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RESEARCH ARTICLE

Identification of new sources of resistance to *Striga gesnerioides* in cowpea *Vigna unguiculata* accessions

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Abstract The parasitic weed, *Striga gesnerioides*, is a major threat to cowpea productivity throughout the savannas of West and Central Africa. The identification of sources of *S. gesnerioides* resistance and their incorporation into breeding programs would be a beneficial strategy to combat the devastation caused by the parasite in cowpea fields. In this study we examined one hundred and ninety-four (194) accessions, four commercial varieties and two controls collected from a mini core collection of cowpea held at the International Institute of Tropical Agriculture genebank for resistance to *S. gesnerioides* race 3 (SG3), the most prevalent race in Nigeria, using phenotypic screening and molecular marker analysis. Our studies identified two cowpea accessions, Tvu-1272 and Tvu-16514, that are resistant to *S. gesnerioides* SG3. Resistance in these lines is associated with the molecular marker SSR1, known to segregate with the gene conferring resistance to *SG3* in the cultivar B301. Phenotypically, resistance in Tvu-1272 and Tvu-16514 is expressed as a hypersensitive response at the site of infection on the roots. Allelism tests indicated that the gene that conferring *SG3* resistance in Tvu-1272 is independent of that conferring resistance in B301. Tvu-1272 and Tvu-16514 will provide additional new sources of resistance to *Striga* and races prevalent in Nigeria.

Keywords Allelic test · Cowpea accessions · Molecular markers · Parasitic weeds · Resistance · *Striga gesnerioides*

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Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.] is an important food legume in West and Central Africa and this region represents over 66 % of the 12.5 million ha grown worldwide. It is a staple food for millions of poor people living in the dry areas of the world where it is difficult to grow any other crop. Its grain contains about 25 % protein and so is a cheap source of protein in the daily diet of rural and urban populations. Its haulms are also an important source of nutritious fodder for livestock in the dry savannas (Bressani

1985; Singh et al. 1997; Tarawali et al. 1997). Despite the potential of the crop in ensuring food security, a number of biotic and abiotic constraints are known to attack cowpea however, most of them are not economically important. There are only a few biological constraints that are considered economically important, including aphids, stem rot, maruca, pod sucking bud, and parasitic weeds. Among these, the parasitic weed caused by *S. gesnerioides* (Wild) Vatke an obligate hemi-parasite of the Orobanchidceae family is the most damaging and dominant weed of cowpea in Nigeria and several countries in Africa resulting in low yield (Emechebe et al. 1991; Muleba et al. 1997). A number of mechanisms ensure the coordination of the parasite' life cycles to that of their hosts. *Striga* seeds germinate after a pre-incubation period of moist and suitable temperatures, and only when they perceive host-derived chemicals, termed 'germination stimulants', released from plant roots, ensuring that only seeds within the host rhizosphere will germinate (Joel et al. 1995, 2006). The parasite seedling radicle grows reach a host root, within a few days. Upon contact with the host root the radicle develops a specialized organ, the haustorium, which adheres to the root, penetrates the epidermis and cortex tissues of the root and ultimately establishes connections to the host vascular system. Successful parasite establishment creates a strong sink for nutrients to the detriment of the host, leading to drastic growth reduction (Keyes et al. 2001; Joel et al. 2006). Yield loss resulting from these parasites ranges from 41 % (Lagoke et al. 1997) to total crop loss of the highly susceptible cultivars (Emechebe et al. 1991). The *Striga* infestation in cowpea is more devastating in areas with sandy soils, low fertility, and low rainfall (Singh and Emechebe 1990). Farmers having crop fields severely infested with *Striga* often resort to abandoning their fields, contributing to an already severe non availability of farm lands (Emechebe et al. 1991). In northeast Nigeria, where cowpea is the most important legume crop, Dugje et al. (2006) reported that more than 97 % of cowpea fields in the dry savannas were infested with *S. gesnerioides*, leading to serious crop losses. *S. gesnerioides* produces as many as 20,000 seeds per plant (Singh and Emechebe 1997). The large numbers of seed produced makes difficult the mechanical control of the parasite. Furthermore, up to 75 % of the crop damage is done underground because the parasite tubercles grow underground for several weeks before producing aboveground flowering shoots (Singh et al. 1991). Thus, the identification of *Striga* resistant genes and their incorporation into breeding programs could be the most successful strategy to combat the parasites. Based on qualitative differential host reactions and genetic diversity analysis within the cowpea growing regions of West and Central Africa (i.e. ability to infect or not infect specific genotypes) seven races of *S. gesnerioides* have been identified within the cowpea growing regions of West and Central Africa (Lane et al. 1997; Botanga and Timko 2006). The use of genetic resistance is the most appropriate, safe and cost-effective way to control the parasite. In Nigeria, like other countries in West Africa, the development of race-specific *Striga*-resistant cowpea (has been recommended as the only sustainable way to manage *Striga* (Singh et al. 1997)). In the last 20 years, significant progress has been made in the development of *Striga* resistant cowpea varieties by IITA in partnership with the National Agricultural research centers. However, most of the cowpea cultivars having resistance to *Striga* races prevalent in Nigeria were developed using B301 or lines derived from it as sources of resistance. Resistance in B301 is governed by a single major gene (vertical resistance), which may not be durable (Gnanamanickam et al. 1999). The breakdown of resistance is common phenomenon in breeding for vertical gene resistance (Gnanamanickam et al. 1999). It has been reported that resistance conferred with major genes frequently failed to provide long-term disease control and the use of such cultivars grown over a broad area potentially lead to serious epidemics (Gnanamanickam et al. 1999). To delay such breakdown, pyramiding of more than one gene from diverse resistance sources into a single genotype would provide a better option. Achieving this goal would go a long way towards providing much needed durability of resistance and also broadening the resistance genetic base.

