African Journal of Biotechnology Vol. 3 (5), pp. 260-265, May 2004
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2004 Academic Journals

Full Length Research Paper

Improving bambara groundnut productivity using gamma irradiation and *in vitro* techniques

H.K. Adu-Dapaah[1]* and R.S. Sangwan[2]

[1]Crops Research Institute, P. O. Box 3785, Kumasi, Ghana.
[2]Director, Androgenesis and Biotechnology Laboratory, Université de Picardie Jules Verne, 33 rue Saint Leu, F-80039 Amiens, France

Accepted 29 March, 2004

In recent times efforts are being made to improve the productivity of bambara groundnut. Studies were initiated (i) to characterise and evaluate landraces and to select superior ones for irradiation, (ii) to induce genetic variation through gamma irradiation and (iii) to use biotechnological approaches to shorten the generation cycle. The results of the study indicated that gamma irradiation induced higher genetic variation of up to four times within the varieties used in the study compared to the unirradiated control. Bambara groundnut yield could be increased through selection for number of pods per plant. Using the *in vitro* plus *in vivo* system and embryo axis explants, over four generations per year were obtained compared to 1 or 2 in the field. All the plants were morphologically normal and fertile. The shorter duration, high efficiency and genotype independency makes this system well suited for wider biotechnological applications in bambara groundnut. This novel approach is being applied to the variants/mutants obtained from gamma irradiation.

Key words: Gamma irradiation, generation cycle, genetic variation, *Vigna subterranean*.

INTRODUCTION

Bambara groundnut (*Vigna subterrenea* (L) Verdc) is a major source of vegetable protein in sub-Saharan Africa. It is well adapted to harsher conditions and constitutes an important part of the local diet, culture and economy (Goli, 1997; Heller et al., 1997). The seed is regarded as a completely balanced food because it is rich in iron 4.9-48 mg/100 g, compared to a range of 2.0-10.0 mg/100 g for most food legumes, protein 18.0-24.0% with high lysine and methionine contents (Rowland, 1993), ash 3.0-5.0%, fat 5.0-7.0%, fibre 5.0-12.0%, potassium 1144-1935 mg/100 g, sodium 2.9-12.0 mg/100 g, calcium 95.8-99 mg/100 g, carbohydrate 51-70%, oil 6-12%, and energy 367-414 kal/100 mg (Rowland, 1993). The gross energy value of bambara groundnut seed is greater than that of several other pulses (Anchirina et al., 2001; Rowland, 1993; Lacroix, 2003; Amarteifio et al., 2002).

Bambara groundnut grains are eaten in many ways. They can be eaten fresh or grilled while immature. In many countries in West Africa, fresh pods are boiled with salt and pepper and eaten as a snack. In East Africa, the beans are roasted, pulverized and used in preparing soup. According to Linnemann (1990), bambara groundnut flour is used to make bread. Bambara milk is preferred to that prepared from other pulses because of its flavour and colour (Goli, 1997). Bambara seed and haulm have been used to feed livestock and poultry (Anchirina, et al., 2001).

Bambara groundnut fixes atmospheric nitrogen in symbiosis with *Bradyrhizobium* strains through a nodulation process (Gueye et al., 1998). The crop requires relatively low inputs and contributes to the sustainability of the cropping systems in West Africa. It is grown mostly by women who intercrop with maize, millet, sorghum, yam, and groundnut (Goli, 1997, Anchirina, et al., 2001). In spite of the importance of bambara groundnut, it has not received much research attention compared to other legumes. The landraces remain unimproved (Doku, 1997).

The ability of bambara to survive and grow under harsh conditions is due to specific adaptive traits which include

*Corresponding author. E-mail: hadapaah@cropsresearch.org.

production of subterranea pods and a combination of physiological regulations: osmotic adjustment, leaf area index reduction, increase leaf reflectivity and changes in leaf orientation and stomatal regulation (Collinson et al., 1999a and 1997b; Chapman et al., 1993). In spite of these specific adaptive traits, bambara groundnut is less competitive compared to other improved major crop species (Lacroix, et al., 2003).

