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Virulence characteristics of a new race of the parasitic angiosperm, *Striga gesnerioides*, from southern Benin on cowpea (*Vigna unguiculata*)

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Abstract

An *in vitro* growth system was used to determine the virulence of two samples of *Striga gesnerioides* from Zakpota in southern Benin. Cowpea variety B301, previously considered resistant to all races of *S. gesnerioides*, was susceptible to both samples of the parasite. Two other cowpea varieties, 58–57 and IT81D–994, were totally resistant. Resistance in 58–57 was associated with a hypersensitive necrosis of infected roots, whilst IT81D–994 supported production of small *S. gesnerioides* tubercles with stems which failed to develop. *Striga gesnerioides* from southern Benin is the fourth race of the parasite to be identified, and the first with virulence on variety B301. The implications for breeding cowpeas with resistance to *S. gesnerioides* are discussed.

Abbreviations: IITA – International Institute of Tropical Agriculture, LARS – Long Ashton Research Station, SAFGRAD – Semi-Arid Food Grain Research and Development.

Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.] is a legume grown widely in the semi-arid regions of Africa for food grains and fodder for animals. Approximately 60 per cent of world cowpea production occurs in West Africa, reflecting the importance of cowpea as a major source of dietary protein in these regions (Rachie, 1985). The parasitic angiosperm, *Striga gesnerioides* (Willd.) Vatke infests cowpea causing yield reductions in the order of 30–50 per cent (Aggarwal & Ouedraogo, 1989). Resistant varieties are probably the most appropriate way for subsistence farmers to control *S. gesnerioides* (Parker, 1991; Lane & Bailey, 1992). The use of most resistant cowpea varieties is limited to certain countries due to the existence of races of *S. gesnerioides* which vary in virulence (Aggarwal, 1991). Cowpea variety 58–57 is resistant to *S. gesnerioides* only in Burkina Faso (Aggarwal, 1991), whereas variety IT81D–994, bred for bruchid resistance by IITA, is resistant in Burkina Faso and Mali (B. Dembele, personal communication). Both cowpeas are susceptible to *S. gesnerioides* in Niger and Nigeria (Aggarwal, 1991). Three races of *S. gesnerioides* have been identified; one from parasite samples collected in Burkina Faso, another from Mali and a third from Niger and Nigeria (Parker & Polniaszek, 1990). However, one cowpea variety, B301, has been found to be completely resistant to *S. gesnerioides* throughout W. Africa (Parker & Polniaszek, 1990). Its resistance is due to a single dominant gene (Singh & Emechebe, 1990) and it is being widely used in national and international breeding programmes. Advanced breeding material (F_6 progeny) incorporating the B301 resistance gene has been recently evaluated in Nigeria (Singh & Emechebe, 1991).

There have been several reports of 'breakdown of resistance' in variety B301. For example, B301 was parasitised by *S. gesnerioides* at Bakura in Nigeria (Parker, 1991). However, the susceptibility of B301 at Bakura was later shown to be due to the use of impure B301 seed (Lane & Bailey, 1992). Also, in 1990, several B301 plants were observed to be infested with *S. gesnerioides* in a field trial at Zakpota in southern Benin; in the following year, about 25 per cent of B301 plants were susceptible at this site (J. Detongnon & N. Muleba, personal communication). The present research aimed to determine the pattern of virulence of *S. gesnerioides* from Zakpota on B301 and on three other cowpea varieties known to differ in their resistance to the three races of *S. gesnerioides*. An *in vitro* growth system, developed to study the infection process of *S. gesnerioides* on resistant and susceptible cowpeas, was used (Lane & Bailey, 1991; Lane et al., 1991, 1993).

Materials and methods

Source and growth of plants

Seeds of cowpea cv. Blackeye were from the USA, those of variety IT81D–994 from IITA in Nigeria and variety 58–57 from SAFGRAD/IITA in Burkina Faso. Three samples of B301 were used: one was propagated at LARS from the original seed source supplied by the Department of Agricultural Research in Botswana while the other two were obtained from IITA, Nigeria and SAFGRAD/IITA, Burkina Faso. Seeds of B301 from Burkina Faso were used unless otherwise stated.

