Collection and Utilization of Germplasm of Cowpea Resistant to *Striga* and *Alectra*

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Abstract

Cowpea is a major food legume in the tropics particularly in sub-Saharan Africa which grows over 75% of the world cowpea area. However, the average yield is very low. Among several yield-reducing factors, the two parasitic weeds, *Striga gesnerioides* (Wild.) Vatke and *Alectra vogelii* Benth. cause substantial damage to cowpea. Therefore, a systematic breeding program has been initiated to develop varieties with combined resistance to *Striga* and *Alectra*. Screening through germplasm lines led to the identification of several sources of resistance to both parasitic weeds which are used for genetic studies and the breeding program. Of these, B 301, a land race from Botswana has shown complete resistance to *Striga* as well as *Alectra*. It was crossed to a susceptible variety IT 84 S-2246-4 which is otherwise resistant to aphids, bruchids and thrips and several diseases with high yield potential. The F_1 plants were backcrossed to IT 84 S-2246-4 and systematic selection and testing of segregating populations enabled to define the mode of inheritance as well as to develop new varieties similar to IT 84 S-2246-4 but with added resistance to *Striga*, and *Alectra*. Screening of 1600 new germplasm lines has provided other sources of resistance which are being used to diversify the genetic base for resistance to *Striga* and *Alectra* in cowpea.

Introduction

Cowpea [*Vigna unguiculata* (L.) Walp] is an important food legume throughout the tropics including Asia, the Far East, Africa, Central and Southern America as well as Southern U.S.A. As it is warm weather crop telerant to drought, it is grown between 20° N to 20° S latitudes particularly in the semi-arid and low rainfall regions. It is consumed as dry seeds, green pods, green seeds, and tender green leaves as spinach. The haulms are fed to cattle and other household animals. Due to its ability to fix atmospheric nitrogen and to its deep root system, it improves the soil fertility. Cowpea is a major crop in Africa where it is grown in mixture with millet, sorghum and maize and forms an integral part of traditional cropping systems. Of the world total area of about 8 million hectares, Africa alone accounts for about 6 million. However, the average yield of cowpea in Africa is very low ranging from 100-300 kg/ha due to numerous biotic and abiotic constraints. Among several yield-reducing factors, two parasitic weeds, *Striga gesnerioides* (Wild.) Vatke, and *Alectra vogelii* (Benth.) of the family Scrophulariacea cause considerable damage to cowpea in the semi-arid regions of Africa. Presently striga is more prevalent in the Sudano-Sahelian belt and *alectra* is more serious in Guinea savanna, but both are rapidly spreading beyond these limits. *Striga* incidence has been recorded in the coastal savanna of Benin Republic and *alectra* is becoming a serious threat in several East and southern African countries particularly Kenya, Zambia, Zimbabwe and Botswana. Total yield loss is observed in heavily infested fields. These parasites are difficult to control by chemical and/or cultural methods due to the large amount of seeds produced by them and their adaptation/dormancy mechanism which allows seeds to stay alive in the soil for several years. Therefore, a major component of a long-lasting control package for these parasitic weeds would be genetic control

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through host plant resistance. International Institute of Tropical Agriculture (IITA) has a worldwide mandate for cowpea improvement and therefore, its present goal is to develop improved cowpea varieties with inherent resistance to *striga* and *alectra*.

Screening cowpea germplasm for sources of resistance

Over the last 20 years, the Genetic Resources Unit (GRU) of IITA has collected a large number of cowpea lines from different parts of the world. At present the total collection comprises about 15,200 lines from over 72 countries (NG, 1990, Table 1). This germplasm base has been used to identify sources of resistance to several diseases, insect pests and viruses as well as for desirable plant traits and seed characteristics.

Initial screening for *striga* resistance was performed by IITA scientists based at Kamboinse, Burkina Faso where a total of 54 cowpea lines were planted in a heavily infested field. Subsequently pot culture technique was developed both for striga and alectra (Emechebe *et al*., 1991) and used for controlled studies. A combination of field and pot culture screening has led to the identification of several resistant sources and also promoted genetic studies for the identification of genes responsible for resistance to *striga* and *alectra* and allelic relationships among different genes (Fig. 1).