Among the constraints militating against the development of bambara groundnuts include lack of genetic improvement, inadequate knowledge on the taxonomy, reproductive biology and on the genetics of agronomic and quality traits, disease and pest infestation (Anchirina, et al., 2001; Lacroix et al., 2003). For effective selection in any enhancement programme, genetic variation must exist. Radiation and other chemical mutagens have been used to induce variability in crop plants (Ahloowalia et al., 1998). To improve upon the productivity of bambara groundnut, strategies like genetic recombination and selection, induced mutation and appropriate biotechnological approaches are some of the techniques that could be used. Novel approaches aimed at shortening the generation cycle for faster breeding of protein legumes such as pea and bambara groundnut have been developed (Ochatt et al., 2002; Lacroix et al., 2003). Literature on tissue culture work on bambara groundnut is scanty. The objectives of this study were (i) to characterize and evaluate landraces and select superior accessions for irradiation, (ii) to study the effects of gamma irradiation as a means of increasing variability, and (iii) to shorten the generation cycle using *in vitro* and *in vivo* strategy.

MATERIALS AND METHODS

Three studies were conducted from 1999 to 2003 and the details are presented below:

Experiment 1

Twenty-three landraces, collected earlier on from farmers were grown at the Crops Research Institute, Kumasi Ghana (6° 43'N, 1° 36'W). A randomized block design with three replications per treatment was followed at the spacing of 0.30 m x 0.50 m on 5 m long plots. Two seeds per hill were sown at 5 cm depth and later thinned to one per hill seven days after emergence. The landraces were characterized using the IBPGR/IITA/GTZ, 1987 index. Data were collected on days from sowing to emergence, days to 50% flowering, days to maturity, vigour index, canopy spread (cm^2), petiole/internode ratio, shelling percentage, number of pods per plant, number of seeds per pod and grain yield (kg/ha). Basic statistics were computed and the best two landraces with superior agronomic traits selected for gamma irradiation.

Experiment 2

Following the establishment of LD_{50} for bambara groundnut, 2000 seeds of two landraces were irradiated by a ^{60}Co gamma ray 220 unit cell at the Ghana Atomic Energy Commission at 150 Gy. The irradiated seeds and their controls were planted on field plots at the Crops Research Institute, Kumasi. The experimental design used was randomized block design with three replications. The plants were spaced 0.30 m x 0.50 m on a 5 m row length. To raise the M_2 plants, twenty seeds from each surviving plant were planted. Seeds from selected M_2 generation plants with desirable agronomic traits were sown to raise the M_3 generation. The following data were collected (i) seedling survival 21 days after planting (ii) seedling height (iii) number of days to flowering and maturity (iv) number of pods per plant (v) seed size and grain yield per plant as well as shelling percentage.

Four of these traits were further evaluated in the M_3 using the genetic parameters: genetic variance, heritability and genetic gain (Allard, 1960) with 10% selection pressure.

Experiment 3

Greenhouse strategy: Four landraces of bambara groundnut from Ghana GB_1, GB_2 and Mali MB_1 and MB_2 were grown in the greenhouse at the University of Picardie, France, with a thermo period of 27±1°C (day) and 25±1°C (night) under 10 h light intensity of 5000 lux. Seeds were sown in 51 cm x 31 cm multi-walled trays. Soil or Perlite was used as the substrate. The seedlings were watered and nourished by capillarity with a nutrient solution. Watering and nutrient supplementation was stopped when the pods were whitish (50-60% dry matter content in the seeds). At maturity, the pods were harvested by hand, threshed and sown following the procedure outlined above. Combination of *in vitro* and *in vivo* strategy: The combination of *in vitro* and *in vivo* techniques is modified from Ochatt et al. (2002). The following four-step approach was employed.

(a) Culture medium: The composition of the medium included Murashige and Skoog (1962), Macro-elements and micro-elements and vitamins of Nitsch and Nitsch (1965), 2% sucrose and 0.6% Difco-Bacto agar. The medium so constituted is referred to as bambara medium (BM) which contained different types and concentration of growth regulators. Half-strength hormone-free BM was used for seed germination. To ensure better root growth 0.5-1.0 mg/l naphthalene acetic acid (NAA) was added to the BM.

(b) Sterilization: Fifty to hundred seeds of bambara groundnut were soaked overnight in 50 ml conical tubes containing 30 ml of distilled water. After soaking over night, the water was decanted and the seeds rinsed 3-4 times with distilled water, surface sterilized with 70% ethanol for 1 min and 5% calcium hypochlorite for 30 min. Pods with matured fresh seeds were detached and surface-sterilized as outlined above.

