

## Genetic Diversity of Rice vampireweed (*Rhamphicarpa fistulosa*) Populations in Rainfed Lowland Rice in West Africa

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Rice vampireweed belongs to the Orobanchaceae and is found in Africa and Australia. It is a hemiparasitic weed of lowland rice genotypes and causes losses of 40 to 100% of rice grain yield. Our study addressed the genetic diversity of rice vampireweed in Benin and Senegal. The specific objectives of this research were to study the genetic diversity of rice vampireweed accessions in Benin and Senegal and the relationship between the different genotypes of rice vampireweed through agroecological areas. To achieve these objectives, the genetic diversity of rice vampireweed accessions using the AFLP technique was studied. Based on our results, dendrogram classification has distinguished four different genetic groups. The populations of Benin and Senegal are genetically diverse. Substantial genetic differentiation ( $G_{ST}$ ) exists among agroecological areas within Benin and Senegal ( $G_{ST} = 0.17$ ). The high genetic diversity of rice vampireweed in Benin and Senegal presents a challenge for the development of resistant rice germplasm.

**Nomenclature:** Rice vampireweed, *Rhamphicarpa fistulosa* (Hochst.) Benth.; rice, *Oryza sativa* L.

**Keys words:** Facultative parasite, weeds, Benin, Senegal, rice vampireweed.

Rice is an important cereal crop worldwide, particularly in many African countries, where its importance is growing considerably. Considering the inherent problems with upland agriculture and lack of water availability, lowland rice production is more suitable because it can be rainfed and practiced with less difficulty and risk. However, farmers in lowland rice production are faced with notable biotic constraints, including infestation by rice vampireweed (Rodenburg et al. 2014). This species is a facultative hemiparasitic weed that inflicts serious damage on rice production. Like other facultative parasitic weeds, such as *Buchnera hispida* Buch.-Ham. ex D. Ron, rice vampireweed develops lateral haustoria that connect the parasite and host root xylem system (Neumann et al. 1998). This weed causes an estimated 40 to 100% loss in yield (Gbehounou and Assigbe 2003; Rodenburg et

al. 2011). The parasite also occurs in other important crops such as millet (*Setaria* sp.), sorghum [*Sorghum bicolor* (L.) Moench], corn (*Zea mays* L.), and cowpea [*Vigna unguiculata* (L.) Walp.] (Cissé et al. 1996; Gbehounou and Assigbe 2003; Kuijt 1969; Maiti and Singh 2004; Neumann et al. 1998; Ouédraogo et al. 1999). Rice vampireweed belongs to the Orobanchaceae and is largely found in Africa, as well as Australia (Rodenburg et al. 2014).

The efficient management of parasitic weeds requires adequate knowledge about their ecology, reproductive biology, and genetic diversity (Adoukonou-Sagbadja et al. 2010; Muller 2007). The reproductive biology of rice vampireweed is not fully understood, but studies have reported nocturnal cross-pollination (Cissé et al. 1996; Parker and Riches 1993) putatively mediated by the Darwin's moth (*Xanthopan morgani praedicta* Rothschild & Jordan) (Fischer et al. 2012). The assessment of genetic diversity in plant species can be performed using phenotypic features or biochemical and DNA-based markers (Cho et al. 2010; Hyun et al. 1999). DNA fingerprinting techniques are preferred for cultivar or genotype identification and genetic diversity analysis because they are not influenced by environmental factors such as geographical location or microclimate (Staub and Meglic 1993). Several DNA fingerprinting techniques are available, of which restriction fragment length polymorphism (RFLP), RAPD, AFLP, inter-simple sequence repeat, and simple sequence repeat (SSR) analyses

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have been successfully used to analyze genetic relationships in a diverse range of plant species (Adoukonou-Sagbadja et al. 2010; Cho et al. 2010; Paul et al. 1997; Sathyanarayana et al. 2011). RFLP and RAPD have now been more and more replaced by AFLP and SSR because of the technical advantages of these latter methodologies, which offer high reproducibility and reliability (Fernández et al. 2002; Kabir and Park 2011; Sathyanarayana et al. 2011; Vos et al. 2011). AFLP is a DNA fingerprinting technique that has been used to analyze genetic relationships in a diverse range of plant species (Sathyanarayana et al. 2011).

In contrast to many other parasitic weeds, such as *Striga* spp., little is known about the genetic diversity of rice vampireweed populations in infested inland valleys or in the natural vegetation of West Africa. In the absence of SSRs, the present study aimed to use the AFLP molecular technique, which does not require any prior information about the genome (Adoukonou-Sagbadja et al. 2007), to study genetic variation in rice vampireweed populations in rainfed lowland rice production in Benin and Senegal. The second objective of this research was to investigate the relationships between the different genotypes of rice vampireweed through agroecological areas.

## Materials and Methods

Our study covers the countries of Benin and Senegal in West Africa.

**Some Concept Definitions.** Accessions in our studies represent plant material of an individual rice vampireweed collected from a sampling site.

Ecotype refers to individuals of a species adapted to a given environment but that are not necessary genetically distinct. They can present morphological differences.

Genotype refers to individuals of rice vampireweed that are genetically distinct.

Geographical origin is the country in which we collected rice vampireweed accessions.

Genetic diversity is the variation in the whole genome of rice vampireweed accessions.

