ORIGINAL ARTICLE

# GENETIC DIVERSITY OF A COLLECTION OF AMARANTH (*Amaranthus spp*) OF BURKINA FASO USING ISSR MARKERS

**| Jacques Ouédraogo *[1] | Mariam Kiébré [1] | Zakaria Kiébré [1] | Boureima Sawadogo [2] | and | Pauline Bationo/Kando [1] |**

[1.] Université Joseph KI-ZERBO | Equipe de Génétique et Amélioration des Espèces | Ouagadougou | Burkina Faso |
[2.] Université de Fada N'Gourma | Institut Supérieur des Sciences du Développement Durable | Fada N'Gourma | Burkina Faso |

| Received 22 August 2019 | | Accepted 21 September 2019 | | Published 26 September 2019 | | ID Article | Jacques-Ref.1-ajira170919 |

## ABSTRACT

**Background**: The assessment of genetic diversity is an asset for the development of any varietal conservation and improvement strategy. **Methods**: This work aims to establish the level and structure of genetic diversity of 54 accessions of amaranth collected in two climatic zones (Sudanese zone and Sudano-Sahelian zone) of Burkina Faso. Fourteen (14) ISSRs markers developed by eurofinmwg/ Operon were used in this study. **Results**: Eight (8) of them were been polymorph and used for data analysis. All eight primers showed 100% polymorphism with an average PIC of 64%. A total of 40 alleles were revealed with an average of 5 alleles by locus. Also, an average of effective allele number of 3.94, a heterozygoty expected of 0.37 and Shannon diversity indexes of 1.3 were recorded. These parameters calculated showed that the markers used are informative and revealed an important genetic variability structured in four groups (A, B, C and D) within the collection amaranth cultivated in Burkina Faso. The influence of "climatic zone" and "morphotype" factors on the level and the organization of genetic diversity is very low. **Conclusion**: 98.80% of total genetic diversity is due to variability intra-population.
***Keywords***: *Amaranth, neglected species, molecular variability, assessment*

## 1. INTRODUCTION

The traditional leafy-vegetables play an important role in the food security for the population of developing country. There are well adapted to the local agro-ecologic conditions and could contribute to fight against poverty and malnutrition. Indeed, leaves of amaranth are rich in vitamin C, β-carotene, folate, calcium, iron and zinc [1, 2, 3]; in this fact, it constitutes a good dietary supplement for pregnant women and growing children [4]. Its nutritional value is higher than many exotic vegetables such as cabbage, eggplant and lettuce. Also, it contains twice more calcium than milk and could use to reduce sicknesses due to calcium deficiency. Finally, leaves of amaranth contain more proteins than cereals and constitute a good source of food against malnutrition. The selling of this vegetable procures money to the horticultures that permitted to improve their standard of living [5].

Nevertheless, at Burkina Faso, the potentialities of amaranth are under-exploited and it diversity is unrecognized by scientists. Also, there are no yet available improved seeds and good cultural practices that are always archaic. For this, it's so necessary to set up a framework of conservation and valorization of amaranth cultivated in Burkina Faso.

The objective of this study is to contribute to a best known of diversity of amaranth grown in Burkina Faso. Specifically, it's to assess the genetic diversity of amaranth accessions and determining the level and structure of this diversity within a collection of amaranth from Burkina Faso using ISSRs markers.

## 2. MATERIALS AND METHODS

### 2.1 Plant material

Fifty-three (53) local accessions from two climate zones of Burkina Faso and one (1) accession of Benin were used in this study. Among the accessions of Burkina Faso, twenty (20) of them are collected in Sudanese climatic zone and the rest are from Sudono-sahelian climatic zone. These accessions are being classified in six (6) morphotype by [6].

