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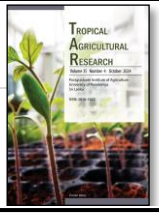

RESEARCH

Assessment of Genetic Parameters and Diversity Organization of Ginger Accessions Cultivated in Burkina Faso as Revealed by Microsatellite Markers

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ABSTRACT

Rhizomes of Ginger (*Zingiber officinale* Rosc.) are widely used as a spice across the world. It has originated from South-east Asia and introduced to many parts of the world, and has been cultivated as a spice and for medicinal purpose. Ginger is a high value crop in Burkina Faso. The genetic diversity organization in ginger species is not well-known in Burkina Faso. Genetic resources are imperative for varietal selection. The importance of genetic resources depends on the levels of their diversity. The present investigation aims to assess the diversity of ginger accessions collected from Burkina Faso. Forty-seven ginger accessions collected from three provinces, Comoé (03), Léraba (14), Kénédougou (30), located in the southwestern zone of Burkina Faso were genotyped for their molecular traits using microsatellite markers. A total of 24 alleles were revealed by electrophoresis. All the simple-sequence repeats (SSR) set primers used revealed high polymorphic rate (100%) with the expected heterozygosity (0.499), showing their ability in assessment of the diversity levels and genetic organization of ginger accessions due to their codominance. The dendrogram obtained by the Neighbor-Joining method revealed three main genetic groups. The genetic differentiation index value (0.313) showed that the accessions of the three provinces were different, with an interesting diversity observed from kénédougou ginger accessions. Information from this study could be used in the development of effective management strategies for the conservation and effective use of the genetic resources within ginger accessions grown in Burkina Faso.

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INTRODUCTION

Ginger (*Zingiber officinale* Rosc.) is grown mainly in tropical and subtropical regions and has been used in traditional oriental medicine since ancient times (Zheng et al., 2008; Preeti et al., 2008; Waki et al., 2013). Among medicinal plants, ginger occupies a prominent place in ancient and modern practices (Singh et al., 2012), and therefore is a real center of interest in the food and pharmaceutical industry. In Burkina Faso, ginger is considered as a minor crop and its production is limited to a small extent (Nandkangre et al. 2015). The loss of genetic resources due to market demand and changing climatic conditions (e.g., drought) mainly leads consequently to narrowing the genetic diversity, that is a reality in Burkina Faso. Thus, study of diversity using agro morphological traits has been undertaken by Nandkangre et al. (2016), and they have shown moderate variability within ginger accessions grown in Burkina Faso. Although morphological traits are relatively easy to study, they are likely to be influenced by environmental conditions, and by the stage of plant development. Molecular characterization based on DNA markers is more reliable in assessing the genetic diversity of a species. The study of diversity revealed by molecular markers provides better precision on diversity for a better conservation strategy.

The molecular markers provide a powerful tool for the breeders in the search of new sources of variation, and in the study of the genetic factors responsible for quantitatively inherited traits (Harisaranraj et al., 2009). The molecular markers mostly applied in the assessment of the genetic diversity of ginger cultivars are random amplified polymorphic DNA (RADP), inter simple sequence repeats (ISSRs) and Simple-sequence repeats (SSRs) (Nayak *et al.*, 2005; Nair et Thomas, 2007; Prem *et al.*, 2008; Pretti *et al.*, 2008; Das et al. 2016; Cui et al. 2019; Ismail et al. 2019). The SSRs markers are renowned for their accuracy in genome fingerprinting of any species (Lee et al., 2007, Cui et al. 2019). They are generally abundant, dispersed throughout the genome, and show a high level of polymorphism compared to other genetic markers (Jatoi *et al.*, 2006).

Genetic characterization using microsatellite markers will make it possible to know accurately the extent of variability within ginger accessions grown in Burkina Faso. This will help to develop conservation strategies, and optimally use the ginger accessions grown in Burkina Faso. The present investigation aims to assess the level of diversity and the organization of genetic variability of the collected ginger germplasm from south western zone in Burkina Faso using SSR markers.

METHODOLOGY

Plant material

Genotyping was carried out with 47 accessions of ginger collected in the Provinces of Comoé (03), Léraba (14) and Kénédougou (30) (Table 1; Figure 1). This ginger germplasm was established at Bérégadougou (10°43'23.7'' N et 004°44'47.1'' W). A piece of rhizome of each accession was grown in pot.