Genetic differentiation is the patterning of genetic diversity on rice vampireweed across multiple populations.

Agroecological area refers to a mapping unit of land resources defined in terms of climate, geomorphology, and soil or vegetation cover, or both, and having a specific range of potentials and constraints

for land use. The concerned agroecological areas of our studies—two in Senegal and three in Benin—include an agroecological area of eastern Senegal and Upper Casamance (SO), the Low and Middle Casamance agroecological area in Senegal (BM), the cotton agroecological area in central Benin (ZC), the west Atacora agroecological area in Benin (ZO), and the cotton agroecological area in northern Benin (ZN).

**Plant Material and Sampling Strategies.** A total of 180 accessions of rice vampireweed individuals were collected from two different geographical origins in West Africa: Benin and Senegal (30 sites in each country and three accessions per sites); 90 samples were collected from each country in selected agroecological areas where the parasite either had been previously identified or was currently observed. Sampling in Benin was based on previously published results (Rodenburg et al. 2011), whereas prospective searches in Senegal were made according to preliminary information about infested areas obtained from research extension offices, policy makers, and farmers. In each country, sampling sites were defined based on known or observed infested inland valleys (where lowland rice was grown) or in natural vegetation. Before sampling, we ensured that rice vampireweed roots were connected to the rice root system. Infested rice plants were completely removed from the soil to facilitate collection of whole rice vampireweed individuals. Collected plant samples were dried in the shade and stored in jars containing blue silica gel. At the time of sampling, informal surveys were conducted to evaluate farmers' perceptions of yields losses due to rice vampireweed in their rice fields; we also considered our own observation in the fields. The global positioning system information for the sampling sites in the different agroecological areas are presented in Figure 1.

**DNA Isolation and AFLP Analysis.** Total genomic DNA was extracted from leaf, stem, or root samples of the collected accessions following the previously reported modified mixed alkyltrimethylammonium bromide protocol (Ndoye et al. 2013). The extracted DNA was purified using DNA Clean & Concentrator<sup>TM</sup> 100 (Zymo Research). DNA concentration ( $\text{ng } \mu\text{l}^{-1}$ ) was estimated visually according to size/mass standards via agarose gel electrophoresis. Quantities of DNA were limiting; this was the main reason for selecting samples for further AFLP analysis. Only 60 accessions were considered for AFLP analysis because we selected

