ARTICLES ORIGINAUX | OORSPRONKELIJKE ARTIKELS
ORIGINAL ARTICLES | ARTICULOS ORIGINALES

Floral Biology and Hybridization Potential of Nine Accessions of Physic Nut (*Jatropha curcas* L.) Originating from Three Continents

L.E. Ahoton[1*] & F. Quenum[1]

Keywords: Intraspecific Hybrids- *Jatropha curcas*- Improvement- Breeding System- Benin

Summary

Jatropha curcas *is a shrub which has an important economic and medicinal role in tropical and subtropical zones of the world. The oil of its kernels can serve as fuel feedstock to produce diesel, indicating its potential as a renewable source of energy. In an attempt to introduce new variation into cultivated* Jatropha curcas*, a program of intraspecific hybridization with several ecotypes originating from Africa, Asia and America was undertaken. Field studies were performed over three years 2009, 2010 and 2011. Before achieving hybridization, the floral ratio and the breeding system of physic nut were studied in Southern Benin ecological conditions. Significant differences (P< 0.05) were observed between the analysed ecotypes regarding the number of female flowers. This study has also confirmed that the breeding system of* Jatropha curcas *is essentially outcrossing and that foraging insects are the main pollination actors of female flowers. New intraspecific hybrid combinations were produced involving nine accessions. Crosses results varied according to the origin and the direction of the hybridization. Crosses between accessions of* J. curcas *originating from Africa and Asia gave hybrids without difficulty. The data obtained confirm that apomixis might play a major role in* J. curcas *a reproductive biology. Considering the high number of female flowers per inflorescence produced by the accession from Ecuador, and the large genetic distance existing between this accession and those from Africa and Asia, it should constitute a valuable genetic stock for the development of F_1 hybrids with local ecotypes of* J. curcas*. However, the use of growth regulators might be necessary to improve the hybridization success rate when it is used as female parent.*

Résumé

Biologie florale et potentiel d'hybridation entre neufs accessions de pourghère (*Jatropha curcas* L.) provenant de trois continents

Le pourghère est un arbuste à potentiel économique et médicinal très important dans les zones tropicales et subtropicales du monde. L'huile provenant de l'amande de la graine peut être transformée en biocarburant ce qui indique que la plante est une source d'énergie renouvelable. Dans le but de créer des hybrides intraspécifiques, un programme de croisements entre plusieurs écotypes venant d'Afrique, d'Asie et d'Amérique a été entrepris. Les travaux ont été conduits sur une période de trois ans de 2009 à 2011. Avant la réalisation des croisements intraspécifiques, le ratio entre fleurs mâles et femelles a été déterminé et le système de reproduction de la plante a été étudié dans les conditions écologiques du Sud Bénin. Une différence significative (p< 0,05) est notée entre le nombre des fleurs femelles des écotypes étudiés. Cette étude a aussi confirmé que Jatropha curcas *est une plante allogame et que les insectes sont les principaux acteurs de la pollinisation des fleurs femelles. De nouveaux hybrides ont été créés à partir des croisements impliquant les écotypes étudiés. Les résultats des hybridations ont varié en fonction du sens des croisements et des provenances. Les croisements entre accessions de pourghère venant d'Afrique et d'Asie ont donné des hybrides sans difficulté. Les résultats obtenus confirment aussi que l'apomixie pourrait jouer un rôle important dans le système de reproduction de* J. curcas*. Compte tenu du nombre élevé de fleurs femelles produites par inflorescence par l'accession équatorienne, et de la distance génétique importante qui existe entre cette accession et celles d'Afrique et d'Asie, elle devrait constituer un matériel génétique de haute valeur pour développer des hybrides F_1 avec les écotypes locaux de* J. curcas*. Cependant, l'utilisation de régulateurs de croissance pourrait être nécessaire pour améliorer le taux de réussite des hybridations quand il est utilisé comme parent femelle.*

[1]Faculty of Agronomic Sciences, University of Abomey-Calavi, Benin. Phone: 00229/90/91/45/42.
[*]Corresponding author, E-mail: essehahoton@yahoo.fr, BP 01 526, Cotonou 01, Bénin.

Received on 21.02.12 and accepted for publication on 21.08.12.

