ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 05, pp.50122-50126, May, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

GENETIC DIVERSITY ANALYSIS OF BURKINA FASO GINGER (*ZINGIBER OFFICINALE ROSC.*) LANDRACES USING MICROSATELLITE MARKERS

[1,*]Hervé Nandkangré, [1]Mahama Ouedraogo, [2]Souleymane Bado, [1]SergeFélicien Zida, [1]AiméSévérin Kima and [3]Mahamadou Sawadogo

[1]Institut del' Environnement et de Recherches Agricoles (INERA), Département Productions Végétales, Laboratoire de Génétique et de Biotechnologies Végétales, 04 BP 8645 Ouagadougou, Burkina Faso
[2]Plant Biotechnology Unit, Department of Biotechnology, University of Natural Resources and Life Sciences, Muthgasse 18, A-1190 Vienna, Austria.
[3]Université de Ouagadougou, UFR–SVT, Département de Biologie et Ecologie Végétales, Equipe de Recherche en Génétique et Amélioration des Plantes, Laboratoire Biosciences, 03 BP 7021 Ouagadougou 03, Burkina Faso

ARTICLE INFO

Article History:
Received 06th February, 2017
Received in revised form
29th March, 2017
Accepted 16th April, 2017
Published online 23rd May, 2017

Key words:

Genetic diversity,
Ginger,
SSR markers,
Burkina Faso.

ABSTRACT

Identification and characterization of germplasm is important for efficient management strategies in conservation and utilization of plant genetic resources. This investigation aimed to assess genetic diversity of 47 ginger landraces from Burkina Faso base on polymorphism of eight SSR primers. Seven of theses primers produced 60 loci out of which 95.3% were found to be polymorphic. The number of loci ranged from two to 15 with an average of 8.571lociperprimer. Values for Nei's genetic diversity or expected heterozygosity (He) varied from 0.123 to 0.454 with an average of 0.314. Whereas, the mean polymorphism information content (PIC) ranged from 0.119 to 0.443 with an average of 0.302. The high polymorphism (95.32%) depicted showed the existence of a genetic variability within the ginger accessions. Dendrogram based on seven SSR markers using Neighbour-Joining method classified all the ginger accessions into three genetic groups with 20, 25 and 2 accessions respectively for groups 1, 2 and 3. The accession gathering in three distinct groups is not according to their origin. This investigation showed the genetic diversity in germplasm of West regions of Burkina Faso. That provides important genetic information for the germplasm conservation and of ginger improvement breeding programs.

Copyright©2017, Hervé Nandkangre et al. *This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.*

Citation: Hervé Nandkangré, Mahama Ouedraogo, Souleymane Bado, Serge Félicien Zida, Aimé Sévérin Kima and Mahamadou Sawadogo, 2017. "Genetic diversity analysis of burkina faso ginger (*zingiber officinale rosc.*) landraces using microsatellite markers", *International Journal of Current Research*, 9, (05), 501221-50126

INTRODUCTION

Ginger, the rhizome of *Zingiber officinale* Rosc. is one of the most widely used spices of the family *Zingiberaceae* (Jayashree *et al.*, 2012). It is thought to be originated from South-east Asia and introduced to many parts of the world, and has been cultivated for thousands of years as spice and medicinal purpose (RafieandOlczyk, 2003; Abdullah *et al.*, 2010; Shariq *et al.*, 2010). Ginger mainly possesses anti-diarrheal, anti-diabetic, anti-inflammatory, anti-bacterial and cardio-protective proprieties (Imtiyaz *et al.*, 2013), anti-oxidant proprieties (Ghasemzadeh *et al.*, 2010; Yeh*et al.*, 2013). The antioxidative and free radical scavenging properties of ginger extracts have been well established (Singh *et al.*, 2008).

***Corresponding author: Hervé Nandkangre**
Institut del'Environnement et de Recherches Agricoles (INERA), Département Productions Végétales, Laboratoire de Génétique et de Biotechnologies Végétales, 04 BP 8645 Ouagadougou, Burkina Faso.

