouélé Zone V                     |
| Zm118 | 118     | Aouélé Zone V                     |
| Zm119 | 119     | Azongnihgon Zone V                |
| Zm120 | 120     | Azongnihgon Zone V                |
| Zm121 | 121     | Azongnihgon Zone V                |
| Zm122 | 122     | Ayéladjou Zone V                  |
| Zm123 | 123     | Ayéladjou Zone V                  |
| Zm124 | 124     | Koutokou Zone V                   |
| Zm125 | 125     | Pounga Zone V                     |
| Zm126 | 126     | Atchakpa Zone V                   |
| Zm127 | 127     | Atchakpa Zone V                   |
| Zm128 | 128     | Atchakpa Zone V                   |
| Zm129 | 129     | Atchakpa Zone V                   |
| Zm130 | 130     | Atchakpa Zone V                   |
| Zm131 | 131     | Gbanlin Zone V                    |
| Zm132 | 132     | Gbanlin Zone V                    |
| Zm133 | 133     | Yaoui Zone V                      |
| Zm134 | 134     | Yaoui Zone V                      |
| Zm135 | 135     | Kassehlo Zone V                   |
| Zm136 | 136     | Kpassatora Zone V                 |
| Zm137 | 137     | Kpassatora Zone V                 |
| Zm138 | 138     | Kpassatora Zone V                 |
| Zm139 | 139     | Kpassatora Zone V                 |
| Zm140 | 140     | Kpassatora Zone V                 |
| Zm141 | 141     | Kpuri Zone V                      |
| Zm142 | 142     | Kpuri Zone V                      |
| Zm143 | 143     | Kpuri Zone V                      |
| Zm144 | 144     | Kpuri Zone V                      |
| Zm145 | 145     | Kpuri Zone V                      |
| Zm146 | 146     | Boue Zone V                       |
| Zm147 | 147     | Boue Zone V                       |
| Zm148 | 148     | Gounin Zone III                   |
| Zm149 | 149     | Gounin Zone III                   |
| Zm150 | 150     | Gounin Zone III                   |
| Zm151 | 151     | Gounin Zone III                   |
| Zm152 | 152     | Gounin Zone III                   |
| Zm153 | 153     | Gounin Zone III                   |
| Zm154 | 154     | Bouynérou Zone III                |
| Zm155 | 155     | Bouynérou Zone III                |
| Zm156 | 156     | Bouynérou Zone III                |
| Zm157 | 157     | Bouynérou Zone III                |
| Zm158 | 158     | Bouynérou Zone III                |
| Zm159 | 159     | Bankounkpo Zone III               |
| Zm160 | 160     | Bankounkpo Zone III               |
| Zm161 | 161     | Bankounkpo Zone III               |
| Zm162 | 162     | Bankounkpo Zone III               |
| Zm163 | 163     | Bankounkpo Zone III               |
| Zm164 | 164     | Bankounkpo Zone III               |
| Zm165 | 165     | Bankounkpo Zone III               |
| Zm166 | 166     | Bankounkpo Zone III               |
| Zm167 | 167     | Bankounkpo Zone III               |
| Zm168 | 168     | Bankounkpo Zone III               |
| Zm169 | 169     | Bankounkpo Zone III               |
| Zm170 | 170     | Bankounkpo Zone III               |
| Zm171 | 171     | Bankounkpo Zone III               |
| Zm172 | 172     | Bankounkpo Zone III               |
| Zm173 | 173     | Bankounkpo Zone III               |
| Zm174 | 174     | Bankounkpo Zone III               |
| Zm175 | 175     | Bankounkpo Zone III               |
| Zm176 | 176     | Bankounkpo Zone III               |
| Zm177 | 177     | Bankounkpo Zone III               |
| Zm178 | 178     | Bankounkpo Zone III               |
| Zm179 | 179     | Bankounkpo Zone III               |
| Zm180 | 180     | Bankounkpo Zone III               |
| Zm181 | 181     | Bankounkpo Zone III               |
| Zm182 | 182     | Bankounkpo Zone III               |
| Zm183 | 183     | Bankounkpo Zone III               |