Genomic DNA extraction

The second leaf of each sample plant was excised, wrapped into Flanders Technology Associate (FTA) plant card saver and placed in a small polythene bag. A pestle was used to press the young leaf sample extract onto the FTA card until the paper was soaked. The FTA card was then hanged on a drying line using a paper clip for an hour, air drying at room temperature, and later stored in an airtight plastic container with silica gel. Then, discs of 1 mm in diameter were sampled from the FTA card using a puncher (Harrison), and placed in 1.5 ml "Eppendorf" tubes. Each disc was washed twice in 200 µl of FTA reagent for purification for 5 min, and then rinsed twice also with the same quantity of TE buffer (Tris-EDTA) 0.5X for five minutes. Discs sample from FTA cards containing DNA were then dried for one hour at room temperature and transferred to polymerase chain reaction (PCR) tubes for DNA amplification.

Table 1: Number and origin of the ginger accessions collected for genotyping

Accession ID.	Province (number of accessions)	Region
ZoC01; ZoC02; ZoC03	Comoé (3)	Cascades
ZoL04; ZoL05; ZoL06; ZoL07; ZoL08; ZoL09; ZoL10; ZoL11; ZoL12; ZoL13; ZoL14; ZoL15; ZoL16; ZoL17	Léraba (14)	Cascades
ZoK18; ZoK19; ZoK20; ZoK21; ZoK23; ZoK24; ZoK25; ZoK26; ZoK27; ZoK28; ZoK29; ZoK30; ZoK31; ZoK33; ZoK35; ZoK36; ZoK37; ZoK38; ZoK39; ZoK40; ZoK41; ZoK42; ZoK43; ZoK44; ZoK45; ZoK46; ZoK48; ZoK49; ZoK50; ZoK53	Kénédougou (30)	Haut-Bassins

ZoC: *Zingiber officinale* from Comoé; ZoL: *Zingiber officinale* from Léraba; ZoK: *Zingiber officinale* from Kénédougou

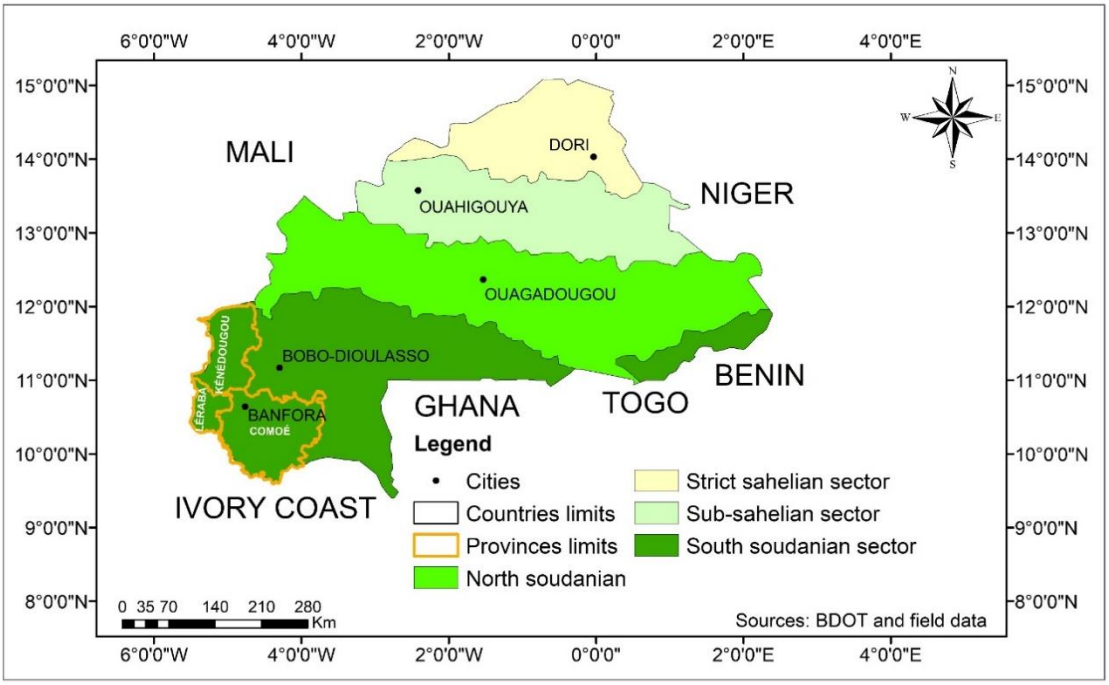

Figure 1. Map showing the different agroclimatic zones of Burkina Faso and the delimitation of the study provinces

