

## Genetic diversity among *Striga gesnerioides* (Willd.) Vatkerevealed by microsatellite markers

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**Abstract:** The efficacy of different strategies for controlling parasitic weeds depends on the level of awareness on the genetic structure of their populations. However, although *Striga gesnerioides* is a major threat to cowpea production in Burkina Faso, very few studies have focused on its genetic diversity at molecular level so far. This study has been designed in order to analyze the genetic diversity of *Striga gesnerioides* using microsatellite markers. Thirty ecotypes of *Striga gesnerioides* were screened with 36 microsatellite markers using the FTA card method for DNA extraction. The PCR products were revealed by electrophoresis on 3% agarose gel. The data were first processed by visual analysis of the migration profiles. The grouping of ecotypes was established using the Neighbour Joining (N-J) approach with Darwin version 6 software. Single and multi-varied statistical methods were used to assess the similarity and diversity between the samples studied and to calculate the genetic parameters of the different populations. The study revealed a moderate diversity within the collection. The fixation index (0.968) indicates high inbreeding. In addition, the low genetic distances between agroclimatic zones indicates genetically close populations. The cluster analysis identified 3 genetic groups. Genetic differences were also revealed between some physiological races. The findings of this study will serve to develop new breeding and varieties dissemination strategies for cowpea with regards to *Striga gesnerioides*.

**Keywords:** *Striga gesnerioides*, genetic diversity, FTA cards, DNA, microsatellite markers.

### Introduction

*Striga gesnerioides* is one of the major constraints to cowpea production and the achievement of food security in Africa, particularly in Burkina Faso. Among *Striga* control measures, genetic resistance appears to be the best strategy to boost cowpea production. Knowing the genetic structure of weed populations, particularly those of *Striga gesnerioides*, is essential for the development of efficient control programme to improve cowpea productivity [1]. Several studies have shown that awareness of genetic diversity affects the effectiveness of different weed control strategies [2] and [3]. Some characters of this diversity can be detected at the phenotypic level, while highlighting others requires the use of adapted techniques, based on modern molecular tools [4]. However, few studies have been undertaken on the genetic diversity of *Striga gesnerioides* using these molecular tools. Recently, [5] and [6] reported on the genetic diversity of *Striga* revealed by microsatellite markers. The previous ones have been carried out using AFLP markers [7]; [1] and ISSR markers or chloroplastic microsatellites [8]. These studies showed relatively low to medium variability within the species. In Burkina Faso, no studies of genetic diversity at the molecular level on *Striga gesnerioides* have yet been carried out. The interest of molecular markers lies in the opportunity they offer to overcome the shortcomings of agro-morphological characterization by allowing the identification of variations between populations at the DNA level [9]. Therefore, the objective of this study is to determine the level and structure of the genetic diversity of *Striga gesnerioides* using microsatellite molecular markers.

### Materials and methods

#### Plant material and microsatellite markers tested

Thirty ecotypes of *Striga gesnerioides* collected from all four (4) agroclimatic zones of Burkina Faso were used for this molecular characterization. The ecotypes in the collection were classified into 5 physiological groups following differential screening (Table 1). Analysis of the results of the differential screening made it possible to classify the ecotypes of the physiological groups obtained into SG1 race, SG5 race, SG Kp race, Biotype 1 and Biotype 2 presented in Table 1 [10]. DNA sampling involved one individual per ecotype, except the ecotype of Ouindingui for which two individuals were sampled, one with a purple flower and the other with a white flower. Thirty-six (36) microsatellite markers were used in this study to characterize the genetic diversity of *Striga gesnerioides* occurring in Burkina Faso. These markers were selected based on the level of polymorphism they revealed between *Striga gesnerioides* populations in Ghana as reported by [5].

