African Journal of Biotechnology Vol. 10(15), pp. 2811-2819, 11 April, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1983
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Assessment of genetic diversity among accessions of two traditional leafy vegetables *(Acmella uliginosa* (L.) and *Justicia tenella* (Nees) T.) consumed in Benin using amplified fragment length polymorphism (AFLP) markers

Adéoti K.[1], Rival A.[2], Dansi A.[3]*, Ahohuendo B.C[4], Santoni S.[5], Beule T.[2], Nato A.[6], Henry Y.[7], Ahanchédé A.[4], Vodouhè R.[8], Hounhouigan D.J.[4] and Sanni A.[1]

[1]Laboratory of Biochemistry and Molecular Biology, Faculty of Sciences and Technology (FAST), University of Abomey-Calavi (UAC), BP 526 Cotonou, Republic of Benin.
[2]Cirad-BioS, UMR DIAPC, Centre IRD. 34394 Montpellier Cedex 15, France.
[3]Laboratory of Agricultural Biodiversity and Tropical Plant Breeding, Faculty of Sciences and Technology (FAST), University of Abomey-Calavi (UAC), 071BP28, Cotonou, Republic of Benin.
[4]Faculty of Agriculture (FSA), University of Abomey-Calavi (UAC), BP 526 Cotonou, Republic of Benin.
[5]Centre INRA, UMR DIAPC, Unité de Génétique et Amélioration des plantes, Atelier de Marquage Moléculaire, 34060 Montpellier Cedex 01, France.
[6]Institut de Génétique et Microbiologie, Bâtiment 360, Université Paris-Sud 11, 91405, Orsay, France.
[7]Institut de Biotechnologie des Plantes, Bâtiment 630, Université Paris-Sud 11, 91405 Orsay, France.
[8]Bioversity International, Office of West and Central Africa, 08 BP 0931, Cotonou, Republic of Benin.

Accepted 18 February, 2011

Amplified fragment length polymorphism (AFLP) markers were used to evaluate the genetic diversity and explore the genetic relationship among accessions of *Acmella uliginosa* and *Justicia tenella*, two leaf vegetables collected from different areas in the northwest and northeast parts of Benin (West Africa). The total number of exploitable amplicons revealed with genomic DNA from *A. uliginosa* was 224 with an average of 50.5% polymorphic amplicons. Using DNA from *J. tenella*, we obtained 34% of polymorphic amplicons from a total of 418. The coefficient of dissimilarity varied from 0.01 to 0.67 and from 0.17 to 0.62 for *Acmella* and *Justicia*, respectively. Low genetic diversity was observed among *Acmella* accessions although three distinct clusters could be differentiated. Contrarily, a great genetic diversity was observed among *J. tenella* accessions. In addition to this, most of the clusters were heterogeneous and showed the relationship between accessions collected from northeast and northwest. Our results confirm the robustness of AFLP techniques for genetic diversity studies and they provide the first set of molecular data for these two species.

Key words: Amplified fragment length polymorphism (AFLP), genetic diversity, leafy vegetable, Benin.

INTRODUCTION

Traditional leafy vegetables (TLVs) are plants whose leaves are socially accepted, used and consumed (Shippers, 2000). In the Republic of Benin, they occur as cultivated and semi-cultivated crops or weedy and wild plants, with ecological, social and cultural values, playing a significant role in the daily food and nutritional requirements of local people mainly in rural areas, but more increasingly in urban zones. Recent surveys implemented throughout the country revealed 187 species of

*Corresponding author. E-mail: adansi2001@gmail.com or agrobreed.fast@gmail.com.

TLVs of interest, among which are *Acmella uliginosa* and *Justicia tenella* (Dansi et al., 2008a, 2009). *A. uliginosa* and *J. tenella* are distributed throughout Africa (Shippers, 2000) where they are known at both cultivated and wild state. In Benin, these neglected and underutilized species (NUS) are locally cultivated mainly in the northern part of the country, where they are intensively consumed (Dansi et al., 2008b; Adéoti et al., 2009). *A. uliginosa* is a key nutraceutical for local rural populations. It is used as an antibiotic, which stimulates milk production and facilitates the elimination of blood clots in women after delivery (Dansi et al., 2009). *J. tenella* is also an appreciated leafy vegetable. The leaves are cooked and consumed as spinach. For medicinal purposes, the leaves are used to treat cardiac disorder, diarrhoea, fever and indigestion (Denton, 2004; Dansi et al., 2008c). The plant possesses anti-inflammatory activity and is also used as antidepressant (Sanmugapriya et al., 2005). Thus, is of paramount importance that the agro biodiversity of these important NUS be precisely characterised with the use of modern molecular tools. Genetic resour-ces must be preserved based on this accurate characterization for the benefit of local populations. The assessment of intraspecific genetic diversity and the understanding of its structure are a prerequisite for any further action. To the best of our knowledge, such information has never been reported on these species.

