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Molecular characterisation for resistance to leaf spot disease in some local groundnut ecotypes of Burkina-Faso

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Leaf spot disease is the major constraints causing yield losses in groundnut. Numerous breeding programmes have been set up to select disease-resistant varieties, but the selection pressure exerted by resistant varieties encourages the development of new biotypes. It is therefore necessary to use biotechnology to identify traits of interest. The general objective of this study is to identify SSR markers associated with resistance to groundnut leaf spot disease. The Rippoll DNA extraction protocol was used to extract DNA from genotypes. Then twenty microsatellite markers were used to genotype the ecotypes. After DNA extraction, the reaction mixture was prepared, amplified in a thermocycler and then migrated on an agarose gel. Development was carried out using ultraviolet (UV) light and photographed using a Canon camera. The data was analysed using DARWIN software. At the end of this study, it was possible to identify a marker associated with susceptibility and a marker associated with resistance to groundnut leaf spot disease. These markers could be used in molecular marker-assisted selection for resistance to this disease.

Key words: SSR, leaf spot, groundnut, Burkina Faso.

INTRODUCTION

Groundnut is a crop of great economic and strategic importance, because it is a food, commercial, industrial, fodder and fertilizer crop (World Bank, 2015). According to statistics from the United States Department of Agriculture (USDA, 2021), groundnut production in Burkina Faso in 2022-2023 was 0.89 t/ha. This low production is subject to phytosanitary constraints, including leaf spot (early leaf spot caused by *Cercospora arachidicola* and late leaf spot caused by *Phaeoisariopsis personata)*, which causes significant yield reductions and harvest losses. These losses can reach 30 to 70% in certain regions of the country (Taita et al., 2005). These diseases reduce the surface area of leaves available for photosynthesis, leading to reduction in photosynthetic

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Table 1. Characteristics of the genotypes used in the experimentation.

Genotype	Origin	Botanical type	Cycle (Day)	Pod	Genotypes resistance
NAMA	Burkina Faso	*Virginia*	120	Small	Resistant
TS32-1	IRHO	*Spanish*	90	average	Sensitive
CN94C	INERA	*Spanish*	90	average	Sensitive
BF1	BF/USA	*Virginia*	120	Small	Resistant
BF2	BF/USA	*Virginia*	120	Small	Resistant
Mayoro	East central/BF	*Spanish*	90	Small	Sensitive
Zampou	East central/BF	*Spanish*	120	Big	Resistant
Dalga	East central /BF	*Spanish*	120	Big	Resistant
Wobgo	North central/BF	*Spanish*	120	Big	Resistant
Balole	North central/BF	*Spanish*	90	Small	Sensitive

BF/USA: Burkina Faso/United State of America; INERA: National Institute for the Environment and Agricultural Research; IRHO: Research Institute for Oils and Oil seeds.

activity, which has a negative impact on dry matter production. Some studies have shown that the application of fungicides can reduce the severity of leaf spot and improve yields in West Africa (Kannaiyan et Haciwa, 1990; Waliyar et al., 2000). These fungicides can be very harmful to human health and the environment, so it is imperative to choose a control method that improves the quality of the environment and preserves natural resources, hence the idea of varietal selection. However, the selection pressure exerted by single-gene resistance varieties encourages the development of new biotype capable of bypassing this resistance, and the complexity of disease resistance makes it difficult to select using conventional methods. Indeed, studies have shown that the genetic determinism of resistance to leaf diseases and that of groundnut yield components is polygenic and has an additive effect (Khedikar et al., 2010). Consequently, phenotypic selection based on conventional breeding techniques will be very limited for improving these resistance traits. In view of the difficulties mentioned earlier for conventional selection for crop improvement, the advent of molecular biology techniques, such as the use of DNA markers for mapping and identifying markers associated with the various traits of interest, is of vital importance. This is the background to this study work, which consists of identifying SSR molecular markers associated with resistance to groundnut leaf spot disease.