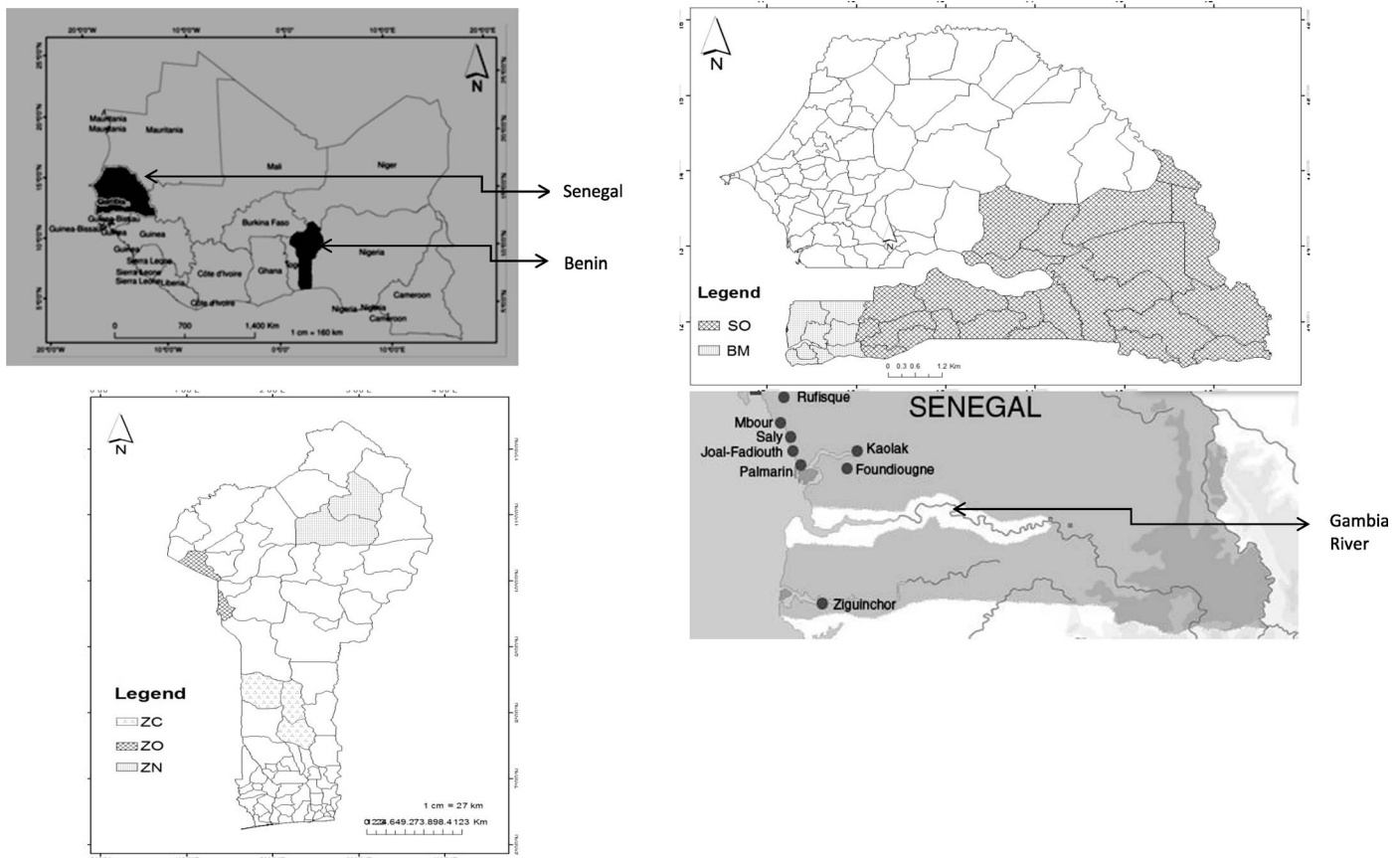


Figure 1. Map indicating the sampling agroecological areas in Benin and Senegal. SO, agroecological area of eastern Senegal and Upper Casamance; ZC, cotton agroecological area in central Benin; ZO, West Atacora agroecological area in Benin; ZN, cotton agroecological area in northern Benin; BM, Low and Middle Casamance agroecological area in Senegal.

one accession per site that presented the highest quantity of pure genomic DNA. AFLP analysis was performed as described previously by Adoukonou-Sagbadja et al. (2007) with minor modifications. Approximately 100 ng of genomic DNA [i.e., 9  $\mu$ l of working solution as recommended by the IRDye<sup>®</sup> 700 AFLP Startup Kit manufacturer's instructions (LI-COR Biosciences)] was digested using the restriction enzymes *EcoRI* and *MseI*. Digested fragments were ligated with double-stranded site-specific adapters using T4 DNA ligase. A preamplification step was carried out before the final and selective amplification. Selective PCR in 10.5- $\mu$ l reactions was performed using primer (*EcoRI* and *MseI*) combinations with three selective nucleotides. The PCR conditions have been described previously (Adoukonou-Sagbadja et al. 2010). Thirty primer combinations were tested on a set of 10 DNA accessions, and based on their ability to generate informative data, 10 were finally selected and used for the fingerprinting of all accessions. Selective amplification products combined with blue urea were separated by electrophoresis on

5.5% polyacrylamide gels using a 4300 DNA Analyzer (LI-COR Biosciences). Fragment sizes were estimated compared with a 50 to 650-bp DNA ladder.

**Data Analysis.** AFLP bands were scored to generate a 1/0 matrix, with 1 and 0 referring to the presence and absence of a band, respectively. AFLP banding pattern consistency was first checked to determine the amount of error and its different sources by examining differences in the banding patterns of duplicate assays (repeated lanes of four selective amplification reactions on different gels) and replicate assays (lanes of two individuals of the same accession run on different gels).

From the 1/0 matrix, pairwise relatedness between rice vampireweed accessions was computed according to the Dice dissimilarity procedure of the DARwin 5.0.158 software package (Perrier and Jacquemoud-Collet, 2006). Based on the dissimilarity matrix obtained, a neighbor-joining tree was constructed, and its reliability and robustness was tested on 1,000 random resamplings before being used on the datasets with a bootstrap procedure of

the software package. The goodness of fit of the tree compared with the basic data matrix was also tested by determining the cophenetic correlation coefficient using the fit criterion procedure of the software package. Additionally, factorial analysis was carried out on the pairwise genetic dissimilarity matrix using the same software.

For comparison, the unweighted pair-group method with arithmetic mean clustering and principal component analysis (PCA) methods were used in parallel on the genetic similarity matrix to determine the genetic structure. Using both methods is recommended to gather the maximum amount of information from the data matrix. These two analyses were carried out using the numerical classification procedure of the R i386 2.15.3 software package with the Ward distance approach (R Core Team 2012).

Finally, diverse genetic population parameters such as number of alleles per locus, Shannon genetic diversity indices, fixation index ( $F_{ST}$ ), and ANOVA were computed to describe the genetic diversity and population differentiation within the collection following the procedures available in the POPGENE 1.32 software package (Yeh et al. 1997).

## Results and Discussion

**Accession Polymorphisms.** The 10 selected AFLP primer pairs yielded a total of 217 polymorphic markers ranging in size from 70 to 250 bp. The number of polymorphic markers generated by each primer combination varied from 19 (M-CAT/E-ACG) to 26 (M-CAC/E-ACC, M-CAG/E-ACA, and M-CAG/E-AGG) with a mean of 21.7. The best combinations that generated the largest number of polymorphic markers and enabled us to distinguish between the different accessions were M-CAC/E-ACC, M-CAG/E-ACA, and M-CAG/E-AGG. In total, 45 (20.7%) markers were specific to a single accession, and only seven (3.2%) AFLP markers frequently covered more than 50% of the accessions. Twenty-nine (13.4%) AFLP markers were specific to accessions from Senegal, whereas 80 (40.6%) were specific to those from Benin.