These are the green and violet morphotypes belonging to the species *Amaranthus cruentus* (Figures 1), the green and purple morphotypes for the species *Amaranthus spinosus* (Figures 2), the purple green morphotype for the species

***Corresponding author Author & Copyright Author © 2019:** | **Jacques Ouédraogo** |. 

*Amaranthus hypochondriacus* (Figures 3), the dark green morphotype for the species *Amaranthus dibius* (Figures 4). These accessions were sown and grown in a greenhouse in some pots. Always, the pots were regularly watered on morning and evening in order to sure good growth of pant development of seedlings during the test (20 days). The young leaves of each accession were removed, weighed immediately and used for of genomic DNA extraction.

Stem Leaves Inflorescence

**A**: Green morphotype.

Stem Leaves Inflorescence

**B:** violet morphotype.

**Figure 1:** The figure presents the morphotypes of species *Amaranthus cruentus* [6].

Stem

Inflorescence

**A**: Green morphotype.

Stem

Leaves

Inflorescence

**B**: Purple morphotype (relative to the color of inflorescence).

**Figure 2**: The figure presents the morphotypes of species *Amaranthus spinosus* [6].

Stem

Inflorescence

**Figure 3:** The figure presents the urple green morphotype for the species *Amaranthus hypochondriacus* [6].

Stem

Leaves

Inflorescence

**Figure 4:** The figure presents the dark green morphotype for the species *Amaranthus dibius* [6].

## 2.2 Genomic DNA extraction

Genomic DNA extraction was conducted at Molecular Biology Unit of Plant Genetics and Improvement Team of University Joseph KI-ZERBO. Young leaves were harvested from 20 days old plants. DNA was extracted from 0.4 g of fresh leaves following by "CTAB" method of [7] adapted to amaranth. Briefly, leaves were ground using a mortar and pestle and dissolved in Tris EDTA Sorbitol (TES). The samples were centrifuged at 10,000 rpm and 4° C for 10 minutes. A volume of 1000 µl of CTAB at 60°C was added in each sample and all samples were placed during three hours to half in water bath at 60 °C before centrifugation. Thus, a volume of 750 µl of CIAA (chloroform-isoamyl alcohol) was added in each sample and all samples were centrifuged during 15 minutes at 10,000 rpm and 4°C. After first centrifugation, the float was collected and put in some new tubes next a volume of isopropanol at -20 °C was added in each sample. Newly, the tubes were centrifuged again during 10 minutes at 10,000 rpm and 4 °C. The pellets of DNA were rinsed with 70% ethanol and centrifuged during 10 minutes at 10, 000 rpm. After centrifugation, the floats were removed and cooled at room temperature. The DNA pellets of samples were dissolved in 150 µl of Tris EDTA and put in freezer at -20 °C.

## 2.3 ISSR markers

In this study, fourteen (14) ISSRs markers were used. These markers were developed by eurofinmwg/ Operon and were successfully tested by [8] for the genetic diversity study of *Cleome gynandra*.

### 2.4 PCR Conditions

For each DNA sample, amplification was performed in 20 µl reaction volume containing 2 µl buffer 10X (10 mM Tris-HCL pH 9, 50 mM of KCl, 1.5 mM of MgCl2), 0.4 µl dNTPs (10 mM), 0.4 µl *Taq* polymerase (2U), 3 µl DNA (5 ng/ µl), 4 µl primer (10 µM) and 10.2 µl ultrapure water. After homogenization, amplification was programmed with the thermocycler to fulfill 40 cycles after an initial denaturation cycle for 4 min at 94 °C. Each cycle consisted of a denaturation step at 94 °C for 1 min, an annealing step at Tm (°C) for 2 min, and an extension step at 72 °C for 2 min, following by a final extension for 7 min at 72 °C.

### 2.5 Detection of PCR products

After amplification, the PCR products were detected using agarose gel electrophoresis (3% in 1×TBE buffer), then stained with ethidium bromide (0.5 ug/ml). The bands were visualized under UV light of transilluminator (DI-01-220) and photographed using a camera brand Canon Power Shot A620, 10 Mega Pixels. The PCR products were separated for 2 h at 90 V in TBE 0.5X. Each band was considered a single locus. Data were scored as (1) for the presence and (0) for the absence of a given DNA band. The size of DNA bands in base pairs was estimated using 1kb DNA standard ladder (Invitrogen, Carlsbad, CA, USA).