Host-specific virulence has been observed in *S. gesnerioides* (Lane et al. 1997; Botanga and Timko 2006). Evolution of host-specific virulence results in the identification of seven races of *Striga gesnerioides.* This leads to breakdown of resistance in the host cultivar either as a result of an increase in the aggressiveness of the *Striga* races or the presence of new *Striga* races. Omoigui et al. 2012 working in northern Nigeria reported differential responses of

cowpea breeding lines to *S. gesnerioides*. Some lines that were reported previously to be resistant to *Striga* in one region were found to be susceptible when grown in Borno leading them to speculate the presence of other races or variant within the population of *S. gesnerioides* in the region. In that study, the authors concluded that the occurrence of new races could complicate breeding cultivars with stable resistance, unless varieties can be developed with resistance to multiple races. Similar host differential response of different cowpea cultivars to *S. gesnerioides* have been reported in Burkina Faso (Tignegre et al. 2013). *S. gesnerioides* seeds are frequently collected from the major cowpea growing areas in West Africa and characterized for virulence and diversity; representative highly-virulent races from different regions are then selected for greenhouse screening (Singh et al. 1997). Although resistance sources have been identified and used in cowpea breeding programs to combat *Striga*, evolution of new virulence of *S. gesnerioides* calls for the identification and utilization of new sources of *Striga* resistance gene(s). The International Institute of Tropical Agriculture (IITA) Genebanks conserve germplasm of cowpea that are considered as reservoirs of traits of economic importance. Characterization of these genetic materials for *Striga* resistance in germplasm repository will facilitate their utilization in commercial breeding programs. However, it is difficult to evaluate large number of accessions in the germplasm collections, especially for the traits like Striga resistance where accessions need to be screened against different races of the parasite. This problem can be solved by selecting and evaluating subset of the germplasm accessions that corresponds to variation available in the entire collection.