**Genetic Relationships and Numerical Classification.** Dice dissimilarity varied from 0.1 to 1, with an overall mean of 0.7, and the Euclidean distance for all accessions was 0.6. Construction of a neighbor-joining phylogenetic tree based on the Dice dissimilarity index revealed the relationships between rice vampireweed accessions collected in

Benin and Senegal. In general, the main and subgroups were supported by low and high bootstrap values, respectively. The high bootstrap values of the subgroups indicated the reliability and stability of the relationships as well as the robustness of the AFLP dataset. This result was confirmed by the high cophenetic coefficient obtained ( $r = 0.9$ ). The numerical classification of all data generated a cluster dendrogram (Figure 2) and PCA map (Figure 3).

Four different genetic groups were obtained with 13% truncation. Group A consisted of accessions collected in mountainous areas (Kédougou) of Senegal. These were associated with damage caused by rice vampireweed in farmers' lowland rice fields, representing losses of 30 to 60% of yield. In this group, we also found rice vampireweed in natural vegetation. Group B samples were mainly collected in Senegal and included just two accessions from Benin. This group largely matched the geographical origin of rice vampireweed accessions and was associated with the highest level of damage caused by rice vampireweed in lowland production according to farmers' perceptions (from 60 to 100% of yield losses). The two accessions from Benin were collected from one area (Dassa), where the parasite had been observed for the first time. Group C comprised Benin accessions and also matched a relatively high level of damage (from 40 to 100% of yield losses). This group was associated with the origin of the rice seeds, because 90% of producers in the collection areas received their seeds from national extension services. Group D contained sets collected in Benin and Senegal, similar to group B. However, the Senegal sampling areas were in the Gambia River water catchment, where low infestation levels of the parasite were recently reported (around 2010) by rice farmers of this areas.

These four groups were identified on the PCA map (Figure 3). Variability among genetic groups A, B, and D was explained by dimension (axis) 2, which represents the severity of damage caused by the parasite on rice production. The rice seed origins are plotted on dimension 1 (axis 1), and differences in this factor explained the variability of group C.

**Genetic Variation, Population Differentiation, and Analysis of Molecular Variance.** Estimates of Nei's gene diversity ( $H$ ) and Shannon's information index ( $I$ ) for each sample and for different groups are summarized in Table 1. The highest genotypic diversity of rice vampireweed evaluated in this study was found in the ZO agroecological area in Benin

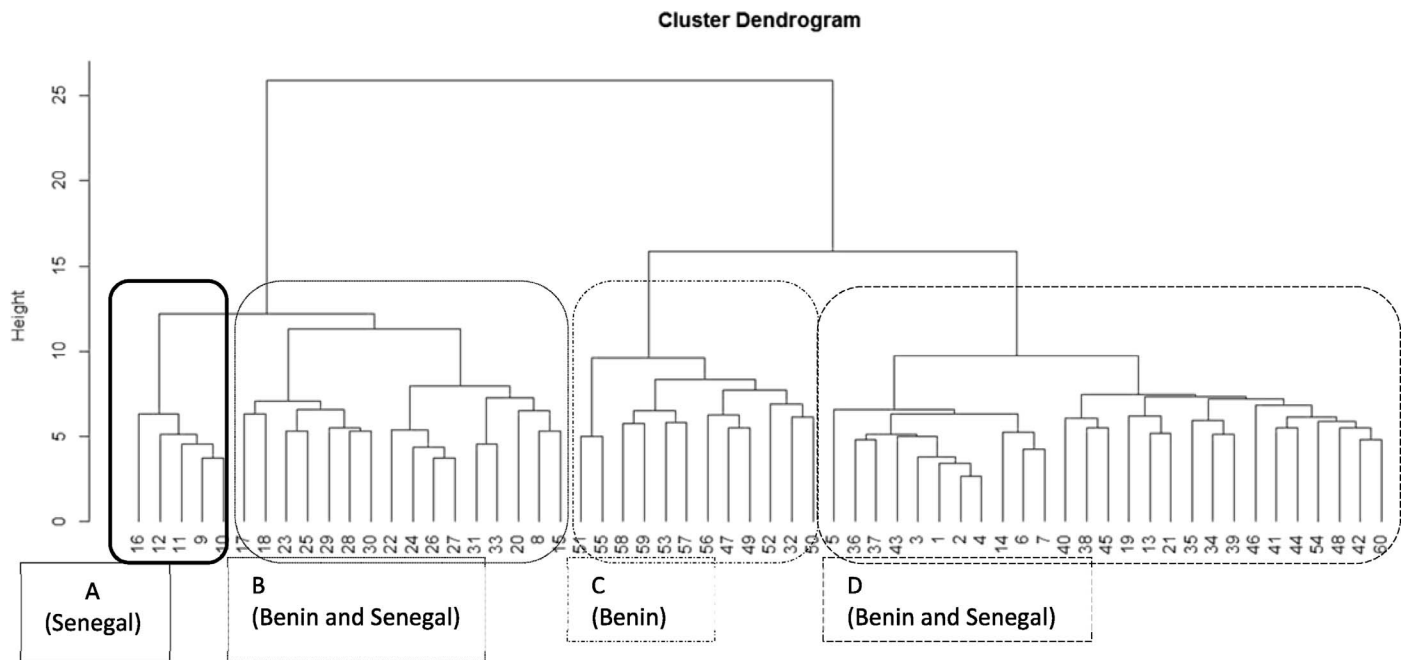


Figure 2. Dendrogram based on numerical classification of rice vampireweed accessions of genetic groups A, B, C, and D. Data are based on AFLP scoring analysis.