There are now a number of molecular markers which have proven very efficient in assessing plant genetic diversity (Santoni et al., 2000). Among them, amplified fragment length polymorphism (AFLP) (Vos et al., 1995) has been found to be the most appropriate in many cases. AFLPs were described as a powerful and efficient approach in population genetics and diversity analysis, molecular taxonomic classification, gene mapping and marker-assisted breeding for various crops (Ayele et al., 1999; Carr et al., 2003; Uptmoor et al., 2003). AFLP analysis provides an effective means of covering large area of the genome in a single assay (Ayad et al., 1997; Milbourne et al., 1997; Zhang et al., 1999; Muminovic et al., 2004). It is highly reproducible and discriminative (Rafalski and Tingey, 1993; Savelkoul et al., 1999; Soleimani et al., 2002), and generate a virtually unlimited number of genetic markers (Blears et al., 1998; Gaudeul et al., 2000). AFLP has been already used to assess genetic diversity in many crops such as hibiscus (Tiang et al., 2003), peach (Xu et al., 2006), linseed (Adugna et al., 2006), soybean (Tara Satyavathi et al., 2006), Rice (Mackill et al., 1996), wheat (Shoaib and Arabi, 2006), sesame (Laurentin and Karlovsky, 2007) and fonio (Adoukonou-Sagbadja et al., 2007).

In this study, we used AFLP markers to assess the genetic diversity and analyse the relationship among accessions of *A. uliginosa* and *J. tenella* collected from different agroecological zones of Benin, West Africa.

MATERIALS AND METHODS

Plant material

The plant materials under study consists of seventeen accessions of *A. uliginosa* and fourteen accessions of *J. tenella* collected from various villages (Table 1) located in the northern part of Benin (Adeoti et al., 2009). Accessions were maintained as field collections at the Biological Control Station of the International Institute of Tropical Agriculture (IITA) based in Cotonou, Benin.

DNA extraction

In order to take into account possible individual genetic variability within each accession, total genomic DNA was extracted from bulked young leaves (100 to 200 mg FW per accession) collected from ten 2- to 3-week-old plants. DNA from freshly collected material was extracted following the MATAB (mixed alkyltriméthylammonium bromide) procedure according to Doyle and Doyle (1990). After RNAse treatment, DNA content was fluorometrically quantified (GENIOS PLUS TECAN Scientific Instruments) using Hoechst 33258 dye and diluted to 25 ng/µl working solution.

AFLP protocol

AFLP analysis was performed as originally described by Vos et al. (1995) with minor modifications. Here, 250 ng of genomic DNA (10 µl of working solution) were digested using *EcoRI* and *MseI* restriction enzymes and the generated fragments were ligated with double-stranded site-specific adapters using T4 DNA ligase. Following ligation, a pre-amplification was carried out with primers containing one selective nucleotide cytosine and adenine for *MseI* and *EcoRI* primers, respectively. PCR was performed for 30 cycles which consisted of 1 min at 94°C, 1 min at 56°C, and 1 min at 72°C with final extension for 3 min at 72°C. Resulting PCR products were 10 times diluted with sterile double distilled water and used as templates for the selective amplification step. This was carried out with a couple of selective primers (*EcoRI*/*MseI*) containing three selective nucleotides at the side. *EcoRI* was labelled with fluorescent dye. Selective amplification was performed on two steps in a total volume of 20 µl containing 5 µl of diluted pre-selective PCR product, 2 µl of each primer at 1 pmole/µl for *EcoANN* and 5 pmole/µl for *MseCNN* and 0.2 µl of 1 U of Taq polymerase. The first step of selective amplification consisted of 12 cycles, 3 min at 94°C, 45 s at 94°C, 45 s at 65°C and 1 min at 72°C for final extension. The second step was performed for 25 cycles which consisted of 45 s at 94°C, 45 s at 56°C, 1 min at 72°C and 3 min at 72°C. The PCR products of selective amplification were diluted 10 times and an aliquot (2 µl) of diluted solution was mixed with 18 µl of a ROX-labeled internal size standard (AMM 524). Then the mixture was denaturated for 5 min at 95°C, loaded and separated on an ABI PRISM 3130X Genetic Analyzer sequencer (Applied Biosystems).

Scoring and analysis of AFLP data

Electrophoregram generated by the sequencer were analysed using the GeneMapper version 3.7 software package (Applied Biosystem, 2004). Clear and unambiguous peaks were considered as AFLP markers and scored as present (1) or absent (0) in order to generate a binary data matrix. The total number of scored markers and the number and percentage of polymorphic markers were determined for each primer pair used. Polymorphic markers were used for further data analysis. With the binary matrix (0, 1) compiled, pairwise relatedness between all accessions was esti-

Table 1. Studied accessions of *A. uliginosa* and *J. tenella* analysed and corresponding collecting sites.