### 2.6 Data statistical analysis

Clear and unambiguous bands were considered for study. Polymorphism of locus (P), total number of alleles (At), number of alleles per locus (A), number of effective alleles (Ae), Shannon's diversity index, calculated according to [9], expected heterozygosity (He) or Nei Genetic diversity index, polymorphism information content (PIC) and minimum distance of Nei between pairs of genetic groups were estimated using GenALEx 6.501. Genetic diversity structuring was carried out using the software DARwin V6.0. It was also used to calculate genetic differentiation between genetic groups based on Fst and generate dissimilarity matrix between accessions according *«simple matching»* method. A dendrogram was then constructed based on the dissimilarity matrix data using Neighbor-Joining method in order to identify genetic relatedness among the accessions.

## 3. RESULTS

### 3.1 Diversity of amaranth of Burkina Faso

Of the fourteen (14) markers tested, eight (08) were polymorphic. Data of polymorphic alleles were therefore used for calculation of genetic parameters (Table 1). A total of 40 alleles, ranging in size from 300 pb to 600 pb, have been identified and all loci were presented a polymorphism rate of 100%. The number of alleles per primer varies from 2 for primer 814.0 and 17898A to 8 for HB13 primer with an average of 5 alleles per primer tested. The effective number of alleles (Ae), ranges from 1.76 (17898A) to 6.30 (HB13) with an average of 3.94. The expected heterozygosity lied between 0.21 for the 814.0 primer and 0.50 for the 844A primer with an average of 0.37. Shannon's diversity Index (I) extended from 0.62 for marker 17898A to 2.10 for marker HB13. Shannon's diversity index (I) ranges from 0.62 for the 17898A primer to 2.10 for the HB13 primer. The polymorphism information content (PIC) varies from 0.41 for the UBC827 primer at 0.98 for the 814.0 primer with an average of 0.64.

**Table 1**: The table presents the genetic diversity amaranth revealed by markers used

| Primers | $A^t$ | Ae | I | He | PIC | P (%) |
|---|---|---|---|---|---|---|
| **HB13** | 8.00 | 6.0 | 2.10 | 0.35 | 0.43 | 100 |
| **HB14** | 6.00 | 4.97 | 1.71 | 0.39 | 0.48 | 100 |
| **814.0** | 4.00 | 2.54 | 0.73 | 0.21 | 0.98 | 100 |
| **844A** | 2.00 | 1.96 | 0.68 | 0.50 | 0.82 | 100 |
| **UBC827** | 6.00 | 4.44 | 1.31 | 0.29 | 0.41 | 100 |
| **17898B** | 6.00 | 4.61 | 1.55 | 0.34 | 0.50 | 100 |
| **17899A** | 6.00 | 4.98 | 1.70 | 0.39 | 0.63 | 100 |
| **17898A** | 2.00 | 1.76 | 0.62 | 0.44 | 0.90 | 100 |
| **Average** | 5.00 | 3.94 | 1.30 | 0.37 | 0.64 | 100 |

**At**: Total number of alleles, **Ae**: number of effective alleles, **I**: Shannon's diversity index, **He**: heterozygosity expected; **PIC**: Polymorphism Information Content, **P**: Rate of polymorphic loci.

### 3.2 Structuring of genetic diversity of the collection of amaranth studied

Table 2 shows low values of genetic parameters for the two climate zones (zone Sudano-Sahelian and Sudanese zone). In general, these parameters were higher in Sudano-sahelian zone than Sudanese zone, with an effective number of alleles of 1.61, an expected heterozygosity of 0.37, an index of Shannon diversity of 0.54 and a

polymorphism of 100%. On the other hand, in the Sudanese zone, the effective number of alleles is 1.51, an expected heterozygosity of 0.32, a Shannon diversity index of 0.46 and a polymorphism of 95%.