Ginger is vegetatively propagated, therefore has a very narrow genetic diversity (Babu *et al.*, 2013). However, the maximum variation within cultivated ginger was found in India with a collection of 659 accessions (Parthasarathy *et al.*, 2012). Ravindran *et al.* (1994) suggested being due to geographical spread from its centre of origin in South-east Asia. Recently, several cultivars officially released were characterized on the basis of phenotypic data such as morphological and biochemical characters and yield potential (Jiang *et al.*, 2006; Zhou *et al.*, 2007). However, these characters fluctuate under varied environmental conditions making characterization of ginger cultivars a laborious task (Nayak *et al.*, 2005). With the advent of molecular biology, DNA-based markers have provided an effective tool for an efficient complement for morphological, cytological, and biochemical characterization in germplasm diversity study. Thus DNA-based markers allow varietal identification, clonal fidelity and genetic variability assessment, validation of genetic relationship, phylogenetic and evolutionary studies, marker-assisted selection, and gene

tagging (Mohanty *et al.*, 2014). Therefore, it represents a powerful tool for breeders to search for new sources of variation for cultivars improvement and to investigate genetic factors controlling quantitatively inherited traits (Palai and Rout, 2007). The limited research on characterization at DNA level has hindered the improvement of cultivated ginger through molecular breeding (Lee *et al.*, 2007). In the past decade, microsatellite has become the marker of choice for genetic characterization of plants. They are generally abundant, dispersed throughout the genome, and also show higher levels of polymorphism than other genetic markers (Jatoi *et al.*, 2006). The genetic diversity among ginger species is not well documented and few studies have been reported so far (Nayak *et al.*, 2005; Harisaranraj *et al.*, 2009; Mohanty *et al.*, 2014). One of the major reasons is the lack of appropriate and specific genetic markers and limited interest in under-utilized taxa such as *Zingiberaceae* in academic research (Jatoi *et al*., 2006).

Most works on genetic variability studies in *Zingiber officinale* Rosc. in family *Zingiberaceae* are mainly from India, China, Thailand and Malaysia (Jatoi and Watanabe, 2013). Recently, in Burkina Faso, studies on ginger have been focused on characterization of the production system (Nandkangré *et al.*, 2015; Nandkangré, 2016), agro morphological characterization (Nandkangré *et al.*, 2016a; Nandkangré, 2016), heritability and genetic advance for identification of top genotypes for further breeding program (Nandkangré *et al.*, 2016b). Thus, this present study was undertaken to consolidate the agro-morphological characters and for assessment genetic diversity of our ginger accessions germplasm for meaningful breeding and conservation purposes.

MATERIALS AND METHODS

Plant material and DNA extraction

A total of 47 ginger accessions composing the germplasm in this study. The accessions were collected in three provinces: Kénédougou (30), Léraba (14) and Comoé (03) located at the South-west of Burkina Faso (Table 1). All ginger accessions were collected directly from producers. The investigated germplasm was grown in pots in Bérégadougou (10°43'23.7'' North 004°44'47.1''West), young leaves were collected for DNA extraction. Total genomic DNA was extracted using the Whatman FTA card. FTA classic card measures 7.5 x 13 cm, and was labelled prior to the day of sampling. Each FTA card was divided in eight parts. The leaves were excised from the plant, wrapped round the FTA paper strip, and placed in a small polythene bag. A pestle was used to press the leaf sample extract onto the FTA paper until the paper was soaked. The FTA card was then hanged on a drying line using a paper clip for one hour air drying, and later stored in an airtight plastic container with silicagel. Discs of 1 mm diameter were sampled on the FTA cards using a puncher (Harrison). Each disc was washed twice with 200 µl of FTA purification reagent for 5 min and as well rinsed twice with 200 µl of Tris EDTA buffer for 5 min. Then, the discs containing the DNA were dried for one hour at room temperature after that they were ready for PCR amplification.

PCR amplification and bands reading

Polymerase chain reaction (PCR) was performed with eight rice SSR primers sets developed by REF.

Table 2 shows each primer set with repeat type and length, product size range and annealing temperature. The final volume of the reaction mixture used for PCR analysis was 20 µl containing 1x Mix, 3 m MMgCl, 1.25 Unit Taq, 0.2 m MdNTPs mix), 2 µl for forward and reverse primersand 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. Amplified products were separated alongside a molecular weight marker plus ladder by 2% agarose gel electrophoresis in 1xTBE buffer stained with ethidium bromide and visualized under UV light. Gel was photographed and the amplification product was evaluated. The variability at each locus was assessed in terms of numbers of alleles.