MATERIALS AND METHODS

Plant

The plant material comprised ten groundnut genotypes, including five local ecotypes, and three control varieties: TS32-1, NAMA, and CN94C (Table 1). After germination of the seeds, young leaves at least two (2) weeks old were harvested and stored at -80°C pending use.

Extraction of genomic DNA

CTAB buffer (2% CTAB, 100 mM Tris Hcl, 20 mM EDTA, 1.4 M Nacl, pH=8) was first pre-warmed to 60°C. 25 to 30 mg of groundnut leaves collected and dehydrated in an oven at 37°C for 48 h were placed in 2 mL tubes containing two sterile metal beads. The leaf tissues were ground in a tissue lyser for 30 s, then 1.6 µL of B-mercaptoetanol was added for 1 mL of CTAB solution. 1 mL of extraction buffer (CTAB) was added to each sample, then the tubes containing the suspension were incubated at 60°C in a water bath for 1 h (with manual shaking 3 to 5 times by inverting the tubes). In addition to this, 200 µLof potassium acetate (3 M or 5 M) was added and all the samples were incubated on ice for 20 min. After this, 500 µL of the chloroform-isoamyl alcohol mixture (24:1) was added and incubated in ambient air for 3 to 5 min. The whole mixture was shaken, centrifuged at 9200 rpm (10000 g) for 10 min and the supernatant transferred to a new 2 mL tube. An addition of 500 µl to the aqueous phase of isopropanol (70°C) was also made and then kept at 20°C for 30 min. Centrifugation of the mixture at 9200 rpm (10000 g) was carried out for 10 min, followed by removal of the supernatant. The resulting pellet was dried at room temperature for 20 min.

Genotyping of DNA extracts using SSR markers

Twenty microsatellite markers were used to genotype the ecotypes in order to study their level of polymorphism. Details of the twenty SSR markers used are shown in Table 2.

PCR amplification

The microsatellite loci were amplified by PCR using the Touch-down programme, in a reaction volume of 25 µL in a thermocycler. Each PCR tube contained 2 µL of marker (1 µl for each primer); 3 µL of liquid PCR premix (the premix contains 1U of Taqpolymerase, 250 µM of dNTPs, 10 mM of Tris-HCl, 30 mM of KCl and 1.5 mM of $MgCl_2$), 17 µL of ultrapure water and finally a volume of 3 µL of liquid DNA obtained after extraction by the CTAB method. The PCR programme (Touch-down) used for all the markers consisted of the following phases: (a) Initial denaturation at 95°C; (b) Denaturation at 94°C; (c) Hybridization at 65°C; (d) Elongation at 72°C; (e) Denaturation at 94°C; (f) Hybridization at 53°C; (g) Elongation at 72°C; (h) Final elongation at 72°C; (i) Holding temperature at 4°C.

Table 2. List of SSR markers used to characterize the 10 groundnut genotypes.

Number	Markers	Repeat pattern	Amplification size	Reference
1	AH193	(AAC)5(GA)24	444	Moretzsohn et al. (2004)
2	GM1009	(CTC)5(CCG)5	411	Nagy et al. (2009)
3	GM1954	(GA)11	115	Nagy et al. (2009)
4	GM2032	(CATA)6(CATA)6	149	Nagy et al. (2009)
5	GM2079	(CAG)6	418	Nagy et al. (2009)
6	GM2301	(TGC)7	137	Nagy et al. (2009)
7	GM2637	(GA)12	131	Nagy et al. (2009)
8	GM2638	(TC)14	107	Nagy et al. (2009)
9	IPAHM108	(TC)18	212	Cuc et al., (2008)
10	IPAHM475	(GT)8(GA)12	300	Cuc et al. (2008)
11	PM179	(GA)15	110	He et al. (2003)
12	S01	(CT)27	200	Wang et al. (2007)
13	S109	(GA)21	425	Wang et al. (2007)
14	Seq8D09	(CTT)13	130	Ferguson et al. (2004)
15	TC1A02	(TC)35	255	Moretzsohn et al. (2005)
16	TC2C07	(CT)23	165	Moretzsohn et al. (2005)
17	TC2D08	(CT)25	300	Moretzsohn et al. (2005)
18	TC4F12	(CT)23	220	Moretzsohn et al. (2005)
19	TC4G02	(TC)27	151	Moretzsohn et al. (2005)
20	TC9F10	(AG)31	263	Moretzsohn et al. (2005)