(c) Culture conditions: For shortening generation cycle, three different treatments were used. (i) Entire seeds – unpeeled control seeds, (ii) Seeds with seed coat removed (peeled seeds), and (iii) Embryonic axes isolated from the peeled seed. Seed coats were removed and embryonic axis isolated under a binocular microscope. Seeds were carefully cut with a scapel to isolate the embryo axis. The seeds and embryonic axis were cultured on half-strength BM in petri dishes (20 ml medium per petri dish) and/or culture tubes. The tubes were kept in a culture room under a short day (5000 lux, for 10 h light) at 27±1°C (day) and 25±1°C (night). The plantlets of 3-4 cm height were transferred to the greenhouse for seed set. Pods containing matured embryos were detached and surface-sterilized. The pods were opened aseptically and the seeds of each pod were randomly selected for sowing. The wet seed coat was carefully opened. Care was taken not to damage the cotyledon, embryo axes and the root tip with cap.

(d) Analysis of ploidy level: The ploidy level of the regenerants was determined using the flow cytometry as outlined by Sangwan

Table 1. Characterisation and evaluation of Bambara groundnut in Ghana.

Trait	Min	Max	Mean	SD[1]	Variance	CV[2]	Skewness	Kurtosis
Days to emergence	7	15	9	1,08	1.15	11.98	1.29	2.86
Days to 50% flowering	38	70	45	5.42	29.94	12.46	1.77	4.3
Days to maturity	91	148	127	14.8	226	11.01	0.32	-0.91
Vigour index	1.0	9.0	4	1.55	2.40	29.9	0.21	-0.30
Canopy speed (cm)	10	80	45	12.61	168.10	28.40	0.31	0.69
Petiole/internode ratio	4.1	8.9	6.8	2.10	4.11	9.11	0.83	-0.81
Shelling percentage (%)	75	52	68	10.11	99.81	19.48	1.60	3.96
No. of pods/plant	10	165	36	20.21	205.12	36	1.72	6.76
No. of seeds/pod	1	2	1.05	0.14	0.04	11.16	2.17	7.16
Grain yield kg/ha	400	1650	839	375.7	133307.1	32.1	0.15	0.98

[1]Standard deviation
[2]Coefficient of variation

et al. (1992). The ploidy level was further confirmed by scoring chloroplasts in stomatal guard cells of the lower epidermis of young leaves as described by Lacroix et al. (2003). To score chloroplast, young leaves were fixed for 1 h in ethanol, acetic acid (3:1 v/v) and stained with potassium iodide solution (comprising 80 ml acetic acid, 10% ethanol and 3% potassium iodide. From a minimum of 25 stomata in each regenerant studied, counts were made.

RESULTS AND DISCUSSION

Experiment 1

The performance of bambara groundnut landraces following characterization and evaluation are presented in Table 1. The basic statistics for quantitative agronomic traits have been summarized. For most of the traits, the range from minimum to maximum scoring of the accessions were provided. The ranges fall within what was reported for similar studies conducted by Goli et al. (1997) and Karikari (2002) on bambara groundnut in Nigeria and Botswana, respectively. The results for most of the traits evaluated were specific to the environment. The humid agro-ecology in Kumasi might have lowered the growth and productivity of the plants. Two of these accessions with high pod yield and desirable agronomic traits but susceptible to *Cercospora* were selected for irradiation studies following characterization and evaluation.

Experiment 2

For selection to be effective in any plant breeding programme, there must, as a necessity exist genetic variation in the accessions under study. Radiation and other mutagens may be used to generate new genetic combination or increase variability in even closed populations. Results of the study to induce genetic variation through gamma irradiation are presented in Table 2. Genetic variance was increased in all characters under study. High increases of up to four times greater than their respective unirradiated control were observed after irradiation in most of the characters (Table 2). This is in agreement with observations by Mensah and Eroutor (1993) and Gregory (1955) in lima beans and peanut, respectively. With reference to the selected plants, the genetic studies indicated that in bambara groundnut, number of pods per plant offered the best opportunity for selection in the M_3 generation followed by seed size and grain yield. This is due to the high heritability exhibited by both the irradiated and control populations. The predicted genetic gain in M_3 progenies were higher in the irradiated than the unirradiated control population (Table 2). Papa et al. (1961) made a similar observation in soybean M_3 generation following irradiation with x-ray and thermal neutrons.