( $H=0.19$ ;  $I=0.30$ ); followed by SO and BM areas, both in Senegal; then ZC and ZN, both in Benin. According to genetic groups, the highest number of polymorphisms was observed in group C ( $H=0.18$ ;  $I=0.28$ ) followed by groups B, D, and A. Genetic diversity was similar in Benin and Senegal, considering the geographical origins of the accessions (Table 2).

The genetic differentiation test ( $G_{ST}$ ) revealed that the most differentiated groups were the genetic groups ( $G_{ST} = 0.31$ ), followed by agroecological areas and geographical origins (Table 3). Estimated gene flow ( $Nm$ ; Table 3) was higher ( $Nm = 9.12$ ) among samples originating in Benin than those originating in Senegal. It could mean that small fragments of rice vampireweed DNA may pass from

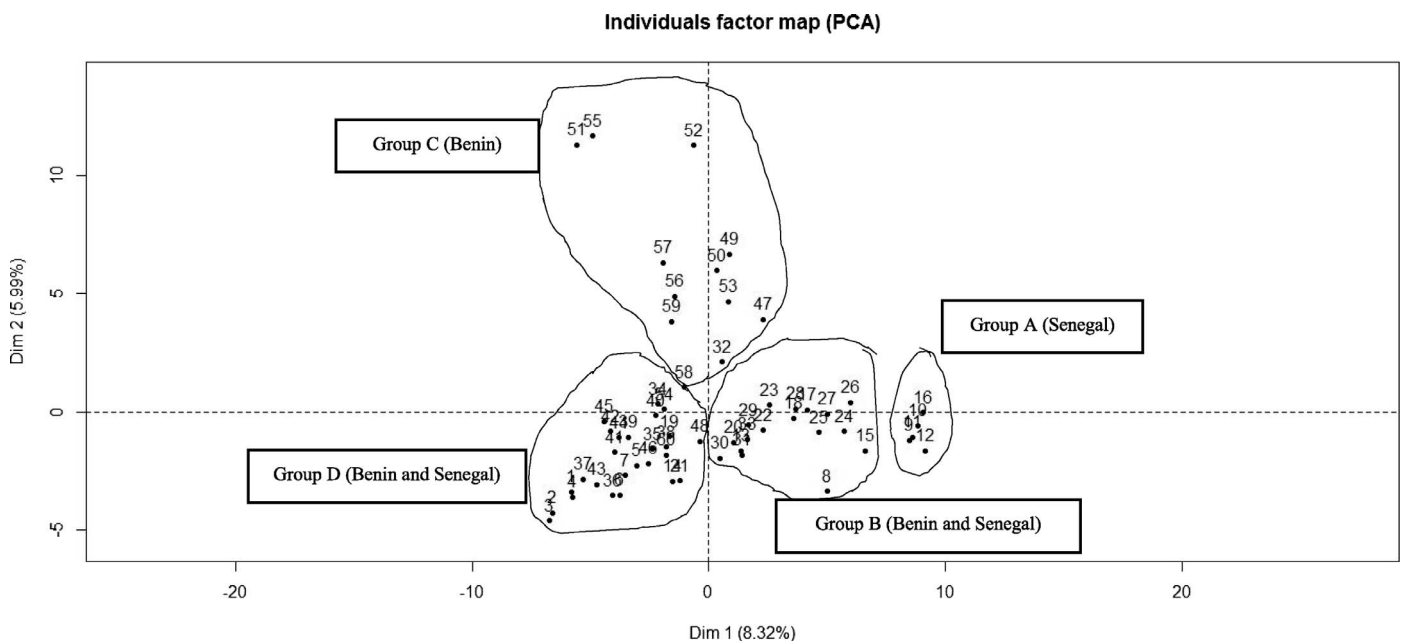


Figure 3. Principal components analysis (PCA) map of AFLP data for genetic groups A, B, C, and D. The graph was produced by the R i386 2.15.3 software package (R Core Team 2012).

Table 1. Descriptive statistics and genetic diversity of rice vampireweed among agroecological area for genetic group (A to D).<sup>a</sup>

	<i>N</i>	<i>Na</i>	<i>Ne</i>	<i>H</i>	<i>I</i>	Ploci
Agroecological area						
BM (Low and Middle Casamance agroecological area)	14	1.53 ± 0.5	1.26 ± 0.3	0.16 ± 0.2	0.24 ± 0.3	53
SO (eastern Senegal and Upper Casamance agroecological area)	16	1.59 ± 0.5	1.29 ± 0.4	0.17 ± 0.2	0.26 ± 0.3	59.45
ZC (cotton agroecological area in central Benin)	13	1.58 ± 0.5	1.21 ± 0.3	0.14 ± 0.1	0.23 ± 0.2	58.06
ZO (west Atacora agroecological area)	14	1.67 ± 0.5	1.30 ± 0.3	0.19 ± 0.2	0.30 ± 0.2	67.28
ZN (cotton agroecological area in northern Benin)	3	1.23 ± 0.4	1.19 ± 0.3	0.10 ± 0.2	0.15 ± 0.3	23.96
Total	60	2	1.29 ± 0.3	0.19 ± 0.1	0.31 ± 0.2	
Genetic group						
A	5	1.23 ± 0.5	1.16 ± 0.3	0.09 ± 0.2	0.13 ± 0.2	23.5
B	16	1.59 ± 0.5	1.27 ± 0.3	0.16 ± 0.2	0.25 ± 0.2	58.99
C	12	1.60 ± 0.5	1.29 ± 0.3	0.18 ± 0.1	0.28 ± 0.2	59.91
D	27	1.79 ± 0.4	1.16 ± 0.2	0.12 ± 0.1	0.22 ± 0.2	79.26
Total	60	2	1.28 ± 0.3	0.18 ± 0.1	0.30 ± 0.2	
Total	60	2	1.28 ± 0.3	0.18 ± 0.1	0.30 ± 0.2	

