Available online at http://www.ifgdg.org

Int. J. Biol. Chem. Sci. 12(2): 668-675, April 2018

ISSN 1997-342X (Online), ISSN 1991-8631 (Print)

International Journal of Biological and Chemical Sciences

Original Paper http://ajol.info/index.php/ijbcs http://indexmedicus.afro.who.int

Resistance evaluation of five varieties of cowpea and their F_1 descendants from a diallel crossing to cowpea aphid-borne mosaic virus in Burkina Faso

Antoine BARRO[1*], Téyouré Benoit Joseph BATIENO[2], Nerbéwendé SAWADOGO[3], Jean Baptiste De La Salle TIGNEGRE [4], Kiswendsida Romaric NANEMA[3], Renan Ernest TRAORE [3] and Mahamadou SAWADOGO[3]

[1] *Université de Dédougou, BP 07, Dédougou; Burkina Faso.*
[2] *Institut de l'Environnement et de Recherches Agricoles, BP 476, Ouagadougou, Burkina Faso.*
[3] *Université Ouaga I Pr Joseph KI-ZERBO, 03 BP 7021, Ouagadougou 03, Burkina Faso.*
[4] *World Vegetable Center (AVRDC), BP 320, Bamako, Mali.*
[*] *Corresponding author; E-mail: antoine.barro@yahoo.fr*

ABSTRACT

Cowpea is a dual-purpose protein crop which has a high nutritional value. However, many problems such as cowpea aphid-borne mosaic virus limit its production. This study aimed at assessing the resistance of varieties of cowpea to cowpea aphid-borne mosaic virus (CABMV). Thus, a complete diallel cross between five cowpea varieties was done in a partially balanced incomplete block plan (alpha design) with three replications. The F_1 descendants obtained and their parents were evaluated using five characters. Results obtained reveal that all characters discriminate the parents and F_1 descendants. All five parents tested revealed their resistance or susceptibility status to CABMV. The F_1 descendants from the cross between local Gorom x KVx640, KVx30-309-6G x KVx396-4-5-2D, KVx61-1 x KVx640, KVx640 x local Gorom and KVx640 x KVx61-1 which have shown a low severity value and a high weight of one hundred seeds are the best. They could be used in Burkina Faso' cowpea breeding program to develop resistant varieties to cowpea aphid-borne mosaic virus.

Keywords: Cowpea, severity, resistance, Virus, Burkina Faso.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is one of the main legumes cultivated in Burkina Faso. It would hail from West Africa, which is considered to be the primary center of diversity and domestication (Ng et al., 1985). It adapts well to hot-humid climate and semi-arid climate (Pandey, 1987; Tignegré, 2010).

As cowpea is a dual-purpose crop used for human and animal's food. Its production contributes to fight against poverty. In Burkina Faso, cowpea is cultivated mainly for its seeds which are consumed in several forms. It is either prepared (boiled) directly or transformed into donuts, couscous, stew, etc. (Burkina Faso' Ministry of Agriculture, 2002). The mature cowpea seed contains 20.5 to 31.7% protein (Onwuliri and Obu, 2002) and is also rich in essential microelements such as iron, calcium and zinc. Its leaves contain 13 to 17% of total nitrogenous matter

DOI: https://dx.doi.org/10.4314/ijbcs.v12i2.5

(Tarawali et al., 1997) and constitute a quality feed for livestock (Akundabweni et al., 1991; Batieno, 2014). Its leaves and green pods can also be consumed and marketed. Thus, cowpea is now both a food crop and a cash crop.

Despite this importance of cowpea, some diseases hinder her production. One of the most dangerous diseases that significantly reduce its size and yield is Cowpea Aphid Borne Mosaic Virus (CABMV). This filamentous virus belongs to the genus Potyvirus and the family Potyvirideae which has been divided into six (6) genera (Fauquet et al., 2005).

Nowadays, several research works are carried out in order to create varieties resistant to this virus (Neya, 2011; Barro et al., 2017) and meeting the expectations of producers and consumers. This study which is part of this framework aimed at evaluating the resistance level and performance of five cowpea parents and their F_1 descendants against CABMV.