**Tableau 2:** The table presents the genetic diversity of collection according to climatical zones.

| Climatical zones | Ae | I | He | P |
|---|---|---|---|---|
| **Sudano-sahelian** | 1.61 | 0.54 | 0.37 | 100 |
| **Sudanese** | 1.51 | 0.46 | 0.32 | 95 |
| **Average** | 1.56 | 0.50 | 0.35 | 97.5 |

**Ae**: number of effective alleles; **I**: Shannon's diversity index; **He**: expected heterozygosity; **P** (95 %): Rate of polymorphic loci.

The minimum distance of Nei (0.012) showed a very small influence of the "zone factor climate" on inter-population differentiation (Table 3). Only about 1.2% of the total variability can be attributed to this factor.

**Table 3**: The table presents the genetic differentiation between climatical zone.

| | Minimum distance of Nei | |
|---|---|---|
| | **Sudano-sahelian** | **Sudanese** |
| **Sudano-sahelian** | 0 | |
| **Sudanese** | 0.012 | 0 |

The highest genetic diversity is observed within the green morphotypes. In fact, this morphotype has an effective number of alleles of 1.60, an expected heterozygosity of 0.37, a Shannon diversity index of 0.53 and a polymorphism of 100%. The purple morphotype presented polymorphism (30%) and Shannon's diversity index (0.21) the lowest. The lowest expected heterozygosity (0.26) is observed in purple morphotype (Table 4).

**Table 4** : The table presents the genetic diversity according to the 5 identified morphotypes.

| Morphotypes | Number of accession | Ae | I | He | P% |
|---|---|---|---|---|---|
| **Green** | 34 | 1.60 | 0.53 | 0.37 | 100 |
| **Purple green-** | 05 | 1.44 | 0.38 | 0.32 | 65 |
| **Dark-green** | 04 | 1.45 | 0.35 | 0.33 | 55 |
| **Purple** | 02 | 1.30 | 0.21 | 0.30 | 30 |
| **Violet** | 08 | 1.37 | 0.35 | 0.26 | 70 |
| **Average** | | 1.43 | 0.36 | 0.31 | 64 |

**Ae**: number of effective alleles; **I**: Shannon's diversity index; **He**: expected heterozygosity, **P** (95 %): Rate of polymorphic loci.

The results of genetic differentiation between the five morphotypes are recorded in Table 5. These results show a low genetic differentiation between morphotypes. The minimum distances of Nei vary from 0.024 to 0.136. The weakest genetic distances are observed between the green and violet morphotype (0.024); purple-green and dark green (0.045), and purple and violet (0.048). The highest values are observed between dark green and violet (0.125), and between dark green and purple (0.136). Not any genetic distance is observed between the green and purple-green morphotype, between the purple-green and purple morphotype, and between the purple-green and violet morphotype. Overall, the factors "climate zone" and "morphotype" have a low influence on the genetic differentiation of the amaranth collection.

**Table 5**: The table presents the genetic differentiation between the 5 identified morphotypes.

| Morphotypes | Minimum distance of Nei | | | | |
|---|---|---|---|---|---|
| | **Green** | **Purple green-** | **Dark-green** | **Purple** | **Violet** |
| **Green** | 0.000 | | | | |
| **Purple green** | 0.000 | 0.000 | | | |
| **Dark green** | 0.051** | 0.045ns | 0.000 | | |
| **Purple** | 0.064** | 0.000 | 0.136** | 0.000 | |
| **Violet** | 0.024ns | 0.000 | 0.125** | 0.048ns | 0.000 |

## 3.3 Organization of genetic diversity of the collection

The matrix of dissimilarities through the Neighbor-Joining method (Figure 5) classified the studied accessions in four (04) groups (A, B, C and D). Groups A and B contain the largest number of accessions and are made up of 20 accessions and 22 accessions respectively. The groups C and D each contain only 06 accessions. Diversity is

structured independently of the collection sites. From Even so, the distribution of groups is independent of morphotypes and species.

