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Research Article

Variation in Nitrogen Fixation among Three Bush Bean Cultivars Grown in Kenya when Inoculated with Three Rhizobia Strains

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ABSTRACT

Nitrogen deficiency is a major factor limiting bean production in Africa. This problem can be alleviated by the use of nitrogen fertilizers which on the other hand adversely affect the environment. The problem can also be alleviated by the use of environmental friendly biological nitrogen fixation technology. In this study, growth experiments were designed to investigate biological nitrogen fixation in three determinate (bush) beans namely Ayenew, GLP 24, and Ecab 0807. Greenhouse and field studies were carried out to determine the most efficient bean cultivar and rhizobia strain in nitrogen fixation. Each bean cultivar was inoculated with the rhizobia strains CIAT 899, USDA 2674, USDA 2676, the three rhizobia strains combined and one was not inoculated. Results from greenhouse studies showed significant statistical differences in nodulation within the bean cultivars. The bush bean cultivar GLP24 was poorly nodulated (40.0 nodules on average) as compared to Ayenew (52.0 nodules on average) and Ecab 0807 (58.0 nodules on average). It was also observed that bush beans inoculated with USDA 2674 strain of rhizobia in the greenhouse produced the highest nodule number although in the field, it was those inoculated with strain CIAT 899. Bush beans inoculated with USDA 2674 gave the highest total plant dry weight. Treatments within each of the cultivars showed differences in total plant dry weight yield in response to rhizobia strain used. Field experiments showed that plant dry weight differed statistically between cultivars at $P<0.05$. Bush beans inoculated with USDA 2674 gave the highest total plant dry weight but there were no significant differences in yield.

INTRODUCTION

Kenya accounts for about 9% of total bean production in Africa making it one of the leading producers (Baudoin *et al*., 2001). In Kenya, the common bean is the most commonly grown grain legume with production of over 414,000 metric tons per year (Abate and Ampofo, 1996). It comes second to maize as a subsistence crop and is a major source of protein in human diets. In Kenya, a major problem with common bean production is low yield. Yield average is between 400-600 kg per hectare (Stoorvogel *et al.,* 1993). As a result, Kenya like many other African countries has the unfortunate reputation of being chronically hungry and ever in need of food aid. According to the World Bank (2008), the demand for food in SSA will have to double by 2015 from its level in 2000 to avoid hunger hence the need to embrace sustainable agriculture. Sustainable agriculture involves the successful management of agriculture resources to satisfy changing human needs while maintaining or enhancing the environment quality and conserving natural resources (TAC, CGIAR, 1988, Kramer *et al.,* 2006 and Badgley *et al.,* 2007). It relies greatly on renewable resources, and on-farm nitrogen contributions are achieved largely through biological nitrogen fixation.

In the light of the aforementioned challenges, this research was designed to investigate Biological Nitrogen Fixation (BNF) in three bush beans namely Ayenew, GLP 24, and Ecab 0807 grown in the East African region. The objectives of this study were two fold:

1. To determine yield in the three bush bean cultivars in response to inoculation with different *Rhizobia* strains.
2. To identify the most effective *Rhizobium* strain in nitrogen fixation with the three bush bean cultivars.

MATERIALS AND METHODS

Field experiments

Field experiments were carried out at the research farm of Jomo Kenyatta University of Agriculture and Technology (JKUAT) at Juja in Central Kenya. The site receives an average annual rainfall of 850 mm in a bimodal pattern with over 55% of the total falling in the long rain season (March-June) and 45% in the short rain season (October-January). Temperatures are moderate ranging from 13 ^{o} C – 26 ^{o} C (Jaetzold and Schmidt, 1983).

Inocula

The *Rhizobium* inocula used was obtained from Microbiological Resource Centre (MIRCEN) of the University of Nairobi.