Cowpea seeds were grown in moist Vermiculite for six days in a Fisons F600H growth cabinet at 30/25 °C (light/dark temperature), 67% RH with a 16 h daylength. Photosynthetic photon flux density was 180 μmol m^{-2} s^{-1}.

Seed of *S. gesnerioides* was collected from a field trial at Zakpota (about 12 km north of Abomey) in southern Benin in 1990. One small sample (LARS accession no. 90–75) came from parasitised cowpea plants of variety B301 and another larger sample (LARS accession no. 90–74) came from an unknown cowpea variety. *Striga gesnerioides* seeds were surface sterilized, washed and imbibed for 19 d in the growth cabinet (Lane et al., 1991).

Inoculation and incubation

Imbibed *S. gesnerioides* seeds were pipetted on to 6 mm discs of glass fibre filter paper, which were then placed in contact with cowpea roots growing on glass fibre filter paper and tissue paper in plastic trays (Lane et al., 1991). The trays were enclosed in a polyethylene bag and surrounded with aluminium foil to exclude light from the roots. Nutrient solution as described by Lane et al. (1991) was added to the filter paper at daily intervals.

After three days, *S. gesnerioides* seedlings were viewed with a stereo-microscope and transferred from the filter paper on to the surface of adjacent cowpea roots using a fine paint brush. All parasite seedlings that germinated up to a maximum of 50 were placed on each cowpea root system. Four cowpea plants of each variety were used to assess each parasite sample. However, on a few cowpea plants, there was no germination of *S. gesnerioides* seeds, so the number of cowpea plants for some varieties had to be reduced to two or three plants. Trays were enclosed as described previously and returned to the growth cabinet. Infections were viewed with a stereo-microscope and the development of *S. gesnerioides* and the responses of the infected roots were assessed 6, 14 and 21 d after inoculation. The number of *S. gesnerioides* tubercles with a stem and their diameter were measured at the third assessment date. A generalised linear model with a binomial distribution and logit link was fitted using Genstat (McCullagh & Nelder, 1989; Payne et al., 1987) to examine tubercle numbers as a proportion of the number of parasite seedlings transferred to host roots. Analysis of variance was used to examine the mean tubercle diameter on each cowpea plant.

Cowpea plants with parasite infections which were not developing normally were removed from trays after the third assessment, placed in pots containing a mixture of John Innes No. 1 compost and sand (3:2) and grown for a further 6–8 weeks to monitor any slowly developing parasite infections.

Results

Virulence of Striga gesnerioides *(90–75)*

Germination of *S. gesnerioides* (90–75) ranged from 0–40% and was similar with all four cowpea varieties. Approximately half of the transferred *S. gesnerioides* seedlings failed to penetrate cowpea roots and died 3–4 d after placing the seedlings on the roots. The remaining *S. gesnerioides* seedlings penetrated the roots of all four varieties within 6 d after inoculation. Most parasite seedlings which penetrated roots of varieties Blackeye and B301 developed successfully. Parasite tubercles with stems formed within 14 d. At 21 d, there was no significant difference in the number of tubercles on both cowpea varieties, although tubercles on the roots of B301 plants were significantly smaller than those on Blackeye (Table 1). Parasite stems started to emerge above the surface of the soil about 14 d after transferring B301 and Blackeye plants to soil and flowering stems were formed within 6–8 weeks (Fig. 1). Variety B301 was also assessed against parasite samples from Burkina Faso and Nigeria and was completely resistant as no parasite tubercles greater than 1mm in diameter formed (Lane, Moore & Child, unpublished data). Varieties 58–57 and IT81D–944 were totally resistant to parasite sample 90–75. Examination of the roots of 58–57 at 14 d revealed that all *S. gesnerioides* seedlings were dead. Parasite death was associated with necrosis of host tissue around the sites of penetration of *S. gesnerioides* radicles. Parasite tubercles formed on the roots of variety IT81D–994, but they remained at less than 1.5 mm in diameter, even if host plants were maintained in the trays after 21 d. Stems formed on the tubercles but failed to enlarge beyond 2–3 mm in length (Fig. 2). There was no further development of parasite infections on the roots of variety IT81D–994 after the cowpea plants were transferred to soil. A repetition of this experiment gave similar results (data not shown).