<sup>a</sup> Abbreviations: *N*, number of accessions; *Na*, number of alleles; *Ne*, number of effective alleles; *H*, Nei's diversity index; *I*, Shannon's diversity index; Ploci, proportion of polymorphic loci.

one individual plant directly into the germline of another in Benin more often than in Senegal. This fact could be transduced by a pathogenic virus or other vector, or deliberately via a human transgenic manipulation (Mallet 2001). Investigations relative to rice vampireweed as a host of pathogenic viruses need to be performed. Pairwise genetic differentiation (Table 4) revealed significant differentiation ( $P < 0.05$ ) between all pairs of different agroecological areas, genetic groups, and geographical origins. This result was consistent with the previous findings and could also help scientists know more about the evolution of rice vampireweed in infested areas. It may be explained by the movement or dispersal of whole organisms or genomes of rice vampireweed from one population to another. After entering a new population, immigrant genomes may become incorporated by sexual reproduction or hybridization, to be gradually broken up by recombination.

Analysis of molecular variance estimates (Table 5) revealed that most genetic variation was attributed to geographical origins, followed by agroecological areas and genetic groups A through D. The variance components for each source of variation were highly

significant ( $P < 0.01$ ; Table 5). Significant differentiation ( $P < 0.01$ ) with approximate values from 8 to 23% of the total variation ( $V = 1.736, 2.135,$  and  $5.476$ ; Figure 4) was also detected among different subgroups, attesting to the fact that substantial differentiation may exist among geographic origins (Benin and Senegal), agroecological areas (SO, ZC, ZO, ZN, and BM), and genetic subgroups (A through D).

This study represents the first attempt to comprehensively investigate the genetic diversity of a large collection of rice vampireweed in Benin and Senegal. AFLP analysis is an important tool of genetic diversity studies, because it enables the generation of multiple molecular markers (Adoukonou-Sagbadja et al. 2007). The present study allowed the screening of many polymorphic loci, providing details of the genetic structure within different groups of this parasitic weed. These results are comparable to those obtained by Welsh and Mohamed (2011), who studied the genetic diversity of *Striga* populations in Ethiopia, and to the study about the use of allozymes for the purple witchweed [*Striga hermonthica* (Delile) Benth.] parasitism of

Table 2. Descriptive statistics and genetic diversity of rice vampireweed among geographical origin (Benin and Senegal).

Geographical origin	<i>N</i>	<i>Na</i>	<i>Ne</i>	<i>H</i>	<i>I</i>	Ploci
Senegal	30	1.72 ± 0.4	1.29 ± 0.3	0.17 ± 0.2	0.27 ± 0.2	72.35
Benin	30	1.85 ± 0.3	1.25 ± 0.3	0.17 ± 0.1	0.28 ± 0.2	84.79
Total	60	2	1.28 ± 0.3	0.18 ± 0.1	0.30 ± 0.2	

<sup>a</sup> Abbreviations: *N*, number of accessions; *Na*, number of alleles; *Ne*, number of effective alleles; *H*, Nei's diversity index; *I*, Shannon's diversity index; Ploci, proportion of polymorphic loci.

Table 3. Genetic diversity indices among rice vampireweed agroecological area, genetic group (A to D), and geographical origin (Benin and Senegal).<sup>a</sup>

Population	$H_T$	$H_S$	$G_{ST}$	$Nm$
Agroecological area	0.18 ± 0.02	0.15 ± 0.01	0.17	2.52
Genetic group	0.19 ± 0.03	0.14 ± 0.01	0.31	1.10
Geographical origin	0.18 ± 0.02	0.17 ± 0.02	0.05	9.12

<sup>a</sup> Abbreviations:  $Nm$ , estimate of gene flow from  $G_{ST}$ ;  $G_{ST}$ , measure of population differentiation;  $H_T$ , total genetic diversity in the pooled populations;  $H_S$ , diversity within each population.

different hosts (Bharathalakshmi and Musselman 1990; Kuiper et al. 1996; Olivier and Leroux 1992).