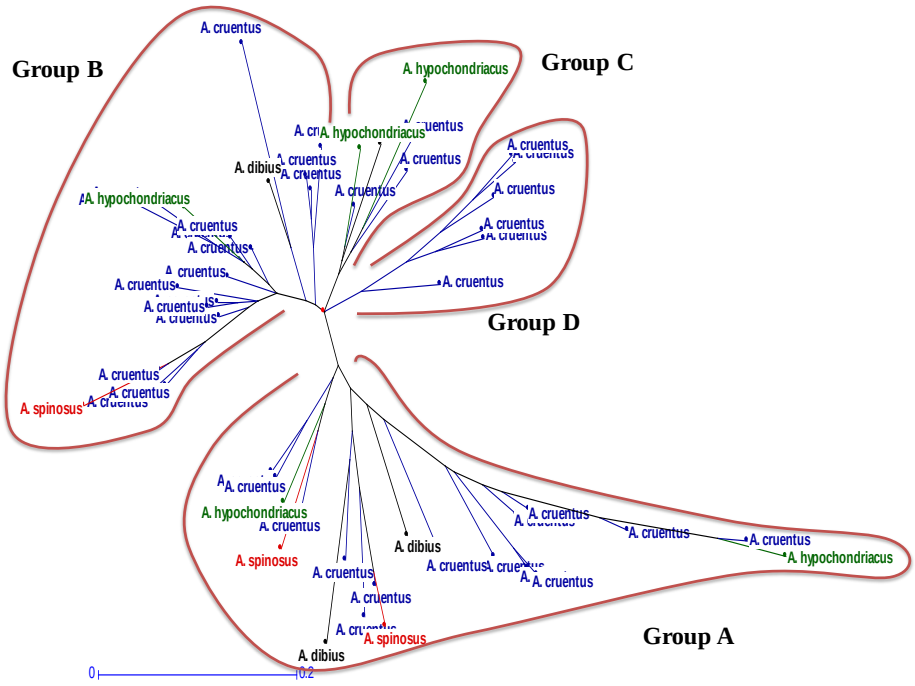


**Figure 5**: The figure presents the hierarchical representation of the dendrogram of the 54 accessions of amaranth constructed from the dissimilarities matrix using the Neighbour-Joining method.

## 3.4 Description of genetic groups

The genetic parameters of the four (04) genetic groups obtained from the matrix of dissimilarities according to the Neighbour-Joining method are recorded in Table 6. Group A and group D which have an effective number of alleles of 1.57 and 1.67, a polymorphism of 0.90, an expected heterozygosity of 0.40 and 0.38, a Shannon diversity index of 0.37 and 0.36 and a Polymorphism information contain of 0.74 and 0.63 respectively records the highest genetic parameters.

Group C with an effective number of alleles of 1.38, a polymorphism rate of 0.65, an expected heterozygosity of 0.28, a Shannon diversity index of 0.23 and a polymorphic information potential of 0.54, has the lowest parameters genetic. Group B, on the other hand, has genetic parameters ranging between those of the other three groups.

**Table 6:** The table presents the characteristics of genetic groups;

| Groups | Ae | I | He | PIC | %P |
|---|---|---|---|---|---|
| **Group A** | 1.64 | 0.36 | 0.38 | 0.74 | 90 |
| **Group B** | 1.48 | 0.28 | 0.29 | 0.51 | 80 |
| **Group C** | 1.38 | 0.23 | 0.28 | 0.54 | 65 |
| **Group D** | 1.57 | 0.34 | 0.40 | 0.63 | 90 |
| **Average** | 1.52 | 0.45 | 0.34 | 0.60 | 81.25 |

**Ae**: number of effective alleles, **I**: Shannon's diversity index, **He**: heterozygosity expected; **PIC**: Polymorphism Information Content, **P**: Rate of polymorphic loci.

## 3.5 Differentiation between genetic groups

The genetic distances between the three genetic groups showed the existence of low intergroup diversity between them (Table 7). Only Group D submitted a difference with other groups. The longest Nei minimum distance (0.04) was observed between groups D and A.