Field screening

Most of the experimental fields at IITA Kano Station are infested with *striga* and *alectra*. One of these fields (0.5 ha) was selected and developed as *striga* infested plot by evenly spreading 20 bags of mature *striga* and 10 bags of mature *alectra* plants in it and incorporating them in the soil by repeated harrowing about 3 weeks before planting. This procedure is being applied for field screening. An additional amount of inoculum is added each year. Sick plots have also been developed/identified in Burkina Faso, Mali, Niger Republic, Nigeria and Benin Republic in collaboration with the national programs. The test lines are planted in these plots along with known susceptible varieties and data on number of emerged *striga/alectra* are taken from 5-6 weeks after planting. The number of days required to the first emergence of *striga/alectra* in each line are recorded and then weekly counts are made to study the pattern of *striga/alectra* emergence. Seeds of lines free from parasitic weeds and those showing delayed and a lower level of emergence in the field are further tested using the pot culture technique in a screenhouse.

Pot culture screening

Plastic pots 13 cm in diameter and 13 cm in depth are used for screening. Each pot contains about 1 ℓ of unsterilized sieved sand and top soil (sandy loam) mixture (1 : 1 v/v) previously inoculated uniformly with about 800 seeds of *striga* or *alectra*. The pots are kept on benches in a screenhouse and planted with test cowpea populations with two plants per pot. The pots are watered daily and weeds other than *striga* and *alectra* are removed. Emergence of *striga* and *alectra* plants in pots containing susceptible plants normally begins from 6 weeks after planting. The experiments are terminated 10 weeks after planting when the differences between resistant and susceptible plants become significant. The level of *striga* and *alectra* infection is determined by observing the attachments of *striga* and/or *alectra* on roots of each cowpea plant. The soil is washed off the plant roots after submerging each pot in a 20 ℓ bucket of water for about 5 min. The roots of each plant are gently separated from one another and the number of *striga* and /or *alectra* plants attached to each plant are counted. Plants showing attachment and healthy development of these parasitic weeds are classified as susceptible and those free of infection or showing only a small number of *striga/alectra* plants are grouped as resistant.

Sources of resistance

The initial lead on cowpea *striga* resistance was associated with the studies carried out by the IITA scientists based at Kamboinsé, Burkina Faso under the IITA/IDRC (International Development Research

Table 1 Cowpea germplasm collection at the Genetic Resources Unit of IITA, and geographical distribution*

Region/Countries	No. of accessions	Region/Countries	No. of accessions
Central and Western Africa		Indonesia	4
Burkina Faso	222	Israel	8
Cameroun	600	Iran	29
Central African Republic	183	Japan	2
Chad	268	Laos	1
Gambia	4	Nepal	3
Ghana	282	Pakistan	7
Guinea	2	Papua New Guines	12
Côte d'ivoire	134	Philippines	114
Liberia	9	Sri Lanka	2
Mali	293	Syria	8
Niger	976	Thailand	1
Nigeria (+IITA)	3,221	Turkey	47
People's Republic of Benin	331	USSR	42
Senegal	290	**America**	
Sierra Leone	13	Argentina	1
Togo	103	Brazil	17
Zaire	15	Canada	182
Eastern and Southern Africa		Columbia	2
Angola	1	Cuba	1
Botswana	1	El Salvador	1
Congo (Brazzaville)	47	Guatemala	11
Ethiopia	7	Honduras	1
Kenya	155	Jamaica	
Madagascar	34	Mexico	23
Malawi	401	Nicaragua	2
Lesotho	42	Paraguay	12
Swaziland	19	Peru	3
Somalia	95	Surinam	14
Tanzania	443	USA	828
Uganda	71	Venezuela	3
Zambia	587	**Europe**	
Zimbabwe	158	UK	282
North Africa		Hangary	36
Algeria	1	Portugal	5
Egypt	374	Italy	78
Asia		**Others**	
Afghanistan	65	Australia	23
Bangladesh	1	Unknown	623
China	35	Mixed	630
India	2,075		
		Total	15,200