Field screening with *Striga* inoculum have been used in selecting cowpea genotypes with resistance to *S. gesnerioides* (Singh et al. 1997; Atokpele et al. 1995; Muleba et al. 1997). However, selection of resistant genotype based on field screening using naturally *Striga* infested field condition or pot culture techniques does not always reflect the true resistance of the genotype due to environmental fluctuation. To increase selection efficiency, phenotypic screening using pot culture in conjunction with DNA marker will help to identify true resistant genotype and avoid pseudo-resistant genotype. Screening for resistance to *S. gesnerioides* under field condition is ineffective because of non-uniform parasite pressure over time and space. It has been reported that environmental stress alters physiological mechanisms of resistance to biotic stresses such as *Striga* attack (Ayres 1984). Studies by Muranaka et al. (2011) on stability of *S. gesnerioides* resistance mechanism in cowpea under high infestation level, low soil fertility and drought stresses under field conditions showed that certain physiological mechanisms such as high seed infestation condition combined with low soil fertility and drought stresses influence resistance to *S. gesnerioides* race *SG3* in cowpea. This suggests that screening under field or using pot technique methods, based on phenotyping only is ineffective in identifying true genotype with resistance to *S. gesnerioides* due to the strong influence of environmental factors on the expression of resistance or susceptibility to the parasite. Genetic characterization with molecular markers technologies offers greater power of detection than do phenotypic methods. This is because molecular methods reveal differences in genotypes variation embodied by DNA sequencing of an individual which is not influenced by environment. In contrast, difference revealed by phenotypic approaches is at the level of gene expression (protein). Molecular markers linked to *Striga* resistance gene have been identified including SCAR and other PCR amplifiable markers capable of tracking most of the major races of the parasite in West Africa (Ouedraogo et al. 2001; Boukar et al. 2004, Timko et al. 2007). Of these, SSR1 which is linked to resistance to *S. gesnerioides* race 3 (SG3) found in Nigeria, has been the most widely applied in marker assisted selection and breeding for improved cowpea varieties. This present study was undertaken to identify new sources of resistance to *S. gesnerioides* from a mini core cowpea accessions.

Materials and methods

Seed source

Seeds of the mini core germplasm accessions ($n = 200$) of cowpea were obtained from the genebank of the International Institute of Tropical Agriculture, Ibadan, Nigeria. The germplasm accessions which comprised of 194 entries, also included were four commercial cultivars (IT98K-573-1-1, IT98K-573-2-1, IT97K-499-35, Danila and IT89KD-391), and B301 a landrace from Botswana (resistance to four

cowpea *Striga* races) and 'TVx3236' (susceptible to all races of *Striga*), as controls (Supplemental Table 1).

Pot experiments for Striga resistance phenotyping

Striga gesnerioides seed were collected from Borno State, Nigeria in 2012. The *Striga* race from this area (designated *SG3*) is the most virulent race in Nigeria (Omoigui et al. 2012).

The pot-screening experiments were conducted at the screenhouse of IITA Research Station in Kano during the 2013 off-season (April 2013 to July 2013). The pot screening was conducted according to the method described by Singh and Emechebe (1990) and Atokple et al. (1995) with slight modification. Parasite Seeds were conditioned for 7 days before sowing the test materials to enhance *Striga* seed germination. Plastic pots measuring 13-cm-diameter were filled with sterilized sieved sand and top soil (sandy loam) mixture (1:1 vol/vol) and inoculated with about 5000 *Striga* seeds per pot. After soil inoculation with *Striga* seeds, the pots were watered for 1 week to precondition the seeds to break their dormancy and ensure optimum germination.

Three seeds each of the 194 accessions, and check were sown in 13-cm-diameter pots filled with about 1 L soil previously inoculated uniformly with about 5000 *S. gesnerioides* The seeds were sown at uniform depth in holes made with the help of the thumb and kept in a greenhouse with temperature ranging from 25 to 38 °C. The experiments were arranged in a completely randomized design with three replicates and two pots per replicate.

Genotyping

Following screenhouse phenotyping, DNA analysis was carried out to validate phenotypic data. For PCR assays, three molecular markers (SSR-1, MahSe2 and C-42-B) were used Total genomic DNA was extracted from young leaf tissue of 2 week-old plants using the FTA® PlantSaver cards for PCR analysis using the methodology of Whatman (2002) and Omoigui et al. (2012). PCR was performed in a total volume of 20 µL using customized Accupower PCR premix tube (BIONEER) to which a purified FTA disc containing the DNA sample and 16 µL of water-Molecular Biology Grade (Lonza) were added. The PCR profile for SSR1 and MahSE2 followed was: one cycle of 95 °C for 4 min; 1 cycles of 95 °C for 30 s min; 55 °C for 30 s; 72 °C for 30 s for 35 cycles and a final extension at 72 °C for 2 min and held for 4 °C for infinity. The annealing temperatures of all primer combination were 55 °C for SSR-1 and MahSE2 except for C42-B primer pair whose annealing temperature was 60 °C. All PCR amplification was performed with the BIO-RADMyCycler™ thermal cycler. The markers fragments were analyzed by electrophoresis on 2 % agarose gels stained with ethidium bromide (10 ng/100 mL of agarose solution in Tris Acetase borate EDT buffer). The gels were run for approximately 1 h 10 min at 200 voltage in 1 × Tris acetic acid (TAE) buffer (45 mmol L^{-1} glacial acetic acid, 0.5 mmol L^{-1} ethylenediaminetetra acetic acid (EDTA), (pH, 8.4). A 100 bp molecular weight ladder was used to determine the molecular weight of each band in a gel of PCR products. The ethidium bromide-stained gel was visualized on an UV trans-illuminator at a wavelength of 320 nm and images photographed using a cannon digital camera.