Introduction

Physic nut (*Jatropha curcas* L.) is a perennial multipurpose small tree or shrub originating from Mexico and Central America (12). Nowadays, the plant has a pantropical distribution (2). *J. curcas* is easily propagated by generative (seeding) and vegetative (cuttings) methods (14). This shrub is cultivated as a medicinal and oil plant in many tropical and subtropical countries. Various extracts from *J. curcas* seeds and leaves showed molluscicidal, insecticidal and fungicidal properties (11, 16). *J. curcas* belongs to tribe *Joannesieae* of *Crotonoidea, Euphorbiaceae* family which contains approximately 200 known species (6). *J. curcas* is a monoecious tree. Inflorescences are formed terminally on branches and are complex, possessing main and co-inflorescences with paracladia (5). Pollination is mainly secured by insects. Physic nut is adapted to a variety of habitats (2). Future improvements in environmental stress-, resistance, agronomic fitness, and quality of oil of physic nut depend on the diversity of the genetic stocks from which new traits can be selected. Species and provenance trials contribute fundamental information for further breeding and genetic improvement (4). Some ecotypes recently introduced in Benin present desirable attributes (3) which can be used for improving local plant materials. The development of improved cultivars through hybridization can contribute significantly to increase productivity and quality in cultivated plants. The key for success of any genetic improvement program lies in the availability of genetic variability for desired traits (6). Hybridization of selected parental lines allows the creation of new forms through genetic recombinations. The resulting hybrids serve as sources of genetic variations on which selection can be imposed. In order to assess the possibility to increase the genetic variability of physic nut and produce genetic stocks adapted to the local conditions, the floral biology and the crossing potential of 9 accessions originating from Africa (5 ecotypes, with one from Benin), Asia (3 ecotypes) and America (1 ecotype) were studied. The results of this work are presented and discussed here.

Materials and methods

Study site and plant material: The experiments were conducted at the Faculty of Agricultural Sciences of Abomey-Calavi University, Benin, (Altitude 17.4 m, 06° 24'N, 02° 20'E) in 2010. Observations on the floral biology and crosses were carried out on two years old plants belonging to nine ecotypes. Five plants of each ecotype were used in the program. The ecotypes used come from: Benin (Abomey-Calavi), Cambodia, India (Hyderabad 1 and 2), Equator (Salinas), Madagascar, Senegal, Democratic Republic of Congo (LA22C3IV and LA23C3V). An optimum package of agronomic practices was followed through various stages of crop growth. Plants were irrigated in dry season. The studied ecotypes were different regarding their flowering precocity. The first to bloom was the ecotype from Senegal (7 months after sowing). It was followed by Benin's ecotype two days later, and by six other ecotypes [India (Hyderabad 1 and 2) Madagascar, Democratic Republic of Congo (LA22C3IV and LA23C3V) and Cambodia]. The ecotype from Equator (Salinas) was the last to bloom (two and half months to three months after Senegal's ecotype). African and Asian ecotypes presented a synchronized flowering in the environment of Southern Benin. The American ecotype flowered much latter than the others. First flower anthesis was observed - 9 to 10 months after sowing for this ecotype instead of 7 to 8 months for the others.

Floral ratio: Ten inflorescences of each ecotype were selected from May to July 2010. The number of male and female flowers was counted in each inflorescence.

Breeding system: In order to estimate self-pollination and outcrossing pollination percentages, three methods were used. 1) Ten inflorescences were covered completely with polythene bags. The number of female flowers on each inflorescence was counted before bagging. At blooming period, the shrub was shaken vigorously with the objective to make fall pollen on stigma. Four days later, the polythene bags were removed. Twenty days after removing the bags, the number of fruits per inflorescence was counted. 2) Ten others inflorescences were also covered with polythene bags the day before flowering. The number of female flowers on each inflorescence was then counted before bagging. The flowering day, the pollen of each inflorescence was artificially applied by rubbing the anthers of male flowers on the stigma of female flowers from the same inflorescence. After artificial auto-pollination, the inflorescence was bagged again and the bags were removed four days later. Twenty days after removing the bags, the number of fruits per inflorescence was counted. 3) To check pollination under the natural conditions, the flowers were left without any intervention (no bag, no artificial pollination). Ten inflorescences were followed in natural conditions.

Hybridization potential: The flowers were emasculated by removing the petals and anthers by hand in the afternoon (5 to 7 pm) before anthesis and covered with polythene bags. Crosses were carried out in the morning of the following day (9 to 12 am) by rubbing the anthers of male parent against the stigma of female parent of another ecotype and the flowers were bagged again. The bags were removed four days after pollination, and the number of produced fruits was counted.