Electrophoresis of PCR reaction products

The amplification products were subjected to electrophoresis on a 2% agarose gel (that is, 2 g of agarose flour per 100 mL of 0.5x TBE (Tris Borate EDTA)). This electrophoresis allowed the nucleic acids to migrate through the gel with ethydium bromide (BET) added. BET is an intercalant that slides between the bases of the nucleic acids, causing the DNA molecule to fluoresce orange under UV illumination. As the speed of migration depended on the mass of the molecule (number of bases in the DNA tested), the presence and size of the amplicons were easily checked on the gel. The migration time varied between 1 h 30 min and 2 h and was carried out in 0.5x TBE at 80 V and 50 mA. The amplification products were revealed using ultraviolet (UV) light from a transilluminator and photographed using a Canon camera. All data were entered using EXCEL 2016 and analysed using Darwin software. The estimated parameters are as follows:

(1) The polymorphism rate (P) is an estimate of the number of polymorphic loci in relation to all the loci studied.

$$P = \frac{\text{Number of polymorphic loci}}{\text{Number of loci studied}}$$

(2) Discrete, binary method describing the presence or absence of a resistance gene
(3) Presence 1, Absence 0.

RESULTS AND DISCUSSION

Polymorphism

Twenty SSR molecular markers were used. A total of 07 SSR molecular markers were amplified, all of which were monomorphic. Figures 1 and 2 show an extract of the results of the polymorphism test, in particular the electrophoretic profile of four microsatellite markers used, including GM1009 and GM2032 on one hand and TC2C07 and TC1A02 on the other hand. The markers used to amplify the DNA of the susceptible and resistant genotypes are monomorphic. In Figures 1 and 2, the markers all show bands of the same size after migration of the PCR products on an agarose gel. Figure 1 shows the presence of the susceptible control, two susceptible ecotypes and the absence of the resistant control and resistant ecotypes in the wells. This marker is therefore potentially associated with susceptibility to leaf spot. Figure 2 shows the absence of the susceptible control and the presence of the resistant control plus the resistant and a few susceptible ecotypes, but the literature has identified this marker as being associated with resistance to leaf diseases, so we can assume that it is associated with resistance to groundnut leaf spot disease. The most promising markers have been the subject of research aimed at identifying genotypes that carry the marker associated with a lower incidence of the disease. The technical development of a rapid and simple test has been successful for markers GM2032 and TC2C07, but has not been successful for markers TC4G02, TC2008, GM1009, TC2008, and S109. As shown in the figure below, a PCR test can be used to determine whether a groundnut genotype carries the Marker associated with susceptibility (S) (Figure 1) or