Polymerase chain reaction amplification

Eight microsatellite primers for ginger developed by REF were used for this molecular characterization. The characteristics of the different primers are presented in Table 2. DNA amplification was performed in a total volume of 20 µL with PreMix composed of Taq polymerase (2.5 U), dNTPs (250 µL), Tris-HCl pH 9 (10 mM), KCl (30 mM), MgCl (1.5 mM), 2 µl of each primer (forward - reverse), and a disk from the FTA card containing the genomic DNA of the accession. The amplification reaction consisted of initial denaturation phase at 94 °C (3 min), then a series of 30 cycles with a denaturation at 94 °C (30 s) /55 °C (45 s)/72 °C (45 s), followed by 20 cycles at 94 °C (30 s)/53 °C (45 s)/72 °C (45 s), and a final extension at 72 °C for 20 min.

Gel electrophoresis

The PCR products separation was done on a 2% agarose gel in which 7.5 µl of Ethidium Bromide (BET) were added for visualization. Electrophoresis was performed under a voltage of 100 V for one hour in a 0.5x Tris Borate EDTA (TBE) buffer stained with ethidium bromide. The amplified DNA fragments were visualized under UV light and gel was photographed to allow further scoring.

The bands were scored as presence (1) and absence (0) and recorded for each individual accession and for all the primers tested. The variability at each locus was assessed in terms of number of alleles and polymorphism in population.

Molecular data analysis

The bands of DNA fragment were identified on the basis of their position on the gel. The presence (1) and absence (0) of the bands were recorded, and only reliable bands were scored for statistical analysis. Data obtained were processed with Gen Alex software to determine the following genetic parameters for each locus tested: the total number of alleles, the rate of polymorphism, the observed, and expected heterozygosities. The coefficient of genetic differentiation was calculated to estimated genetic divergence among populations (Weir and Cockerham, 1984) using GENETIX 4.04 software (Belkhir et al., 2002). Aptitude of the different loci to show diversity organisation between the accessions was carried out using DARwin V5.0.158 software (Perrier and Jacquemoud-Collet, 2006). The dendrogram was constructed based on Neighbour-Joining method from dissimilarity matrix.

RESULTS AND DISCUSSION

Genetic diversity of ginger accessions from Sudanian zone in Burkina Faso

The estimated genetic parameters for assessment of the level of diversity was calculated with the eight microsatellite primers (Table 3). A total of 24 alleles were revealed by electrophoresis. The number of alleles range from two (GB-ZOM-033; GB-ZOM-103; GB-ZOM-107; GB-ZOM-140) to seven (GB-ZOM-064) with an allelic richness of three alleles per locus. These results showed a low number of alleles compared to the findings of Lee et al. (2007) with 20 ginger accessions collected in Malaysia, Thailand and Indonesia, giving a total of 34 alleles with an average of 4.3 alleles per locus for the same SSR markers set. Jatoi et al. (2006) reported 141 alleles with an average of 17.6 alleles per locus with only 10 ginger accessions collected from seven Asian countries. The large number of alleles could be due to the diverse origin and a wide range of ecological conditions of the ginger germplasm. Furthermore, genotyping of 16 ginger cultivars from India yielded a total of 145 alleles using RAPD markers with an average of 7.5 alleles per locus (Nayak et al., 2005). India being one of the main centers of ginger diversity could explain this significant diversity observed (Akram et al., 2011). In this study, all the primers used were polymorphic with polymorphism rate of 100%. This high level of polymorphism shows that these markers are interesting in the evaluation of genetic diversity in ginger. Polymorphism detection is largely due to the use of highly informative primers that are identified during primer screening (Kishore et al., 2013). The level of polymorphism within a population is governed by a number of factors, which include population size, habitat type, type of markers used, and the impact of human communities (Das et al., 2013). The last factor listed could be the cause of the high level of polymorphism detected in the accessions of our collection. The proximity of the study provinces to the neighboring countries of Côte d'Ivoire and Mali would favor the introduction of ginger accessions from these countries. Although vegetatively propagated species are assumed to have low genetic diversity, the high polymorphism rate recorded in germplasm of the present study would likely imply the existence of genetic diversity within cultivated ginger accessions in Burkina Faso.