Results

Floral ratio

J. curcas produces flowers in racemose inflorescencs with dichasial cyme pattern. The flowers are unisexual. The average number of male flowers per inflorescence varied from 78 to 291, with a mean of 159.9 ± 37.51 while the number of female flowers per inflorescence varied from 2 to 20, with a mean of 7.9 ± 2.82 (Table 1). The mean ratio of female to male flowers is thus 8/160. This ratio varies according the provenance of the plants. The analysis of variance regarding male and female flowers frequencies showed that there was a significant difference among ecotypes for the number of female flowers per inflorescence. The provenance of Equator (Salinas) which has the highest mean number of female flowers comes from America, the continent of origin of the physic nut. There were no significant differences concerning the mean number of male flowers. Provenances from India showed the lowest number of female flowers.

Breeding system and hybridization potential

The results of self-pollination and hand-pollination (Table 2) showed that *J. curcas* is self-compatible and that outcrossing is common. The result obtained by artificial auto-pollination with male flowers from the same tree is not different compared to the data obtained by free pollination in natural conditions. The lowest mean rate of pollination success (7%) was obtained from bagged inflorescences without castration. This indicates that wind pollination is unlikely for *J. curcas*.

Fruit set was not observed for all the crosses carried out. The rate of pollination success varied from ecotype to ecotype (Table 2) and according to the direction of the cross (data not shown). The hybrid seed number per fruit obtained is lower than three, the usual number of seeds produced in a capsule of *J. curcas* (data not shown).

Discussion

Physic nut is monoecious and the terminal inflorescences contain unisexual flowers. The ratio of male to female flowers we observed ranges from 13:1 to 26:1. These results are similar to those obtained by Teweri (18). Divakara *et al.* (6), observed in their report that this ratio varies and changes drastically from 13:1 to 108:1 with the fall in temperature. The total average numbers of female (eight) and male (one hundred and sixty) flowers is comparable to the result obtained by Chang-wei *et al.* (5). The total average number of female flowers per inflorescence numerated by Wijaya *et al.* (19) and Ghosh and Singh (7) was respectively 5.2 and 5.8 in the two accessions they analyzed. The average numbers of female flowers we observed are higher than those mentioned by these authors. This study showed that in the growing conditions of Southern Benin main rainy season the studied *Jatropha* accessions carry a high number of female flowers. Rao *et al.* (15) argue that the low number of female flowers is one of the factors causing low yield. Chang-wei *et al.* (5) and Ghosh and Singh (7) observed that this ratio varies according to climatic characteristics, nutrition conditions and the origin of the accessions. We noticed in our experiment that the plants flower almost throughout the year because they were irrigated during the dry season. The same observation was made by Ghosh and Singh (7). Most

Table 1
Mean number of male flowers, female flowers and mean ratio of male to female flowers per inflorescence and provenance

Provenances	Number of observed inflorescences	Mean number of female flowers (standard deviation)	Maximum	Minimum	Mean number of male flowers (standard deviation)	Maximum	Minimum	Mean Ratio of male to female flowers
Abomey-Calavi (Benin)	10	8.7 ± 1.25 ab	10	7	152.6 ± 30,78 a	177	86	18: 1
Cambodia	10	6.8 ± 2.04 a	11	4	157.5 ± 30.12 a	209	107	23: 1
Hyderabad 1 (India)	10	6.4 ± 1.67 a	10	4	157.7 ± 41.97 a	243	75	25: 1
Hyderabad 2 (India)	10	6.5 ± 2.15 a	10	2	143.2 ± 30.53 a	205	103	23: 1
LA22C3IV (Kivu , DRC)	10	6.1 ± 1.30 a	9	4	158.7 ± 27.34 a	206	126	26: 1
(LA23C3V (DRC)	10	7.7 ± 1.88 ab	10	5	164.5 ± 32.39 a	218	99	21: 1
Madagascar	10	8.9 ± 2.15 b	14	6	175.2 ± 47.30 a	279	102	20: 1
Salinas (Ecuador)	10	12.7 ± 3.61 c	20	8	167.9 ± 49.57 a	291	95	13: 1
Senegal	10	7.2 ± 1.66 ab	10	5	162.2 ± 26.80 a	195	123	23: 1
General mean		7.9 ± 2.82	-	-	159.9 ± 37.51	-	-	20: 1
F test		8.74			0.59			
Probability		0.000*			0.782 ns			
CV		5%			12%			

Significance level of F-test *= (P< 0.05), ns= no significant (P< 0.5) CV= coefficient of variation, the means followed by the same letter are not different statically.
DRC= Democratic Republic of Congo.