Data analysis

Amplified DNA fragments were recorded for the presence (1) or absence (0) of bands and only reliable bands were scored. Genetic diversity within the collection was performed as the total number of loci, the effective number of loci, the percentage of polymorphic alleles, gene diversity/expected heterozygosity (He) and the polymorphic information content (PIC) by using GenALEX software. The coefficient of genetic differentiation (Gst) was calculated to estimated genetic divergence among populations through the estimator of Weir and Cockerham (1984) using GENETIX 4.04 (Belkhir *et al.*, 2002). Aptitude of the different loci to structure diversity between the genotypes 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

This study on the genetic characterization of ginger accessions using rice microsatellite markers is unique in the genotype identification and estimation of genetic diversity of ginger germplasm from Burkina Faso. The genetic diversity analysis was conducted with seven polymorphic primers out of the eight previously stated. The locus RM1 presented an illegible profile, therefore was not included in the analysis. A total of 60 loci were found with seven SSR primer sets across the 47 ginger accessions (Table 3). The number of loci ranged from two (RM171) to 15 (RM153) with a mean of 8.571 loci per locus. These values are higher than those of Lee *et al.* (2007), which characterized 20 accessions of ginger and detected in total 34 loci with an average of 4.3 loci per primer set.

Palai and Rout (2007) also found a low number of loci (55) with eight ginger varieties collected in India using 12 RAPD markers. The low number of loci obtained by these authors could be due to the small sample size of their samples. That consideration is supported by the work of Kishore *et al.* (2013) on 75 ginger accessions with 13 ISSR markers revealed 110 loci. However, Jatoi *et al.* (2006) used 12 rice SSR primer sets, and found 141 loci with an average number of 17.6 loci per primer set with 10 cultivars of ginger collected through seven countries of Asia. Although the numbers of cultivars used in their study were low, the distance origin of the accessions could explain the high diversity found in this study.

Table 1. Origin of the ginger accessions collected

Accession N°	Origin
ZoC01; ZoC02; ZoC03	Comoé
ZoL04; ZoL05; ZoL06; ZoL07; ZoL08; ZoL09; ZoL10; ZoL11; ZoL12; ZoL13; ZoL14; ZoL15; ZoL16; ZoL17;	Léraba
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

Table 2. Characteristics of the eight rice SSR primer sets

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

Table 3. Genetic diversity parameter across 47 accessions of *Z. officinale*

Loci	Number of loci			He	PIC
	Total	Effective	Polymorphic		
RM117	8	5.223	8 (100%)	0.214	0.209
RM125	8	5.939	6 (75%)	0.282	0.276
RM131	13	7.568	12 (92.3%)	0.123	0.119
RM135	8	7.340	8 (100%)	0.454	0.443
RM153	15	10.978	15 (100%)	0.271	0.255
RM154	6	5.261	6 (100%)	0.418	0.408
RM171	2	1.775	2 (100%)	0.438	0.408
Total	60	44.084	57	2.20	2.118
Average	8.571	6.297	6.07 (95.32%)	0.314	0.302

Legend: He: expected heterozygosity; PIC polymorphic information content

Table 4. Diversity parameters and differentiation index between the provinces

Factors	N	A	Ae	He	P%(0,95)	Gst
Total collection	47	60	-	-	95.33	
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	

N:Number of accessions; A: Total number of bands; Ae: Effective allele; He: Expected heterozygosity; P:Polymorphism;Gst:Genetic differentiation index

