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Comparative study of genetic diversity parameters within ginger ecotypes from Burkina Faso using microsatellite markers developed on rice and ginger

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Abstract

Ginger is a valuable plant, used generally as spice across the world, which have a great nutritional, medicinal, social and economic potential. However, it still remains neglected by research works in Burkina Faso. This study experiments were carried out to evaluate a genetic diversity level of 47 ginger's accessions from Burkina Faso using an SSR markers set specific for rice and ginger. All the primers set used showed an important genetic diversity among the ginger accessions grown in Burkina Faso. Comparison between RM SSR and ZOM SSR reveal that ZOM SSR primers set are more efficient to determine genetic variation between the ginger germplasm with a genetic diversity of Nei (D) and Shannon information index (I) value respectively 0.459 and 0.806. This study allowed a better understanding of genetic diversity level of Burkina Faso's ginger collection and it provide useful data in determination of effective management strategies in order to improve the genetic resources of the species.

Keywords: Ginger, SSR primers, Diversity, Burkina Faso

1. Introduction

Ginger is one of the most popular spices across the world (Dohroo *et al.*, 2012) and is used in traditional medicinal practices (Preeti *et al.*, 2008; and Kubra and Rao, 2012). It is also appreciated for its horticultural

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importance (Joshi *et al.*, 2012) and used in religious rituals (Daimei and Kumar, 2013). The oils extracted from the rhizome are used in cosmetics, pharmacy and perfumery (Vernin and Parkanyi, 2005). It contains many biochemical compounds such as gingeroles, gingerdioles and gingerdiones (Ghasemzadeh et al., 2010 and Colleen *et al.*, 2012) which possess high antioxidant activity (Singh *et al.*, 2008; and Akbarian *et al.*, 2011). In traditional Chinese medicine, ginger is used to treat stomach ache, nausea and indigestion (Azam *et al.*, 2014), rheumatism, nervous diseases, asthma, constipation, diabetes, etc. (Soong *et al.*, 2011). Ginger therefore represents a plant of major interest for the pharmacopoeia. Despite the potential offered by this plant, research work is almost non-existent in Burkina Faso, where it appears to be a minor and neglected crop. However, many researches have been carried out on ginger variability in Asia (Jatoi and Watanabe, 2013). A better knowledge of the diversity of ginger will make it possible to lay milestones for a good strategy of conservation, management and improvement. It is therefore, imperative to characterize and preserve the diversity of cultivated ginger in order to avoid genetic erosion of the species. The loss of genetic resources and, consequently, the genetic diversity they represent is a reality. The work carried out by Nandkangré *et al.* (2016) using morphological markers showed that there is a moderate variability within ginger accessions grown in Burkina Faso. Although these markers are important in the study of diversity, they can be influenced by environmental factors and by the stage of plant development. Molecular characterization based on DNA markers is more reliable in the genetic characterization of a species. The assessment of the genetic diversity of ginger cultivars was carried out using the RADP and ISSR molecular markers which are the most widely used (Nayak *et al.*, 2005; Nair and Thomas, 2007; Prem *et al.*, 2008; and Pretti *et al.*, 2008). However, the SSR markers developed for ginger are very limited in number. These markers are renowned for their accuracy in study the extent of genetic diversity of any species (Lee *et al.*, 2007). They are generally abundant, dispersed across the genome and show a high level of polymorphism compared to other genetic markers (Jatoi *et al.*, 2006). Genetic characterization using microsatellite markers makes it possible to know precisely the level of diversity existing within a species. Identification of more discriminating primers provides a new, more precise tool for the breeder in the search for new sources of variation and in the study of the genetic factors responsible for quantitatively inherited traits (Harisaranraj *et al.*, 2009). The present study aims to know with precision the level of genetic diversity of the collection of accessions of ginger and to identify which primers set is suitable for ginger molecular characterization. This could make it possible to develop strategies for the conservation and optimal management of the intra-specific genetic diversity of ginger accessions cultivated in Burkina Faso.