The numerical classification of AFLP data identified four genetic groups: A, B, C, and D. Group A, contained accessions from Senegal, where rice vampireweed is found in natural mountainous vegetation. This highlights the importance of not generalizing rice vampireweed as a parasitic plant of wetlands, although it undoubtedly grows best in lowland areas. Our results are also consistent with the results of Gbehounou and Assigbe (2003), who related that rice vampireweed is naturally found at the tops of hills. Accessions of group B were collected in parts of Benin, where a large group of farmers are members of the transhumant Fulani community, which is a common ethnic group in Africa. When women of this community have been worked on rice farms, children and men lead cattle through the fields to graze on rice vampireweed plants. We hypothesized that rice vampireweed seeds could be transported in the dung of cattle and distributed from one infested part of the inland valley to another. The origins of rice seeds were another factor in the infestation of the parasite in new inland valleys in group C because most farmers (90%) obtained their seeds from the same infested inland valleys; the best quality of harvested rice grains will be the seeds of the next production in the same or another inland valley. Another source of infestation of rice vampireweed is the contamina-

tion of rice seeds with parasitic plant seeds, which occurs when rice is threshed on the ground. Moreover, fields then become contaminated by oxen and donkeys during the transportation of seeds to other areas throughout much of the African inland valleys. Rice vampireweed seeds can adhere to crop harvested from infested inland valleys, be sold at market, and integrated into the production system of new, previously uninfested fields when they are sown. Ethiopian farmers have been instructed to pull up and burn *Striga* plants to prevent them from setting seed (Welsh and Mohamed 2011). This compares with the less effective practice of rice vampireweed being pulled up and dumped after setting seed. It is likely to spread the infestation to new areas through run off, as exemplified by genetic group D, which was approximately evenly divided between Benin and Senegal. Group D accessions from Senegal derived from the Gambia River catchment area were genetically identical to those from Benin. Thus, water played an important role in the distribution of rice vampireweed seeds.

Most of the time, rice vampireweed was found in temporary flood areas and in soils with a high content of expansive clay (Kabiri et al. 2014; Rodenburg et al. 2014). We hypothesized that water indirectly helped seeds move from one point to another within a river's catchment area. This agrees with results from an earlier study that it is important to consider the river's catchment area in the control of rice vampireweed in inland valleys. It is also supported by a study that attributed Lake Victoria as a vector in the *Striga* infestation in Kenya (Gethi et al. 2005).

According to the PCA map (Figure 3), dimension 1, which we named "the severity of damage axis," better explained the existence of genetic groups D and B, whereas dimension 2, which we named "seed origin axis," explained the existence of group C. Greater genetic variability was observed within but not among different agroecological areas; conse-

Table 4. Pairwise genetic differentiation and Nei's genetic distance among rice vampireweed agroecological area, genetic group (A to D), and geographical origin.

	Agroecological area						Genetic group				Geographical origin		
	BM(R)	SO(U)	ZC(V)	ZO(W)	ZN(X)		A(1)	B(2)	C(3)	D(4)	Senegal (Y)	Benin (Z)	
R	—	0.975	0.973	0.961	0.941	1	—	0.925	0.866	0.845	Y	—	0.977
U	0.060	—	0.966	0.953	0.930	2	0.209	—	0.942	0.946	Z	0.076	—
V	0.081	0.111	—	0.970	0.941	3	0.340	0.172	—	0.955			
W	0.104	0.131	0.068	—	0.945	4	0.450	0.207	0.165	—			
X	0.109	0.135	0.115	0.040	—								

Table 5. Analysis of molecular variance according to AFLP data set results.

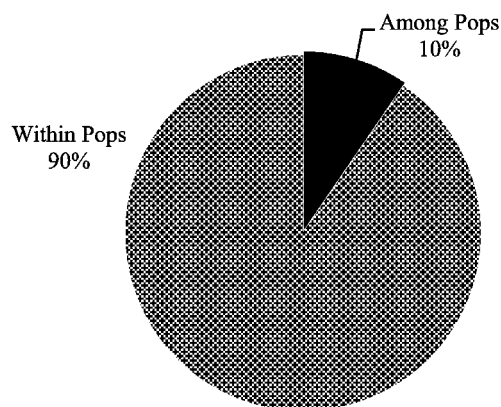
Source of variation	Analysis of molecular variance <sup>a</sup>					
	df	Sum of squares	Estimated variance ( <i>V</i> )	% of variance	ØTP value	P value
Among agroecological areas	4	179.44	2.13	10	0.10	0.003
Within agroecological areas	55	1,110.28	20.19	90		
Among genetic groups	3	277.47	5.48	23	0.83	0.001
Within genetic groups	56	1,012.27	18.08	77		
Among geographical origins	1	73.07	1.74	8	0.08	0.001
Within geographical origins	58	1,216.67	20.98	92		

<sup>a</sup> Abbreviations: df, degrees of freedom; ØTP, genetic differentiation of population.

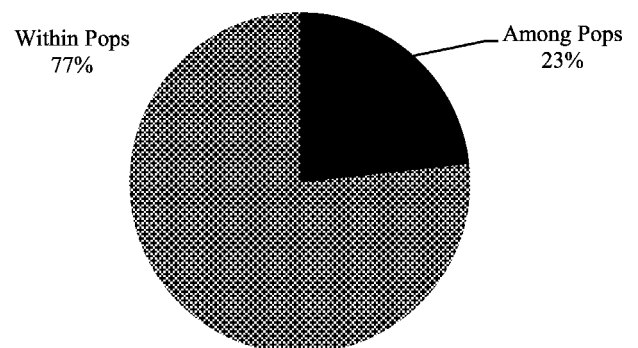
quently, ecotypes didn't exist, because the same agroecological areas contained more than one genetic group. Previous studies on purple witchweed showed that agroecological areas play an important role in establishing genetic differences within populations (Koyama et al. 1999; Musselman et al. 1998), followed by dispersal of some

genotypes through seed or pollen flow to different areas. The genetic differentiation analysis revealed that most genetic differentiation was observed within groups A to D defined by cluster analysis ( $G_{ST} = 0.3$ ; Table 3). A  $G_{ST}$  value between 0.2 to 0.3 is required to document significant genetic differentiation within a population (Wright 1978).