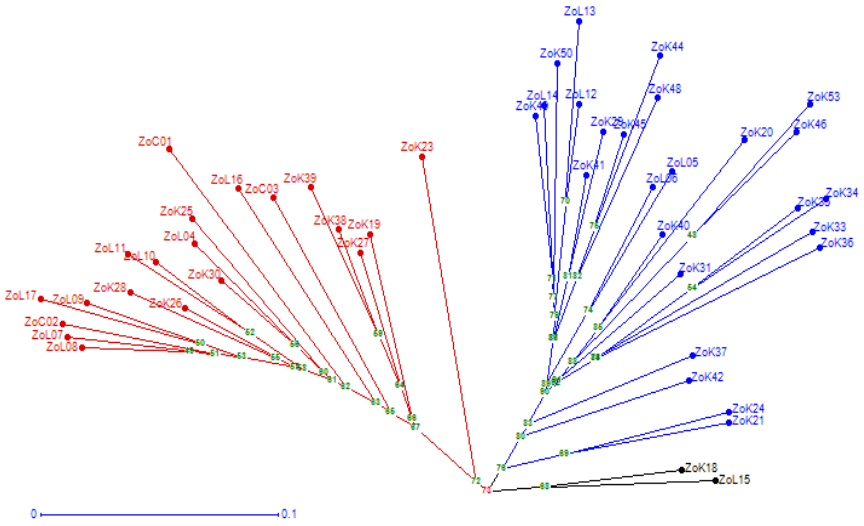

Figure 1: Dendrogram based of Neighbour-joining methodbase on seven rice SSR markers showing the clustering of 47 *Z. officinale* landraces from Burkina Faso in three major groups: group 1 in red, Group 2 in blue and group 3 in black

Moreover, Nayak *et al.* (2005) by genotyping 16 ginger cultivars collected in India observed 145 loci using 20 RAPD markers with an average of 7.5 alleles per primer. This large diversity could result in the fact that India is believed to be the centre of diversity of ginger (Akram *et al.*, 2011). From our study the smallest and the largest value of effective loci was found respectively with RM171 (1.775) and RM153 (10.978) (Table 3). The average PIC per locus was 0.302 within a range of 0.119 (RM131) to 0.443 (RM135). According to Lee *et al.* (2007), microsatellites have been proven as one of the most powerful markers, which can accurately assess the level of genetic diversity within a germplasm collection of any crop species. This assertion is supported by the results of the present study. The seven rice SSR markers used to reveal the genetic diversity of Burkina Faso ginger germplasm were found to be polymorphic at 95.32%.

This finding is similar to Jatoi *et al.* (2006) which obtained 99.5% polymorphism. In contrary, Nayak *et al.* (2005) and Jatoi *et al.* (2008) found a low value with, respectively 69.6% and 71%. According to Das *et al.* (2013), the differential rate of polymorphism at the intra specific level is generally dependent on various factors such as breeding system, habitat specialization, impact of human communities, preferential selection and the type of molecular marker used. Species with vegetative propagation mode are supposed to have a low genetic diversity. However, this is not necessarily true in practice, according to Jatoi *et al.* (2008). The rate of polymorphism (95.32%) in the present study implies that there exists a genetic variation within the gingers accessions cultivated in Burkina Faso. The knowledge of the level of polymorphism in a population is important for efficient management strategies in germplasm conservation and utilization. Moreover, amplification of polymorphic bands and specific fragments to the species show that the rice microsatellite markers are adapted for the molecular characterization of ginger cultivars. This finding highlights the interest of SSRs for the genetic diversity study in ginger. Values for Nei's genetic diversity or expected heterozygosity (He) varied from 0.123 (RM131) to 0.454 (RM135) with an average of 0.314 showed a moderate diversity within ginger accessions cultivated in Burkina Faso. Our results aligned with same observation of Lee *et al.* (2007) who recorded moderated diversity (He = 0.449) with eight SSR markers and 20 ginger accessions.

Genetic Differentiation and diversity structuration

Genetic diversity of the accessions according to their province of origin showed that ginger accessions from Kénédougou observed the best performances (Table 4). In total, 57 alleles were revealed with 1.419 effective loci found. Expected heterozygosity and the rate of polymorphism are respectively 0.290 and 93.33%. This diversity is higher than that obtained by Palai and Rout (2007) with eight ginger accessions collected in the district of Orissa in India. The genetic variability in a gene pool is normally considered as being the major resource available to breeders for ginger improvement program (Harisaranraj *et al.*, 2009). For this purpose, it is important to develop an efficient management strategy for the conservation of the cultivars of ginger in Kénédougou, which abound in high variability. Ginger being facultative vegetative propagation species, it is almost impossible to improve its agro-morphological properties by using classical methods such as inbreeding.