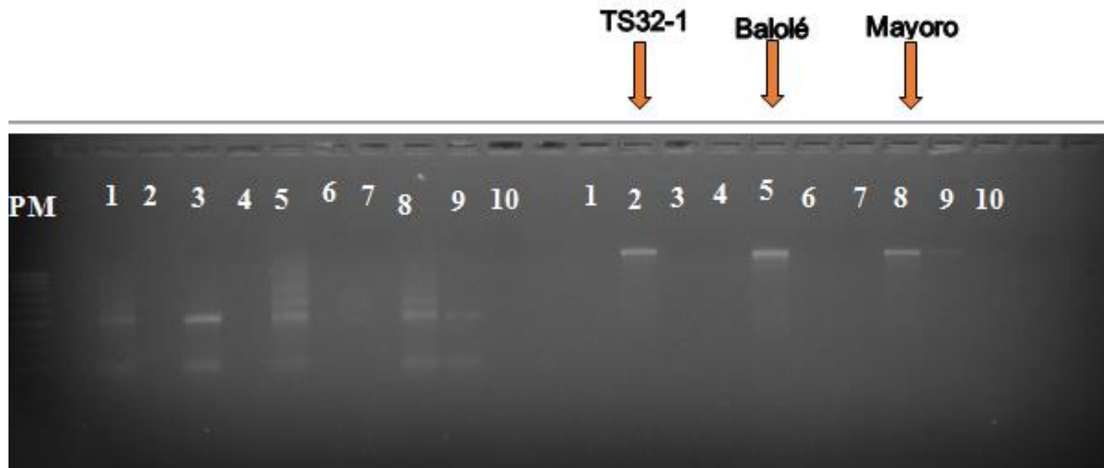

Figure 1. Photograph of agarose gel electrophoretic profile of PCR products ofDNA extracts (Absence/Presence type) from susceptible (TS32-1) and resistant (Nama) varieties with microsatellite markers 2 and 3.

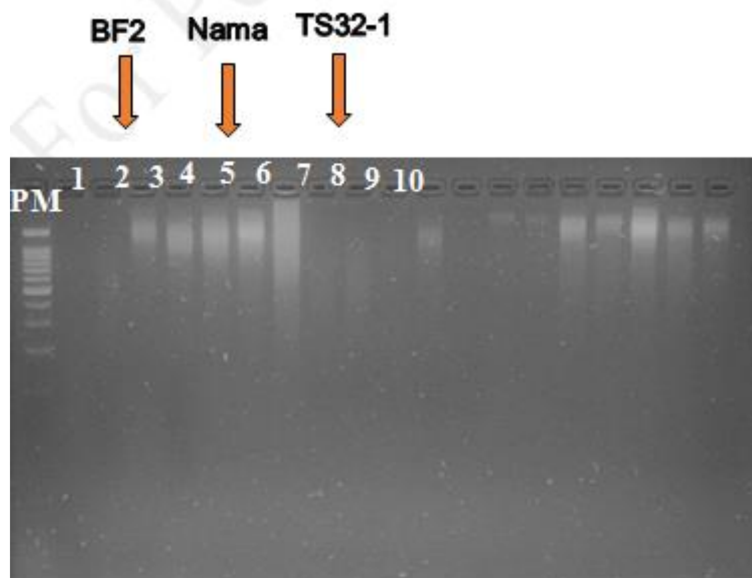

Figure 2. Photograph of agarosegel electrophoretic profile of PCR products ofDNA extracts (Absence/Presence type) from susceptible (TS32-1) and resistant (Nama) varieties with microsatellite markers 8 and 9.

resistance (R) (Figure 2).

Genetic distance between the different lines and ascending hierarchical classification (AHC)

The results of the genetic distances between individuals are shown in Table 3. The greatest genetic distances were between Wobgo and BF1, BF2 and BF1, Wobgo and Zampou, and BF2 and Zampou. The greatest genetic distances were noted between the leaf spot resistant varieties BF2, BF1, Zampou, and Wobgo. It can also be observed that the Zampou genotype is genetically distinct from all the other genotypes. Analysis of the dendrogram based on genetic distances reveals two main groups of genotypes. A first group with Balolé, Dalga, CN94C, and BF1, then the second group divided into two sub-groups, one sub-group made up of sensitive genotypes TS32-1 and Mayoro and a second sub-group made up of resistant genotypes Nama, BF2, and Wobgo (Figure 3).

Table 3. Matrix of genetic distances between genotypes.