Table 2
Rate of fruit set according to the pollinating method

Provenances (ecotypes)	Self-pollination without castration by covering completely the inflorescences with polythene bags and shaking vigorously the shrub			Artificial auto-pollination by rubbing the anthers of male flowers against the stigma of female flowers of the same plant.			Free pollination in natural conditions			Crossing potentialities for each ecotype when used as female parents[1]		
	Number of flowers counted before bagging of 10 inflorescences	Number of fruits obtained	Rate of fruit set (%)	Number of flowers counted before hand-pollination. of 10 inflorescences	Number of fruits obtained	Rate of fruit set (%)	Number of flowers counted before free pollination of 10 inflorescences	Number of fruits obtained	Rate of fruit set (%)	Number of crosses carried out	Number of fruits obtained	Rate of fruit set (%)
Abomey-Calavi (Benin)	77	7	9	66	62	94	68	65	96	C (33) + M (10) + H_2 (18) = 61	C (8) + M (4) + H_2 (6) = 18	29.5
Cambodia	60	2	3	53	49	92	64	56	88	20 (B) = 20	B (0) = 0	
Hyderabad 1 (India)	62	2	3	65	59	91	66	60	91	4 (S) = 4	S (0) = 0	
Hyderabad 2 (India)	49	3	6	54	49	91	58	50	86	S (8) + B (9) + M (2) = 19	S (2) + B (8) + M (1) = 11	57.9
LA22C3IV (Kivu, DRC)	74	7	9	75	67	89	70	63	90	B (8) + S (10) = 18	B (4) +S (0) = 4	22.2
LA23C3V (DRC)	68	6	9	65	62	95	62	56	90	S (12) = 0	S (0) = 0	-
Madagascar	60	5	8	71	64	90	68	62	91	-	-	-
Salinas (Equator)	108	4	4	91	87	96	93	81	87	B (23) = 0	B (0) = 0	-
Senegal	92	8	9	86	82	95	83	75	90	B (20) + H_2 (5) + C (17) + K_1 (5) + Sa (14) = 61	B (8) + H_1 (4) + C (7) + K_1 (0) + Sa (0) = 19	31.1
total	650	44	7	626	581	93	632	568	90	218	52	23

(1) The data presented here were generally obtained from different crossing combinations. For the same mother parent the pollen parents varied according to the flowering synchronization of both parents. The different male parents used are designated as follows: B= Benin; C= Cambodia; H_2= Hyderabad 2 (India); K_1= Kivu 1 (Democratic Republic of Congo); M= Madagascar; S= Senegal; Sa= Salinas (Equator). Data between brackets represents either the number of crosses carried out or the number of fruits obtained by using the male parent whose designation is placed just before the brackets.

of the plants start producing seeds 7 months after sowing in our conditions. This is coherent with the observations made by Divakara *et al.* (6) who report that in good conditions plants can start producing within 7 months after planting.

Breeding systems are recognized as being of prime importance in regulating the genetic structure and evolutionary dynamics of plant populations. There are three kinds of mechanisms in plant breeding systems; geitonogamy, xenogamy and apomixis (10). The breeding system of *Jatropha curcas* is incrossing and outcrossing but the rate of the first is low compared to the second (5). The results of the present study also support this conclusion. The results obtained in Southern Benin conditions using free pollination (89.87%) showed that most of the pollination in *J. curcas* depended on entomophilous pollination. The results we obtained for natural and artificial cross-pollination (89.87% and 92.81% respectively show that the main breeding system of *J. curcas* is xenogamy. Insects play an important role in the pollen flow. The result obtained by bagging inflorescences without castration (7% for the average fruit set rate) point out that the wind does not play an important role in the pollination of *J. curcas*. Several publications confirm that wind pollination is almost impossible for *J. curcas* (5). The fruit set we obtained in case of bagging of the inflorescence before anthesis of the female flowers could be the result of apomixis. Many authors have reported apomixis percentages in their works: 4% for Abdelgadir *et al.* (1), and 12% for Chang-wei *et al.* (5). Many insects intervene in the pollination of physic nut and their nature varies according to the cultivation zones.