2. Material and methods

2.1. Plant material and DNA extraction

Genotyping was performed with 47 ginger accessions from Comoé (03), Kénédougou (30) and Léraba (14) in the south western area of Burkina Faso (Table 1). The investigated germplasm was grown in pots in Bérégadougou (10°43′23.7′′ North 004°44′47.1′′ West), Genotyping was carried out at the plant genetics and biotechnology laboratory of the Institute for the Environment and Agricultural Research. Fresh tender ginger leaves were excised to isolate genomic DNA using Whatman FTA (Flanders Technology Associate) card method (Nandkangré, 2016).

Table 1: Origin of the ginger accessions collected		
N° Accession	**Province**	**Region**
ZoC01; ZoC02; ZoC03	Comoé	Cascades
ZoL04; ZoL05; ZoL06; ZoL07; ZoL08; ZoL09; ZoL10; ZoL11; ZoL12; ZoL13; ZoL14; ZoL15; ZoL16; ZoL17	Léraba	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	Haut-Bassins

2.2. Ginger SSR primers set and rice SSR primers set amplification

Polymerase chain reaction (PCR) was performed with eight primers developed on the rice (RM SSR primers set) and eight primers developed on the ginger (ZOM SSR primers set) by REF. Table 2 shows each primer set with repeat type and length, product size range and annealing temperature. For the ZOM SSR primers set, the final volume of the reaction mixture used for PCR analysis was 20 µl containing 1x Mix, 3 mM MgCl, 1.25 Unit Taq, 0.2 m MdNTPs mix), 2 µl for forward and reverse primers and one FTA disc containing DNA template. DNA amplification was carried out in thermal cycler (My Cycler, BioRad), with flowing PCR amplification conditions: 94°C (3 min), then 30 cycles 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. For the eight rice SSR primers set developed on rice, the PCR proceeded as follows: an initial denaturation at 94°C (5 min), followed by 35 cycles at 94°C for 1 min, annealing for 1 min, and an extension at 72°C for 90s. A final extension was performed at 72°C for 10 min followed by cooling to 10°C. Amplified products were electrophoresed by 2% agarose gel in 1xTBE buffer

Table 2: Details of RM SSR primers and ZOM SSR primers set used in this study					
Primer type	Primer	Sequence	Repeat	T (°C)	Size (pb)
ZOM SSR primers set	ZOM-033	F: CAGCAGATTTTTGCTCCG R: GTCGCGTTCGTGGAAAT	$(GTC)_6$	55	86-239
	ZOM-040	F: TCTCCCTCTCGGATCCAT R: ATCCATTGCCTGATGGTG	$(GGC)_5$	60	172-181
	ZOM-055	F: GTGAGCAGAAAACAGCCG R: TCGCCAATTGAAGACCAC	$(CTG)_5$	58	270-288
	ZOM-064	F: CGTAGGATCTTCCCGACC R: CGAGTGAACCCATGGAGA	$(GGA)_5$	60	252-258
	ZOM-103	F: GCTGCGGACTAAATGCTG R: ACGCTAGGGAACAGGGAG	$(GTG)2 (CTC) (GTG)_4$	63	117-246
	ZOM-107	F: TCCAAAGGTGCTGTTGCT R: CTGTGTTTTTCTTCGCCG	$(GTG)2 (CTC) (GTG)_4$	58	181-184
	ZOM-111	F: TAACCGGGAGAAAACCGT R: ACTCGTCCGATTCCGATT	$(GA)9 (GAG)_4$	52	279-291
	ZOM-140	F: AGGGGGCAGTGGAGAG R: ACGTTCCTGCACTTGACG	$(GGA)_4$	55	100-280
RM SSR primers set	RM1	F: GCGAAAACACAATGCAAAAA R: GCGTTGGTTGGACCTGAC	ACAA (AG)26 CCAC	55	113
	RM117	F: CGATCCATTCCTGCTGCTCGCG R: CGCCCCCATGCATGAGAAGACG	(AG)7	60	203-207
	RM125	F: ATCAGCAGCCATGGCAGCGACC R: AGGGGATCATGTGCCGAAGGCC	(GCT)8	55	124-136
	RM131	F: TCCTCCCTCCCTTCGCCCACTG R: CGATGTTCGCCATGGCTGCTCC	(CT)9	60	209-217

Table 2 (Cont.)