Greenhouse work

Greenhouse work was carried out to assess nodulation ability of the bean cultivars when inoculated with different rhizobia strains under controlled conditions. Nitrogen free plant nutrient solution was prepared as described by Somasegaran *et al.,* (1985). Bean seeds, which were undamaged, uniform in colour and size, were selected. They were surface sterilized by immersing them in 3% sodium hypochlorite. The seeds were rinsed with 8 changes of sterile distilled water, soaked in clean, sterile distilled water and allowed to imbibe it for 1 hour. The seeds were then transferred aseptically to 2% water agar plates with a spoon-shaped spatula. The plates with the seeds were incubated upside down for 3 days at 28 ^{o} C to enable the radicals to grow away from the water agar. Seedlings whose radicals had attained a length of 1-2 cm after incubation were considered ready for planting in the polythene bags filled with sterile vermiculite. In each bag, 50 ml of plant nutrient solution was added. A pair of flame-sterilized forceps was used to prepare two holes in the rooting medium in each bag. Seeds with radical length of 1-2 cm were then picked up with sterile forceps and placed two per hole with the radicals facing downwards. Inoculation of the seeds was carried out by pipetting 2 ml of inoculum onto the radical base. Each treatment with *Rhizobium* strain was replicated 4 times. The controls were left uninoculated. The seedlings were then covered with vermiculite and polythene bags arranged on greenhouse benches in a Complete Randomised Block Design (CRBD). After every four days, 50ml of nitrogen free plant growth media was added.

Harvesting in the greenhouse

The plants were harvested at flowering stage by uprooting twenty-eight days after emergence. During harvesting, the stems were separated from the roots and put in brown bags. The roots were then removed gently from the vermiculite by cutting open the polythene bag and putting them onto a course sieve. Rooting medium was washed from the roots using a gentle stream of water. All the nodules were carefully detached from the roots and their number recorded. The nodules were placed in reagent bottles for drying. Roots were then put in the same brown bags with the shoots. They were then dried at 70^{o} C to a constant dry weight. The total plant dry weights were determined using Sartorius weighing balance type H 160.

Field experiments

Field experiments were carried out to assess the effect of inoculation on the nodulation, dry weight yield and seed dry weight yield of the bean cultivars. The experimental design used was Complete Randomised Block Design (CRBD). The block was sub-divided into

60 plots measuring 2 m by 2 m. The treatments for each bean cultivar were as follows:

i) Not inoculated.
ii) Inoculated with CIAT 899.
iii) Inoculated with USDA 2674
iv) Inoculated with USDA 2676 and
v) Inoculated with CIAT 899, USDA 2674 and USDA 2676.

Plant spacing was 50cm by 10cm which is the recommended spacing.

Before planting, beans for the inoculated treatments were moistened with 5 ml of gum arabic solution to help inoculant carrier material (peat) to stick on the seeds. Inoculation with the respective rhizobia was carried out by addition of 1 gram of the peat based inoculant to the moistened seeds. Seeds were dressed with furadan (active ingredient 5% carbofuran) at the rate of 5g per kg of seed to control soil pests. Two seeds were planted per hole.

The plants were randomly sampled at flowering stage and at physiological maturity. At each stage, two plants were sampled per sub-plot. The plants were uprooted carefully to avoid root breakages. The nodules were carefully detached from the roots and their number per plant recorded. Each plant was then separated into roots and shoots and put in brown bags separately. At physiological maturity, all the pods were detached from each plant and their number recorded. The seeds were then removed from the pods and their number per plant also recorded. The nodules, shoots and roots were then oven dried in their brown bags at 70 ^{o}C to a constant dry weight. The dry weights were then determined and recorded. During the final harvest, the number of plants per plot was determined and seed yield in kilograms determined.

Laboratory experiments

Laboratory experiments were carried out to establish characteristics of rhizobia isolated from the bean cultivars. Yeast Extract Mannitol Agar (YEMA) was used to grow and culture root nodule bacteria. It was prepared according to Beck *et al*., 1993.

Ten nodules from the field were collected from each treatment at random. The intact nodules were surface sterilized by immersing them in 95% solution of calcium hypochlorite for three minutes. After this, they were rinsed in seven changes of sterile distilled water. They were left in the final rinse for one hour. The sterilized nodules were crushed with a pair of blunt-tipped forceps in a large drop of sterile water in a Petri-dish. A sterile inoculation loop full of the resulting suspension was streaked on Yeast Extract Mannitol Agar (YEMA) plates. The inoculated Petri-dishes were incubated at 28 ^{o}C for colonies to appear. The plates were observed daily for the appearance of colonies typical of rhizobia.

YEMA slants

YEMA slants in screw cap McCartney bottles were used for storage of pure rhizobia. Pure isolates were transferred to the screw cap McCartney bottles to form stock cultures and were stored at 4 ^{o}C in a refrigerator. Sub-culturing was carried out after every three months to avoid loss of viability of the isolates.