Species	Accession number	Collecting sites	County	Region
Acmella uliginosa	AA1	Boukoumbé	Atacora	Northwest
	AA2	Koutagou	Atacora	Northwest
	AA3	Cobly	Atacora	Northwest
	AA4	Souomou 1	Atacora	Northwest
	AA5	Tiélé	Atacora	Northwest
	AA6	Tchakalakou	Atacora	Northwest
	AA7	Péporiyakou 1	Atacora	Northwest
	AA8	Pam-Pam	Atacora	Northwest
	AA9	Dangoussar	Atacora	Northwest
	AA10	Bajoudè	Donga	Northwest
	AA11	Koupagou	Atacora	Northwest
	AA12	Péporiyakou 2	Atacora	Northwest
	AA13	Souomou 2	Atacora	Northwest
	DA1	Djougou	Donga	Northwest
	DA2	Belléfoungou	Donga	Northwest
	DA3	Borondy	Donga	Northwest
	DA5	Kawado	Donga	Northwest
Justicia tenella	AJ1	Nouagou	Atacora	Northwest
	AJ4	Péporiyakou	Atacora	Northwest
	BJ1	Sonoumon	Borgou	Northeast
	BJ2	Bori	Borgou	Northeast
	BJ3	Wèrèkè	Borgou	Northeast
	BJ4	Boroyerou	Borgou	Northeast
	BJ5	Ina	Borgou	Northeast
	DJ1	Nalohoun I	Atacora	Northwest
	DJ2	Dangoussar	Atacora	Northwest
	L1S	Gogounou	Borgou	Northeast
	LJ2	Toumè	Borgou	Northeast
	LJ3	Sérou	Borgou	Northeast
	LJ4	Guéssou-sud	Borgou	Northeast

mated using Dice index of similarity (Dice, 1945). Using DARwin5 software package Version 5.0.158 (Perrier and Jacquemoud-Collet, 2006) and the Neighbor-joining method, a dendrogram was generated with the aim of analysing the relationship between accessions. The binary matrix was also used to undertake a factorial coordinate analysis (FCA) with the same software in order to obtain a graphical representation of the genetic diversity patterns existing among accessions.

RESULTS

Diversity among *A. uliginosa* accessions

A total of four pairs of primer combinations were screened. Among these, only three generated useful amplification products with a high polymorphism; these were selected for DNA profiling. Sequences of selected primers, total number of generated markers and associated polymorphism are shown in Table 2. The number of amplicons obtained per primer combination ranged from 51 to 88 and the percentage of polymorphic amplicons generated by each primer combination ranged from 30 to 71% (Table 2). A total of 224 amplicons (50 to 500 pb) was generated, from which 50.50% (114 amplicons) were found to be polymorphic. The genetic dissimilarity index calculated between accessions ranged from 0.01 to 0.67 (Table 3). The lowest value was obtained between AA12 collected at Perporiyakou 2 and AA11 collected at Koupagou, while the highest value was calculated between AA1 and AA7 sampled at Boukoumbé and Péporiyakou1, respectively. The generated dendrogram based on the dissimilarity matrix (Table 3) using the

Table 2. Number of AFLP amplicons and corresponding rate of polymorphism for the two species under study.

Species	Primer combination	Number of amplicons	Number of polymorphic amplicons	Percentage of polymorphism (%)
A. uliginosa	EcoACT FAM / MseCAT	88	27	30
	EcoAAG HEX / MseCTC	85	61	71
	EcoACG TAM / MseCAG	51	26	50
J. tenella	EcoACT FAM / MseCAT	92	15	16
	EcoACG TAM / MseCAG	92	45	49
	EcoACG / MseCTA	93	31	33
	Eco ACA / MseCAA	81	27	33
	EcoAGG / MseCTC	60	26	43

Table 3**.** Dissimilarity matrix between accessions of *A. uliginosa* as revealed by AFLP markers.

	AA1	AA2	AA3	AA4	AA5	AA6	AA7	AA8	AA9	AA10	AA11	AA12	AA13	DA1	DA2	DA3
AA2	0.41															
AA3	0.41	0.04														
AA4	0.51	0.08	0.10													
AA5	0.48	0.05	0.09	0.06												
AA6	0.52	0.22	0.19	0.23	0.23											
AA7	**0.67**	0.49	0.45	0.50	0.49	0.37										
AA8	0.48	0.09	0.10	0.11	0.08	0.23	0.50									
AA9	0.40	0.03	0.03	0.07	0.06	0.19	0.45	0.07								
AA10	0.43	0.03	0.07	0.08	0.05	0.23	0.50	0.08	0.05							
AA11	0.43	0.02	0.04	0.08	0.06	0.22	0.50	0.09	0.03	0.03						
AA12	0.44	0.03	0.05	0.09	0.08	0.23	0.50	0.09	0.04	0.05	0.01					
AA13	0.46	0.07	0.10	0.07	0.06	0.24	0.48	0.09	0.07	0.08	0.07	0.07				
DA1	0.49	0.07	0.10	0.05	0.05	0.24	0.51	0.09	0.07	0.08	0.07	0.07	0.03			
DA2	0.46	0.05	0.07	0.05	0.03	0.22	0.50	0.08	0.04	0.05	0.04	0.05	0.04	0.04		
DA3	0.49	0.07	0.09	0.03	0.04	0.23	0.50	0.10	0.06	0.07	0.06	0.08	0.05	0.04	0.02	
DA5	0.39	0.05	0.04	0.09	0.08	0.19	0.45	0.09	0.02	0.06	0.04	0.06	0.09	0.09	0.06	0.08

neighbour-joining approach of the UPGMA method showed three distinct clusters (Figure 1):