Experiment 3

Mean germination percentage and plantlet development on half-strength BM medium for 3 treatments are presented in Tables 3 and 4. Mean germination percentage and plantlet development on half-strength BM medium for three treatments during a 3-week incubation period are presented in Table 3. Peeled seeds germinated 7 days after culturing, while the unpeeled control took 14 days to germinate. After 21 days, however, the germination percentage and plantlet growth were comparable among the peeled and unpeeled seeds (Table 3). Significant differences in number of days from sowing to emergence between the peeled and the unpeeled seeds calls for seed priming to soften seed coat and enhance moisture imbibition for seeds grown on the field. Among the treatments, embryo axis explant gave the highest germination percentage throughout the period of culturing with germination percentage of 38.2, 62.5 and

Table 2. Population mean, genetic variance, heritability and predicted genetic gain for some agronomic traits in M_3 progenies of the two bambara groundnut varieties grown from seeds treated with gamma rays GR and untreated UT.

Trait	Population Mean		Genetic Variance		Heritability		Genetic Gain	
	UT	GR	UT	GR	UT	GR	UT	GR
Nkoranza								
Pp	66.80±2.5	79.8±3.1	1.50	3.50	89.6	90.2	14.1	15.7
Ss	58.10±1.7	61.2±2.3	1.20	5.71	73.8	85.1	12.1	13.9
Gy	83.40±3.1	97.1±2.7	3.78	9.00	65.0	70.5	11.0	12.0
Sp	54.00±1.1	58.6±1.8	2.70	3.35	59.2	62.0	7.8	8.7
Yoromba								
Pp	62.1±1.5	70.8±2.3	4.10	9.70	85.10	86.31	9.7	11.9
Ss	51.0±2.4	62.1±3.1	6.10	9.30	84.60	85.21	13.6	14.7
Gy	76.2±1.9	94.5±2.7	3.20	7.00	82.40	86.71	10.2	11.8
Sp	55.3±1.1	58.2±1.0	5.10	10.20	27.10	28.10	8.3	9.2

Pp: No. of pods per plant
Ss: Seed size (100 seed weight)
Gy: grain yield
Sp: ?

Table 3. Mean germination percentage and plantlet development on half-strength BM medium for different treatments of explants.

Duration (days)	Treatments		
	Unpeeled Seeds (%)	Peeled Seeds (%)	Embryo Axis (%)
7	0	15.3±1.8	38.2±4.2
14	14.8±1.6	35.8±2.6	62.5±3.6
21	49.3±3.4	58.8±7.6	96.2±2.6

Table 4. Mean performance of three explant types on half-strength BM medium after four weeks of culture.

Trait	Explant Types Treatments		
	Unpeeled Seed	Peeled Seeds	Embryo Axes
% Germination	48.2±4.2	51.4±3.6	95±4.6
Plant size	6.8±0.8	7.1±1.2	1.9±0.6
Root length	17.4±2.1	18.3±1.8	3.6±0.8
Stem height	1.5±0.6	2.2±0.8	2.1±0.6
No. of stem/branches	1.2±2.0	1.3±2.0	4.0±1.0

96.2% at seven, fourteen and twenty one days respectively after culturing. During culturing, it was observed that root growth and plantlet development were normal on the BM medium. The addition of 0.5 to 1 mg/l NAA enhanced root growth better than BM without growth regulators.

Mean performance of the three explant types on half-strength BM medium after culturing for four weeks are presented in Table 4. Appreciable differences in performance/development were observed among plantlets derived from embryo axis explants and those from either peeled or unpeeled seeds. Plantlet size of the embryo axis explants were about one quarter that of the peeled or unpeeled. This might be due to adequate nutrient reserves stored in the cotyledon of the peeled and unpeeled seeds with the embryonic axis explant having little stored nutrient reserves. The embryo axis explant however had the highest germination percentage of 95% compared to 48.2% for the unpeeled and 51.4% for the peeled seeds. The embryo axis explant produced more branches/stems (about three times) than the other two explant types. Ochatt et al. (2002) made similar observations in their studies on bambara groundnut explant types. They observed that differences in initial plantlet size did not influence number of days to flowering and seed set.