*Ng. 1990

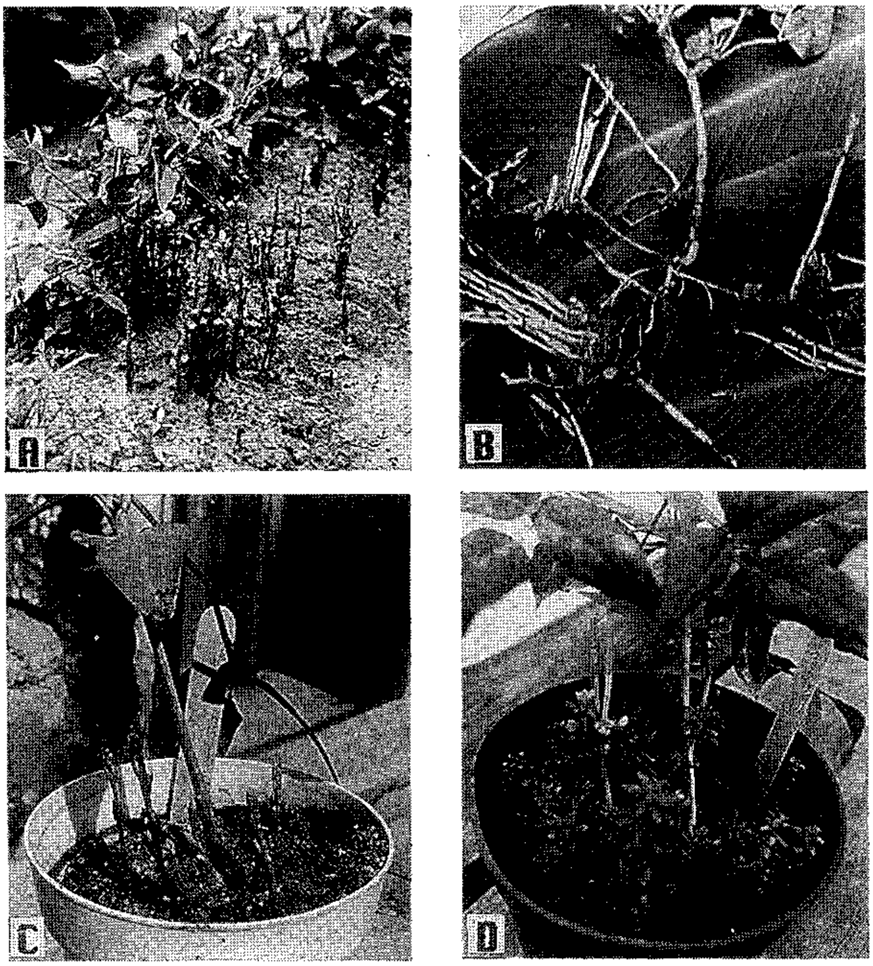

Fig. 1 Field and pot culture screening : A. *Striga* in the field ; B. *Striga* attachment on susceptible roots ; C. pot culture of *Striga* ; D. pot culture of *Alectra*

Centre, Canada)/Burkina Faso and IITA/SAFGRAD (Semi-arid Food Grain and Development, Organization of African Unity) project. Field screening of 54 cowpea varieties at Kamboinsé in 1981 indicated that two varieties, 'Gorom Local' from Burkina Faso and '58-57' from Senegal were resistant to *striga* (IITA, 1982). These two varieties showed minimal or no *striga* emergence compared to a large number of emerged *striga* on other varieties. These resistant varieties along with other breeding lines were then evaluated under the IITA/SAFGRAD project in many locations in Burkina Faso, Mali, Republic of Niger, Cameroon and Nigeria during the period 1983-86 to determine the stability of *striga* resistance across West African savanna. Unfortunately, 'Gorom local' and '58-57' showed a high level of resistance to *striga* only in Burkina Faso but were susceptible in other countries indicating the presence of different strains (Aggarwal, 1985). Therefore, the identification of additional sources of resistance continued and two new resistant sources, B 301 and IT 82 D-849, were indentified in 1987. They showed stable resistance to *striga* across Burkina Faso, Mali, Rep. of Niger and Nigeria (Aggarwal, 1991 ; Emechebe *et al*., 1991). B 301, a local germplasm line from Botswana was initially found by Riches (1987) to be resistant to *Alectra vogelii* in Botswana. In addition to these two lines, several lines have also been identified which were parasitized by a smaller number of *striga* plants and showed delayed emergence of *striga* (Singh and Emechebe, 1991). Some of these lines are listed in Table 2. IT 86 D-534, IT 86 D-371 and IT 84 D-666 are moderately resistant to *striga* and highly resistant to *alectra* whereas B 301 is completely resistant to both. IT 82 D-849 is completely resistant to *striga* but susceptible to *alectra*. Suvita-2, which is resistant to *striga* in Burkina Faso, is mderately susceptible to *striga* in Nigeria but highly susceptible to *alectra*. Among the lines highly susceptible to *striga*, some are also susceptible to *alectra*. These data indicate that the yield loss