Fig. 1. Parasitism of cowpea variety B301 by *S. gesnerioides* (90–75). The cowpea plant was grown using the *in vitro* system for 21 d prior to transferring to soil for a further 42 d. Bar = 60 mm.

Virulence of Striga gesnerioides *(90–74)*

Germination of the second *S. gesnerioides* sample (90–74) ranged from 30–50%. *Striga gesnerioides* radicles penetrated the roots of all four varieties within 6 d of placing parasite seedlings on cowpea roots. As with the other parasite sample, about half of the *S. gesnerioides* seedlings failed to penetrate the roots and were dead about 3–4 d after placing the seedlings on host roots. Numerous parasite tubercles with stems formed on the roots of varieties Blackeye and B301 within 14 d. At 21 d, there was no significant difference in the number of parasite tubercles on the roots of varieties Blackeye and B301, although tubercles on the roots of B301 plants were significantly smaller than those on the roots of cv. Blackeye (Table 1). Parasite stems emerged above the surface of the soil on varieties Blackeye and B301 about 14 d after transferring cowpea plants to soil. Similar results were obtained on repetition of the experiment (data not shown). In parallel experiments, variety B301 was also totally resistant to *S. gesnerioides* from Mali and Nigeria (Lane, Child & Moore, unpublished data).

No *S. gesnerioides* tubercles formed on the roots of variety 58–57. Parasite seedlings died with necrosis of host tissue associated with the sites of parasite penetration. On the roots of variety IT81D–994, parasite tubercles remained at less than 1.5 mm in diameter. There was only very limited development of *S. gesnerioides* stems which were less than 3 mm in length. There was no further growth of *S. gesnerioides* on IT81D–994 plants following transfer of the cowpea plants to pots containing soil.

A second source of B301, from Botswana, was compared with that from SAFGRAD/IITA against the larger sample of *S. gesnerioides* (90–74). Parasite tubercles formed on the roots of both samples of B301 and on cv. Blackeye (Table 2). The number and size of tubercles on the roots of variety Blackeye and on the two sources of B301 were not significantly dif-

Table 1. Development of *S. gesnerioides* (90–75 and 90–74) on four cowpea varieties

S. gesnerioides	Cowpea variety	Number of seedlings transferred	Number of seedlings penetrated	Number of tubercles	Development[a] (approx 95% CI)	Mean tubercle diameter (mm)
90–75	Blackeye	37	20	17	45.9 (13.6, 82.1)	3.8
	B301	58	21	13	22.4 (5.5, 59.0)	2.9
	58–57	73	24	0	0	–
	IT81D–994	27	16	0	0	–
90–74	Blackeye	152	72	67	44.1 (25.5, 64.5)	4.4
	B301	187	102	88	47.1 (29.6, 65.2)	3.0
	58–57	163	69	0	0	–
	IT81D–994	140	65	0	0	–

The number of *Striga* seedlings transferred to each cowpea plant varied between 7 and 30 according to the size of the root system. [a] *Striga* development was assessed at 21 d as the number of tubercles greater than 1.5 mm in diameter per plant divided by the number of parasite seedlings placed on to each root system.
CI = Confidence interval SED = 0.36, df = 9.

ferent. As found previously, *S. gesnerioides* tubercles on the roots of B301 plants were slightly smaller than those on cv. Blackeye, but the difference was not statistically significant. An additional sample of variety B301 that had been selected for morphological purity by IITA was also susceptible to the 90–74 sample (data not shown). All samples of B301 seed were resistant to parasite samples from Burkina Faso and Nigeria (Lane, Child & Moore, unpublished data; Lane et al., 1993).