**Table 1: Collection sites of *Striga gesnerioides* ecotypes tested**

Physiological races	Provinces	Localities	Ecotype code	Agroclimatic zones
Race SG1 (Group 1)	Bazèga	Ipélcé	BI	North-soudanian
	Bazèga	Toécé	BTo	North-soudanian
	Fada N'Gourma	Diapangou	FD	North-soudanian
	Ganzourgou	Zam	GZR	North-soudanian
	Kadiogo	Kamboinsin	Kb	North-soudanian
	Kouritenga	Baskouré	KKB	North-soudanian
	Kouritenga	Sapaga	KS	North-soudanian
	Kadiogo	Tintilou	KT	North-soudanian
	Namentenga	Dargo	NDY	South-sahelian
	Nayala	Toma	NTP	North-soudanian
	Oubritenga	Manèga	OM	North-soudanian
	Oubritenga	Zitenga	OZN	North-soudanian
	Sourou	Lankoué	SL	South-sahelian
	Soum	Pobé-Mengao	SPM	North-sahelian
	Sanmatenga	Pibaoré	SPO	South-sahelian
Race SG 5 (Group 2)	Sanguié	Tita	SPT	South-soudanian
	Ziro	Sapouy	ZSF	South-soudanian
	Fada N'Gourma	Kouaré	FK	North-soudanian
Race SG Kp (Group 3)	Nahouri	Po	NP	South-soudanian
	Séno	Bani	SB	North-sahelian
	Séno	Dori	SDN	North-sahelian
Biotype 1 (Group 5)	Zoundwéogo	Nobéré	ZN	South-soudanian
	Boulgou	Tenkodogo	BTe	North-soudanian
Biotype 2 (Group 4)	Fada N'Gourma	Boulontou	FFB	North-soudanian
	Balé	Poura	BPB	South-soudanian
Biotype 2 (Group 4)	Boulkiemdé	Saria	BSG	North-soudanian
	Houet	Bama	HB	South-soudanian
	Loroum	Titao	LO	South-sahelian
Biotype 2 (Group 4)	Sourou	Toénié	STD	South-sahelian
	Yatenga	Séguénéga	YS	South-sahelian

**Genomic DNA extraction methods**

The FTA card technique is economical and time-saving, as it offers the possibility to switch directly from extraction to PCR amplification [11] and [12]. In addition, it is adapted to DNA sampling even in the field and allows samples storage over a long period of time. This method was used to extract genomic DNA (Figure 1). Fresh young leaves of each *Striga gesnerioides* ecotype were collected, placed over the card, overlaid with a plastic film and crushed with a pestle. Thereafter, the FTA cards were air dried at room temperature for 24 hours. Small disks of 02 mm in diameter were punched out of the FTA cards and placed in PCR tubes for purification. The punch was systematically disinfected with alcohol before punching each sample to avoid contamination. The purification consisted of

washing twice each disk in 200  $\mu$ l ethanol solution, 70% concentrated, for five minutes. An equal volume of Tris EDTA (TE) buffer 1% concentrated was used to rinse twice the disks and solubilize the DNA. Afterward, the disks were dried at room temperature before DNA amplification.