Data scoring and genetic analysis

SSR-1, MahSE2 and C42-B markers exploit differences in DNA sequences based on the presence or absence of polymorphisms in the primer site. In this case, there is sequence polymorphism that provides an amplicon in resistance genotypes that is missing in susceptible genotypes. The bands were counted by starting from the top of the lances to the bottom. All visible and unambiguously scorable fragments amplified by the primers were scored under the heading of total scoreable fragments. Amplified profiles of the cowpea accessions were compared with each other and bands of DNA fragments were scored as present or absent. The genotypes that were selected based on phenotypic screening and confirmed with molecular markers were tested for allelic relationship.

Data collection

Data were collected on days to first *Striga* emergence at 7, 8, 9 and 10 weeks after planting (WAP), *Striga* height, plant height, days to 50 % flowering of cowpea plants, *Striga* dry biomass, number of *Striga* shots per cowpea plant, and number of *Striga* haustorial attached to cowpea root. Other data collected were

Striga shoots and haustorial weights. The experiment was terminated at 70 days after planting; pots were washed and examined for *Striga* haustorial attachment. Plants which support *Striga* emergence and haustorium attachment were classified as susceptible while those that were free of *Striga* haustorial attachment were classified as resistant.

Data were analyzed using the general linear model statistical procedures with the SAS system for Window (SAS Institute 2014). Means were separated using LSD.

To test for allelic relationship between resistance sources, segregation ratios for each resistant × resistant (R × R) progeny were computed. Genetic hypotheses were tested for significance using the Chi squared goodness-of-fit test to determine the deviation of observed frequencies from the hypothesized ratios.

Results

Screening for *S. gesnerioides* resistance

The first pot screening experiment was conducted to quickly eliminate the highly susceptible cowpea accessions to *S. gesnerioides*. Combined analysis of variance (ANOVA) showed significant mean squares for year, cowpea accessions. Significant interaction was only detected for plant root dry weight (Table 1). The mean *Striga* count ranged from 0 to 15 per pot. Some of the highly susceptible accessions were killed by *Striga* before flowering. Out of the 194 accessions assessed for *S. gesnerioides* resistance in the first pot experiment, 89 cowpea accessions were found resistant or moderately resistant. The 89 accessions that appeared resistant were further phenotyped and genotyped with three markers tightly linked to the region of *S. gesnerioides* resistance gene. *Striga* shoots plant count per plant at 70 was not significant, and ranged from 0 to 6 with a mean of 2.4. *Striga* shoots per cowpea plant in the second experiment. The resistant accession and checks did not support *Striga* emergence or root attachment to the cowpea plant compared to susceptible accessions and checks that supported *Striga* shoots and haustorial attachment. Many of the susceptible cowpea accessions were characterized by stunted growth, severe leaf chlorosis, and partial leaf senescence. Some plants developed these symptoms but *S. gesnerioides* did not emerge from the soil. The top best accessions with two checks are presented in Table 2. Two of the cowpea accessions, Tvu 1272 and Tvu 16514 had the highest plant root weight and plant shoot dry weight.