Code	Genotype	1	2	3	4	5	6	7	8	9	10
1	Dalga										
2	TS32-1	0.82									
3	BF1	0.34	0.15								
4	Nama	0.56	0.79	0.89							
5	Balolé	0.19	0.80	0.52	0.54						
6	Wobgo	0.84	0.38	1.17	0.33	0.82					
7	BF2	0.84	0.38	1.17	0.33	0.82	0.00				
8	Mayoro	0.50	0.50	0.83	0.47	0.48	0.76	0.76			
9	CN94C	0.00	0.82	0.34	0.56	0.19	0.84	0.84	0.50		
10	Zampou	0.46	0.97	0.80	0.71	0.45	0.99	0.99	0.65	0.46	

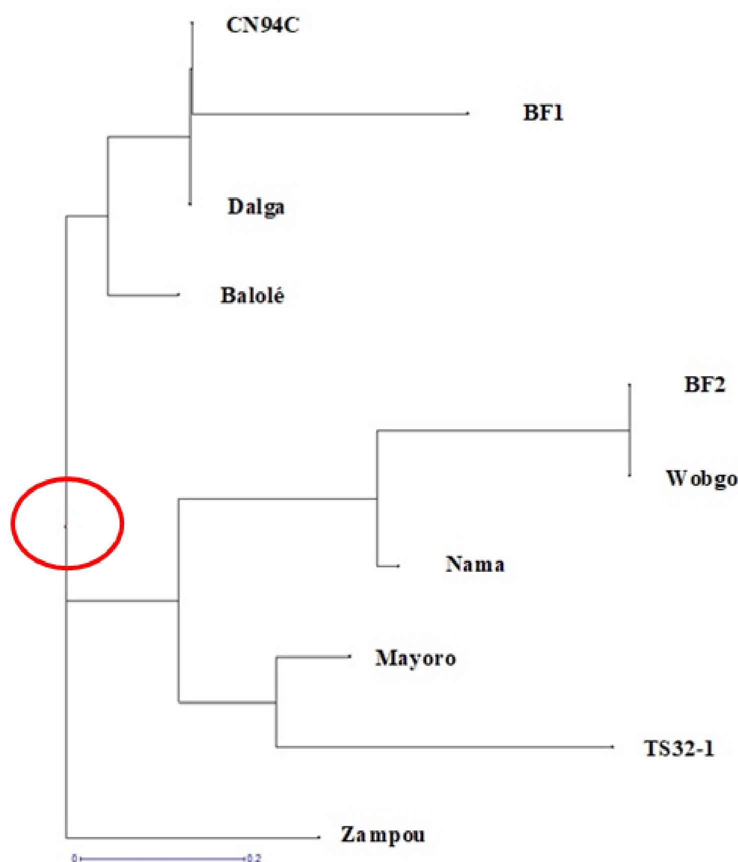

Figure 3. Dendrogram showing the distribution of the 10 genotypes using the Neighbor-Joining method.

To make better use of the ecotypes and varieties existing in a region, it is important to know their genetic diversity and agronomic potential in order to develop and disseminate those that meet growers' needs (Adoukonou-Sagbadja et al., 2014).

The microsatellites used to study molecular variability in this study proved to be monomorphic (a single allele) for most of the species studied, and the polymorphism rate was 14.28%. Of the twenty SSR markers used, seven were amplified, one of which proved to be polymorphic. However, it should be remembered that these results were obtained from one plant per cultivar of each species and when the SSR marker does not amplify; this may be a question of genotype. These