Jatoi et al. (2008) reported that the low level of genetic diversity in vegetatively propagated species is not always the rule in practice. Furthermore, amplification of polymorphic bands and species-specific fragments indicates the importance of SSR primers for the molecular characterization of ginger accessions. Jatoi et al. (2006) reported a polymorphism rate of 99.5%; contrary to Nayak et al. (2005) who observed a lower polymorphism rate of 69.6% with RAPD markers on 16 cultivars. The observed heterozygosity (Ho) per locus was zero for four loci. The highest Ho value was recorded for the GB-ZOM-033 locus (0.681) The expected heterozygosity (He) or genetic diversity of Nei ranged from 0.386 for the GB-ZOM-111 locus to 0.799 for GB-ZOM-064 with an average value of 0.499. The mean values of

the expected heterozygosity and the average allelic richness per locus (3) reflect a moderate diversity of the ginger accessions grown in south western of Burkina Faso. Similarly, Lee et al. (2007) found a moderate diversity with an expected heterozygosity rate of 0.449. Nandkangré et al. (2017) detected 60 alleles with an average of 8.571 alleles per locus, with eight rice SSR markers on ginger. SSR primer sets developed on rice have strong DNA amplification ability compared to the GB-ZOM primer type developed on ginger.

Organization of the genetic diversity of ginger accessions

The dendrogram constructed by the Neighbor-Joining method highlighted three genetic groups (Figure 2). That permitted to objectively assess the links between the different accessions. The genetic parameters of the three genetic groups are listed in Table 5. Group 1 consisted of 15 accessions subdivided into two subgroups 1a (07 accessions from Kénédoudou and 01 from Léraba) and subgroup 1b composed exclusively of accessions from Kénédougou. The group 2 had eight accessions originated from Kénédougou with the lowest number of alleles (18). The group 3 contained the largest number of accessions (24) from the three provinces and was subdivided into two subgroups (3a and 3b).

Table 2: Characteristics of the eight SSR primers used for ginger accessions genotyping

Locus	Primer sequences	Repeats	T (°C)	Size (pb)
GB-ZOM-033	F: CAGCAGATTTTTGCTCCG R: GTCGCGTTCGTGGAAAT	$(GTC)_6$	55	86–239
GB-ZOM-040	F: TCTCCCTCTCGGATCCAT R: ATCCATTGCCTGATGGTG	$(GGC)_5$	60	172–181
GB-ZOM-055	F: GTGAGCAGAAAACAGCCG R: TCGCCAATTGAAGACCAC	$(CTG)_5$	58	270–288
GB-ZOM-064	F: CGTAGGATCTTCCCGACC R: CGAGTGAACCCATGGAGA	$(GGA)_5$	60	252–258
GB-ZOM-103	F: GCTGCGGACTAAATGCTG R: ACGCTAGGGAACAGGGAG	(GTG)2 (CTC) $(GTG)_4$	63	117–246
GB-ZOM-107	F: TCCAAAGGTGCTGTTGCT R: CTGTGTTTTTCTTCGCCG	(GTG)2 (CTC) $(GTG)_4$	58	181–184
GB-ZOM-111	F: TAACCGGGAGAAAACCGT R: ACTCGTCCGATTCCGATT	(GA)9 $(GAG)_4$	52	279–291
GB-ZOM-140	F: AGGGGGCAGTGGAGAG R: ACGTTCCTGCACTTGACG	$(GGA)_4$	55	100–280

Source**:** Lee et al. (2007)

Table 3: Diversity parameters of 47 of *Zingiber officinale* (Rosc.) accessions based on SSR primers.

Primers	Total bands	% of polymorphic	Observed heterozygoty	Expected heterozygoty
GB-ZOM-033	2	100%	0.681	0.453
GB-ZOM-040	3	100%	0.000	0.507
GB-ZOM-055	3	100%	0.000	0.566
GB-ZOM-064	7	100%	0.029	0.799
GB-ZOM-103	2	100%	0.658	0.447
GB-ZOM-107	2	100%	0.000	0.438
GB-ZOM-111	3	100%	0.000	0.386
GB-ZOM-140	2	100%	0.538	0.399
Total	**24**	**100%**	**1.906**	**3.995**
Mean	**3**	**100%**	**0.238**	**0.499**