Out of 89 cowpea accessions phenotyped, only 3.4 % (TVu 1272, TVu 9343, and TVu 16514) were completely resistant to *Striga* with no symptoms observed on the leaves. On the other hand, all the other cowpea accessions were either tolerant or susceptible to *Striga* About 20 % were moderately resistant having chlorosis on leaves with limited leaf senescence, while 77 % were considered to be susceptible to *S. gesnerioides*. It was also observed that some commercial varieties included as check (TVx 3236, IT84S-2246-4, Danila, IT89KD-391) were highly susceptible to *Striga* except B301 that was resistant to both parasites. IT97K-573-1-1, IT97K-573-2-1, and IT97K-499-35 exhibited differential responses to *Striga* infestation on the field and in the screenhouse.

Genotyping

The 89 cowpea accessions were screened for presence of genes conferring resistance to *S. gesnerioides* using the three different molecular markers for *Striga* resistance (Fig. 1). The three markers gave reproducible and scorable bands with B301. The first two

Table 1 *P* values for the analysis of variance (NOVA) for measured parameters of cowpea accessions combined environment

Effect	Pht3wk	Stcn42d	Stcn49d	Stcn56d	Stcn66d	Stcn70d	Prtdwt	Pshdwt	Strpt	Strat
Year (Y)	<0.0001	<.0001	<0.0001	<0.0001	<0.0001	0.0063	0.0001	<0.0001	<0.0001	<0.0001
Accession (A)	<0.0001	0.7526	0.0008	0.0025	0.0187	0.1032	0.0575	0.0015	0.2048	0.7162
Y*A	0.7759	0.6138	0.5395	0.6422	0.4402	0.1713	0.0004	0.6064	0.4998	0.9113

Pht3wk Plant height at 3 weeks, *Stcn Striga* count, *Pshdwt* plant shoot dry weight, *Prtdwt* Plant root dry weight, *Strdwt* Striga root dry weight, *Strp* Striga root plant, *Trat* Striga attachment

Table 2 Mean number of *Striga* shoot per plant, plant root dry weight and plant shoot dry weight of the top 10 cowpea accessions and checks

Cowpea accessions	Number of *Striga* shoots per plant				PRDWT (g)	PSDWT (g)
	7wap	8wap	9wap	10wap		
Tvu 3739	0.0	0.0	0.0	0.0	6.5	8.5
Tvu 1272	0.0	0.0	0.0	0.0	10.5	14.1
Tvu 16514	0.0	0.0	0.0	0.0	10.5	13.5
Tvu 12431	0.0	0.0	0.0	0.0	8.2	11.7
Tvu 9232	0.0	0.0	0.0	0.0	4.5	6.5
Tvu 4622	0.0	0.0	0.0	0.0	5	6.8
Tvu 16576	0.0	0.3	0.0	0.0	3	9.9
Tvu 9343	0.0	0.0	0.0	0.0	5.2	9.9
Tvu 16505	0.0	0.0	0.0	0.0	4.5	13.4
Tvu 10100	0.0	0.3	0.0	0.0	3.1	9.4
B301	0.0	0.0	0.0	0.0	4.8	11.5
IT97K-499-35	0.0	0.0	0.0	0.0	6.5	10.5

PRDWT plant root dry weight, *PRDWT* plant shoot dry weight, *wap* weeks after planting