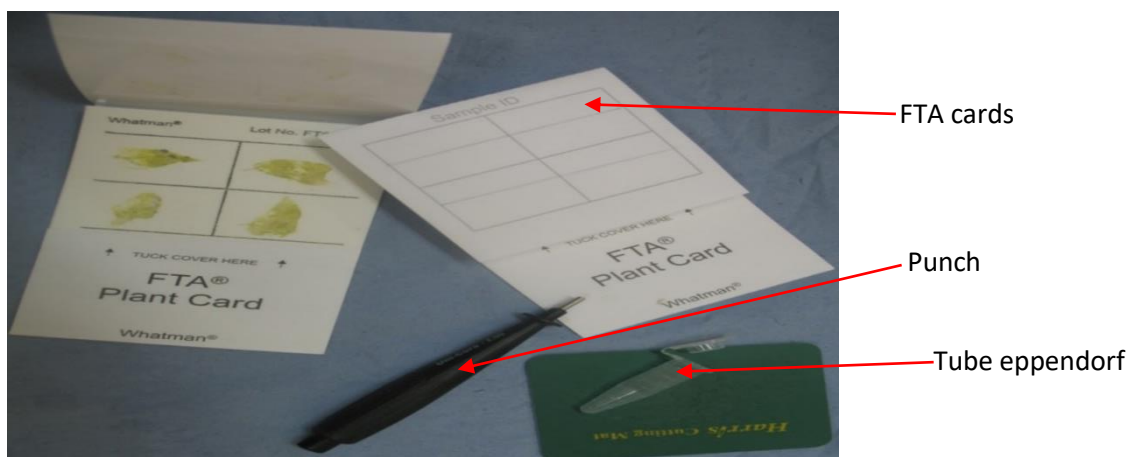


Figure 1: FTA cards

### Polymerase chain reaction (PCR)

PCR reactions were performed in a final volume of 25  $\mu$ l containing 1  $\mu$ l of each primer (10  $\mu$ M per primer), 5  $\mu$ l of PCR premix (1U Taq polymerase, 250  $\mu$ M dNTPs, 10 mM Tris-HCl, 30 mM KCl and 1.5 mM MgCl), 18  $\mu$ l of ultrapure water and a disk from the FTA card containing the genomic DNA to be amplified. The PCR amplification consisted of an initial denaturation phase at 94°C for 5 minutes, followed by series of 35 cycles composed of a denaturation phase at 94°C for 30 seconds, hybridization at the required temperature (°C) of each primer for 45 seconds and an extension at 72°C for 30 seconds. At the end of the 35 cycles, a final extension at 72°C for 10 minutes was performed, followed by cooling to 4°C until the PCR product was deposited on the agarose gel for electrophoresis.

### Electrophoretic and tape reading

The PCR products were subjected to electrophoretic on 3% agarose gel prepared with a 0.5X solution of Tris Borate EDTA (TBE) in which 5  $\mu$ l of Ethidium Bromide (BEt) was incorporated as fluorescent dye. The amplification products and ladders (100 to 1500 bp) were loaded into the gel wells and subjected to 80 V for 1 h 30 minutes in a 0.5 X TBE buffer for migration. An ultraviolet transilluminator combined with an 18.0 mega pixels Canon EOS 1300D a camera was used to visualize and take gel profiles. A binary coding, where the absence of bands was noted as 0 and the presence of bands 1, was used for gels recording.

### Analysis of genetic diversity

A binary matrix table generated based on band sizes of gel profiles. The intra-population and inter-population genetic diversity of *Striga gesnerioides* populations were analyzed at level. The data were first processed by visual analysis of the migration profiles. The grouping of ecotypes was established using the Neighbour Joining (N-J) approach with Darwin version 6 software. GenAlEx6.41 and GENETIX software were used to calculate the genetic diversity parameters of *Striga gesnerioides*. Single and multi-varied statistical methods were used to assess the similarity and diversity between the samples studied and to calculate the genetic parameters of the different populations.

### Intra-population genetic diversity

Intra-population genetic diversity parameters were estimated for each locus and the average was over all loci, using GenAlEx6.41 software. The following parameters were calculated:

- the total number of alleles ( $A_t$ ), expresses the total number of alleles in the population or sample studied
- the number of effective alleles ( $A_e$ ), corresponds to the number of alleles taking into account their difference in frequency
- the number of distinct or private alleles ( $A_P$ ) is the number of alleles that are specific or unique to each population;
- the rate of polymorphism ( $P\%$ ) is the percentage of polymorphic loci in the sample studied;
- the observed heterozygous rate ( $H_o$ ) is the proportion of heterozygous individuals at a locus  $K$ ,
- the expected unbiased heterozygosity rate ( $H_e$ ), is the probability that at a given locus, any two randomly selected alleles in the population are different.
- Fixation index ( $F_{is}$ ), this parameter measures the difference between the population of individuals found in the heterozygous state ( $H_o$ ) and the expected heterozygous rate ( $H_e$ ), it is also called the panmixed difference. The fixation index varies from -1 to +1 and makes it possible to know the heterozygous deficit per population, per locus and for all loci. It is positive when the population has a heterozygous deficit in relation to the panmictic equilibrium and negative in the opposite,
- the diversity index or Polymorphic Information Content (PIC). It demonstrates the ability of a marker to detect a polymorphism in a population. Its value is between 0 and 1.