**Table 7**: The table presents the differentiation between genetic groups.

| | Minimum distance of Nei | | | |
|---|---|---|---|---|
| **Groups** | **Group A** | **Group B** | **Group C** | **Group D** |
| **Group A** | 0.000 | | | |
| **Group B** | 0.000 | 0.000 | | |
| **Group C** | 0.033 | 0.000 | 0.000 | |
| **Group D** | 0.040 | 0.005 | 0.020 | 0.000 |

## 4. DISCUSSION

The present study revealed the existence of genetic diversity in the collection of amaranth of Burkina Faso which genetic parameters were often higher than those of others studies. For example, the number of effectives alleles (3.94) and the PIC value (0.64) obtained in this study are higher than theses obtained (2.91 and 0.33 respectively) on a collection of *Cleome gynandra* by Kiébré, (2016) using the same markers [8]. The PIC value obtained for the all markers showed that these ISSR are informative for genetic diversity studied of amaranth. This study revealed that the tri-nucleotide repeat sequences were less informative than di-nucleotides repetition (CT and CA) in intraspecific diversity study of amaranth. These results are similar to those of [10, 11, 12].

The total of 40 alleles and the average of 5 alleles per locus observed in this study could be justified by the nature of the ISSR markers. Thus, ISSR markers amplified inter-microsatellites region and have advantage to be more polymorphic [13, 14]. The polymorphism rate of 100% for the all markers obtained is in accord of the results of [15] and [16] on *Amaranthus spp* (99 and 98.4 respectively).

The value of expected heterozygote and average of the allelic richness showed a moderate genetic diversity in the collection. The random structuration of the collection in 4 genetic group without regarding collection site could be due to farmers seed management. Indeed, at Burkina Faso, farmers seed management such as exchange or donation have been evoked by previous work [17, 8, 18] on traditional leafy and fruity vegetables.

The intra-morphotype genetic study revealed that green morphotype presented the higher value on expected heterozygote (He= 0.37) than the others. These results could be explained by the preference of those morphotype by consumers that influenced farmer's production in the two climatic zones. Kahane et al. (2005), showed that the green morphotype is the most widely consumed in Africa precisely in tropical region [19]. Diouf et al., (2007), also shown that green type amaranth accessions have the preferred characteristics of women producers in Senegal [20]. According to [6] as in Senegal, Burkina Faso, market gardeners orient amaranth production towards consumer needs. This observation was also made by Kiébré, (2016) on the species *Cleome gynandra* [8].

The low intra-accession and intra-group distance observed in this study could due to the technique or the type of the markers that were developed on species of *Cleome droserifolia*. These results could also due to mode of reproduction of Amaranths. Indeed, *Amaranthus* species are preferentially allogame that conduced to reduce inter-population diversity due to the gene flow [21].

The low values of genetic distance between accessions and genetic groups could be due to the technique and the type of markers used because these markers were developed on the *Cleome droserifolia*. They may therefore be unable to discriminate against species of amaranths. These low values could be also explained by the reproduction mode of amaranth species [11]. Indeed, according to [22], amaranth is an allogamous plant, which tends to reduce inter-population genetic diversity [21] due to the flow important genes between individuals. In fact, widely distributed species such as amaranth, with a very high gene flow, shows differentiations between populations very low, falling to less than 4%. Also, according to [23], the populations of self-pollinated species are further subdivided and differentiated on the each other than populations of allogamous or mixed species.

## 5. CONCLUSION

This study allowed showing a moderate genetic diversity within the collection of amaranth grown in Burkina Faso. The markers used in this study were polymorphic and informative on genetic diversity. Still, 8TG primer (CT) is presented as a better marker in the study of the intra-specific diversity of amaranth species. These markers permitted to grouping the accession in four heterogeneous genetic groups. Overall, the factors "climatical zone" and "morphotype" showed very little influence on the genetic differentiation of the amaranth collection. In fact, the future selections working would concerned to development of varieties that presented interests characters and adapted to the all climatical zones of country.

**Acknowledgments:** This research has been supported by International Foundation for Science (IFS), Sweden, through a scholarship given to Boureima SAWADOGO (D5923-1).