Table 2 Performance of cowpea lines under *striga* and *alectra* infestation in field (Kano, 1989)*

Variety	Number of			Yield kg/ha Kano
	Days to 50% *striga* infection	Parasitic weeds per plot*		
		striga	*alectra*	
IT 86D-534	66	135	1	656
B 301	—	0	0	599
Suvita-2	46	98	110	413
IT86D-472	66	56	0	559
IT86D-371	50	160	3	428
IT84D-666	50	92	0	410
IT82D-849	—	0	63	292
IT82D-957	35	324	20	35
IT86D-843	43	362	25	70
Vita-3	34	439	3	35
LSD-5%	11	196	20	228

* Average of 4 plots, 6 m^2 each. Singh and Emechebe (1991)

due to parasitic weeds is significant and that breeding for *striga* resistance alone in cowpea is not enough as *alectra* can then cause severe damage, as evidenced by the performance of IT 82 D-849 and Suvita-2. Therefore, resistance to both parasitic weeds must be incorporated into improved varieties.

Manifestation of resistance in different cowpea varieties

Field and pot culture studies have revealed major difference in the expression of resistance in different varieties. Lack of emergence or delayed and minimal emergence are noticed in resistant and moderately resistant lines compared to the severe infestation of susceptible lines (Fig. 2). In the pot culture tests, the two- and four-week washing of B 301 roots indicated that this line stimulates the germination of *striga* as well as of *alectra* seeds and promotes the attachment which haustorial formation and further growth are inhibited. The parasite primordia subsequently die and distintegrate so that B 301 roots appear as if they had never been infected, suggesting the presence of a hypersensitive type of reaction. Lane *et al*. (1991) reported a similar observation from *in-vitro* culture studies on the mechanisms of resistance to *striga* in B 301. The expression of resistance to *striga* in IT 82 D-849 is slightly different from that of B 301. This line also promotes *striga* seed germination and attachment and inhibits haustorial development as B 301 but about 10% of the plants show some haustorial development and support limited *striga* growth with occasional emergence of 1 or 2 *striga* plants which are very weak and die before reproductive maturity. However, unlike B 301, IT 82 D-849 is highly susceptible to *alectra* permitting normal attachment and growth of *alectra* plants. The variety IT 81 D-994 is moderately resistant to *striga* and *alectra*. It allows the establishment of a few *striga* and *alectra* plants (3 to 5/plant) but delays their emergence. The emerged *alectra* plants are weak and seldom reach maturity. However, few *striga* plants do reach maturity but cause little damage to the plants. The reactions of Suvita-2 to *striga* from Burkina Faso and *alectra* are similar to that of IT 82 D-849. However, the cultivar is susceptible to the *striga* strain from Nigeria.

Genetics of resistance to *striga* and *alectra*

Good progress has been made in elucidating the genetics of resistance to *striga* and *alectra* in cowpea. The initial studies on the mode of inheritance of *striga* resistance in Suvita-2 indicated a simple mode of iheritance (Aggarwal *et al*., 1984). Singh and Emechebe (1990 a) revealed the presence of a single domi-

Fig. 2 A. Pot culture screening: Resistant to *alectra* (left), moderately resistant to *alectra* (middle); susceptible to *alectra* (right), B. Improved cowpea variety with combined resistance to *striga* and *alectra*

nant gene 'Rsg' which is responsible for resistance to *striga* in B 301. They also observed duplicate dominant genes for resistance to alectra in B 301 (Singh and Emechebe, 1990 b; Singh *et al*., 1993). The genes for *striga* and *alectra* resistance in B 301 are independent and segregate into 45 : 15 : 3 : 1 in F_2, (Atokple *et al*., 1993). The resistance to *striga* in IT 82 D-849 is also controlled by a single dominant gene which, based on the test of allelism was found to be different from that in B 301. The source of resistance in IT 82 D-849 is derived from its parent Emma 60, a line from Uganda.