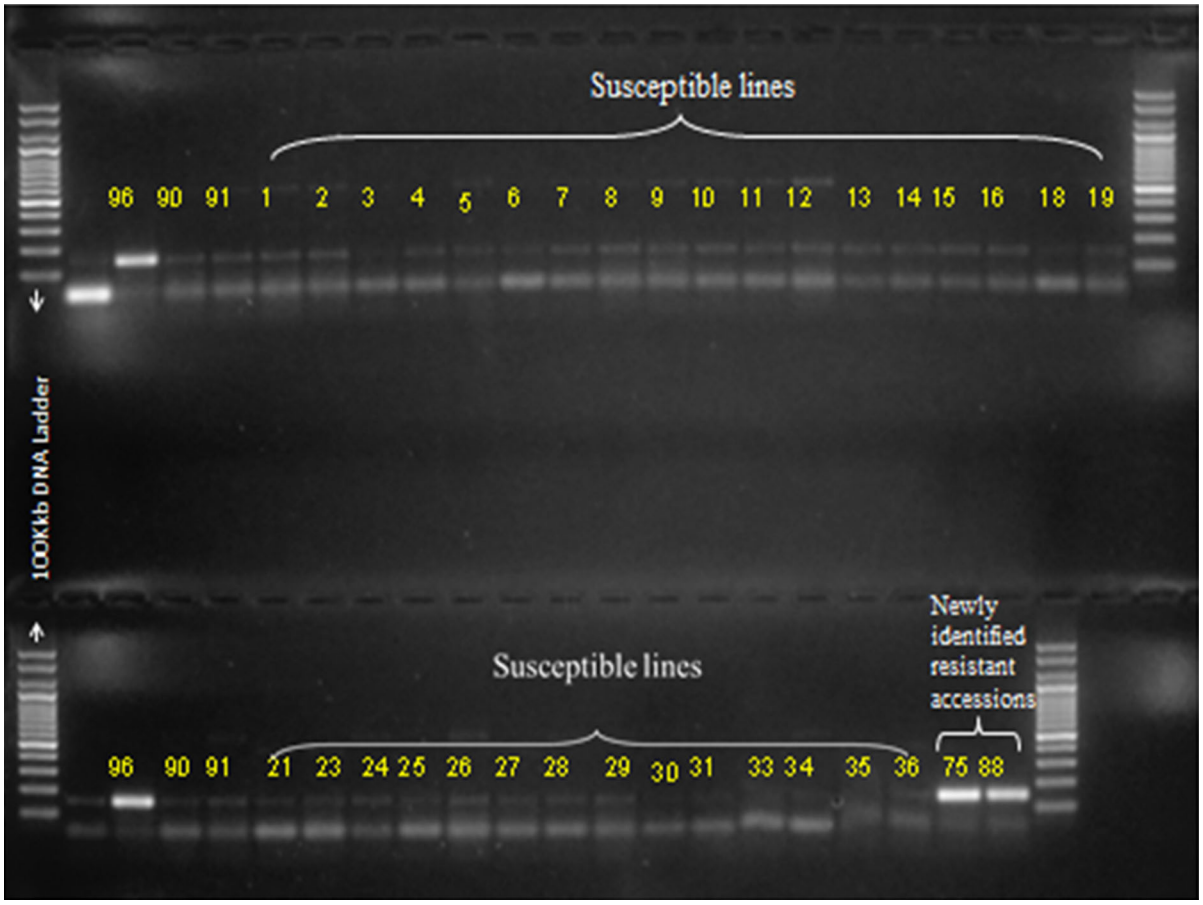

Fig. 1 Analysis of SSR1 marker linked to SG3 resistance in various cowpea accessions. Shown is a representative picture of resolution of the SSR1 marker on on 2 % agarose gel. Presence of the 150 bp product indicates the presence of the resistance allele

groups (SSR-1 and MahSE2) consists of markers associated with genes conferring resistance to *Striga* race 1, 2, 3, and 4 while C42-B marker is linked to *S. gesnerioides* race 5. The gel shows that the two lines TVu 16514 and TVu 1272 both contain the SSR1 resistance marker which was consistent with those having resistance to *SG3* from Nigeria. Since Mahse2 and C42B are loosely linked with *SG3* resistance it is not surprising that these bands may or may not be present in the *SG3* resistant lines.

However, the marker did not amplify one (TVu 9343) of the 3 accessions previously found to be resistant in the pot culture techniques (Table 3). The three markers (SSR-1, MahSE2, and C42-B) were amplified for 2 accessions (TVu 16514 and TVu 1272) out of 89 accessions tested. This result was also confirmed in field trial as the cowpea accession TVu 9343 supported severe *Striga* shoots attachment (data not shown) underscoring the effectiveness of molecular markers in identifying resistant genotype as compared to phenotypic scoring alone. Cowpea accession TVu1272 was highly diverse from B301 on the basis of SSR-1 band size difference. The amplification shifted away from the predicted molecular weight SSR amplicon in B301. The molecular weight amplified in B301 was 100 bp while that in TVu 1272 was 120 bp suggesting a novel gene from that in B301.