## 6. REFERENCES

1. Rangarajan A, Chenoweth WA, Kelly JF, Agee KM. Iron bioavailability from *Amaranthus* species: 2—Evaluation using haemoglobin repletion in anaemic rats. *J. Sci. Food and Agric.* 1998; 78 (2): 274-280. Available on: https://doi.org/10.1002/(SICI)1097-0010(199810)78:2<274::AID-JSFA115>3.0.CO;2-Z

2. Berger A, Monnard I, Dionisi F, Gumy D, Hayes KC, Lambelet P. Cholesterol-lowering properties of amaranth flakes, crude and refined oils in hamsters. *Food Chem.* 2003; 81: 119 – 124. Available on: https://doi.org/10.1016/S0308-8146(02)00387-4

3. Sogbohossou OED, Achigan-Dako EG, Komlan FA, Ahanchede A. Diversity and Differential Utilization of *Amaranthus spp*. along the Urban-Rural Continuum of Southern Benin. *Economic Botany*. 2015 ; 69 (1): 1- 17. Available on: http://www.researchgate.net
4. FAO. L'État de l'insécurité alimentaire dans le monde. *EN BREF*. 2015 ; 8 p. Available on: http://www.fao.org/3/a-i4646f.pdf
5. Somtore H, Sawadogo B, Bationo-Kando P, Kiebre M, Ouedraogo J, Kiebre Z. Ethnobotanical Investigation of Amaranth (*Amaranthus spp*) Cultivated in Burkina. *International Journal of Applied Agricultural Sciences*. 2019; 5 (2): 50-55. Available on : http://www.sciencepublishinggroup.com/j/ijaas
6. Somtore H. Etude de la Diversité Agromorphologique d'Accessions d'Amarante (*Amaranthus spp*) du Centre-Ouest et de l'Ouest du Burkina Faso. Master Professionnel en Sélection et Valorisation des Ressources Phyto-Génétiques, Univ. Ouaga. 2017: 49 p.
7. Doyle JJ. DNA protocols for plants. In: Hewitt G.M. Johnston A.W.B., Young J.P.W. (eds) Molecular Techniques in Taxonomy. NATO ASI Series (Series H: Cell Biology). 1991; 57, Springer, Berlin, Heidelberg: 283–293. Available on : https://doi.org/10.1007/978-3-642-83962-7_18
8. Kiébré Z. Diversité génétique d'une collection de Caya blanc (*Cleome gynandra* L.) du Burkina Faso. Thèse de doct. Unique, Univ. Ouaga. 2016: 126 p.
9. Martynov SP, Dobrotvorskaya TV, Dotlacil L, Stehno Z, Faberova I, Bares I. Genealogical approach to the formation of the winter wheat core collection. *Russian J. Genet.* 2003; 39 (8): 917-923. Available on: https://doi.org/10.1023/A:1025382807351
10. Singh B, Pandey S, Kumar J. A comparative study of Inter Simple Sequence Repeat (ISSR), Random Amplified Polymorphic DNA (RAPD) and Simple Sequence Repeat (SSR) loci in assessing genetic diversity in *Amaranthus*. Indian Journal of Genetics and Plant Breeding. 2013; 73 (4): 411- 418. Available on: http://www.indianjournals.com
11. Oduwaye OA, Ojo DK, Popoola AR, Daniel IO, Miroslav D. Genetic Diversity Assessment in Amaranth Germplasm using AFLP and ISSR Markers. *Journal of Crop Improvement*. 2014; 28: 4 (4): 518 – 529. Available on: http://dx.doi.org/10.1080/15427528.2014.921262
12. Štefúnová V, Bežo M, Žiarovská J, Ražná K. Detection of the genetic variability of *Amaranthus* by RAPD and ISSR markers. *Pak. J. Bot.* 2015; 47 (4): 1293-1301. Available on: http://www.researchgate.net
13. Zietkiewicz E, Raflski A, Labuda D. Genome fingerprinting by simple sequence repeat (SSR)- anchored Polymerase Chain Reaction Amplification. *Genomics*. 1994; 20: 176 – 183. Available on : http://doi.org/10.1006/geno.1994.1156