Discussion

Three sources of cowpea variety B301, including progeny from the original collection in Botswana (Riches, 1987), were found to be completely susceptible to *S. gesnerioides* collected from parasitised B301 plants and from another variety from the same trial in southern Benin. There was also no significant difference between the development of the two parasite samples on varieties Blackeye and B301. These results confirm the observed susceptibility of variety B301 in field trials at Zakpota (J. Detongnon & N. Muleba, personal communication). There were similar numbers of parasite tubercles on the roots of B301 plants and on the susceptible cv. Blackeye, though parasite tubercles were sometimes significantly smaller on B301 plants compared with those on cv. Blackeye. This may have been due to the slower growth rate of variety B301, which is an unimproved landrace (Riches, 1987). No hypersensitive response was shown by B301 following penetration by *S. gesnerioides* from Zakpota, as was observed when other parasite races were inoculated on to the roots of variety B301 (Lane et al., 1993).

The pattern of virulence of the samples of *S. gesnerioides* from southern Benin is unique, indicating that there is a new race of the parasite which is distinct from the three races previously reported from W. Africa (Parker & Polniaszek, 1990). The distribution of the new race would appear to be very localised because it was not identified in samples of *S. gesnerioides* from northern Benin nor from six other W. African countries (Lane, Moore, Child & Cardwell, unpublished data). Similarly, a sample of *S. gesnerioides* from Abomey (about 12 km south of Zakpota) was also not virulent on variety B301 (Steel, 1992).

Variety 58–57 showed the same hypersensitive resistance reaction to *S. gesnerioides* from Zakpota as that described earlier following inoculation with *S. gesnerioides* from Burkina Faso (Lane & Bailey, 1991). Variety IT81D–994 expressed a novel resistance response to *S. gesnerioides* from Benin; development was restricted to the production of small tubercles with small shoots. This represents a greater development of the parasite than that which occurs when B301 resists other races of *S. gesnerioides*, where tubercles form but do not produce stems (Lane & Bailey, 1992; Lane et al., 1993). This suggests that these two cowpeas and varieties based on their resistance genes could be successfully grown in southern Benin and Burkina Faso,

Table 2. Development of *S. gesnerioides* (90–74) on two sources of variety B301

Cowpea variety	Number of seedlings transferred (plants)[a]	Number of seedlings penetrated	Number of tubercles	Development[b] (approx. 95% CI)	Mean tubercle diameter (mm)
Blackeye	105 (4)	59	46	43.8 (25.2, 64.3)	4.0
B301 (1)	116 (5)	43	34	29.3 (14.8, 49.7)	3.2
B301 (2)	81 (4)	46	39	48.1 (26.5, 70.5)	3.3

The number of *Striga* seedlings transferred to each cowpea plant varied between 5 and 49 according to the size of the root system. Two sources of variety B301 were used; Burkina Faso (1) and Botswana (2). [a] Number of cowpea plants, [b] Assessed as described in Table 1. CI = Confidence interval, SED = 0.44 for Blackeye against B301 (1) and 0.44 for other comparisons, df = 10.