Biochemical tests

A number of morphological and biochemical tests were carried out to establish the characteristics of the rhizobia isolated from beans grown in the field. Various differential media were used. These included Gram's stain, Congo red, Bromothymol blue, Litmus milk and Peptone agar.

Data analysis

Data collected was subjected to two way analysis of variance (ANOVA) to test significance between the treatments. Means were separated using Least Significance Difference (LSD). Genstat version 6.8 statistical package was used.

RESULTS

Nodulation in the greenhouse

The number of nodules were significantly different ($P \leq 0.05$) in the three bush cultivars (Table 4.1). The variety with the lowest nodule number was GLP 24 (40.0 nodules on average). The number of nodules formed in response to inoculation also differed significantly ($P \leq 0.05$). Uninoculated plants produced the lowest nodule number (4.0 on average). Among the inoculated plants, beans inoculated with USDA 2676 produced the lowest nodule number (50.0 on average) and those inoculated with USDA 2674 the highest (70.0 on average). Among the different treatments within a cultivar, statistically significant differences in nodule number were noted at $P \leq 0.05$. The highest nodule number (90.0 ± 13.0 on average) in Ayenew cultivar resulted from inoculation with USDA 2674. In cultivar GLP 24 the highest nodule number (69.0 ± 21.0 on average) resulted from inoculation with CIAT 899 while multistrain inoculated cultivar Ecab 0807 produced the highest average nodule number of 93.0 ± 22.0 on average (Table 4. 1).

Total plant dry weight.

The total plant dry weight differed statistically at $P \leq 0.05$. The cultivar Ecab 0807 had the highest average dry weight of 1.0 mg per plant (Table 4.2). The different inoculant treatments within each cultivar gave statistically different average dry weights at $P \leq 0.05$. Multistrain inoculation in Ayenew gave the highest average dry weight of 1.0 mg ± 0.1. Among cultivar GLP 24 treatments, plants inoculated with USDA 2676 had the highest average dry weight of 0.9 mg ± 0.03 while in Ecab 0807 the highest total plant dry weight was in plants inoculated with USDA 2676 whose average dry weight was 1.2 mg ± 0.1. These results also confirmed that inoculation increased plant dry weights in the bean cultivars under controlled conditions of the greenhouse.

Biochemical tests

These results confirmed that the isolated bacteria were rhizobia and there was no contamination. The results also showed that there were different rhizobia populations that nodulated the bean cultivars in the study area as confirmed by acid and alkaline production.

The rhizobia isolated from the control treatments were the indigenous bacteria that the bean cultivars picked from the soil at the research site.

Field studies

Nodulation at flowering

Nodule number among the bush bean cultivars differed significantly at $P \leq 0.05$. Ayenew had the lowest average nodule number of 43.0 while GLP 24 and Ecab 0807 had averages of 59 and 74.0 nodules respectively. Beans inoculated with CIAT 899 had the largest number of nodules 77.0 on average and were statistically higher than all the other inoculant treatments. In Ayenew and GLP 24 cultivars, the use of different inoculants did not lead to significant differences ($P \leq 0.05$) in nodule number (Table 4.4). However, in cultivar Ecab 0807, inoculation with CIAT 899 resulted in significantly higher nodule number (128.0 ± 41.0) at $P \leq 0.05$ than those inoculated with USDA 2674, USDA 2676 or left uninoculated (Table 4.4). From these results, uninoculated Ayenew and GLP 24 cultivars produced more nodules than the inoculated treatments. This showed that the native soil rhizobia at the research site were more competitive than the inocula used. They were therefore able to form more nodules.

Total plant dry weight at flowering

There were no significant differences in total plant dry weight among the three bush bean cultivars at $P \leq 0.05$. Cultivars inoculated with USDA 2674 gave the highest average total plant dry weight of 8.5 mg and uninoculated cultivars had the lowest dry weight of 6.6 mg. Cultivar Ayenew inoculated with CIAT 899 produced the highest total plant dry weight of 8.8 mg ± 1.4. In cultivars GLP 24 and Ecab 0807, the highest dry weights were produced by beans inoculated with USDA 2674 and were statistically higher than the others at $P \leq 0.05$ (Table 4.5).