results are in agreement with those of Burow et al. (2001) who found that cultivated groundnuts show low diversity at the molecular level. This could be explained by the recent monophyletic origin of the cultivated tetraploid species and subsequent domestication. However, the results of the present study disagree with those obtained by Mace et al. (2007) who observed a polymorphism rate of 99.4% by studying the polymorphism of two parents using twenty-two SSR markers. A 76.5% polymorphism rate was also obtained by Mondal and Badigannavar (2009) between parental lines in their study for the identification of SSR markers associated with resistance to early cercosporiosis. The same applies to Roomi et al. (2014) and Cuc et al. (2008),who obtained polymorphism rates of 50 and 44%, respectively when studying genetic diversity between genotypes. It also emerged that the TC2CO7 marker is associated with resistance to cercosporiosis and that the GM2032 marker is associated with susceptibility to cercosporiosis. These markers include TC2CO7, which was identified by Sarvamangala (2009) as being associated with resistance to peanut leaf diseases. In addition, it was possible to validate the ability of this genetic marker to identify genotypes less prone to the disease. Observation of the dendrogram revealed two gene pools, one of susceptible genes and one of resistant genes. The molecular analysis of our genotypes supports the agro-morphological study carried out with these cultivars.

This is in line with the results of Zongo (2015) and de Neya (2017) who also obtained two different groups for respectively 6 lines studied using twenty SSRs and six lines characterised on the basis of fifty eight SSRs. The pool of resistant genes constitutes a reservoir that can be used in hybridisation programmes to improve resistance to cercosporioses. The pool of susceptible genes is made up of productive genotypes and could therefore be used in breeding programmes to improve productivity. Also in the dendrogram obtained, we can see that the Zampou genotype is genetically distinct from all the other genotypes. This genotype is geographically isolated from the rest of the genotypes analysed, and is the only one of the five to have been collected in Garango. This genotype would be made up of unique genetic material because of its geographical location, which could be an obstacle to any exchange of seeds between different farmers, and because of its adaptation to the environmental conditions of its region of origin. These results corroborate those of Bogyo et al. (1990) who believed that the most effective way of capturing genetic diversity is to collect the genotype in contrasting environments.

Conclusion

The molecular characterisation of the 10 genotypes of the present study has enabled us to acquire new knowledge about them, particularly at the molecular level. In this study, leaf spot resistant and susceptible genotypes were used. These genotypes were the subject of an agro-morphological characterisation, which this molecular characterisation study supports. The study confirmed the presence of two gene pools: a pool of resistant genes with the BF2, NAMA and Wobgo genotypes, and a pool of susceptible genes represented by the TS32-1 and Mayoro genotypes. Despite Mayoro susceptibility to disease, this genotype has good productivity.

These genotypes are very interesting sources of genes for improving leaf spot resistance and productivity in groundnut. The genetic distance between the different ecotypes and then the construction of the resulting dendrogram show that they are quite far apart, especially between susceptible and resistant varieties,

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Adoukonou-Sagbadja H, Missihoun AA, Sedah P, Dagba RA, Kinhoegbe G, Ahanhanzo C, Agbangla C (2014). Variabilité génétique des accessions d'igname *Dioscorea alata* L. introduites au Bénin à partir des Îles du Sud-Pacifique. Journal of Applied Biosciences 73:5966-5978.

Bogyo TP, Porceddu E, Perrino P (1990). Analysis of sampling strategies for collecting genetic material. Economic Botany 34:160-174.

Burow MD, Simpson CE, Starr JL, Paterson AH (2001). Transmission genetics of chromatin from a synthetic amphidiploid to cultivated peanut (*Arachis hypogaea* L.). broadening the gene pool of a monophyletic polyploid species. Genetics 159(2):823-837.

Cuc LM, Mace ES, Crouch JH, Quang VD, Long TD, Varshney RK (2008). Isolation and characterization of novel microsatellite markers and their application for diversity assessment in cultivated groundnut (*Arachis hypogaea*). BMC plant biology 8:1-11.

Ferguson ME, Burow MD, Schulze SR, Bramel PJ, Paterson AH, Kresovich S, Mitchell S (2004). Microsatellite identification and characterization in peanut (*A. hypogaea* L.). Theoretical and Applied Genetics 108:1064-1070.

He C, Poysa V, Yu K (2003). Development and characterization of simple sequence repeat (SSR) markers and their use in determining relationships among Lycopersicon esculentum cultivars. Theoretical and Applied Genetics 106:363-373.

