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 grown in 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
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 gingerols, gerdioles and gerdiones (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 Prettì 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 precisely 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 MgCl₂, 1.25 Unit Taq, 0.2 mM dNTPs 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 90 s. 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     | ZOM-033   | F: CAGCAGATTTTTGCTCCG     | (GTC)₆ | 55     | 86-239    |
|             |           | R: GTGCGGTTCGTGGAAAT      |        |        |           |
| ZOM-040     |           | F: TCTCCCTCTCGGATCCAT     | (GGC)₅ | 60     | 172-181   |
|             |           | R: ATCCATTGCGTGGGTG       |        |        |           |
| ZOM-055     |           | F: GTGAGCAGAAACACGGCG     | (CTG)₅ | 58     | 270-288   |
|             |           | R: TCCGCAAATTGAAACCCAC    |        |        |           |
| ZOM-064     |           | F: CGTACGGATCCTCCGGACC    | (GGA)₅ | 60     | 252-258   |
|             |           | R: CGAGTGAAACCACTGAGA     |        |        |           |
| ZOM-103     |           | F: GCTGCCGACTAATGCTG      | (GTG)₂ (CTC) (GTG)₄ | 63    | 117-246   |
|             |           | R: ACCTAGGGGAACGGGAG      |        |        |           |
| ZOM-0107    |           | F: TCAAAAAAGGTGTGGTGTG    | (GTG)₂ (CTC) (GTG)₄ | 58    | 181-184   |
|             |           | R: CTGTGTTTTTTTTCTCGCCG   |        |        |           |
| ZOM-111     |           | F: TAACCCGGGAGAAAACCGT    | (GA)⁹ (GAG)₄ | 52    | 279-291   |
|             |           | R: ACGCTAGGGGAGCTG        |        |        |           |
| ZOM-140     |           | F: AGGGGGCACTGGAGAG       | (GGA)₄ | 55     | 100-280   |
|             |           | R: ACGTTTCTGCACTTGACG     |        |        |           |
| RM SSR      | RM1       | F: GGGAAAAACACAAATGAAAAAA | ACA (AG)₂⁶ CCAC | 55    | 113       |
|             |           | R: GCGGTGGTGGACCTGAC      |        |        |           |
| RM117       |           | F: CGATCATTCTCTGTCTGCGG   | (AG)⁷  | 60     | 203-207   |
|             |           | R: CGCCCCCATGATGAGAAGACG  |        |        |           |
| RM225       |           | F: ATCAGCAAGCCATGGACGACC  | (GCT)₈ | 55     | 124-136   |
|             |           | R: AGGGGATCATGTGCCGAGGGCC |        |        |           |
| RM131       |           | F: TCTCCTCCTCTCCGGCCACTG  | (CT)⁹  | 60     | 209-217   |
|             |           | R: CGATGATGCGGACATGGGCTCC |        |        |           |
stained with ethidium bromide and visualized under UV light. Gel was photographed were obtained using Canon Power Shot A 620 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 genetical diversity among 47 accessions of ginger collection from three different provinces Comoé (03), Kénédougou (30) and Léraba (14) were polymorphic. The locus RM 1 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 (RM 153) 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|         | 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 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.

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.

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) respectively.
# 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 [Kénédougou (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

| 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 |

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

**Note**: N – Sample size; At – Total number of alleles; Ae – Effective number of alleles; P – Polymorphism; D – Genetic diversity of Nei; and Gst – Genetic differentiation index.
Table 6: Diversity parameters and differentiation index between the provinces using RM SSR primers set

| Primer type        | Factors | N   | A<sub>t</sub> | A<sub>e</sub> | 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; A<sub>t</sub> – Total number of alleles; A<sub>e</sub> – Effective number of alleles; P – Polymorphism; D – Genetic diversity of Nei; and Gst – Genetic differentiation 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.

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