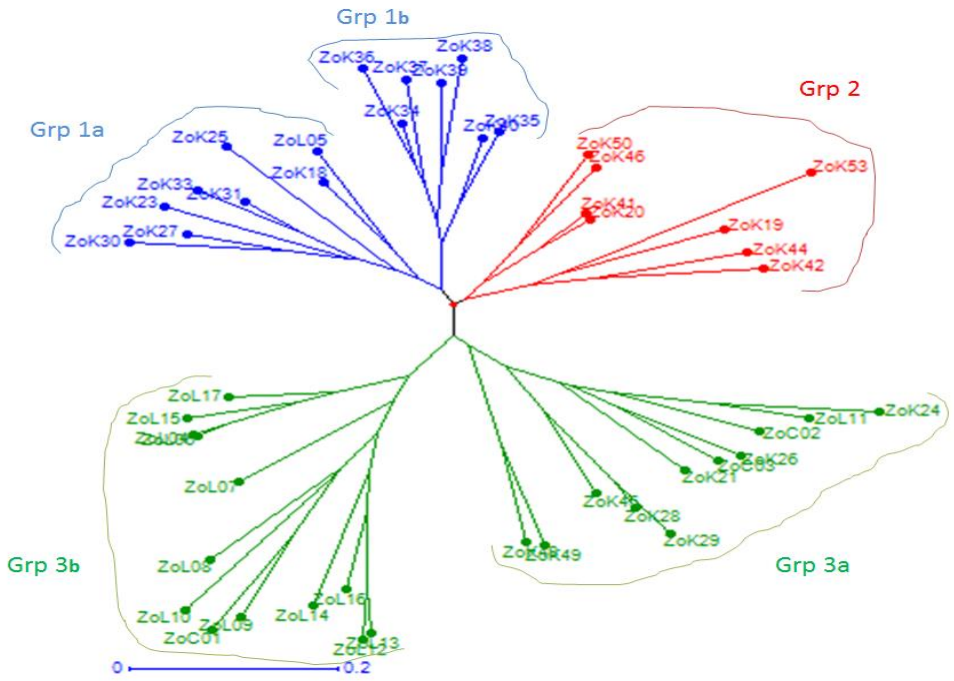

Figure 2. Dendrogram for all 47 accessions of ginger from the South-West zone of Burkina Faso using SSR markers with the Neighbor-Joining method.

Table 5: Parameters of genetic diversity and differentiation of the different genetic groups of ginger accessions from Burkina Faso.

Genetic group	Sample size	Total band	Effective allele	Observed heterozygoty	Expected heterozygoty	Genetic differentiation index
Collection	47	24	-	-	-	**0.320**
Group 1	15	19	1.859	0.282	0.387	
Group 2	8	18	1.936	0.344	0.425	
Group 3	24	19	1.689	0.176	0.360	

In depth assessment of inter- and intra-specific genetic variability is essential for the conservation of plant genetic resources, and the development of appropriate breeding strategies. In addition, it allows to understand with precision, the genetic relationships between individuals within species. The accessions of the group 1 recorded 19 alleles. The effective number of alleles was 1.859 per locus with an expected heterozygosity of 0.387. The group 2 recorded the highest values of expected heterozygosity (0.425) and effective number of alleles of 1.936 per locus. The third group recorded 19 alleles with the lowest effective number of alleles of 1.689 per locus and also the lowest expected heterozygosity of 0.360 (Table 5). The subgroup 3a essentially constituted by accessions from Léraba and the subgroup 3b included accessions from the three provinces.

The genetic differentiation index (0.320) between the three main groups shows that these groups are genetically different. To this extent, it is crucial to conserve accessions in order to preserve the existing diversity within ginger accessions. Ginger being a vegetatively propagated species, it is impossible to improve its agro-morphological properties using conventional breeding techniques. Ginger improvement would be based on selection, by determination of one or more specific gene(s) responsible for the desired traits. The gathering of the accessions into three genetic groups with sub-group reveals a relatively high genetic variability within the collection. Palai and Rout (2007) reported a

population organization into three distinct genetic groups with eight accessions of ginger. Whereas, Harisaranraj et al. (2009) revealed two main genetic clusters in eight ginger accessions collected from Coimbatore in India with 12 RAPD markers. Moreover, Nayak et al. (2005) also observed a structuration in two groups with 16 accessions from the region of Orissa in India using 20 RAPD markers. These differences with our findings might be due to the ability of the different markers to structure diversity and the number of accessions involved in the study.

CONCLUSION

This study represents an important step for gingers germplasm management in Burkina Faso. It reveals the existence of a relatively significant genetic variability within ginger accessions cultivated in Burkina Faso. Thus all of the SSR markers used showed a high rate of polymorphism and distinguished three main genetic groups from the dissimilarity matrix by the Neighbor Joining method. A genetic diversity was also observed between the accessions of ginger from the three provinces. Molecular techniques play a critical role in the studies of phylogeny for understanding the distribution and to some extent of genetic variation within and between species. So, they constitute an effective tool in the study of diversity, and might confirm the variation observed at agro-morphological level. This work permits a better understanding of the level of organization of the ginger germplasm collection. Thus, findings will benefit effective management strategies for the conservation and use of the genetic resources of the species for further breeding and cultivar improvement.

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