MATERIALS AND METHODS

Genetic resources

Genetic resources used in this study are constituted of five release cowpea varieties from Burkina Faso and F_1 hybrids from 5x5 full diallel crosses. F_1 populations were obtained from crosses after manual emasculation to ensure purity of the descendants. Lines used in these crosses were chosen based on their reaction against CABMV. The characteristics of five lines involved in the crosses are presented in Table 1. They all come from the long-term storage germplasm of the cowpea breeding program at Kamboinsé, research station in Burkina Faso.

Experimental site

The tests were conducted in the Environmental, Agricultural and Training Research Center (CREAF) of INERA / Kamboinsé, located at 12 km from Ouagadougou on the Ouagadougou-Kongoussi axis. It is located in the northern Sudanian sector (Guinko, 1984) at 12 ° 28 N latitude, 1 ° 32 W longitude and 296 m altitude. The soils are sandy, ferruginous, tropical, reworked and are very sensitive to erosion. Their texture is predominantly sandy-clayey on the surface and deeply clayey (Tignegré, 2000). The annual rainfall at Kamboinsé during the season is 904.9 mm in 66 days of rainfall (Figure 1).

Experimental design

Twenty (20) F_1 hybrids and their parents were planted in pots and arranged in randomized blocks design with three replications. Each replication is composed of 25 entries with 5 blocks and 5 entries per block making a total of 75 plots. Each pot contained one plant and the plants were sprayed to avoid contamination from aphids.

Cultural operation

Each plant received 45 kg of P_2O_5 per hectare from NPK fertilizer (14-23-14-6S-1B formula). Insecticide spray was done using a mixture of two weeks after planting at a dose of 2 ml per liter of water. One week after planting all the plants were inoculated using extract of leaves from CABMV serotype D grinded based on weight/volume proportion (p/v) =1/10 (Neya, 2011).

Mechanic inoculation

The inoculum from infected seedlings of a CABMV susceptible cowpea variety, Gorom local was homogenized in sodium phosphate buffer 0.01M, pH 7.4 under a crushing ratio p / v 1/10. The extract was filtered through gauze and placed in melting ice. Before inoculation, the leaves of young plants cowpea older than a week of three replications were dusted with carborundum 600 mesh, an abrasive product. Using a cotton swab dipped pestle or in the extract, the upper leaf surface was rubbed gently. The symptoms of CABMV were recorded between the 6 and 21^{th} day after inoculation. The inoculated plants have evolved in the greenhouse, protected from insects including aphids.

Parameters measured

Five parameters related to cycle, yield and scope of the virus attack were determined. These are:

- the number of days between sowing and the first flower (DAPF) for each variety;

- the number of days between sowing and the first pod (DAPG);
- the weight of 100 grains (P-100gr);
- the severity assessment using rating scale 6 classes (0-5) which is a strength criterion in CABMV (Barro et al., 2016);
- the area under disease curve progress (AUDPC) proposed by Shaner and Finnay (1977) using the following equation AUDPC = $\sum_{i=1}^{n}$[(Xi+1 + Xi) / 2][ti+1 – ti], where n is total number of cases; Xi: the first observation of disease by day; Xi + 1: the second observation of disease by day; ti: time in days from the first observation of disease and ti + 1: time in days for the second observation of the disease. It is a study of a disease development rate of a given culture. This parameter selects the best lines in terms of their ability to slow the progression of the disease.

Statistical analysis

The collected plant data was entered into an Excel 2007 spreadsheet and verified using PivotTables. This reduces the input errors. GenStat 15.1 was used to process collected or calculated data and for analysis of variance (ANOVA) in order to assess the level of variability of plant material. The area under disease curve progress (AUDPC) was calculated according Shaner and Finnay (1977) method and was analysed using the Genstat computer package.

Table 1: List of genotypes and their characteristics.

Varieties	Status	Weight of 100 seeds (g)	Origin
Gorom local	Susceptible	15,45	Burkina Faso
KVx30-309-6G	Susceptible	17,21	Burkina Faso
KVx61-1	Moderately susceptible	10,4	Burkina Faso
KVx640	Resistant	15,97	Burkina Faso
KVx396-4-5-2D	Resistant	15,62	Burkina Faso