#### Structuring genetic diversity

In order to describe the genetic diversity between populations, the following parameter was estimated:

- genetic distance, the genetic distance between two samples is defined as the proportion of genetic elements they do not have in common,  $D = 1$  if only the two samples do not have genetic elements in common and takes the value 0 if the populations are identical,
- Differentiation index ( $F_{st}$ ) or Wright's statistical  $F$ , it is equal to 0 if there is no genetic divergence between sub-populations and 1 for totally different populations. The  $F_{st}$  is commonly used to determine whether there is gene flow between populations. According to [13]:

$0 < F_{st} < 0.05$ : weak differentiation  
 $0.05 < F_{st} < 0.15$ : moderate differentiation  
 $0.15 < F_{st} < 0.25$ : significant differentiation  
 $F_{st} > 0.25$ : very important differentiation

## Results

### Genetic diversity revealed by microsatellite markers

All 36 markers were able to amplify the DNA of the samples. Four markers (FA1/2, FA7/8, FA11/12 and FA15/16) out of the 36 geldid not generate readable bandand were therefore dropped. Band sizes varied from 150 to 900 bp. A total number of 100 alleles was obtained for all the polymorphic markers. The number of alleles varied from two to five with an average of three (3) alleles per locus (table 2). The number of effective alleles ( $A_e$ ), varied from 1.37 (FA39/40; FA151/154) to 4.20 (FA131/132) with an average of 2.32. The observed heterozygosity ranged from 0 to 0.32 with a mean of 0.015. Only FA3/4 (0.03), FA127/128 (0.32) and FA141/142 (0.12) showed observed heterozygosity different from zero. The expected unbiased heterozygosity ( $H_e$ ) ranged from 0.27 (FA151/154) to 0.77 (FA131/132) with a mean of 0.54. The expected unbiased heterozygosity ( $H_e$ ) varied from 0.27 (FA151/154) to 0.77 (FA131/132).

The average value of Shannon diversity index ( $I$ ) 0.91. The minimum and maximum indices were 0.44 and 1.51 for markers FA151/154 and FA131/132 respectively. The polymorphism information content (PIC) varied from 0.27 (FA39/40) to 0.76 (FA131/132) with an average of 0.54. The Fixation Index ( $F_{is}$ ) showed values of 0.33; 0.68; 0.95 for FA127/128; FA141/142; FA3/4 and 1 for the remaining markers. These values of the fixation index indicate a deficit of heterozygotes in the study population.

Table 2: Genetic diversity parameters of each of the 32 markers tested

Nº	Markers	At	Ae	H0	He	I	PIC	Fis	P(%)
1	FA3/4	4	3.06	0.032	0.68	1.15	0.67	0.95	100
2	FA31/32	3	2.90	0	0.66	1.08	0.66	1	100
3	FA31/34	2	1.84	0	0.46	0.65	0.46	1	100
4	FA35/36	2	1.77	0	0.44	0.62	0.44	1	100
5	FA37/38	2	1.84	0	0.46	0.65	0.46	1	100
6	FA39/40	3	1.37	0	0.27	0.53	0.27	1	100
7	FA51/52	3	2.35	0	0.58	0.93	0.57	1	100
8	FA51/56	3	1.68	0	0.41	0.71	0.41	1	100
9	FA63/64	4	3.19	0	0.69	1.25	0.69	1	100
10	FA65/62	3	2.03	0	0.51	0.85	0.51	1	100
11	FA67/62	3	1.91	0	0.48	0.75	0.48	1	100
12	FA81/76	3	2.06	0	0.52	0.88	0.52	1	100
13	FA91/92	3	2.49	0	0.60	0.98	0.60	1	100
14	FA93/94	4	2.32	0	0.58	1.02	0.57	1	100
15	FA105/116	3	2.12	0	0.53	0.81	0.53	1	100
16	FA121/122	2	1.94	0	0.49	0.67	0.49	1	100
17	FA123/122	4	2.54	0	0.62	1.13	0.61	1	100
18	FA125/126	4	2.67	0	0.63	1.13	0.63	1	100
19	FA127/128	4	1.91	0.320	0.48	0.92	0.48	0.33	100
20	FA131/132	5	4.20	0	0.77	1.51	0.76	1	100
21	FA133/134	4	2.90	0	0.66	1.20	0.66	1	100
22	FA131/138	3	2.95	0	0.67	1.09	0.66	1	100
23	FA141/142	3	1.70	0.129	0.42	0.74	0.41	0.68	100
24	FA149/142	2	1.94	0	0.49	0.68	0.49	1	100
25	FA151/154	2	1.37	0	0.27	0.44	0.27	1	100
26	FA155/156	4	3.19	0	0.69	1.24	0.69	1	100
27	FA157/158	2	1.94	0	0.49	0.68	0.49	1	100
28	FA159/158	2	1.53	0	0.35	0.53	0.35	1	100
29	FA83/76	4	3.37	0	0.71	1.29	0.7	1	100
30	FA5/6	3	2.40	0	0.59	0.94	0.58	1	100
31	FA9/10	3	2.48	0	0.60	0.99	0.6	1	100
32	FA13/14	4	2.38	0	0.59	1.08	0.58	1	100
	<b>Mean</b>	3.12	2.32	0.015	0.54	0.91	0.54	0.96	100