Primer type	Primer	Sequence	Repeat	T (°C)	Size (pb)
	RM135	F: CTCTGTCTCCTCCCCCGCGTCG	(CGG)10	63	119-131
		R: TCAGCTTCTGGCCGGCCTCCTC			
	RM153	F: GCCTCGAGCATCATCATCAG	(GAA)9	55	189-204
		R: ATCAACCTGCACTTGCCTGG			
	RM154	F: ACCCTCTCCGCCTCGCCTCCTC	(GA)21	60	165-169
		R: CTCCTCCTCCTGCGACCGCTCC			
	RM171	F: AACGCGAGGACACGTACTTAC	(GATG)5	55	318-343
		R: ACGAGATACGTACGCCTTTG			

stained with ethidium bromide and visualized under UV light. Gel was photographed were obtained using Canon Power Shot A620 and the amplification product was evaluated. The difference for each locus was assessed in terms of numbers of alleles.

3. Data analysis

Amplified DNA fragments were scored as present (1) or absent (0) of bands and only reliable bands were scored and used for statistical analysis. Descriptive statistical analysis was performed using genetic parameters to compared the two primers set. These are essentially the total number of alleles (At), the effective number of alleles (Ae). In addition, the polymorphism of amplification products (P), expected heterozygosity (He) or Nei's gene diversity index (D) as well as Shannon's information index (I) were calculated. The polymorphism (P) was determined using GENETIX 4.04 software (Belkhir *et al.*, 2002). The other parameters were calculated using GenALEX software. The genetic differentiation index (Gst) was calculated to estimated genetic divergence between ginger accessions from the three provinces through the estimator of Weir and Cockerham (1984) using GENETIX 4.04 software.

4. Results and discussion

4.1. Genetic diversity analysis of gingers germplasm

The RM SSR primers and ZOM SSR primers set used to analyze genetiçc diversity among 47 accessions of ginger collection from three different provinces Comoé (03), Kénédougou (30) and Léraba (14) were polymorphic. The locus RM1 presented an illegible profile, therefore, was not included in the analysis. Then, genetic diversity analysis was conducted with seven RM primers set out of the eight previously stated. A total number of 15 primers, i.e., eight ZOM SSR primers set and seven RM SSR primers set were screened on 47 gingers germplasm collection and revealed, respectively 24 and 60 alleles by electrophoresis. All the primers set were shown to be polymorphic with a polymorphism rate of 100% and 95.32%, respectively for ZOM SSR primers and RM SSR primers. The high polymorphism rates in ginger accessions grown in Burkina Faso shows a strong adaptability of these cultivars. Nayak *et al.* (2005) with RAPD markers on 16 ginger cultivars and Jatoi *et al.* (2008) obtained respective polymorphism rates of 69.6% and 71%. The number of alleles (At) ranged from two (ZOM-033; ZOM-103; ZOM-107; ZOM-140) to seven (ZOM-064) with an average of three alleles per locus for ZOM SSR primers set. The effective number of alleles (Ae) has shown a low value (1.613) with the primer ZOM-111 and a high value (4.702) with ZOM-064 with average of 2.199 (Table 3).

Contrariwise, RM SSR primers set displayed number of alleles (At) ranging from two (RM171) to 15 (RM153) with an average of 8.571 (Table 4). The effective number of alleles (Ae) ranged from 1.775 (RM171) to 10.978 (RM153) with 6.297, as average. ZOM SSR primers set showed genetic diversity of Nei (D) and Shannon's information index (I) value of 0.499 and 0.806, respectively. When using RM SSR primers set, genetic diversity of Nei (D) and Shannon's information index (I) values were 0.314 and 0.459, respectively. The present study results showed an important genetic diversity among the ginger accessions grown in Burkina Faso. Vegetatively