Ginger breeding could be based on the selection, by determination of specific genes responsible for the interesting traits. The accessions of Comoé observed the lowest values. Total number of loci, number of effective loci and rate of polymorphism were respectively 18; 1.227 and 28.33%. Genetic differentiation index (Gst = 0.126) between provinces showed that genetic variation occurred among the accessions of the three provinces. The dendrogram based on Neighbour-Joining method showed three major genetic groups (Figure 1). The gathering of the accessions in three clusters was not based to their germplasm collection origin. The first group comprised 20 accessions collected from the three provinces. The second and third groups are formed by 25 and two accessions, respectively. These groups are formed by accessions collected from Kénédougou and Léraba. The organization of genetic diversity revealed by the genotypic data was different to the results of Palai and Rout (2007) and Harisaranraj *et al.* (2009), who obtained each one only two genetic groups with eight ginger varieties by using 12 RAPD markers.

Conclusion

This study revealed genetic diversity in the ginger accessions cultivated in Burkina Faso with similar level as in ginger germplasm grown worldwide. The clustering shows three distinct groups without origin linkage. This investigation enable to assessment of the genetic variation within ginger cultivated in Burkina Faso. That provides an important breeding data for efficient management strategies in ginger conservation, utilization and for further cultivar improvement.

Acknowledgements

This work was supported by the McKnight Foundation of United Stated of America

REFERENCES

Abdullah, S., Abidin, S.A.Z., Murad, N.A., Makpol, S., Ngah, W.Z.W., Yusof, Y.A.M.2010. Ginger extract (*Zingiber officinale*) triggers apoptosis and G0/G1 cells arrest in HCT 116 and HT 29 colon cancer cell lines.*African J. Biochemistry* Research, 4(4): 134–142.

Babu, K.N., Suraby, E.J., Cissin, J., Minoo, D., Pradeepkumar, T., Parthasarathy, V.A., Peter K.V.2013. Status of transgenics in Indian spices. *Journal of Tropical Agriculture,*51 (1–2): 1–14.

Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N., Bonhomme, F. 2002. Genetix 4.04, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II, Montpellier, France. http://www.univ–montp2.fr/*genetix/genetix/genetix.htm.

Das, A., Kesari, V., Satyanarayana, V.M., Parida, A., Mitra, S., Rangan L. 2013. Genetic diversity in ecotypes of the scarce wild medicinal crop *Zingiber moran* revealed by ISSR and AFLP marker analysis and chromosome number assessment.*Plant Biosystems,* DOI: 10.1080/11263504.2013.795197.

Ghasemzadeh, A., Jaafar, H.Z.E.,Rahmat, A. 2010.Antioxidant Activities, Total Phenolics and Flavonoids Content in Two Varieties of Malaysia Young Ginger (*Zingiber officinale* Roscoe).*Molecules,*15: 4324–4333.

Harisaranraj, R., Suresh, K., Saravanababu S. 2009. DNA Finger Printing Analysis among Eight Varieties of *Zingiber*

officinale Rosc. By Using RAPD Markers.*Global Journal of Molecular Sciences*, 4(2): 103–107.

Imtiyaz, S., Rahman, K., Sultana, A., Tariq, M., Chaudhary, S.S. 2013.*Zingiber officinale Rosc*.:Atraditional herb with medicinal properties. *Review*, TANG Humanitas Traditional Medicine. 7p.

Jatoi, S.A., Kikuchi, A., Mimura, M., Yi,S.S.,Watanabe, K.N.2008. Relationship of *Zingiber* species and genetic variability assessment in ginger (*Z. officinale*) accessions from ex–situ genebank, on–farm and rural markets.*Breeding Sciences*, 58: 261–270.

Jatoi, S.A., Kikuchi, A., Yi, S.S., Naing, K.W., Yamanaka, S., Watanabe, J.A., Watanabe, K.N. 2006.Use of rice SSR markers as RAPD markers for genetic diversity analysis in *Zingiberaceae*.*Breed. Sci.*, 56: 107–111.

Jatoi, S.A., Watanabe, K.N. 2013.Diversity analysis and relationships among ginger landraces. *Pak. J. Bot*., 45(4): 1203–1214.

Jayashree, E., Kandiannan, K., Prasath, D., Pervez, R., Sasikumar, B.S., Kumar, C.M., Srinivasan, V., Suseela, B.R., Thankamani, C.K. 2014. Ginger(Extension Pamphlet). ICAR–Indian Institute of Spices Research, Kozhikode, Kerala.10p.