Breeding for resistance to *striga* and *alectra*

Systematic breeding program for resistance to *striga* and *alectra* using B 301 as resistant source was undertaken in 1987. Since this landrace from Botswana has very small seeds and shows a prostrate growth habit with late maturity, it is not suitable for West Africa. This resistant line was crossed to a susceptible variety, IT 84 S-2246-4 which is otherwise a high-yielding variety with resistance to aphids, bruchids, thrips and several diseases. The F_1 was backcrossed to IT 84 S-2246-4 and the resistant BC_1 F_1

plants were grown in a green house to maturity in 1988. The BC_1 F_2 families were planted at Ibadan in the off season and a large number of plants with desirable agronomic characters were selected at maturity and threshed individually. The selected BC_1 F_3 progenies were then screened for resistance in 1989 at Kano in a field heavily infested with *striga* and *alectra*. Individual plants were again selected based on the resistance as well as on agronomic characters. The selected BC_1 F_4 progenies were then multiplied at Ibadan in the off season. Individual F_4 plants were again selected based on agronomic characters. The selected BC_1 F_5 progenies were then screened in 1990 at Kano in the field as well as at Samaru in pot culture. The remaining seeds of the selected F_4 and F_5 lines were tested for resistance to aphids and bruchids. Several F_6 breeding lines were then selected which were very similar to IT 84 S-2246-4 and exhibited combined resistance to aphids, bruchids, thrips, *striga* and *alectra* and several diseases (Singh and Emechebe, 1991). They have been evaluated for yield and other characters in replicated trials in the 1991-92 cropping seasons, and have performed well (Table 3, Fig. 2). The yield of the *striga*-resistant breeding lines is much higher than that of IT 84 S-2246-4 which was used as a genetic base for improvement. These lines have been distributed to various natinal programs in Africa. In the meantime, these lines are being used as parents in crossing programs involving local varieties and other selected parents in order to develop a range of varieties differing in plant type, maturity and seed characteristics that are suitable for different cropping systems and regional preferences.

New sources of resistance

Due to the strain diversity of *striga*, it is desirable to secure genetically diverse sources of resistance so that stable resistance can be developed in new improved cowpea varieties. Therefore, 1,600 cowpea germplasm lines were screened in 1992 in the field at IITA Kano station. Each line was planted in a *striga* infested plot in 3 m long rows which were 1.5 m apart. Two plants per hill were maintained within the rows with a hill to hill distance of 20 cm. The number of days required to the first *striga* emergence and the number of emerged *striga* plants per plot were recorded each week, 5 weeks after planting. At maturity, 104 lines remained free from *striga* infestation. These lines are being further tested in a screenhouse using pot culture technique and also multiplied for multi-location testing and for genetic studies to determine whether they harbour the same genes as B 301 and 849 or different ones.

Future prospects

The recent work on breeding for resistance to parasitic weeds in cowpea has indicated that it may be possible to develop improved varieties with complete resistance to *striga* as well as *alectra*. Availability of diverse sources of resistance and moderately resistant lines may enable to broaden the genetic base of resistance which will ensure stability of resistance in time and space. There is a need to study the nature

Table 3 Performance (kg/ha) of *striga* resistant cowpea varieties at different locations in Nigeria 1991

Variety	Kano	Gumel	Maiduguri	*Striga* reaction*
IT90K-59-5	1289	1653	1763	1
IT90K-59-3	1055	1544	1171	1
IT90K-101-1	1164	1081	1117	2
IT90K-102-6	1089	1657	1027	2
IT90K-82-2	1104	1320	778	1
IT90K-76-7	1114	1106	976	1
IT84S-2246-4	1028	583	733	4
LSD 5%	337	474	475	

*1 = completely resistant 5 = Highly susceptible of the resistance and chemical factors involved. Such studies will provide a deeper understanding of the mechanism of resistance and also may provide a chemical tag for fast screening against these parasitic weeds which will facilitate breeding programs and accelerate the pace of progress.

References

1) Aggarwal, V. D. (1985): Cowpea *striga* Research p. 335-340. In: Singh, S. R. and Rachie K. O. (Eds). Cowpea Research, Production and Utilization. John Wiley and Sons.

2) Aggarwal (1991): Research on cowpea striga resistance at IITA p. 90-95. *In*: Kim, S. K. (Ed). Combating striga in Africa. Proceeding of the International workshop organised by IITA, ICRISAT and IDRC 22-24 Aug. 1988. IITA Ibadan, Nigeria.

3) Aggarwal, V. D., Muleba, N., Drabo, I., Souma, J. and Mbewe, M. (1984): Inheritance of striga *gesnerioides* resistance in cowpea. p. 143-147. *In*: Parker, C., Musselman, L. J., Polhill, R. M. and Wilson, A. K. (ed.) Proceedings of 3 rd International Symposium on Parasitic Weeds. ICARDA, Aleppo, Syria, 7-11 May, 1984.