Table 3: Genetic diversity parameter across 47 ginger accessions using ZOM SSR primers set

Primer type	Primer	At	Ae	P	D	I
ZOM SSR primers set	ZOM-033	2	1.815	2 (100%)	0.453	0.641
	ZOM-040	3	1.998	3 (100%)	0.507	0.777
	ZOM-055	3	2.263	3 (100%)	0.566	0.904
	ZOM-064	7	4.702	7 (100%)	0.799	1.646
	ZOM-103	2	1.790	2 (100%)	0.447	0.633
	ZOM-107	2	1.761	2 (100%)	0.438	0.624
	ZOM-111	3	1.613	3 (100%)	0.386	0.645
	ZOM-140	2	1.649	2 (100%)	0.399	0.582
	Total	**24**	**17.591**	**-**	**3.995**	**6.452**
	Mean	**3**	**2.199**	**3**	**0.499**	**0.806**

Note: At – Total number of alleles; Ae – effective number of alleles; P - Polymorphism; D - Genetic diversity of Nei; and I – Shannon's index.

propagated species are assumed to have low genetic diversity, due to their clonal reproduction. However, the high polymorphism rate was recorded in the present study imply the existence of genetic diversity within cultivated ginger accessions in Burkina Faso. Jatoi *et al.* (2008) reported that vegetatively propagated species

Table 4: Genetic diversity parameter across 47 ginger accessions using RM SSR primers set

Primer type	Primer	At	Ae	P	D	I
RM SSR primers set	RM117	8	5.223	8 (100%)	0.214	0.352
	RM125	8	5.939	6 (75%)	0.282	0.407
	RM131	13	7.568	12 (92.3%)	0.123	0.214
	RM135	8	7.34	8 (100%)	0.454	0.633
	RM153	15	10.978	15 (100%)	0.271	0.401
	RM154	6	5.261	6 (100%)	0.418	0.589
	RM171	2	1.775	2 (100%)	0.438	0.618
	Total	**60**	**44.084**	**-**	**2.2**	**3.213**
	Mean	**8.571**	**6.297**	**6.07 (95.32%)**	**0.314**	**0.459**

Note: At – Total number of alleles; Ae – effective number of alleles; P – Polymorphism; D – genetic diversity of Nei; and I – Shannon's index.

are supposed to display low genetic variation, that is not always observed in practice. Lee *et al.* (2007) indicated the existence of moderate level of genetic diversity among 20 ginger genotypes with the same ZOM SSR primers set.

The comparison of the two types of primers set showed that the RM SSR presented high values for the parameters, total number of alleles (At) (60 # 24) and number of effective alleles (Ae) (44.084 # 17,591) compared to ZOM SSR. RM SSR primers set have a better ability to detect different variations at the gene level. However, the genetic diversity of Nei (D) and Shannon index (I) displayed a lower mean value (0.314 # 0.499) and (0.459

0.806) in comparison with ZOM SSR primers set. These two diversity parameters (D and I) with high values for ZOM SSR primers set showed that ZOM SSR primers set are more efficient to determine genetic variation between the ginger germplasm.