At: total number of alleles, Ae: number of efficient alleles, Ho: observed heterozygosity; He: expected heterozygosity, PIC: Polymorphism Information Content, I: Shannon diversity index, Fis: fixation index; P: rate of polymorphic loci

**Genetic diversity of *Striga gesnerioides* within agroclimatic zones**

Results presented in Table 3 shows that the values of genetic diversity parameters are relatively high in the North-sudanian zone, intermediate in the South-sahelian and South-sudanian zones, and low in the North-sahelian zone for most parameters. The ecotypes of *Striga gesnerioides* characterized according to the climatic zones presented an average number of efficient alleles of 2, a high average rate of polymorphism (P=91.41%), an average Shannon Index (I=0.70) and relatively low average rates of observed and expected heterozygosity which are respectively 0.014 and 0.483. The fixation index (Fis), which evaluates intra-population differentiation, showed a very high value of 0.965, which corresponds to a low open pollination rate of 3.5%.

Table 3: Genetic differentiation within climatic zones

Climatic zones	N	At	Ae	I	Ho	He	F	P (%)
North-sahelian	3	1.781	1.650	0.484	0.010	0.402	0.948	71.88%
North-soudanian	15	3.000	2.327	0.902	0.016	0.556	0.969	100.00%
South-sahelian	7	2.594	1.996	0.757	0.004	0.496	0.988	100.00%
South-soudanian	6	2.281	1.914	0.677	0.026	0.480	0.951	93.75%
Mean	7.469	2.414	1.972	0.705	0.014	0.483	0.965	91.41%

N: number of ecotypes; At: total number of alleles, Ae: number of efficient alleles, Ho: observed heterozygosity; He: expected heterozygosity, I: Shannon's diversity index, P: rate of polymorphs.

Comparison of the minimum Nei distance and the genetic differentiation index (Fst) between pairs of agroclimatic zones showed relatively low values of genetic differentiation (Table 4). In fact, the molecular analysis of variance showed no significant difference between climatic zones. Thus, the highest value of the genetic distance of Nei was observed between the South-sahelian and South-soudanian zone (0.141) and the lowest distance between the North-sahelian and South-soudanian zone (0.067). With regard to the genetic differentiation index Fst, the highest was observed between the North-sahelian zone and the South-soudanian zone (0.120) and the lowest was observed between the North-soudanian zone and the South-soudanian zone (0.066).

Table 4: Genetic differentiation among agro-climatic zones

Climatic zones	GENETIC DISTANCE OF NEI				DIFFERENTIATION INDEX Fst			
	North-sahelian	North-soudanian	South-sahelian	South-soudanian	North-sahelian	North-soudanian	South-sahelian	South-soudanian
North-sahelian	0				0			
North-soudanian	0.127ns	0			0.117	0		
South-sahelian	0.070ns	0.131ns	0		0.107	0.080	0	
South-soudanian	0.078ns	0.067ns	0.141ns	0	0.120	0.066	0.106	0

ns: not significant

Description of the genetic diversity of *Striga gesnerioides* according to physiological groups

The analysis of the intra-population diversity of physiological groups showed relatively high values. Shannon's diversity index values ranged from 0.641 for group 1 to 1.036 for group 2. The mean rates of unbiased expected heterozygosity and observed heterozygosity were 0.581 and 0.556 respectively, while the mean rate of polymorphism was 98.13% (Table 5).