Among the inoculants used, USDA 2674 ,USDA 2676 and combined (in Ayenew) USDA 2676 (in GLP 24) and CIAT 899, USDA 2676 and Combined (in Ecab 0807) had lower total plant dry weights than the uninoculated plants. This meant that their nodules were not as effective in nitrogen fixation as those formed by the native soil rhizobia. However, it was also noted that inoculating bush bean cultivars with USDA 2674 generally gave higher plant dry weights which suggested that it was able to form more effective nodules than the other inoculant strains used. These results showed that the uninoculated plants produced higher seed dry weights in GLP24 and Ecab 0807. Uninoculated Ayenew produced the lowest seed dry weight though it was not statistically different from the other treatments. These results showed that there was no need of inoculation.

DISCUSSION

Results from the greenhouse indicated differences in nodulation between the three bean cultivars. The results also showed different cultivars preferred different rhizobia strains when grown under controlled conditions. The same observation was noted from the field results where bean cultivars inoculated with CIAT 899 produced the highest nodule number. Among cultivars Ayenew and GLP 24 treatments, there were no significant differences between the different rhizobia inoculants used. This was in contrast with Ecab 0807 treatments as those inoculated with CIAT 899 formed the highest nodule number. Uninoculated beans were also able to form as many nodules as the inoculated.

These results suggested that different cultivars of beans had preference for certain rhizobia and indigenous rhizobia were as good as some of the inoculants used. This supports observations by Graham, (1981); Kremer and Peterson, (1983), Pacovsky *et al.,* (1984) and Mostasso *et al.,* (2002), who noted that though high nitrogen fixing strains of *Rhizobium* have been identified, they often do not provide an agronomic benefit in the field because they are excluded from the nodules of the host plant by the soil indigenous strains which are often more competitive for nodulation than inoculant strains.

Total plant dry weight was used to estimate the rate of dry matter yield. No significant differences in dry matter yield in response to the inoculant used were noted among the three beans in the greenhouse and in the field. However, beans inoculated with USDA 2674 accumulated higher dry weights. Under the controlled conditions of the greenhouse, highest dry matter yield was noted among multistrain inoculated Ayenew. This was in contrast with cultivars GLP 24 and Ecab 0807 whose highest dry matter yield was from those inoculated with USDA 2676. In the field, the results were different as no significant differences were noted among Ayenew and GLP 24 treatments as opposed to Ecab 0807 whose highest dry weight was from multistrain inoculated beans. These results supported observations by Burgos *et al.,* (1999) and Aguilar *et al*., (2001), who noted that native rhizobia bacteria that exist in the fields, often out-compete the inoculant strains that only occupy a small proportion of nodules as observed in some areas of Latin America but contradicted the observation made by Hungria *et* *al.,* (2000) and Mostasso *et al.,* (2002), who noted that bean inoculation with *R. tropici* in Brazil was successful and *Rhizobium* inoculated onto beans enhanced their yields.

CONCLUSION

From this research, differences in nodulation between the three bush bean cultivars was noted. The bean cultivars preferred different strains of rhizobia both under controlled conditions and in the field. In the greenhouse, USDA 2674 inoculated beans produced the highest number of nodules while in the field; it was those inoculated with CIAT 899. The native soil rhizobia were found to be competitive and efficient as compared to the inoculants strains used. This therefore meant that inoculating the bean cultivars did not lead to significantly higher yields.

Table 1. Effect of inoculation on nodule number in the greenhouse

	Nodule number (greenhouse) in different beans (Mean ±SD)		
Inoculum	Ayenew	GLP 24	Ecab 0807
Control	7.0 ±5.0a	2.0 ±1.0a	4.0 ±3.0a
CIAT 899	55.0 ±10.0bc	69.0 ±21.0de	52.0 ±30.0bc
USDA 2674	90.0 ±13.0ef	42.0 ±9.0bc	80.0 ±23.0def
USDA 2676	54.0 ±16.0bc	35.0 ±8.0b	61.0 ±21.0cd
Combined	63.0 ±15.0cd	54.0 ±12.0bc	93.0±22.0f
$LSD_{(0.05)}$ 23.2			

CIAT 899, USDA 2674 and USDA 2676 are the rhizobia inocula used. Ayenew, GLP 24 and Ecab 0807 are bean cultivars used under the different treatments. Values followed by the same letters did not show statistical difference ($p<0.05$)