**Agroecological areas: Percentages of Molecular Variance (P=0.003)**



**Genetic groups: Percentages of Molecular Variance (P=0.001)**



**Geographical origins Percentages of Molecular Variance (P=0.001)**

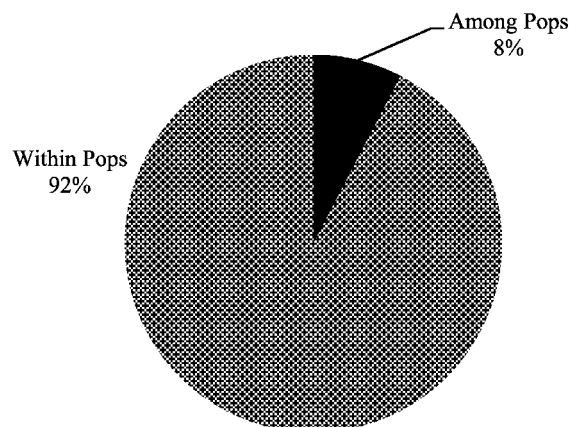


Figure 4. Genetic structure, variability, and population differentiation (●) with proportion of molecular variance among groups (●) and proportion of molecular variance within populations. Data are based on AFLP scoring analysis.



Significant genetic differentiation also suggested that substantial differentiation exists among agroecological areas within Benin and Senegal. Previously, moderate differentiation ( $G_{ST} = 0.05$ ) was observed between the countries Benin and Senegal (Wright 1978). In the case of purple witchweed, geographical origin appears to play the greatest role in establishing genetic differences between populations (Welsh and Mohamed 2011). In this previous case, the effect of isolation by distance was not as strong as the effect of geographic barriers on dispersal. By contrast, a strong correlation was observed between genetic and geographic distance ( $r = 0.6$ ) for the autogamous species witchweed [*Striga asiatica* (L.) Kuntze] (Botanga and Timko 2005).

The high genetic diversity of rice vampireweed in Benin and Senegal presents a challenge for the development of resistance in its crop host. Genetic variability identified in our studies are particularly interesting for breeders and are consistent with those of obligate outcrossed species (Bharathalakshmi and Musselman 1990; Safa et al. 1984). Previously, it was concluded that the high genetic diversity within purple witchweed populations may make it difficult to find reliably resistant varieties of the host plant (Welsh and Mohamed 2011). As rice vampireweed assumes the same ecological role in lowland cereals production as *Striga* does in upland cereals production (Rodenburg et al. 2011), overcoming its parasitism through deployment of resistant host varieties may prove equally challenging (Rodenburg et al. 2011). Moreover, whereas *Striga* is an obligate parasite, *Rhamphicarpa* is a facultative parasite. This complicates the case of *Rhamphicarpa* parasitism because it could be difficult to identify a resistant or tolerant variety of the host (Rodenburg et al. 2011). A truly resistant host would escape parasitism even in heavily infested fields; in contrast with a tolerant host, which would be subject to parasitism but possess some physiological means of limiting its damage on rice growth and productivity relative to the wild-type control. Additionally, a single *Rhamphicarpa* plant can produce more than half a million seeds annually, and those seeds can maintain dormancy in the soil for a long time (Ouedraogo et al. 1999). Thus, a tolerant or resistant host variety will be challenged by the rice vampireweed diversity present in the seed bank. In Benin and Senegal, soils are highly infested with rice vampireweed diversity present in the seed banks. Moreover, our studies have also found *Rhamphicarpa* in upland areas. This fact could seriously handicap future upland rice

production because Kabiri et al. (2014) also identified rice vampireweed in upland cereal production as an important phytosanitary problem.

Substantial genetic differentiation exists among agroecological areas and infested inland valleys within Benin and Senegal. Several different genetic plants of rice vampireweed were introduced into different agroecological areas. The existence of genetic diversity between the populations of Benin and Senegal was also confirmed.

Considering the genetic diversity of this parasite, an integrated approach will be the best option to manage it. When rice vampireweed management practices were ranked by rice farmers in Benin, Cote d'Ivoire, and Tanzania, we identified, in decreasing order of frequency: hand weeding, hand-hoe weeding, soil fertility management, herbicide use, water control, use of clean seeds, transplanting, and the use of resistant or tolerant rice varieties (N'Cho et al. 2015). Gbehounou and Assigbe (2003) related that rice vampireweed can be controlled with the POST herbicide 2,4-D. Mineral fertilizer (particularly nitrogen) also has a proven suppressive effect on rice vampireweed and a positive effect on rice yields of rice vampireweed-infected plants (Rodenburg et al. 2011; Sikirou et al. 2002). It is also important to consider river catchment areas in the control of rice vampireweed in inland valleys. The ecological niches of rice vampireweed need further investigation to determinate indicator species. The relationship between genetic diversity and virulence of rice vampireweed also needs to be investigated.

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