Results of the mean generation length (days) of four bambara groundnut landraces used in the study are presented in Figure 1. The *in vitro* culturing of the four landraces under greenhouse conditions revealed that they all produced fewer pods (2-4/plant). To shorten the generation cycle in bambara groundnut it is essential to reduce the vegetative development in order to produce few seeds. Ultimately, the strategy is to integrate this technique into the single seed descent (SSD) selection procedure which requires one or two seeds per plant (Ochatt et al., 2002).

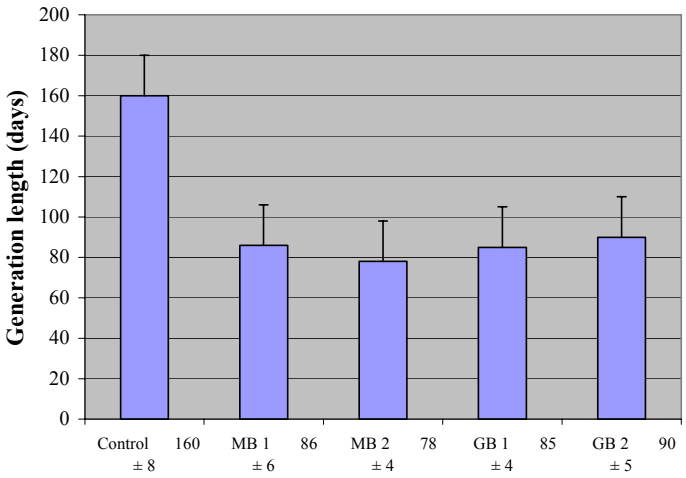

Figure 1. Landraces and mean generation length (days).

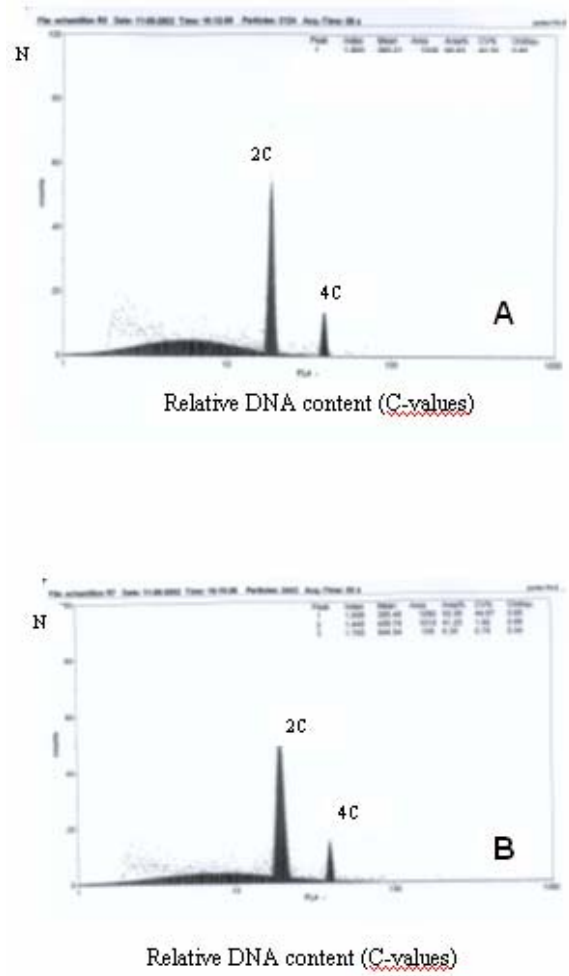

Figure 2. Flow cytometry analysis of DNA-related fluorescence in nuclei released by chopping of diploid control and regenerated diploid line in Bambara groundnut. On the abscissa the log intensity scale is also converted to DNA content as C-values. N- the number of nuclei; the proportion in each peak is shown. **A:** Flow cytometry of (control) seed-derived diploid plants grown in greenhouse. **B:** Flow cytometry of regerants, *in vitro* embryo-derived plants grown in greenhouse.