1. Cluster I contains accessions collected at Djougou,Borondy, Bellefoungou (County of Donga) and at Souo-mou, Tiele and Pam Pam (East of the County of Atakora).
2. Cluster 2 groups together accessions from Dangous-sar, Cobly and Kawado located along the Benin/Togo border.
3. Cluster 3 joins individuals collected at Perporiyakou1, Boukoumbé and Tchakalakou situated at the centre of the County of Atakora.

Axis 1 of the factorial coordinate analysis (FCA) separates the accessions analysed into two groups (Figure 2) of which one is exactly cluster 2 and the second one, the other accessions (Cluster 1 and Cluster 3) which are not structured into differentiated groups.

Diversity among *J. tenella* accessions

Five primers combinations were used to assess the genetic diversity among *J. tenella* accessions (Table 2). A total of 418 fragments were produced among which, only 144 were found to be polymorphic. The average polymorphic rate was 34%. The number of amplicons per primer combination ranged from 60 to 93 and the percentage of polymorphic amplicons varied from 16 to 49% (Table 2). The dissimilarity indexes among accessions varied from 0.17 to 0.62 (Table 4). The lowest

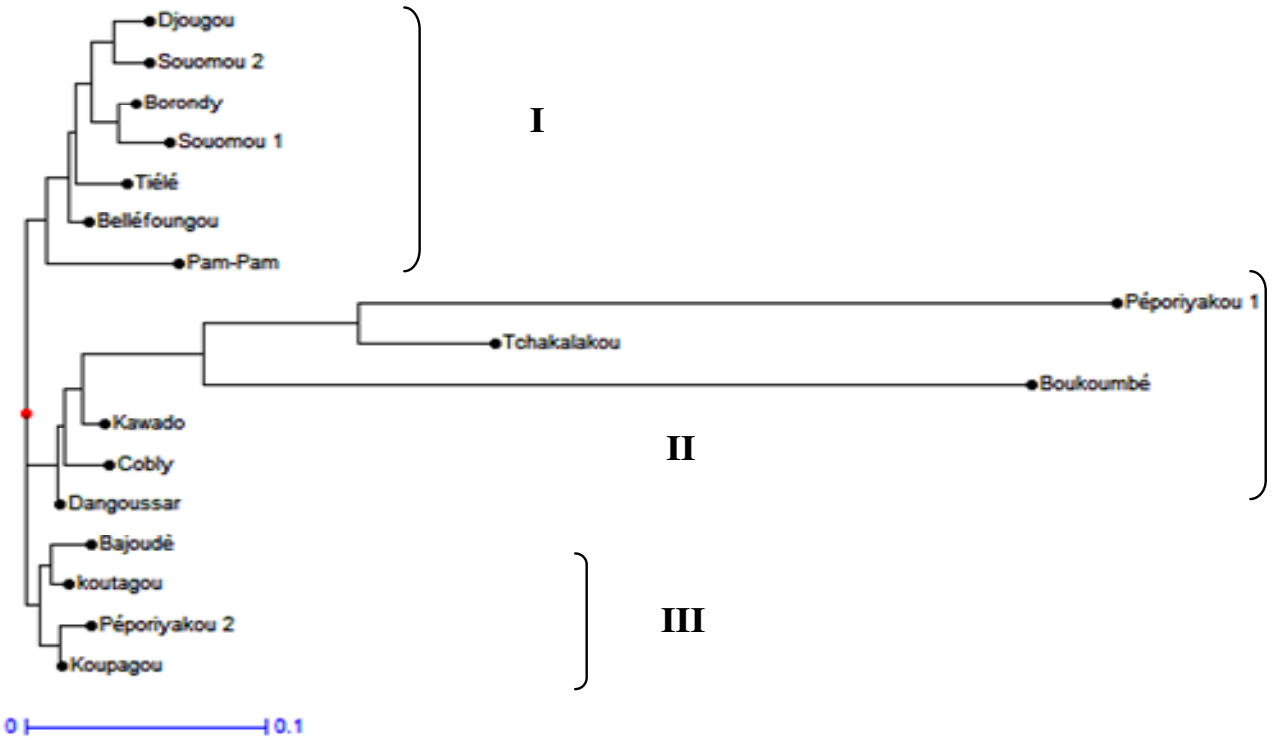

Figure 1. Neighbor-joining analysis of *A. uliginosa* accessions. The dendrogram was generated from UPGMA cluster analysis of dissimilarity data.