Table 2. Effect of inoculation on dry weight in the greenhouse

	Plant dry weight (mg) of bush beans (greenhouse) (Mean ± SD)		
Inoculum	Ayenew	GLP 24	Ecab 0807
Control	0.7 ±0.2ab	0.6 ±0.1a	0.8 ±0.1abcde
CIAT 899	0.8±0.1bcde	0.8 ±0.1abcde	0.9 ±0.2cde
USDA 2674	0.9 ±0.2de	0.8±0.1bcde	1.1 ±0.1ef
USDA 2676	0.7±0.1abc	0.9±0.03cde	1.2 ±0.1f
Combined	1.0 ±0.1e	0.8 ±0.1abcd	1.1 ±0.2ef
$LSD_{(0.05)}$ 0.2			

CIAT 899, USDA 2674 and USDA 2676 are the rhizobia inocula used. Ayenew, GLP 24 and Ecab 0807 are bean cultivars used under the different treatments. Values followed by the same letters did not show statistical difference ($p<0.05$)

Table 3. Biochemical reactions of the isolated rhizobia from the field

Isolate from treatment	Congo red	Bromothymol blue	Milk litmus	Gram stain	Peptone agar
b1 Control	X	Y	P	-ve	-
b1 CIAT 899	X	Y	P	-ve	-
b1 USDA 2674	X	Y	P	-ve	-
b1 USDA 2676	X	Y	P	-ve	-
b1 Combined	X	B	B	-ve	-
b2 Control	X	Y	P	-ve	-
b2 CIAT 899	X	Y	P	-ve	-
b2 USDA 2674	X	Y	P	-ve	-
b2 USDA 2676	X	Y	P	-ve	-
b2 Combined	X	Y	P	-ve	-
b3 Control	X	Y	P	-ve	-
b3 CIAT 899	X	Y	P	-ve	-
b3 USDA 2674	X	Y	P	-ve	-
b3 USDA 2676	X	Y	P	-ve	-
b3 Combined	X	Y	P	-ve	-

b1 refers to cultivar Ayenew, b2 GLP 24 and b3 Ecab 0807. Each bean received five treatments (uninoculated, inoculated with CIAT 899, USDA 2674, USDA 2676 and the three inoculants combined). B is blue colour, P is pink colour, X is pale/Translucent colony and Y is yellow colour. (-) is lack of growth and (-ve) is Gram negative

Table 4. Effect of inoculation on nodule number in the field

	Nodule number in bush bean varieties (Mean ±SD)		
Inoculum	Ayenew	GLP 24	Ecab 0807
Control	51.0 ±9.7abcd	66.0 ±15.0bcd	45.0 ±7.0abc
CIAT 899	31.0±11.0ab	72.0 ±29.0cd	128.0 ±41.0e
USDA 2674	45.0 ±25.0abc	51.0 ±27.0abcd	47.0 ±14.0abc
USDA 2676	30.0±17.0a	58.0 ±15.0abcd	66.0 ±46.0cd
Combined	58.0 ±24.0abcd	49.0 ±22.0abcd	84.0 ±23.0d
$LSD_{(0.05)}$ 35.1			

Note: First column indicates rhizobia inocula used while columns two, three and four indicate nodule numbers on the three bean cultivars as influenced by the different treatments. Values followed by the same letters did not show statistical difference ($p<0.05$)

Table 5. Effect of inoculation on plant dry weight in the field

	Plant dry weight (mg) in bush beans grown in the field (Mean ± SD)		
Inoculum	Ayenew	GLP 24	Ecab 0807
Control	7.7 ± 1.8abcd	5.6±1.5a	6.7 ±0.9abc
CIAT 899	8.8± 1.4cd	6.3±0.8ab	6.0±1.0a
USDA 2674	7.5±1.4abcd	8.6 ±1.7bcd	9.3±3.1d
USDA 2676	6.2 ±1.7ab	5.3±2.5a	6.1±1.1a
Combined	6.5 ± 1.7abc	7.8 ±1.0bcd	6.1±3.2a
$LSD_{(0.05)}$ 2.4			

First column indicates rhizobia inocula used while columns two three and four indicate dry weights of the three bean cultivars when inoculated with different rhizobia. Values followed by the same letters did not show statistical difference ($p<0.05$).