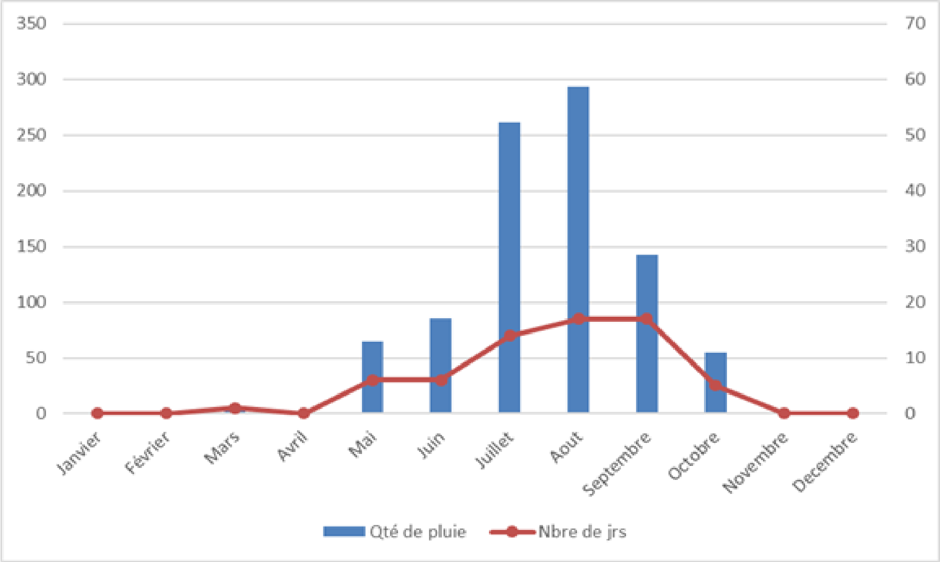

Figure 1: Rainfall during the rainy season 2015 in Kamboinsé.

RESULTS

Agronomic and resistance parameters to Cowpea Aphid-Borne Mosaic Virus of the five parents

Data of Table 2 reveal that the five characters discriminate the five parents. KVx30-309-6G has the lowest value of the cycle while KVx640 has the longest cycle. Resistant parent KVx396-4-5-2D and susceptible parent Gorom local have respectively the lowest and the highest value of AUDPC and severity**.** High values of one 100 seed weight were obtained with cowpea varieties KVx30-309-6G (17.21 g) and KVx640 (15.97 g). The severity ranged from 0 for parent KVx396-4-5-2D to 4 for the local cowpea variety Gorom, evidence susceptible to cowpea aphid-borne mosaic virus with a mean severity of 2.26.

Agronomic and resistance parameters to Cowpea Aphid-Borne Mosaic Virus of the F_1 descendants

The performances of F_1 descendants presented in table 3 show that three traits discriminate them. The F_1 descendants such as Gorom local x KVx640), KVx61-1 x KVx640 (17.91 g), KVx640 x Gorom local (17.93 g), KVx640 x KVx61-1 (17.93 g), KVx30-309-6G x KVx396-4-5-2D have the highest 100 seeds weight. Their 100 seeds weight is greater than their parents. They have also relatively low value of AUDPC than their parents. All F_1 descendants from the cross between resistant parent and susceptible parent or between resistant parents have a very low value of severity. Only 3% of F_1 descendants show a severity close to that the moderately susceptible parent KVx61-1 (3.33). However, the highest values of AUDPC were obtained with the F_1 descendants resulting from the cross between the two sensitive relatives such as Gorom local x KVx30-309-6G (31.67), KVx30-309-6G x Local Gorom (31.67).

Comparison of all population

Except characters related to the cycle which significantly discriminate parents and F_1 descendants, the others characters measured are very significantly (Table 4). Except severity and AUDPC (area under disease curve progress), which showed high coefficient of variation (> 20%), the other three parameters have relatively low values (<20%).

The F1 descendants and the most resistant parents were KVx640 x KVx396-4-5-2D (0), local Gorom x KVx396-4-5-2D (1), KVx396-4-5-2D x KVx30-309- 6G (1), KVx396-4-5-2D (0), KVx640 (1) and the most sensitive were local Gorom x KVx30-309-6-G (4), local Gorom (4).

Table 2: Performance of the five parents for the five measured traits.