Kishore, G., Pandey, A., Dobhal, R., Gupta, S., 2013. Population Genetic Study of Fagopyrumtataricum from Western Himalaya Using ISSR Markers. *Biochemical Genetics*, 51: 750–765.

Lee, S.Y., Fai, W.K., Zakaria, M., Ibrahim, H., Othman, R.Y., Gwag, J.G., Rao, V.R., Park, Y.J. 2007. Characterization of polymorphic microsatellite markers, isolated from ginger (*Zingiber officinale* Rosc.). *MolecularEcology Notes*, **7**: 1009–1011.

Nandkangré, H. 2016.Caractérisation génétique et identification d'accessions de gingembre (*Zingiber officinale* Rosc.) adaptées au système de production au Burkina Faso. Thèse de Doctorat Unique, Université Ouaga I Pr Joseph KI–ZERBO, Ouagadougou, Burkina Faso, 101p.et annexes.

Nandkangré, H., Ouedraogo, M.,Sawadogo, M. 2015.Caractérisation du système de production du gingembre (*Z. officinale* Rosc.) au Burkina Faso : potentialités, contraintes et perspective. *Int. J. Biol. Chem.* Sci.,9(2): 861–873.

Nandkangré, H., Ouedraogo, M., Sawadogo, M., Bado, S.,Sawadogo, N. Ouoba, A., Konaté, N.M., Sawadogo, M. 2016.Morphometric and agronomic characterization of 56 ginger landraces in Burkina Faso. *J. Appl. Biosci*.,100: 954–9556.

Nandkangré, H., Ouedraogo, M., Nanema, K.R., Bado, S., Ouedraogo, N., Sawadogo, M. 2016. Variability, correlations, heritability and genetic advance of rhizomeyield and yield related traits in ginger (*Zingiberofficinale* Rosc.)Landraces from Burkina Faso.*J. Appl. Environ. Biol. Sci*., 6(8): 54–60.

Nayak, S., Naik, P.K., Qcharya, L., Mukherjee, A.K., Panda, P.C. Das, P. 2005. Assessment of genetic diversity among 16 promising cultivars of ginger using cytological and molecular markers.*Z. Naturforsch*, 60c: 485–492.

Palai, S.K., Rout G.R. 2007. Identification and genetic variation among eight varieties of ginger by using random amplified polymorphic DNA markers. *Plant Biotechnology,* 24: 417–420.

Parthasarathy, V.A., Srinivasan, V., Nair, R.R., John, Z.T., Kumar, A., Prasath, D. 2012. *Ginger:Botany and Horticulture*. Indian Institute of Spices Research Indian Council of Agricultural Research, Kerala, India.

Rafie, A.R., Olczyk, T.2003. Hydroponic production of fresh ginger roots (*Zingiber officinale*) as an alternative method for south Florida.*Proc. Fla. State Hort. Soc*.,116: 51–52.

Ravindran, P.N., Sasikumar, B., George, J.K., Ratnambal, M.J, Babu, K.N., Zachariah, J.T., Nair, R.R. 1994 Genetic resources of ginger (*Zingiber officinale* Rosc.) and its conservation in India, *Plant Genet Resour. Newsl.,*98:1–4.

Shariq, S., Tajuddin,Afaq S.H. 2010. Spice and Medicine: Zingiber Officinale. *Intern.J. pplied Biol. and Pharm. Techn.*, 1(3): 968–973.

Singh, G., Kapoor, I.P.S., Singh, P., Heluani, C.S., Lampasona, M.P., Catalan, C.A.N.2008.Chemistry, antioxidant and antimicrobial investigations on essential oil and oleoresins of Zingiber officinale.*Food and Chemical Toxicology,*46: 3295–3302.

Weir, B.S., Cockerham, C.C. 1984.Estimating F–statistics for the analysis of population structure.*Evolution*, 38: 1358–1370.

Yeh, H.Y., Chuang, C.H., Chen, H.C., Wan, C.J., Chen, T.L. Lin,L.Y. 2013. Bioactive components analysis of two various gingers (*Zingiber officinale* Roscoe) and antioxidant effect of ginger extracts. *LWT-Food Science and Technology*.,1–6.

****