Khedikar YP, Gowda MVC, Sarvamangala C, Patgar KV, Upadhyaya HD, Varshney RK (2010). A QTL study on late leaf spot and rust revealed one major QTL for molecular breeding for rust resistance in groundnut (*Arachis hypogaea* L.). Theoretical and Applied Genetics 121:971-984.

Mace ES, Varshney RK, Mahalakshmi V, Seetha K, Gafoor A, Leeladevi Y, Crouch JH (2007). *In silico* development of simple sequence repeat markers within the aeshynomenoid / dalbergoid and genistoid clades of the Leguminosae family and their transferability to

Arachis hypogaea, groundnut. Plant Science 174:51-60.
Mondal S, Badigannavar AM (2009). Molecular diversity and association of SSR markers to rust and late leaf spot resistance in cultivated groundnut (*Arachis hypogaea* L.). Plant Breeding 129(1):68-71.
Moretzsohn MDC, Hopkins MS, Mitchell SE, Kresovich S, Valls JFM, Ferreira ME (2004). Genetic diversity of peanut (*Arachis hypogaea* L.) and its wild relatives based on the analysis of hypervariable regions of the genome. BMC Plant Biology, 4:1-10.
Moretzsohn MC, Leoi L, Proite K, Guimaraes PM, Leal-Bertioli SCM, Gimenes MA, Martins WS, Valls JFM, Grattapaglia D, Bertioli DJ (2005). A microsatellite-based, gene-rich linkage map for the AA genome of Arachis (Fabaceae). Theoretical and Applied Genetics 111:1060-1071.
Nagy E, Timár I, Hegyi Z, Spitkó T, Marton LC (2009). SSR markers as tools in maize breeding for high starch content. Maydica 54(2):253.
Neya BF (2017). Héritabilité de la résistance aux cercosporioses de l'arachide, (*Arachis hypogaea* L.) et de quelques caractères associés au rendement. Thèse de Doctorat, UFR/SVT, Université de Ouagadougou, BP 7021, 202 p.
Roomi S, Sabiha B, Iqbal A, Suleman M, Muhammad I, Zia MA, Ahmad MZ, Rashid F, Ghafoor A, Tabbasam N (2014). SSR based genetic diversity analysis in a diverse germplasm of groundnut (*Arachis hypogaea* L.) from Pakistan. Australian Journal of Crop Science 8(1):55-61.
Sarvamangala SC (2009). Construction of genetic linkage map and qtl analysis for foliar disease resistance, nutritional quality and productivity traits in groundnut (*Arachis hypogaea* L.) Ph D University of Agricultural Sciences, DHARWAD – 580 005, 144 p.
Taita P, Sankara P, Guinko S (2005). Peanut leaf spot: Evaluation of the incidence of two leaf spots on peanuts. Technical sheet n° 5, INERA/CRREA-Ouest (Farako-Bâ station), B.P. 910, Bobo-Dioulasso.
USDA (2021). World agriculture production. Room 326-W, Whitten Building, 1400 Independence Avenue, SW, Washington, D.C. 20250-9410 or call (202):40 p. **https://www.usda.gov/oce/commodity/wasde/wasde0724.pdf**
Wang H, Chen P, Liu D (2007). Assessment of genetic diversity of Yunnan, Tibetan, and Xinjiang wheat using SSR markers. Journal of Genetics and Genomics 34(7):623-633.
World Bank (2015). Etude Diagnostique de la Chaîne de valeurs arachide au Sénégal. 1818 H Street NW, Washington, DC 20433, USA, fax 202-522-2422 http://www.copyright.com/
Zongo A (2015). Analyse génétique et identification de marqueurs moléculaires SSR associés à la résistance à la cercosporiose précoce de l"arachide (*Arachis hypogaea* L.). These de doctorat, Université de Ouagadougou BP. 7021, 148 p.