Varieties	DAPF	AUDPC	DAPG	P-100 gr	Severity
Gorom local	44.33	38.33	45.33	15.45	4.00
KVx30-309-6G	43.00	26.67	44.00	17.21	3.000
KVx396-4-5-2D	46.00	8.33	47.00	15.62	0.000
KVx61-1	46.67	25.00	47.67	9.50	3.333
KVx640	48.67	15.00	49.67	15.97	1.000
Mean	46	22.67	47	14.75	2.26
Min	40	5	41	9.09	0
Max	50	40	51	17.44	4
Pr. (5%)	0.034*	0.001**	0.034*	0.001**	0.001**
CV (%)	3.9	18.9	3.8	6.1	11.4

(*): significantly at 5%; (**): significantly at 1% ; DAPF : Number of day between sowing first flower, DAPG : Number of day between sowing and first pod, AUDPC : area under disease curve progress; P-100gr : weight of 100 grains, CV : coefficient of variation, Pr. : probability, Min: minimum, Max : maximum.

Table 3: F_1 descendants performance for five measured characters.

Varieties	DAPF	AUDPC	DAPG	P-100gr	Severity
Gorom local x KVx30-309-6G	45.00	31.67	46.00	18.08	3.667
Gorom local x KVx396-4-5-2D	44.33	16.67	45.33	17.48	1.000
Gorom local x KVx61-1	45.33	25.00	46.33	14.94	3.000
Gorom local x KVx640	44.00	18.33	45.00	17.57	1.000
KVx30-309-6G x Gorom local	45.00	31.67	46.00	22.64	3.333
KVx30-309-6G x KVx396-4-5-2D	47.33	20.00	48.33	19.05	1.333
KVx30-309-6G x KVx61-1	46.33	21.67	47.33	13.28	1.667
KVx30-309-6G x KVx640	47.67	18.33	48.67	16.10	1.333
KVx396-4-5-2D x Gorom local	45.33	20.00	46.33	15.10	2.000
KVx396-4-5-2D x KVx30-309-6G	47.00	15.00	48.00	17.78	1.000
KVx396-4-5-2D x KVx61-1	47.00	23.33	48.00	13.89	2.000
KVx396-4-5-2D x KVx640	42.67	11.67	43.67	14.11	0.667
KVx61-1 x Gorom local	44.33	25.00	45.33	16.37	2.667
KVx61-1 x KVx30-309-6G	44.67	21.67	45.67	12.39	2.000
KVx61-1 x KVx396-4-5-2D	43.67	20.00	44.67	15.85	2.333
KVx61-1 x KVx640	44.33	20.00	45.33	17.91	1.000
KVx640 x Gorom local	47.33	15.00	48.33	17.93	0.667
KVx640 x KVx30-309-6G	43.67	16.67	44.67	17.99	1.333
KVx640 x KVx396-4-5-2D	45.33	15.00	46.33	14.55	0.000
KVx640 x KVx61-1	44.00	18.33	45.00	17.93	1.333
Mean	45	20,25	47	16,55	1,67
Min	40	10	41	7,5	0
Max	50	35	51	25	4
Pr (5%)	0.078^{ns}	0,001**	0.078^{ns}	0,048*	0,001**
CV (%)	4.2	22,3	4.1	18,1	48,6

(*): significantly at 5%; (**): significantly at 1% ; DAPF : Number of day between sowing first flower, DAPG : Number of day between sowing and first pod, AUDPC: area under disease curve progress ; P-100gr : weight of 100 grains, CV : coefficient of variation, Pr. : probability, Min: minimum, Max : maximum.

Table 4: Results of analysis of variance of parents and F_1 descendants for the measured characters.

Parameters	DAPF	AUDPC	DAPG	P-100gr	Severity
Mean	45	20,73	46	16,19	1,787
Min	40	5	41	7,5	0
Max	50	40	51	25	4
Pr. (5%)	0,023*	0,001**	0,023*	0,002**	0,001**
CV (%)	4,3	21,58	4,21	16,74	40,85

(*): significantly at 5%; (**): significantly at 1% ; DAPF : Number of days between sowing and first flower, DAPG : Number of day between sowing and first pod, AUDPC : area under disease curve progress ; P-100gr : weight of 100 grains, Min: minimum, Max : maximum.