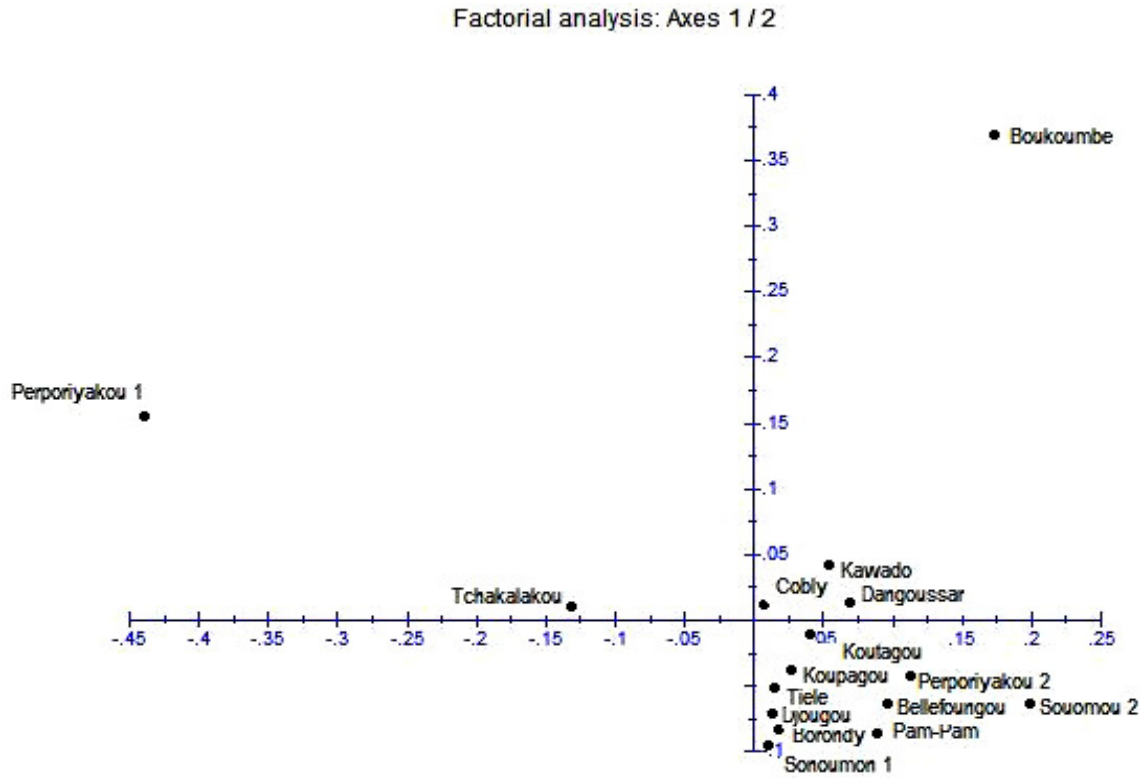

Figure 2. Factorial coordinate analysis for *A. uliginosa* accessions as generated by DARwin software using dissimilarity coefficient matrix calculated from AFLP data.

Table 4. Dissimilarity matrix of *J. tenella* accessions based on AFLP data.

	AJ1	AJ4	BJ1	BJ2	BJ3	BJ4	BJ5	DJ1	DJ2	L1S	LJ2	LJ3	LJ4
AJ4	0.52												
BJ1	0.52	**0.17**											
BJ2	0.52	0.40	0.37										
BJ3	0.51	0.35	0.30	0.37									
BJ4	0.51	0.37	0.32	0.42	0.34								
BJ5	0.51	0.46	0.39	0.54	0.45	0.41							
DJ1	0.51	0.44	0.45	0.48	0.47	0.44	0.45						
DJ2	0.59	0.54	0.53	0.53	0.52	**0.62**	0.60	0.55					
L1S	0.48	0.51	0.49	0.50	0.48	0.51	0.53	0.51	0.50				
LJ2	0.56	0.23	0.24	0.44	0.37	0.38	0.45	0.48	0.55	0.50			
LJ3	0.53	0.19	0.21	0.39	0.31	0.36	0.44	0.43	0.55	0.51	0.27		
LJ4	0.55	0.26	0.29	0.46	0.36	0.39	0.47	0.50	0.55	0.52	0.32	0.27	
LJ5	0.48	0.46	0.39	0.51	0.46	0.44	0.31	0.46	0.57	0.52	0.46	0.44	0.46

similarity index (the highest dissimilarity) was found between the accession collected at Boroyerou (County of Borgou) and the one sampled at Dangoussar (County of Atakora). The highest similarity index was calculated between the individual taken at Péporiyakou (Atakora) and the one collected at Sonoumon (Atakora). The dendrogram revealed three genetic groups, designated as I, II and III (Figure 4). Apart from the group III which joins two accessions from the County of Borgou, the other two groups are composed of samples from both Borgou and Atakora.