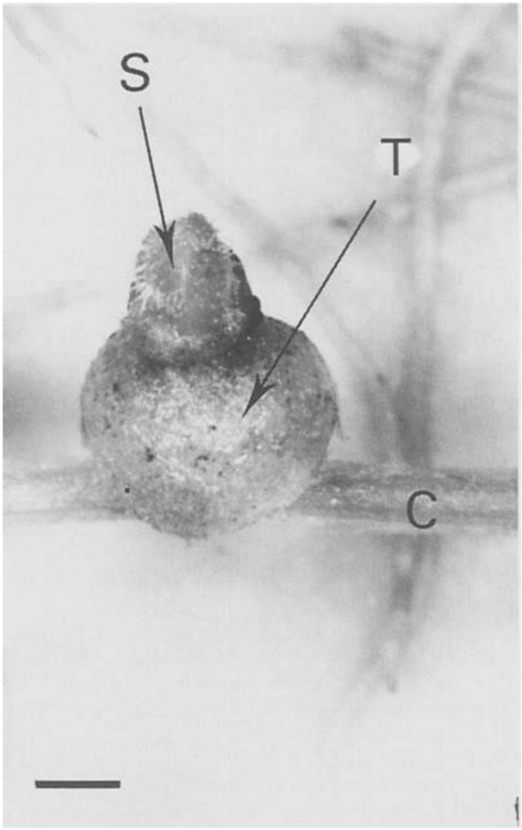

Fig. 2. Resistance of cowpea variety IT81D–994 to *S. gesneroides*. Restricted development of tubercle and stem, 21 d after placing parasite seedlings on to the roots of the cowpea plant. C = cowpea root, S = *S. gesnerioides* stem, T = *S. gesnerioides* tubercle. Bar = 0.5 mm.

and IT81D–994 could also be used in Mali. However, neither is resistant to the parasite race present in the major cowpea growing areas of Niger and Nigeria (Parker & Polniaszek, 1990).

Variety B301 was parasitised by *S. gesnerioides* during the first year that it was grown at Zakpota. It seems likely, therefore, that the parasite race already existed at this site rather than evolving from a non-virulent race by host-selection pressure. In Benin, *S. gesnerioides* frequently parasitises *Tephrosia* species and it is possible that these *Striga* plants may have been the origin of the new race (Gbehounou et al., 1991). However, a sample of *S. gesnerioides* collected from *Tephrosia* species in Benin failed to develop on B301. It induced similar resistance responses in variety B301 to those observed with races of *S. gesnerioides* collected from cowpea (Steel, 1992).

The identification of a new race of *S. gesnerioides* with virulence on B301 has several implications for cowpea breeding programmes. Firstly, it demonstrates that resistance genes from cowpeas other than B301 will be needed to control *S. gesnerioides* in southern Benin. Resistance genes from variety 58–57 or IT81D–994 should be effective. The genetic basis of their resistance is not fully understood, though limited studies have suggested that the resistance of variety 58–57 to the Burkina Faso race of *S. gesnerioides* is due to a single dominant gene (Aggarwal, 1991). Secondly, these findings raise questions about the durability of varieties based on the B301 resistance gene. For many other pathogens, resistance based on a single gene, although highly effective and easy to incorporate into a desirable phenotype, will often 'breakdown' as new virulent races are selected and spread rapidly. This is a well-known phenomenon with obligate plant pathogenic fungi such as mildews and rusts. By analogy with fungal pathogens, it has been argued that resistance to *S. gesnerioides* will be equally 'liable to breakdown' (Kim, 1992). A comparison of the biology of these pathogenic fungi with *Striga* suggests that this scenario seems unlikely. The essential features necessary for 'breakdown' to occur are an extremely large pathogen population, very rapid rates of reproduction and dissemination, and a large population of susceptible host plants. All these factors operate in the development of new virulent races of rusts and mildews. In contrast, *S. gesnerioides* produces seed only once or twice a year. Furthermore, *S. gesnerioides* is a root pathogen, so that the chances of infecting a host and reproducing are also very low compared with airborne fungal spores. A better analogy for assessing the impact and potential spread of new races of *S. gesnerioides* may be the development of herbicide-resistant weeds. Herbicide-resistant weeds have been known for many years, but they have so far failed to become a serious problem in agriculture (Rubin, 1991). Taken together, all these factors suggest that 'breakdown' of resistance to *S. gesnerioides* is unlikely to become a major problem in the development of new *Striga*-resistant cowpeas. It is to be expected, therefore, that, except in regions where virulent races of *S. gesnerioides* already exist, *Striga*-resistant cowpeas based on the B301 resistance gene, possibly also in combination with resistance genes from varieties IT81D–994 and 58–57, will provide an effective and durable means of controlling this important parasite.

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