The mean time span from one generation to the another generation for the four landraces are presented in Figure 1. Genotype MB_1 gave a mean time span of 86.6±6.2 days, while in MB_2 it was 78.1±4.3 days. The two landraces from Ghana gave a mean time span of 85.4±3.9 days in GB_1 and 87.7±3.2 days in GB_1. In the field MB_2 had a mean cycle length of 170 days in Mali (Unpublished data from Prof. Bretaudeau, personal communication) and GB_2 140 days in Ghana (Unpublished data from Dr. H.K. Adu-Dapaah). The field data for number of days to maturity from Ghana and Mali indicate that bambara groundnut could be grown 1 to 2 generations per year with the attendant environmental constraints.

It is evident from the results that in bambara groundnut breeding, the *in vitro* plus *in vivo* approach would enhance efficiency, ease of execution and cut down on cost since at least 4 generations could be grown a year. Plants obtained from the *in vitro* plus *in vivo* strategy were morphologically normal and fertile. The progenies of such plants were also normal. These observations were in consonance with earlier reports by Ochatt et al. (2002) in pea and Lacroix et al. (2003) in bambara groundnut.

As we were interested in using this *in vitro* and *in vivo* system both for shortening of generation cycle and large-scale production of Bambara groundnut plants for breeding purpose, the ploidy level of the regenerants was an important consideration. Cytometry analyses were performed on 100 randomly chosen regenerants plants following transfer to the greenhouse (Figure 2). All the specimens chosen for flow analyses were normal and without vitrification. Frequency distribution of the nuclear DNA contents of leaves from the regenerants showed two distinct peaks (Figure 2B) corresponding to nuclei in the G0/G1 and G2 phases of the cell cycle. A similar profile was obtained using the control plants (seed-derived plants, Figure 2A). Thus, our flow cytometry analyses indicated that landraces GB_1 and GB_2 were diploid and fertile. In a related study using the two landraces from Ghana, Lacroix et al. (2003) observed that using the analysis of chloroplast numbers, none of the 55 randomly selected regenerants exhibited any changes in ploidy level. Ochatt et al. (2001) made similar observations in grass pea *(Lathyrus sativus* (L) in their flow cytometry studies. The shorter duration, high efficiency and genotype independency of the *in vitro* plus *in vivo* strategy makes it amenable for wider biotechnological applications in bambara groundnut. This novel approach is currently being applied to the mutants/variants obtained following gamma irradiation.

It is concluded from the studies that (i) gamma irradiation induced higher genetic variation than untreated control and could be a means of improving bambara groundnut productivity (ii) Seed yield in bambara groundnut could be increased through selection for higher number of pods/plant (iii) Efficient *in vitro*

regeneration system for mass multiplication of improved bambara groundnut varieties/mutants has been developed and (iv) Shortening of generation cycle is the technology for efficient bambara groundnut improvement since at least 4 cycles per year is possible.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the technical support of the Legume Breeding Division of the Crops Research Institute (CRI), Kumasi, Ghana, as well as skillful technical assistance by M.G. Vasseur and Ms. A. Louis of Labo. Androgenese et Biotechnologie, Université de Picardie Jules Verne, France. The financial support given by FAO/IAEA Vienna under the Coordinated Research Programme is gratefully acknowledged.

REFERENCES

Ahloowalia BS, Maluszynski M, Nitchterlein K, van Zanten L, Weck E (1998). Induced mutations and in vitro culture techniques for improvements of horticultural plants. Proc. Second International Crop Science congress. Crop Productivity and sustainability, Shaping the future. V.L Chopra., R. B, Singh and Anupam Varma (eds), pp. 405-412.

Allard RW (1960). Principles of Plant Breeding. John Wiley and Sons, New York, pp. 52-96.

Amartefio JO, Karikari SK, Modise JO (2002). The proximate and mineral composition of six landraces of bambara groundnut. Trop. Sci. 42:188-191.

Anchirina VM, Yiridoe EK, Bennett-Lartey SO (2001). Enhancing sustainable production and genetic resource conservation of Bambara groundnut. A survey of indigenous agricultural knowledge systems: Outlook in Agriculture 30 (4):281-288.

Chapman JC, Ludlow MM, Blamey FPC, Fisher KS (1993). Effect of drought during early productive development on growth of cultivars of groundnut (*Arachis hypogea* L.) In Utilization of radiation and water during drought. Field Crops Res. 32: 193-210.