J. curcas is still considered as a wild plant which exhibits great variability in production and quality characteristics because no careful breeding program with systematic selection has yet been carried out (2, 6). Improved varieties with desirable traits for specific growing conditions are not available, which makes growing *Jatropha* a risky business (8). An approach to increase the productivity of physic nut is to exploit the hybrid vigor that could be expressed by the F_1 progenies issued from adequate crossing combinations. Application of heterosis breeding can boost the jatropha yield. In order to have high yielding plants producing high quantity oil, it is necessary to create and select best suitable germplasm. Obtaining F_1 hybrids (intraspecific hybrids) between ecotypes of physic nut was not difficult. The crosses realized showed that it is possible to develop new intraspecific hybrids. Similar conclusion was made by Tar *et al.* (17). The achievement of 218 hybridizations on 8 accessions used as female parent, gave 52 fruits and 126 seeds. Thirty four seeds germinated and 29 plants were well established and grew to reproductive maturity. The rate of germination was very low. This could be due to the low viability of the seeds perhaps related to their high oil contain or to a phenomenon of dormancy. Seed germination can be improved by the use of growth regulators and by soaking in water (9). Another method for overcoming the dormancy is to keep freshly harvested seeds over a month time at ambient temperature (6). The hybridization success is higher with some ecotypes than others. In some cases, no hybrid seeds could be obtained. This could be due to the provenance of ecotypes. Hybridizations between African and Asian ecotypes gave easily seeds, but it was difficult to obtain seeds when the South American accession was used. This could be explained by the low genetic variation found between African and Indian accessions (13) and by the higher genetic distance existing between South American accessions and ecotypes from the rest of the world (13, 15). Using 225 accessions collected from 30 countries in Asia, Africa and Latin America, Montes-Osorio *et al.* (13) found low genetic variation in African and Indian accessions and high genetic variation in Guatemalan and other Latin American accessions. The morphology of the F_1 plants is very similar because variability between most of the accessions used in this study might not be very wide. The provenance of Ecuador (Salinas) which is morphologically different from other accessions (3) with the highest floral ratio (13:1), did not produce any fruit through crossing with plants originating from Africa or Asia. The analysis of the progeny obtained from crosses carried out between plants showing very different phenotypes should permit to verify the existence of a high rate of apomixis in *Jatropha*.

Conclusion

This study showed that the floral ratio varies according to the provenance of the genetic stocks of *J. curcas*. The breeding system of physic nut is incrossing and outcrossing with foraging insects playing a major role in its pollination. The showy attractive flowers are clearly adapted for insect-mediated cross-pollination, whereas the production of fruits by self-pollination proves that the plants are capable of autogamy. Crosses between African and Asian accessions of physic nut gave hybrids without difficulty. The first attempts to produce hybrids by crossing African and Asian ecotypes to the accession from Ecuador failed. Because of the rather high rate of apomixis registered in different parts of the world and the indices of apomixis occurring in the plants we studied, all the plants produced by crossing different *J. curcas* parents might not be true hybrids. This point will have to be considered in the development of breeding programs aiming at improving the crop.

Considering the high number of female flowers per inflorescence produced by the accession from Ecuador, and the large genetic distance existing between this accession and those from Africa and

Asia, it should constitute a valuable genetic stock for the development of F_1 hybrids with local ecotypes of *J. curcas*. However, the use of growth regulators might be necessary to improve the hybridization success rate when it is used as female parent.

Acknowledgements

The authors warmly thank Prof. F. Flambert for providing the ecotypes originating from Ecuador and India used in their breeding program.