| Zm184 | 184     | Bankounkpo Zone III               |
| Zm185 | 185     | Bankounkpo Zone III               |
| Zm186 | 186     | Bankounkpo Zone III               |
| Zm187 | 187     | Bankounkpo Zone III               |
| Zm188 | 188     | Bankounkpo Zone III               |
| Zm189 | 189     | Bankounkpo Zone III               |
| Zm190 | 190     | Bankounkpo Zone III               |
| Zm191 | 191     | Bankounkpo Zone III               |
| Zm192 | 192     | Bankounkpo Zone III               |
| Zm193 | 193     | Bankounkpo Zone III               |
| Zm194 | 194     | Bankounkpo Zone III               |
| Zm195 | 195     | Bankounkpo Zone III               |
| Zm196 | 196     | Bankounkpo Zone III               |
| Zm197 | 197     | Bankounkpo Zone III               |
| Zm198 | 198     | Bankounkpo Zone III               |
| Zm199 | 199     | Bankounkpo Zone III               |
| Zm200 | 200     | Bankounkpo Zone III               |
| Zm201 | 201     | Bankounkpo Zone III               |
| Zm202 | 202     | Bankounkpo Zone III               |
| Zm203 | 203     | Bankounkpo Zone III               |
| Zm204 | 204     | Bankounkpo Zone III               |
| Zm205 | 205     | Bankounkpo Zone III               |
| Zm206 | 206     | Bankounkpo Zone III               |
| Zm207 | 207     | Bankounkpo Zone III               |
| Zm208 | 208     | Bankounkpo Zone III               |
| Zm209 | 209     | Bankounkpo Zone III               |
| Zm210 | 210     | Bankounkpo Zone III               |
| Zm211 | 211     | Bankounkpo Zone III               |
| Zm212 | 212     | Bankounkpo Zone III               |
| Zm213 | 213     | Bankounkpo Zone III               |
| Zm214 | 214     | Bankounkpo Zone III               |
| Zm215 | 215     | Bankounkpo Zone III               |
| Zm216 | 216     | Bankounkpo Zone III               |
| Zm217 | 217     | Bankounkpo Zone III               |
| Zm218 | 218     | Bankounkpo Zone III               |
| Zm219 | 219     | Bankounkpo Zone III               |
| Zm220 | 220     | Bankounkpo Zone III               |
| Zm221 | 221     | Bankounkpo Zone III               |
| Zm222 | 222     | Bankounkpo Zone III               |
| Zm223 | 223     | Bankounkpo Zone III               |
| Zm224 | 224     | Bankounkpo Zone III               |
| Zm225 | 225     | Bankounkpo Zone III               |
| Zm226 | 226     | Bankounkpo Zone III               |
| Zm227 | 227     | Bankounkpo Zone III               |
| Zm228 | 228     | Bankounkpo Zone III               |

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 | Motif | Sequence (5ʻ-3ʼ) |
|--------------|-----|-------|------------------|
| Umc1222      | 1.01| (AG) 20| For: CTCAGAACAGAAGCCATCAAAGC<br>Rev: CGTCTTCGAGAGACATCGTG |
| Umc1335      | 1.06| (AG) 24.| For: ATGGCATGCATGTGTTTGTTTTAC<br>Rev: ACAGACGTGCTAATTCCGAAAG |
| Umc1327      | 8.01| (GCC) 4| For: AGGTTTTTGCTCTTGGAAATCTCTC<br>Rev: GAGGAAGGAGGAGGATCGTATCGT |

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

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).
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 dendrogram 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. |
|               | Cluster 1| Large height plant, good husk cover and late flowering. |
|               | Cluster 2| Medium height and bad husk cover. |
| North-Benin   | 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 medium husk 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 dendrogram 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.

The 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
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, in contrast 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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