DISCUSSION

The amplified fragment length polymorphism (AFLP) analysis revealed a high level of similarity between accessions of *A. uliginosa*. This is an indication of low genetic diversity among the collected accessions. Cluster II of the UPGMA dendrogram (Figure 1) based on the dissimilarity matrix assembles individuals from Dangoussar, Kawado and Cobly, three geographically distinct bordering villages of Togo (Figure 3). Somehow, these results are in agreement with the farmers' assumptions which depict this species as originating from Togo (Adéoti et al., 2009). Three accessions from this cluster which were collected at Péporiyakou, Boukoumbé and Tchakalakou seem to be genetically different. They probably originate from another ancient introduction of the species into Benin from Burkina Faso. A second hypothesis suggests the existence of pure specimen or hybrids from *Acmella oleracea*, a wild relative of *A. uliginosa* which is well known by farmers. Nevertheless, a precise morphological examination of these three samples by taxonomists from the National Herbarium of Benin confirmed their identity as *A. uliginosa*, which makes more plausible our first hypothesis. In fact, the Wama, Ditamari and Natimba ethnic groups from these villages are all originating from both Burkina Faso (mainly) and Togo (Adam and Boco, 1993). The use of this species by the Gourmantché ethnic group (from Burkina Faso), the recent findings on the multiple origins of fonio in Benin (Adoukonou-Sagbadja et al., 2006; Adoukonou-Sagbadja et al., 2007; Dansi et al., 2010) as well as the grouping of Benin ethnic groups based on their origin with regard to the leafy vegetables species they consume (Dansi et al., 2008a), concomitantly support this hypothesis. Then diversification observed among *A. uliginosa*' accessions could be related to people migration through this part of country.

Contrary to *A. uliginosa*, no clear genetic structuring could be obtained within the accessions of *J. tenella* (Figure 4) despite the high level of amplicons generated per primer. Other AFLP markers could be tested to confirm once again this result. The two major clusters assemble individuals collected from Atakora as well as those from Borgou. Therefore, the accessions collected from the Northwest were not genetically very different from the ones collected from the Northeast. This result was rather expected when our previous report is considered (Adéoti et al., 2009). Indeed, we described this species as mainly located in the Northeast with a spread towards the Western region resulting from the migration of people.

Conclusion

Results from the present study confirm the robustness and the suitability of the AFLP approach for plant diversity analysis and for the assessment of genetic relationship among individuals of a given species

Figure 3. Sampling sites for *A. uliginosa* (L.) and *J. tenella* (Nees) T in Northern Benin.