Table 5: Genetic differentiation among physiological groups

Physiological group	N	At	Ae	Ap	I	Ho	He	P (%)
Group 1	18	3.43	2.61	0.21	1.03	0.53	0.61	100
Group 2	3	2.15	1.83	0	0.64	0.63	0.51	93.75
Group 3	2	2.59	2.25	0	0.82	0.63	0.64	100
Group 4	3	2.93	2.43	0.03	0.94	0.50	0.65	100
Group 5	4	2.25	1.83	0.03	0.64	0.47	0.48	96.88

Mean 6 2.67 2.19 0.05 0.81 0.55 0.58 98.13

*N*: number of ecotypes, *At*: total number of alleles, *Ae*: number of efficient alleles, *Ho*: observed heterozygosity, *He*: expected heterozygosity, *I*: Shannon's diversity index, *P*: rate of polymorphs.

Inter-population genetic diversity parameters between pairs of groups showed low values. Thus, zero genetic distances were observed between group 1 and group 3, group 4 and group 5 (Table 6). The genetic differentiation index *Fst* varied from 0.029 between groups 1 and 4 to 0.188 between group 2 and group 5. On the other hand, group 2 and group 4 showed moderate genetic distance (0.198) and genetic differentiation index (*Fst* = 0.145).

Table 6: Genetic differentiation between physiological groups

Physiological group	GENETIC DISTANCE OF NEI					DIFFERENTIATION INDEX <i>Fst</i>				
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 1	Group 2	Group 3	Group 4	Group 5
Group 1	0					0				
Group 2	0.144ns	0				0.101	0			
Group 3	0.000ns	0.000ns	0			0.062	0.093	0		
Group 4	0.000ns	0.198ns	0.008ns	0		0.029	0.145	0.096	0	
Group 5	0.005ns	0.290ns	0.085ns	0.000ns	0	0.052	0.188	0.132	0.062	0

ns: non-significant difference at the 5% threshold

Genetic diversity structure of *Striga gesnerioides* ecotypes

The genetic structuring of *Striga gesnerioides* ecotypes using the 32 polymorphic markers using the "Neighbor-Joining" method (Figure 2) resulted in a distribution of the ecotypes into three genetic groups A, B and C consisting of 2, 15 and 14 ecotypes respectively. The two ecotypes in group A come from two contrasting agroclimatic zones, the South-sahelian and the South-sudanian zones. Groups B and C are composed of ecotypes from all four agroclimatic zones, with a majority of ecotypes from the South-sahelian zone for group B and the North-sudanian zone for group C. This organization is independent of both agro-climatic zones and physiological groups.