Literature

1. Abdelgadir H.A., Johnson S.D. & Van Staden J., 2009, Pollinator effectiveness, breeding system, and tests for inbreeding depression in the biofuel seed crop, *Jatropha curcus*. Journal of Horticultural Science & Biotechnology, **84**, 3, 319-324.
2. Achten W.M.J., Verchot L., Frankeen Y.J., Mathijs E., Singh V.P., Aerts R. & Muys B., 2008, *Jatropha* bio-diesel production and use. Biomass and Bioenergy, **32**, 12, 1063-1084.
3. Ahoton L.E., Quenum F. & Mergeai G., 2011, Evaluation agromorpho-logique et sélection des meilleures accessions de Pourghère (*Jatropha curcas* L.) introduites au Bénin Int. J. Biol. Chem. Sci. **5**, 4, 1619-1627.
4. Burley J. & Woodn P.J.A., 1976, Manual on species and provenance research with particular reference to the Tropics. Tropical Forestry Papers N°10. Department of Forestry, Commonwealth Forestry Institute, University of Oxford.
5. Chang-wei L., Kun L., You C. & Yong-yu S., 2007, Floral display and breeding system of *Jatropha curcas* L. For. Stud. China, **9,** 2, 114-119.
6. Divakara B.N., Upadhyaya H.D., Wani S.P. & Gowda C.L.L., 2010, Biology and genetic improvement of *Jatropha curcas* L.: a review. Applied Energy, 87, 732-742.
7. Ghosh L. & Singh L., 2008, Phenological changes in *Jatropha curcas* in subhumid dry tropical environment. Journal of Basic and Applied Biology, **2**, 1, 1-8.
8. Jongschaap R.E.E., Corre W.J., Bindraban P.S. & Brandenburg W.A., 2007, Claims and facts on *Jatropha curcas* L. Wageningen, The Netherlands: Plant Research International, <www.facfuels.org/media *Jatropha*_WUR session=isgklbna 58j7grrfst888n5r7>, (20 /12/11).
9. Kumari M., Patade V.Y., Arif V.M. & Ahmed Z., 2010, Effect of IBA on seed germination, sprouting and rooting in cuttings for mass propagation of *Jatropha curcus* L. Strain DARL-2. Res. J. Agric. Biol. Sci. **6**, 6, 691-696.
10. Les D.H., 1988, Breeding systems, population structure and evolution in hydrophilous angiosperms. Ann. Mo. Bot. Gard. 75, 819-35.
11. Makkar H.P.S. & Becker K., 1997b, *Jatropha curcas* toxicity: identification of toxic principle(s). Proceedings 5th International Symposium on Poisonous Plants, San Angelon, Texas, USA, May 19-23.
12. Makkar H.P.S. & Becker K., 1999, Plant toxins and detoxification methods to improve feed quality of tropical seeds Review. Asian-Aus. J. Anim. Sci. **12**, 3, 467-480.
13. Montes-Osorio L.R, Azurdia C., Jongschaap R.E.E, Van Loo E.N., Barillas E., Visser R. & Mejia L., 2008, Global evaluation of genetic variability in *Jatropha curcas*,<www.pri.wur.nl/NR/rdonlyres/413C2933685/70112/PosterMontesHR.pdf>, (20/12/11).
14. Openshaw K.A., 2000, Review of *Jatropha curcas*: an oil plant of unfulfilled promise. Biomass and Bioenergy, 19, 1-15.
15. Rao G.R., Korwar G.R., Arun K.S. & Ramakrishna Y. S., 2008, Genetic associations, variability and diversity in seed characters, growth, reproductive phenology and yield in *Jatropha curcas* (L.) accessions. Trees, 22, 697-709.
16. Solsoloy A.D. & Solsoloy T.S., 1997, Pesticidal effacacy of formulated *J. curcas* oil on pests of selected field crops. *In:* Gübitz, G.M., Mittel-bach, M., Trabi, M. (Eds), Biofuels and industrial products from *Jatropha curcas*. Dbv-Verlag, Graz, 216-226.
17. Tar M.M., Patcharin T. & Peerasak S., 2011, Heterosis of agronomic characters in *Jatropha* (*Jatropha curcas* L.). Kasetsart J. Nat. Sci. 45, 583-593.
18. Tewari D.N., 2007. *Jatropha* and biodiesel. 1st ed., Ocean Books Ltd. New Delhi, India, 227 p.
19. Wijaya A., Tidiana S., Harun M.U. & Hawalid H., 2009, Flower characteristics and the yield of *Jatropha* (*Jatropha curcas* L.) accessions. Hayati Journal of Biosciences, **16**, 4, 123-126.

L.E. Ahoton, Beneneese, Doctorate in Agronomic Sciences and biological engineering of Gembloux University (Belgium), 2002, Researcher and Professor at the Faculty of Agronomic Sciences, University of Abomey-Calavi, Benin.

F. Quenum, Beneneese, Doctorate in Agronomic Sciences of Zhejiang University (China), 2002, Researcher and Professor at the Faculty of Agronomic Sciences, University of Abomey-Calavi, Benin.