4) Atokple, I. D. K., Singh, B. B. and Emechebe, A. M. (1993): Independent inheritance of striga and alectra resistance in cowpea genotype B 301. Crop. Sci. 33: 000-000. (In press).

5) Balley, J. A. and Terry, P. J. (1990): Resistance of cowpea to striga *gesnerioides* and alectra *vogelii*, X 0075. progress report. Long Ashton College, Bristol, U. K.

6) Emechebe, A. M., Singh, B. B. Leleji, O. I. Atokple, I. D. K. and Adu, J. K. (1991): Cowpea striga problems and Research in Nigeria. p. 18-28. *In*: Kim, S. K. (ed.). Combating striga in Africa. Proceeding of the International workshop organised by IITA, ICRISAT and IDRC. 22-24, Aug. 1988. IITA, Ibadan, Nigeria.

7) IITA (1982): International Institute of Tropical Agriculture. Screening for resistance to striga. p. 148. Annual Report for 1981, IITA, Ibadan, Nigeria.

8) Lane, J. A., Bailey, J. A. and Terry, P. J. (1991): An in vitro growth system for studying the parasitism of cowpea (*Vigna unguiculata*) by striga *gesnerioides*. Weed Research, 31, 211-217.

9) NG, N. Q. (1990): Recent developments in cowpea germplasm collection, conservation, evaluation and research at the genetic resources unit: IITA p. 13-20. *In*: NG, N. Q. and L. M. Monti (Eds.). Cowpea Genetic Resources. IITA, Ibadan, Nigeria.

10) Riches, C. R. (1987): The identification of resistance to alectra *vogelii* Benth. (Scrophulariaceae) in cowpea. p. 701-708. *In*: H. C. Weber and Forstreuter, (ed.). Parasitic flowering plant. Proceedings of the 4 th International Symposium on Parasitic Seed Plants. Philips University, Marburg Germany. Aug. 1-7, 1987.

11) Singh, B. B. and Emechebe, A. M. (1990 a): Inheritance of striga resistance in cowpea genotype B 301. Crop Science, 30, 879-881.

12) Singh, B. B. and Emechebe, A. M. (1990 b): Combined resistance to striga and alectra in cowpea. Agronomy Abstracts p. 109-110.

13) Singh, B. B. and Emechebe, A. M. (1991): Breeding for resistance to striga and alectra in Cowpea. p. 303-305. *In*: Ransom, J. K., Musselman, L. J., Worsham, A. D. and Parker, C. (ed.). Proceedings of the 5 th International Symposium on Parasitic Weeds, Nairobi, Kenya. 24-30 June, 1991.

14) Singh, B. B., Emechebe, A. M. and Atokple, I. D. K. (1993): Inheritance of alectra resistance in cowpea genotype B 301. Crop Sci., 33, 70-72.

Discussion

Matsunaga, R. (Japan): 1. Are the erect-type of varieties suitable for the farmers? 2. Is there any difference in the extent of *striga* damage between monocropped and intercropped cowpea varieties?

Answer: 1. The erect-type of variteies are more suitable for pure cropping and the spreading ones for intercropping. We are developing a range of varieties with different plant types to suit the needs of the farmers in various countries. 2. Monocrop cowpea permits rapid build-up of *striga* compared with intercrop cowpea, mainly due to the fact that there is a larger number of cowpea plants in pure corpping.

Ganashan, P. (Sri Lanka) : Did you study the resistance mechanism to both *striga* and *alectra* in the cowpea landrace B 301 from Botswana?

Answer : The resistance may be due to a mechanism of hypersensitivity. It is an acute phenomenon. B 301 stimulates the germination of *striga* seeds and attachment but inhibits haustorial development. Further work on the mechanism of resistance is being carried out at Long Ashton College, UK.

Fujimaki, H. (Japan) : The genetic diversity of cowpea is considerable. Is this wide diversity covered by a single botanical species?

Answer : Cowpea taxonomy is very complex but most of the cultivated cowpea varieties belong to one species, *Vigna unguiculata* (L.) Walp. The cowpea germplasm maintained at IITA displays a great deal of genetic diversity and all belongs to that species.

Rana, R. S. (India) : What is the mechanism of bruchid resistance in cowpea?

Answer : We do not know the mechanism of resistance. Initial studies carried out in UK suggested that the level of trypsin inhibitor was increased. These findings have not been confirmed by researchers at Purdue University.