4.2. Genetic diversity according to the ginger provinces of origin and genetic differentiation

Ginger accessions from Kénédougou present the greatest diversity parameters with ZOM SSR primers set (Table 5). A total of 21 alleles (At) and 2,020 effective alleles (Ae) were recorded. The genetic diversity of Nei (D) value and the polymorphism (P) rate were 0.454 and 87.5% respectively. The high genetic diversity observed within Kénédougou ginger accessions compared to the others two provinces would be due to the number of accessions and its geographical position. Kénédougou is very close to Sikasso in Mali, and benefit exchanges of accessions with producers and traders. Ginger accessions from Comoé had the lowest values. In these accessions, a total of 10 alleles (At) were detected with 1,123 effective alleles (Ae) and a low polymorphism (P) rate (37.5%). Ginger accessions from Léraba showed intermediate diversity parameters with, however, a high polymorphism (P) rate (87.5%). Use RM SSR primers set, ginger accessions from Kénédougou always present the greatest value for number of alleles (At = 57) and effective number of allele (Ae = 1.419) (Table 6), following by accessions of Léraba. The total number of allele (At), effective number of alleles (Ae) and genetic diversity of Nei (D) with ZOM SSR primers set increase with the number of accessions from the province [Kénédoudou (30) > Léraba (14) > Comoé (03)]. Das *et al.* (2016) reported similar results of genetic diversity among the ginger cultivars from different regions of the eastern coast of India (Odisha) using ISSR and SSR type of markers. Use the RM SSR primers set, we also founded similar trends of genetic diversity among the province with total number of alleles (At), effective number of alleles (Ae) and polymorphism rate (P). However, accessions from Léraba (14) had the greatest value of genetic diversity of Nei (D = 0.302) following by those of Kénédougou (30) (D = 0.290). The variability observed within a gene pool is the main resource available for the breeders in ginger breeding programs (Harisaranraj *et al.,* 2009). It is very important to conserve accessions in order to preserve the existing diversity within ginger accessions from the three provinces. This conservation could be done by planting cultivars over the years. Ginger being a vegetatively propagated species, it is quiet impossible to improve its agronomic traits by conventional breeding methods. One of the most effective ways to improve this crop would be based on selection, by identification of one or more specific gene(s) responsible for the desired traits in the species.

The genetic differentiation index (Gst = 0.313) has shown an important differentiation between ginger accessions from the three provinces with ZOM SSR primers set. On the over side, the genetic differentiation index using RM SSR primers set recorded between the accessions from three provinces (Gst = 0.126) implies a moderate differentiation. The accessions from the three provinces are genetically different. Molecular diversity assessment of inter- and intra-specific genetic variability is essential for the conservation of plant genetic resources and the development of appropriate plant breeding strategies. ZOM SSR primers set could be utilized

Table 5 : Diversity parameters and differentiation index between the provinces using ZOM SSR primers set

Primer type	Factors	N	At	Ae	D	P (%)	Gst
ZOM SSR primers set	Collection	47	24	-	-		
	Provinces						0,313
	Comoé	3	10	1.123	0.187	37.50	
	Kénédougou	30	21	2.020	0.454	87.50	
	Léraba	14	16	1.692	0.365	87.50	

Note: N – Sample size; At – Total number of alleles; Ae – Effective number of alleles; P – Polymorphism; D – Genetic diversity of Nei; and Gst – Genetic differention index.

Table 6 : Diversity parameters and differentiation index between the provinces using RM SSR primers set							
Primer type	**Factors**	**N**	**At**	**Ae**	**D**	**P (%)**	**Gst**
RM SSR primers set	Collection	47	60	-	-	-	-
	Provinces						0,126
	Comoé	3	18	1.227	0.192	28.33	
	Kénédougou	30	57	1.419	0.290	93.33	
	Léraba	14	41	1.418	0.302	68.33	

Note: N – Sample size; At – Total number of alleles; Ae – Effective number of alleles; P – Polymorphism; D – Genetic diversity of Nei; and Gst – Genetic differention index.

for further assessment of genetic diversity within a large germplasm collection of ginger in Burkina Faso. In addition, it makes it possible to understand with precision the genetic relationships between individuals of the same species, even the most geographically distant.

5. Conclusion

This molecular characterization study allowed to highlight significant genetic variability among 47 ginger accessions cultivated in Burkina Faso. There is a difference between the ginger accessions of the three provinces. The both ZOM SSR primers and RM SSR primers set used showed a high rate of polymorphism and permitted to show differentiation between the ginger accessions from the three provinces. RM SSR primers set were revealed to be more efficient in ginger accessions characterization for displaying high number of total and efficient alleles, however, ZOM SSR primers were had shown high value of of genetic diversity of Nei and Shannon's index. This work has allowed a good understanding of the genetic diversity of the gingers germplasm. In addition, they provide important data in determination effective management strategies for the conservation and use of the genetic resources of the species.

Acknowledgement

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