DISCUSSION

The existence of significant differences between varieties and descendants for all measured traits indicates the existence of diversity. In addition, the cycle sowing-flowering of all individuals is less than 65 days. Individuals tested would be short cycle because according to Pandey (1987), short cycle varieties have a number of days between sowing-flowering of 60 to 65 days. These varieties are increasingly requested because of climate change and irregular rainfall. Moreover, the viral attack would delay the appearance of flowers and pods. Indeed, the mosaic virus transmitted by aphids causes the fall of flowers, pods and stunting of plants (Tignegré, 2000; Neya, 2002; Barro et al., 2016). According to Barro et al. (2016), the number of days between the date of appearance of the flowers and that of the pod remains constant. The F_1 Descendants which have a faster apparition of flowers would be more resistant

The high weight of the seeds of the majority of the F_1 descendants would show a very important heterosis effect. Indeed, heterosis is very important when cross parents are genetically distant. However, the resistance level of cowpea is independent of the weight of one 100 seeds (Barro et al., 2016). This is confirmed by the sensitivity to CABMV of local Gorom and KVx30-309-6G despite their high 100-seed weight. The low production found in some parents and F_1 descendants may be attributed in part to a decline in chlorophyll production. Singh et al. (1985) showed also that infected plants use less space and nutrients than healthy plants. F_1 descendants with low symptom severity could be considered resistant to CABMV and used in breeding programs. According to Tignégré (2010), the large seeded lines have a good yield and meet the market criteria.

The highly significant difference between individuals for symptom severity would reflect different levels of CABMV resistance among parents and their F_1 descendants. The resistance of F_1 descendants from the cross between resistant parents and between resistant and susceptible parents is explained by the intervention of a gene with a dominant effect. Previous studies (Barro et al., 2016) reported too that cowpea resistance is controlled by two dominant non-allelic genes. The severity of all resistant varieties and descendants tested were in classes 0, 1, 2, which confirms that their status of resistance to virus (Neya, 2011). F_1 descendants with a severity similar to KVx61-1 are also intermediate resistance varieties to CABMV.

Varieties with the lowest values of AUDPC and severity are resistant varieties to CABMV. According to Neya (2002), the AUDPC is a component of epidemiology that takes into account the progression of the disease over time. It implies a notion of installation, growth and the final incidence of the disease. The AUDPC assessment is used to select the best varieties for their ability to slow the progression of the disease (Orawu, 2007). Ndiaye et al. (2010) also used this parameter to evaluate the effects of compost on the development of *Macrophomina phaseolina* coal rot in cowpeas and showed that coal is more severe in the dry season than in the rainy season.

Conclusion

This study revealed resistance to CABMV of all F_1 descendants from the cross of two resistant parents or a resistant and susceptible parents. It also showed that F_1 descendants have a high seed weight and a relatively low date of appearance of the first flower. The best F_1 descendants are local Gorom x KVx640, KVx30-309-6G x KVx396-4-5-2D, KVx61-1 x KVx640, KVx640 x local Gorom, KVx640 x KVx61-1 due to their good agronomic performance and their resistance to CABMV.

The diallel analysis of the agronomic and resistance parameters in the parents and their F_1 descendants would allow a better exploitation of the variability of the crossing on the basis of the genetic determinism of these parameters and their mode of transmission.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. AB wrote the protocol, wrote the first draft of the manuscript and performed the statistical analysis. TBJB and NS reviewed the experimental design and all drafts of the manuscript. JBDLST and MS managed the analyses of the study and designed the study. AB, KRN and RET read and approved the final manuscript.

ACKNOWLEDGMENTS

This research has been not suported. Thanks to the heads of "Laboratoire de Génétique et Biotechnologies Végétales" of CREAF for the technical support and the members of the "Laboratoire Biosciences" of the "Université Ouaga I Pr Joseph KI-ZERBO" for the corrections of manuscript.

REFERENCES

Akundabweni LS, Paul CP, Singh BB. 1991**.** Evaluation de lignées d'élite de niébé pour un usage double (feuilles/fourrage et graines). In : la recherche à Institut International d'Agriculture Tropicale IITA N°3, pp. 6-7.