REFERENCE

Abate, T. and Ampofo, J.K.O. (1996). Insect pests of beans in Africa. Their Ecology and Management. *Annual Reviews of Entomology 1996* 41: 45-73.

Aguilar, O.M., Lopez, M.V. and Riccillo, P.M. (2001). The diversity of rhizobia nodulating beans in North West Argentina as a source of more efficient inoculant strains. *Journal of Biotechnology* 91: 181-188.

Badgley, C., Moghtader, J., Quintero, E., Zakem, E., Chappell, M.J., Avilés-Vàzquez, K., Samulon, A., Perfecto, I. (2007). Organic agriculture and the global food supply. *Renewable Agriculture and Food Systems* 22, 86-108.

Baudoin, P.,Vanderborght, T. ,Kimani, P.M. and Mwangombe, A.W.(2001).Crop Production in

Tropical Africa. In: Directorate general for international co-operation. Ministry of Foreign Affairs, External Trade and International Co-operation. Kluwer Academic Press , Dordrecht Brussels-Belgium.

Beck, D.P., Materon, L.A. and Afandi, F. (1993). Practical *Rhizobium* legume technology manual. Manual No. 9. International Centre for Agricultural Research in the Dry Areas, Allepo.

Burgos, P.A.,Castellanos, J. Mora,Y. and Mora, J. (1999). Field inoculation of common bean (*Phaseolus vulgaris* L.) with high efficiency *Rhizobium* strains. In Highlights of Nitrogen Fixation Research. Eds E. Martinez and G. Hernandez. Pp. 255-257. Kluwer Acandemic/Plenum publishers, New York.

Graham, P.H. (1981). Some problems of nodulation and symbiotic N2 fixation in *Phaseolus vulgaris* L. *Field Crops Research* 4: 93 - 112.

Hungria, M. Vargas, M.A.T., Campo, R.J., Chueire, L.M.O. and Andrade, D. de S. (2000). The Brazilian experience with the soybean (*Glycine max*) and the common bean (*Phaseolus vulgaris*) symbioses. In Nitrogen Fixation: From Molecules to Crop Productivity. Eds. F.O. Pedrosa, M. Hungria, G. Yates and W.E. Newton. Pp. 515. Kluwer Academic Publishers, Dordrecht, the Netherlands.

Jaetzold, R. and Schmidt, H. (Eds) (1983). Farm management handbook of Kenya, Vol. 11/C. Kenya. National conditions and farm management information. Ministry of Agriculture GAT Nairobi and GTZ Eschborn, Rossdorf.

Kramer, S. B. , Reganold, J. P., Glover, J. D., Bohannan, B. J. M., Mooney, H. O. (2006). Reduced Nitrate Leaching and Enhanced Denitrifier activity and Efficiency in Organically Fertilized Soils. Proceedings of the National Academy of Sciences of The USA 103, 4522-4527.

Kremer, R.J. and Peterson, H. L. (1983). Field evaluation of selected *Rhizobium* in an improved legume inoculant. *Agronomy Journal* 75: 139-143.

Mostasso, L. Mostasso, F.L. Dias, B.G., Vargas, M.A.T. and Hungria, M.(2002). Selection of bean (*Phaseolus vulgaris* L.) rhizobial strains for the Brazillian Cerrados. *Field Crop Research*. 73: 121-132.

Pacovsky, R.S., Bayne, H.G. and Bethlenfalvay, G. J. (1984). Symbiotic interactions between strains of *Rhizobium phaseoli* and cultivars of *Phaseolus vulgaris* L. *Crop science* 24: 101-105.

Somasegaran, P., Hoben, H. and Halliday, J. (1985). The NifTAL manual for methods in legume-*Rhizobium* technology.

Stoorvogel, J.J., Smalling, E.M.A. and Janssen, B.H. (1993). Calculating soil nutrient balances in Africa at different scales. In: An Agro-ecological framework for integrated nutrient management with special reference to Kenya. PhD Thesis (E.M.A. Smalling). Agricultural University, Wageningen, pp 250.

TAC, CGIAR. (1988). Sustainable agricultural production: Implications for international agricultural research. CGIAR (Consultative-Group on International Agricultural Research) meeting, Berlin.

World Bank (2008). World Development Report 2008. The World Bank, Washington DC, USA.

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