Collinson ST, Berchie JN, Azum-Ali SN (1999). The effect of soil moisture on light interception and the conversion coefficient for three landraces of bambara groundnut (Vigna subterranea) Journal of Agric Science (Cambridge) 133: 151-157.

Collinson ST, Clawson EJ, Azam-Ali SN, Black CR (1997). Effects of soil moisture deficits on the water relations of bambara groundnut (*Vigna subterranea* L. Verde.). J. Exp. Bot. 48:877-884

Doku EV (1997). Bambara groundnut (*Vigna subterranea* (L) Verdc) production in Ghana. Promoting the use of neglected and under-utilized crops. Proceedings of the workshop on conservation and improvement of Bambara Groundnut. Heller J., Begemann F and Mushanga J. (eds.). 14-16 November 1995, Harare, Zimbabwe.

Goli AE (1997). Bibliographical review. In: *Bambara groundnut*. *Vigna subterranea* (L.) Verdc. Proceedings of the workshop on Conservation and Improvement of Bambara Groundnut (*Vigna subterranea* (L.) Verdc.). Heller J., Begeman F. and Mushonga, J., eds. 14-16 November 1995, Harare, Zimbabwe, pp. 4-10.

Gregory WC (1955). X-ray breeding of peanut *Arachis hypogaea* L. *Agron. J*. 47:314-318.

Gueye M, James EK, Kierans M, Sprent JL (1998). The development and structure of root nodules on bambara groundnut (*Voandzeia (Vigna) subterranea*). World J. Microbiol. Biotechnol. 14: 177-184.

Heller F, Begemann F, Mushonga J (1997). Bambara groundnut: *Vigna subterranea* (L) Verdc. Promoting the conservation and use of underutilized and neglected crops, 9, IPGRI, Rome/Gatersleben, p. 165.

IBPGR/IITA/GTZ (1987). Descriptors for bambara groundnut. International Board for Plant Genetic Resources, Rome 23 p.

Karikari SK (2002). Variability in local and exotic bambara groundnut (Vigna suterranea (L) Verdc) landraces in Botswana. In: Begemann, F., Mukema, I. and Obel-Lawson (eds.) Promotion of bambara groundnut: Latest developments of bambara groundnut research. Proceedings of the 2nd International Workshop of International Bambara Groundnut Network (BAMNET) 23-25 September 1998 CSIR, Accra. Pp 38-45.

Lacroix B, Assoumou Ndong Y, Sangwan RS (2003). Efficient *in vitro* direct shoot regeneration systems in bambara groundnut (*Vigna subterranea* L. Verdc.). Plant Cell Rep. 21: 1153-1158.

Linnemann AR (1990). Cultivation of bambara groundnut in Western Province, Zambia. Report of a Field Study. Trop. Crops Comm. No. 16, Wageningen Agricultural University.

Mensah JK, Eroutor (1993). Genetic variation in agronomic characters of lima beans induced by seed irradiation. Trop. Agric. 70:342-344.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.

Nitsch PP, Nitsch C (1965). Néoformation de fleurs *in vitro* chez une espéce de jours courts. *Plumbago indica L.* Ann. Physiol. Vég. 7:251-258.

Ochatt SJ, Durieu P, Jacas L, Pontecaille C (2001). Protoplast. Cell and tissue cultures for the biotechnological breeding of grass peas. Lathyrus Newsletter 2: 35-38.

Ochatt SJ, Sangwan RS, Marget P, Assoumou Ndong Y, Rancillac M, Perney P (2002). New approaches towards the shortening of generation cycles for faster breeding of protein legumes. Plant Breed. 121: 436-440.

Papa KE, Williams JH, Hanway DG (1961). Effectiveness of selection for quantitative characters in the third generation following irradiation of soybean seed with X-ray and thermal neutrons. Crop Sci. 1:87-90.

Rowland JRJ (1993). Bambara groundnut. In: Rowland, J.R.J. (ed). Dryland Farming in Africa, MacMillan Ltd., London, pp. 278-282.

Sangwan RS, Bourgeois Y, Brown S, Vasseur G, Sangwan-Norreel BS (1992). Characterization of competent cells and early events of *Agrobacterium*-mediated genetic transformation in *Arabidopsis thaliana*. Planta 188: 439-456.