Barro A, Batieno BJ, Tignegré JBS, Diéni Z, Sidibé H, Sawadogo M. 2017. Diallel analysis of cowpea populations for resistance to cowpea aphid-borne mosaic virus disease (CABMV) in Burkina Faso. *Journal of Plant Breeding and Crop Science,* **9**(7): 90-97. DOI: 10.5897/JPBCS2016.0628

Barro A, Sawadogo M, Kiébré Z, Neya BJ. 2016. Evaluation de la résistance de quelques lignées et écotypes de niébé (Vigna unguiculata (L.) Walp.) Au Cowpea Aphid-Borne Mosaic Virus au Burkina Faso. *International Journal of Innovation and Applied Studies,* **15**(2): 387-394. http://www.ijias.issr-journals.org/

Barro A, Tignegré JBS, Diéni Z, Kiébré Z, Poda L, Sawadogo M. 2016. Inheritance and the Allelic Relationship of Resistance to Cowpea Aphid Borne Mosaic Virus (CABMV) in Two Cowpea Genotypes, KVx640 and KVx396-4-5-2D, in Burkina Faso. *International Journal of Current Microbiology Applied Sciences,* **5**(8): 285-292. Doi: http://dx.doi.org/10.20546/ijcmas.2016.508.030

Batieno TBJ (2014). Breeding for Drought Tolerance in Cowpea [*Vignaunguiculata* (L.) Walp.] using Marker Assisted Backcrossing. Doctor of philosophy, University of Ghana, Legon, West Africa Centre for Crop Improvement, p. 153.

Guinko S. 1984. Végétation de Haute -Volta. Thèse de Doctorat ès sciences naturelles. Université de Bordeaux III.T1, p. 318.

Diaye M, Termorshuizen AJ, Van Bruggen AHC. 2010. Effects of compost amendment and the biocontrol agent Clonostachys rosea on the development of charcoal rot (Macrophomina phaseolina) on cowpea. *Journal of Plant Pathology,* **92**: 173-180.

Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA. 2005. *Virus Taxonomy. Eighth Report of the International Committee on Taxonomy of Viruses.* Elsevier Academic Press: San Diego; 1259.

Ministère de l'agriculture SP/CPSA. 2002. Marchés du niébé au Burkina et dans des pays du golfe de guinée: diagnostic et priorisation des interventions, rapport final. Burkina Faso, p.106.

Neya J. 2002. Variabilité sérologique et aspects épidémiologiques du virus de la mosaïque du niébé (L.)WALP.) Transmis par pucerons au Burkina Faso.

Mémoire de DEA., Université de Ouagadougou, p. 54.

Neya J. 2011. Sérologie, pathologie, épidémiologie et contrôle de la mosaïque Cowpea Aphid Borne Mosaic Virus (CABMV) du niébé (Vigna unguiculata (L.) Walp.) Transmise par des pucerons (Aphid crassivora, A. gossypii) au Burkina Faso. Thèse de Doctorat, Université de Ouagadougou, p. 153.

Ng NQ, Marechal R. 1985. Cowpea taxonomy, origin and germplasm. In *Cowpea Research, Production and Utilization*, Singh SR, Rachie KO (eds). John Wiley and Sons: Londres; 11-21.

Onwuliri VA, Obu JA. 2002. Lipids and other constituents of Vigna unguiculata and Phaseolus vulgaris grown in northern Nigeria. *Food Chemistry*, **78** (1): 1-7.

Orawu M. 2007**.** Occurrence of cowpea aphid-borne mosaic virus and prospects of improving resistance in local cowpea landraces in uganda. Thesis, University of Kwazulu-Natal, p.137.

Pandey RK. 1987. *A Famer's Primer on Going Cowpea on Riceland*. IITA; 218p.

Shaner G, Finney RE. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. *Phytopathology*, **67**: 1051-1056.

Singh SR, Jackai LEN. 1985. Insect pests of cowpea in Africa: Their life cycle, economic importance and potential of control. In *Cowpea Research, Production and Utilization*, Singh SR, Rachie KO (eds). John Wiley and Sons Ltd.; 218-231.

Tarawali SA, Singh BB, Peters M, Blade SF. 1997. Cowpea haulms as fodder. In *Advances in Cowpea Research*, Singh BB, Mohan Raj DR, Dashiell KE, Jackai LEN (eds). Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). IITA Ibadan, Nigeria; 313-325.

Tignegré JB. 2000. Etude de la transmission du cowpea Aphid-Borne Mosaic Virus CABMV chez le niébé Vigna unguiculata (L.) WALP. Mémoire de DEA. Université de Ouagadougou, p. 57.

Tignegré JB. 2010. Genetic study of cowpea (Vigna unguiculata L. Walp) resistance to Striga gesnerioides (willd.) Vatke in Burkina Faso. Doctor of phylosophy, University of Kwazulu-Natal, p. 176.