14. Konate I. Diversité Phénotypique et Moléculaire du Caroubier (*Ceratonia siliqua* L.) et des Bactéries Endophytes qui lui sont Associées. Thèse De Doctorat Université Mohammed V-Agdal Faculté Des Sciences Rabat. 2007; 196 p. Available on: http://www.researchgate.net
15. Fangxiu X, Mei S. Comparative Analysis of Phylogenetic Relationships of Grain Amaranths and Their Wild Relatives (*Amaranthus*; *Amaranthaceae*) Using Internal Transcribed Spacer, Amplified Fragment Length Polymorphism, and Double-Primer Fluorescent Inter Simple Sequence Repeat Markers. *Molecular Phylogenetics and Evolution.* 2001; 21 (3): 372–387. Available on: http://www.idealibrary.com
16. Ray T, Roy SC. Phylogenetic Relationships between Members of *Amaranthaceae* and *Chenopodiaceae* of Lower Gangetic Plains Using RAPD and ISSR Markers. *Bangladesh J. Bot.* 2007; 36 (1): 21-28. Available on: http://doi.org/10.3329/bjb.v36i1.1544
17. Bationo-Kando P, Sawadogo B, Nanema KR, et al. Characterization of *Solanum aethiopicum* (Kumba group) in Burkina Faso. *International Journal of Sciences and Nature*. 2015; 6 (2): 169 – 176, ISSN 2278 – 9103. Available on: http://www.researchgate.net
18. Ouédraogo MH. Etude de la diversité génétique des gombos [*Abelmoschus esculentus* (L.) MOENCH] cultivés au Burkina Faso. Thèse de doc. Unique Univ. Ouaga. 2016: 158 p.
19. Kahane R, Temple L, Brat P, DE BON H. Les légumes feuilles des pays tropicaux : diversité, richesse économique et valeur santé dans un contexte très fragile. *Colloques Angers*. 2005: 9 p. Available on: http://www.agritrop.cirad.fr
20. Diouf M, Lo C, Gueye M, Mbengue NB. Sélection participative de nouveaux cultivars de quatre (4) espèces de légumes-feuilles (*Hibiscus sabdariffa* L., *Amaranthus spp* L., *Vigna unguiculata* (L.) WALP et *Moringa oleifera* Lam) au Sénégal. *Africa Journal of food Nutrition agriculture and development*, 2007; 7 (3): 17 p. Available on : http://www.bioline.org.br
21. Hubert-Vincent F. Diversité génétique et adaptation des espèces aquatiques en milieu anthropisé. Direction Centre de Nantes Département Biogéochimie et Ecotoxicologie Laboratoire Ecotoxicologie, *ifremer*. 2007: 36 p. https://archimer.ifremer.fr/doc/00000/3889/
22. Grubben GJH, Denton OA. Plant Resources of Tropical Africa 2. Vegetables. PROTA Foundation, Wageningen, Netherlands / Backhuys Publishers, Leiden, Netherlands / CTA, Wageningen, Netherlands. 2004: 668 p. Available on: https://www.edepot.wur.nl › ...
23. Ouédraogo M, Maquet A, Baudoin JP. Étude comparative de la diversité et de la structure génétique de populations sauvages de *Phaseolus lunatus* L. à l'aide des marqueurs enzymatiques et microsatellites. *Biotechnol. Agron. Soc. Environ*. 2005; 9 (3): 195 – 205. Available on: https://popups.uliege.be:443/1780-4507/index.phd?id=1477.

**Cite this article: Jacques Ouédraogo, Mariam Kiébré, Zakaria Kiébré, Boureima Sawadogo and Pauline Bationo/Kando.** GENETIC DIVERSITY OF A COLLECTION OF AMARANTH (Amaranthus spp) OF BURKINA FASO USING ISSR MARKERS. *Am. J. innov. res. appl. sci. 2019; 9(3): 284*-292.