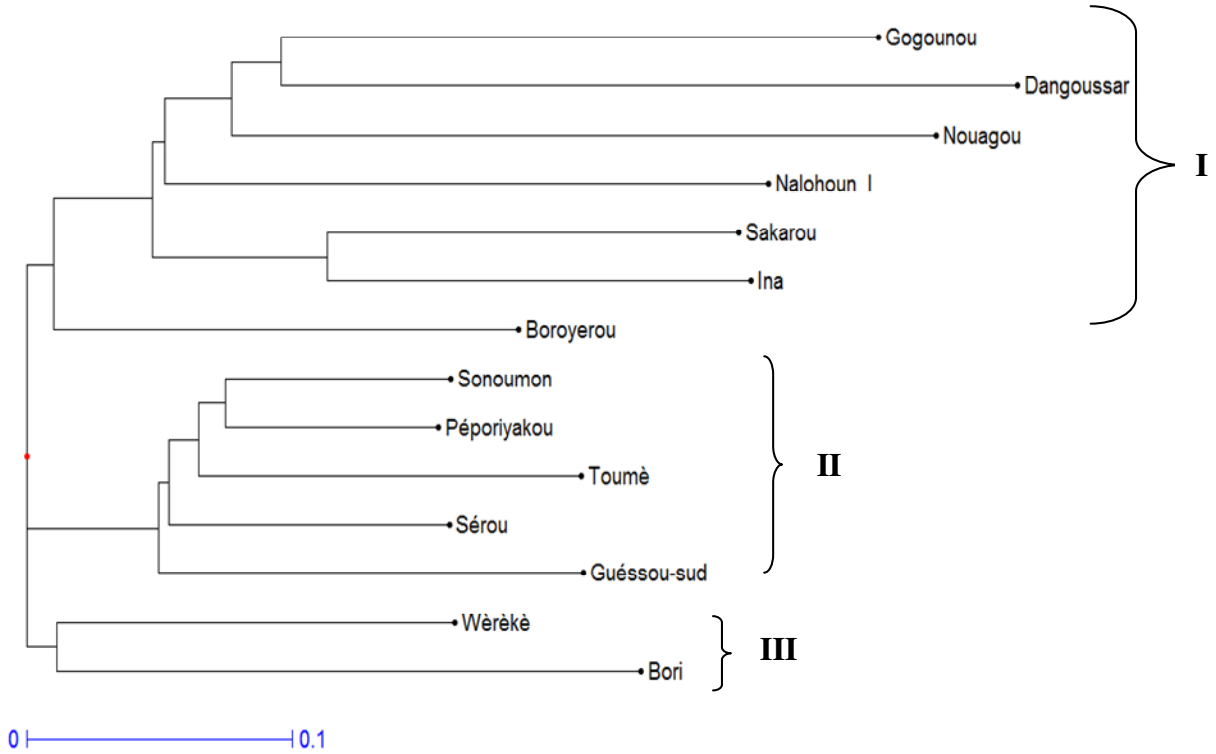

Figure 4. Neighbor-joining analysis of *J. tenella* accessions. The dendrogram was generated from UPGMA cluster analysis of dissimilarity data.

collected from different locations. We have applied this technique to two different neglected and underutilized species from Benin (*A. uliginosa* L. and *J. tenella* (Nees) T.) for the first time. More investigations are needed to clarify the origin (single or multiple) of *A. uliginosa* in Benin taking into account accessions from Togo and Burkina Faso. Likewise, other molecular markers such as ISSR or microsatellites could be useful to deepen the genetic relationship among accessions of these two species. The present study will be extended to two morphologically close species of TLVs (*Sesamum radiatum* and *Ceratotheca sesamoides*) in order to examine their genetic relationship and to explore the relationship between ecology (soil, climate) and genetic diversity.

Abbreviations

AFLP, Amplified fragment length polymorphism; **TLVs,** traditional leafy vegetables; **NUS,** underutilized species.

REFERENCES

Adam S, Boko M (1993). Le Bénin. Les éditions du Flamboyant/Edicef. p. 96.

Adeoti K, Dansi A, Ahoton L, Kpeki B, Vodouhe R, Ahanchede A, Ahohuendo B, Hounhouigan J, Sanni A (2009). Selection of sites for the *in situ* conservation of four traditional leafy vegetables (*Ceratotheca sesamoides, Sesamum radiatum, Acmella uliginosa* and *Justicia tenella*) consumed in Benin. Int. J. Biol. Chem. Sci. 3(6): 1357-1374.

Adoukonou-Sagbadja H, Wagner C, Dansi A, Ahlemeyer J, Daïnou O, Akpagana K, Ordon F, Friedt W (2007). Genetic diversity and population differentiation of traditional fonio millet (*Digitaria* spp.) landraces from different agro-ecological zones of West Africa. Theor. Appl. Genet. 115: 917-931.

Adoukonou-Sagbadja H, Dansi A, Vodouhè R, Akpagana K (2006). Indigenous knowledge and traditional conservation of fonio millet (*Digitaria exilis* Stapf, *Digitaria iburua* Stapf) in Togo. Biodivers. Conserv. 15: 2379-2395.

Adugna W, Labuschagne MT, Viljoen CD (2006). The use of morphological and AFLP markers in diversity analysis of linseed. Biodivers. Conserv. 15: 3193-3205.

Ayad WG, Hodgkin T, Jaradat A, Rao VR (1997). Molecular genetics techniques for plant genetic resources. Rep. IPGRI Workshop, 9-11 October 1995, Int. Plant Genet. Res. Inst., Rome, Italy.

Ayele M, Tefera H, Assefa K, Nguyen HT (1999). Genetic characterization of two *Eragrostis* species using AFLP and morphological traits. Hereditas, 130: 33-40.

Blears MJ, Grandis SA, Lee H, Trevors JT (1998). Amplified fragment length polymorphism (AFLP): A review of the procedure and its applications. J. Ind. Microbiol. Biotechnol. 21: 99-114.

Carr J, Xu M, Dudley JW, Korban SS (2003). AFLP analysis of genetic variability in New Guinea impatiens. Theor. Appl. Genet. 106: 1509-1516.

Dansi A, Adjatin A, Adoukonou-Sagbadja H, Faladé V, Yedomonhan H, Odou D, Dossou B (2008a). Traditional leafy vegetables and their use in the Benin Republic. Genet. Resour. Crop Evol. 55: 1239-1256.

Dansi, A, Adjatin A, Adoukonou-Sagbadja H, Akpagana K (2008b) Production and traditional seeds conservation of leafy vegetables in Benin rural areas. Bulletin de la Recherche Agronomique du Benin,