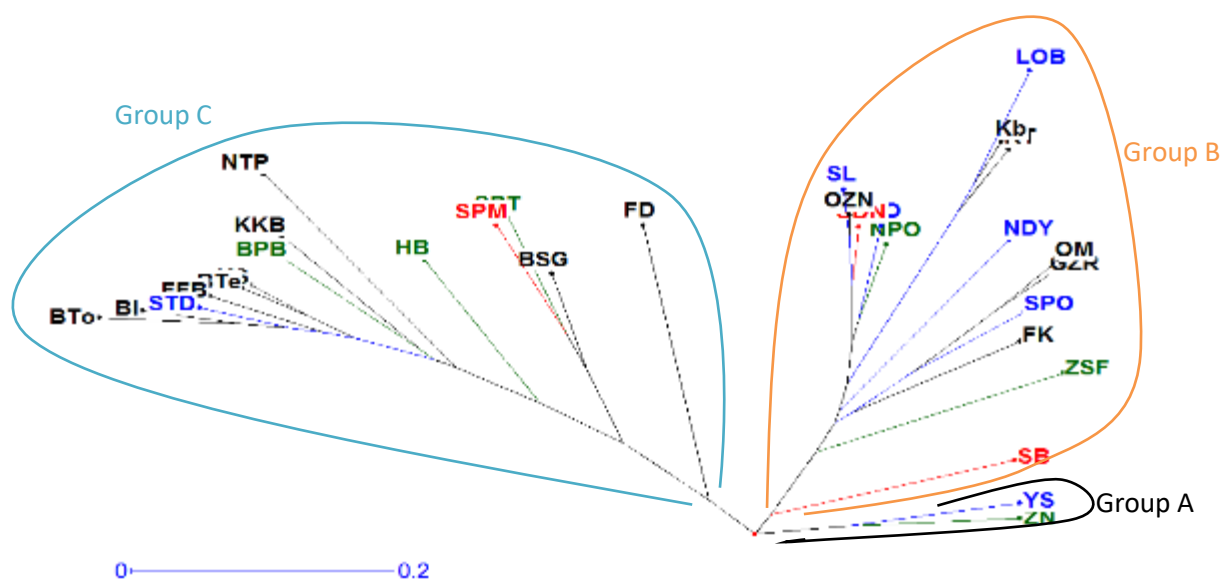


Figure 2: Tree representation of the dendrogram of the 30 ecotypes generated using the Neighbor-Joining method.

Description of the genetic groups of *Striga gesnerioides*

The genetic parameters of the three genetic groups of *Striga gesnerioides* presented in Table 7 reveal that genetic group A has the lowest genetic parameters, while genetic groups B and C have approximately same values except for the polymorphic loci rate which is 84.38% for group B compared to 96.88% for group C.

Table 7: Diversity parameters of the different genetic groups of *Striga gesnerioides*

Genetic groups	N	At	Ae	Ap	I	Ho	He	Fis	P (%)
Group A	2	1.156	1.144	0.000	0.104	0.016	0.099	0.733	15.63
Group B	15	2.250	1.674	0.625	0.563	0.011	0.361	0.974	84.38
Group C	14	2.313	1.662	0.750	0.580	0.019	0.379	0.941	96.88
Mean	9.958	1.906	1.493	0.458	0.416	0.015	0.280	0.939	65.63

N: number of ecotypes; At: total number of alleles, Ae: number of efficient alleles, Ho: observed heterozygosity; He: expected heterozygosity, I: Shannon's diversity index, Fis: fixation index; P: rate of polymorphs

Genetic distance and genetic differentiation between genetic groups

The genetic differences between the three genetic groups revealed by the minimum Nei distance and genetic differentiation (Table 8) showed that these three groups differed from each other in a highly significant way. Groups A and C are the furthest apart with a genetic differentiation index (Fst) of 0.412, followed by groups A and B with a differentiation index (Fst) of 0.353. On the other hand, groups B and C are the closest with a differentiation index (Fst) of 0.328. Referring to the scale established by [13], the genetic differentiation between the three genetic groups can be qualified as very important because the Fst values are greater than 0.25.

Table 8: Genetic differentiation between genetic groups

Genetic groups	Genetic distance of Nei			Genetic differentiation index		
	Group A	Group B	Group C	Group A	Group B	Group C
Group A	0			0		
Group B	0.427*	0		0.353	0	
Group C	0.704*	0.835*	0	0.412	0.328	0

\* Significant genetic distance at the 5% threshold

Discussion

The maximum (100%) polymorphism rate observed for all polymorphic markers combined with the value of the Polymorphic Information Content (PIC =0.54) indicate a high level of polymorphism of the microsatellite markers used. This can be explained by the fact that the microsatellite markers used were selected based on the results of previous diversity study of *Striga gesnerioides* conducted in Ghana by [5]. The medium mean values of expected heterozygosity (He), allelic diversity and polymorphic information content (PIC) implies moderate genetic diversity ecotypes of *Striga gesnerioides* collected from the four phytogeographical zones of Burkina Faso. In addition, a low number of heterozygous individuals was observed as compared to the expected average heterozygosity rate.