59: 59-70.
Dansi A, Adjatin A, Adoukonou-Sagbadja H, Adeoti K, Vodouhe R, Akpagana K, Yedomohan P, Akouegninou A (2008c). Biodiversité des légumes feuilles traditionnels consommés au Bénin, ISBN: 978-99919-68-63, 6: p. 178.
Dansi A, Adjatin A, Adoukonou-Sagbadja H, Adomou A, Faladé V, Yedomonhan H, Akpagana K, De Foucault B (2009). Traditional leafy vegetables in Benin: Folk nomenclature, species under threat and domestication. Acta Botanica Gallica, 156(2): 183-199.
Dansi A, Adoukonou-Sagbadja H, Vodouhe R (2010). Diversity, conservation and related wild species of Fonio millet (*Digitaria spp.*) in the northwest of Benin. Genet. Resour. Crop Evol. 57(6): 827-839.
Denton OA (2004). *Justicia ladanoides* Lam. In: Grubben GJH & Denton OA (Editors). PROTA 2: Vegetables/Légumes. [CD-Rom]. PROTA, Wageningen, Netherlands.
Rafalski JA, Tingey SV (1993). Genetic diagnostics in plant breeding: RAPDs, microsatellites and machines. Trends Genet. 9: 275-280.
Gaudeul M, Taberlet P, Till-Bottraud I (2000). Genetic diversity in an endangered alpine plant, *Eryngiumalpinum* L. (Apiaceae), inferred from amplified fragment length polymorphism markers. Mol. Ecol. 9: 1625-1637.
Laurentin H, Karlovsky P (2007). AFLP fingerprinting of sesame (*Sesamum indicum* L.) cultivars: identification, genetic relationship and comparison of AFLP informativeness parameters. Genet. Resour. Crop. Evol. 54: 1437-1446.
Mackill DJ, Zhang Z, Redona ED, Colowit PM (1996). Level of polymorphism and genetic mapping of AFLP markers in rice. Genome, 39: 969-977.
Milbourne D, Meyer R, Bradshaw J, Baird E, Bonar N, Provan J, Powell W, Waught R (1997). Comparison of PCR-based marker systems for the analysis of genetic relationships in cultivated potato. Mol. Breed. 3: 127-136.
Muminovic J, Melchinger A, Lubberstedt T (2004). Genetic diversity in cornsalad (*Valerianella locusta*) and related species as determined by AFLP markers. Plant Breed. 123: 460-466.
Perrier X, Jacquemoud-Collet JP (2006). DARwin software, http://darwin.cirad.fr/darwin
Sanmugapriya E, Shanmugasundaram P, Venkataraman S (2005). Anti-inflammatory activity of *Justicia prostrata* gamble in acute and sub-acute models of inflammation. Inflammopharmacology, 13(5-6): 493-500.
Santoni S, Faivre-Rampant P, Prado E, Prat D (2000). Marqueurs moléculaires pour l'analyse des ressources génétiques et l'amélioration des plantes. Cahiers Agric. 9: 311-327.
Savelkoul P, Aarts H, Dehaas J, Dijkshoorn L, Duim B, Otsen M, Rademaker J, Schouls L, Lenstra J (1999). Amplified-fragment length polymorphism analysis: the state of an art. J. Clin. Microbiol. 37: 3083-3091.
Shippers RR (2000). African indigenous vegetables: An overview of the cultivated species. Chatham, UK. Natural resources Institute/ACP-UE Technical Centre for Agricultural and Rural Cooperation.
Shoaib A, Arabi MIE (2006). Genetic diversity among Syrian cultivated and landraces wheat revealed by AFLP markers. Genet. Resour. Crop Evol. 53: 901-906.
Soleimani VD, Baum BR, Johnson DA (2002). AFLP and pedigree-based genetic diversity estimates in modern cultivars of durum wheat [*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.]. Theor. Appl. Genet. 104: 350-357.
Tara Satyavathi C, Bhat KV, Bharadwaj C, Tiwari SP, Chaudhury VK (2006). AFLP analysis of genetic diversity in Indian soybean [Glycine max (L.) Merr.] varieties. Genet. Resour. Crop Evol. 53: 1069-1079.
Tiang T, Yang Z, Shuguang J, Suhua S (2003). Genetic diversity of *Hibiscus tiliaceus* (Malvaceae) in China assessed using AFLP markers. Annals of Botany, 92: 409-414.
Uptmoor R, Wenzel W, Friedt W, Donaldson G, Ayisi K, Ordon F (2003). Comparative analysis on the genetic relatedness of Sorghum bicolor accessions from Southern Africa by RAPDs, AFLPs and SSRs. Theor Appl. Genet. 106: 1316-1325.
Vos P, Hogers R, Bleeker M, Reijans M, Van De Lee T, Hornes M, Frijters A, Pot J, Pelemen J, Kuiper M, Zabeau M (1995). AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23: 4407-4414.
Xu DH, Wahyuni S, Sato Y, Yamaguchi M, Tsunematsu H, Ban T (2006). Genetic diversity and relationships of Japanese peach (*Prunus persica* L.) cultivars revealed by AFLP and pedigree tracing. Genet. Resour. Crop Evol. 53: 883-889.
Zhang LH, Ozias-Akins P, Kochert G, Kresovich S, Dean R, Hanna W (1999). Differentiation of bermudagrass (*Cynodon* spp.) genotypes by AFLP analysis. Theor. Appl. Genet. 98: 895-902.