This would be related to the predominantly self-pollinated status of *Striga gesnerioides*. The high level of self-pollination of *Striga gesnerioides* is confirmed by the very high and positive value of the fixation index (Fis= 0.968), which indicates the existence of strong inbreeding in the *Striga gesnerioides* collection leading to a loss of genetic variability. Similar results have been reported, in fact, these authors explained the difference between the observed and expected heterozygotes by the mode of reproduction characterised by strong autogamy or an evolutionary



force, mainly a selection that would favour certain genotypes[14]. In contrast to *Striga gesnerioides*, *Striga hermonthica* is a cross-pollinated plant exhibiting excess of heterozygotes individuals[15].

The distribution of genetic diversity according to agroclimatic zones shows a genetic differentiation between the ecotypes in the collection. In fact, the high average values of genetic parameters such as the Shannon diversity index ( $H = 0.705$ ) and the rate of polymorphic loci ( $P = 91.41\%$ ), between the ecotypes of the four agroclimatic zones, indicate the existence of intra-population genetic diversity. The index of genetic differentiation between agroclimatic zones revealed high polymorphism within agroclimatic zones but relatively low between zones. For example, the highest inter-zone differentiation index of 0.12 was observed between the North-sahelian zone and the South-sudanian zone. This  $F_{st}$  differentiation coefficient of 0.12 reveals that 12% of the genetic diversity is found at the inter-zone level, compared to 88% at the intra-zone level. The matrix of genetic distances between the agro-climatic zones indicates a variation from 0.067 between the North-soudanian and south-sudanian zones to 0.141 between the south-sahelian and south-sudanian zones. These low genetic distances infer that the populations are genetically close and belong to the same genetic group[16]. On the other hand, this distinction between the ecotypes of the North-sahelian zone and those of the South-sudanian zone could be explained by geographical isolation. Thus, according to[17], more populations are genetically divergent farther away they are. However, the genetic structure of the different ecotypes of *Striga gesnerioides*, obtained in this study, based on their genetic distance was built regardless of their geographical origins. In fact, three differentiated genetic groups were obtained with the 32 microsatellite markers. Thus, the parameters evaluating intra-population polymorphism are characterized by a high degree of heterogeneity inferring the existence of intra-population diversity. [6] Mentioned important intra-population diversity among the five races of *Striga gesnerioides* occurring in Ouest Africa. The heterogeneity of the intra-population diversity indices was also highlighted for other self-pollinated plants like *Phaseolus lunatus* and *Sorghum bicolor* analyzed [18] and [19]. An analysis of the three genetic groups shows a random distribution of ecotypes between the groups. This distribution could be related to *Striga* seeds dissemination across agro-climatic zones, mainly through contaminated cowpea seeds along with population movements. Gene flow or migration directly affects the degree of organization of plant genetic diversity[20].

On the other hand, low genetic distances were observed between physiological races meaning that there is low variability between them.[21] asserted that there is a high degree of genetic proximity between *Striga* races resulting in low genetic variability between races of *Striga gesnerioides*. Previous studies have also reported moderate genetic variation between populations of *Striga gesnerioides* in Ghana [22]; [1] and [5]. However, [6] found significant genetic differences ( $F_{st}$  between 0.22 and 0.88) between populations of *Striga gesnerioides* but with ecotypes from seven different countries including Burkina Faso. Therefore, they explained the low genetic variation between races observed in the other studies by the relative proximity of *Striga* collection sites in these studies.

According to [16], the value of genetic distance reflects the degree of divergence between populations. In addition, the genetic differentiation index showed values that indicate low to moderate genetic differentiation between the five (05) biotypes or races based on scale of [13]. All this confirms the existence of genetic divergence between the physiological races of *Striga gesnerioides*.

## Conclusion

The genetic diversity study revealed moderate diversity among the collection of *Striga gesnerioides*. The genetic diversity was more important at intra-population level as compared to the inter-population diversity. The overall genetic diversity was structured into three genetically distinct groups. In addition, genetic diversity of *Striga gesnerioides* was more determined by the ecotype of *Striga* rather than the agroclimatic zones where they were collected in and physiological races. The study also revealed genetic differences between some physiological races of *Striga gesnerioides*. The results can serve as a guide for determining new strategies in cowpea varietal selection for resistance to *Striga gesnerioides*. A better knowledge of *Striga* diversity will contribute to its better control and therefore enhance cowpea production.

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