Testing allelic relationship between resistant genotypes

After the identification of TVu 1272 and TVu-16514 as new sources of resistant to Striga, allelic relationship between resistant genotypes was tested using the resistant landrace B301. B301 was crossed with TVu 1272 and TVu-16514 to generate F_1 and F_2 populations. One hundred individual plants each of the F_2 populations were planted and screened in artificially infested pots with *Striga* seeds. Twenty F_1 seeds from both the straight and reciprocal cross, twenty seeds of parents: TVu 1272, Tvu-16514 and B301 were included as checks. Twenty plants of each parent, and F_1 individuals were assessed. One hundred F_2 individual plants of the different crosses were assessed.

The allelic relationship between *Striga* resistance gene in B301 and other resistance genes identified in the accession; TVu 1272 and TVu 16514 are presented in Table 4. In the F_2 cross derived from B301 x TVu 1272, the segregation for *S. gesnerioides* resistance in the allelism test was 92 resistant and 8 susceptible, which exhibited the action of dominant genes conferring resistance to B301 and TVu 1272. The Chi square χ^2 values showed a good fit for a segregation ratio of 15 resistant to 1 susceptible, which demonstrates the presence of two independent dominant genes. This result supported the hypothesis that the gene conferring resistance to *S. gesnerioides* in TVu 1272 is independent Rsg3-301, harboured in B301. On the other hand, in the cross between B301 $\times$ TVu 16514, no susceptible plants were observed in the F_2 population. There was no segregation (100 resistant: 0 susceptible), which indicated that the resistance gene in the two cultivars co-segregate and are either in same locus or are allelic genes.

The mechanism of resistance to *S. gesnerioides* in TVu 1272 was found to be similar to that in B301. The *Striga* seeds germinated and the radicles attached to the roots of resistant and susceptible plants but the resistant cowpea roots did not permit penetration of the cortex by the haustorium development. The *Striga* seedling dies leaving the resistant plants completely healthy and productive. On the other hand, penetration of the host root cortex and attachment of the parasite to the host vascular system occurs on susceptible cultivars permitting *Striga* to complete its lifecycle.

Discussion

Striga gesnerioides resistance in cowpea is conferred by a single dominate gene which is a typical of vertical resistance, the *Striga* trait therefore will be expressed as a discrete trait. That implies that there will be a clear distinction between a resistance and susceptibility on the basis of supporting or non-supporting *Striga* shoots plant as proposed by Flor (1955). All the susceptible cowpea accession had several *Striga* shoots plant and suffered significant reduction in plant shoot dry weight, and severe chlorosis in the infested pots. Out of the 194 cowpea accessions screened only two accessions were completely resistant to *Striga* infestation. The two accessions TVu 1272 and TVu 16514 had neither *Striga* shoots plant nor supported haustorial attachment. The two accessions were also resistant as B301. Striga shoot count at different date showed significant difference among cowpea accession except

Table 3 Presence of markers linked to gene conferring resistance to *S. gesnerioides* in 89 cowpea accessions with known resistance and susceptible cultivars

S. no.	Accession	Phenotypic score	Genotypic score			S. no.	Accession	Phenotypic score	Presence of marker^{+}		
		Phenotypic	SSR1	Mahse2	C42-B			Phenotypic	SSR1	Mahse2	C42-B
1	Tvu-8	S	–	–	–	46	Tvu-9842	S	–	–	–
2	Tvu-21	S	–	–	–	47	Tvu-9848	S	–	–	–
3	Tvu-43	S	–	–	–	48	Tvu-9866	S	–	–	–
4	Tvu-109	S	–	–	–	49	Tvu-10100	S	–	–	–
5	Tvu-113	S	–	–	–	50	Tvu-10373	S	–	–	–
6	Tvu-132	S	–	–	–	51	Tvu-10910	S	–	–	–
7	Tvu-374	S	–	–	–	52	Tvu-10919	S	–	–	–
8	Tvu-415	S	–	–	–	53	Tvu-11048	S	–	–	–
9	Tvu-443	S	–	–	–	54	Tvu-11082	S	–	–	–
10	Tvu-492	S	–	–	–	55	Tvu-11382	S	–	–	–
11	Tvu-497	S	–	–	–	56	Tvu-11488	S	–	–	–
12	Tvu-527	S	–	–	–	57	Tvu-11500	S	–	–	–
13	Tvu-1280	S	–	–	–	58	Tvu-11682	S	–	–	–
14	Tvu-1775	S	–	–	–	59	Tvu-11955	S	–	–	–
15	Tvu-1780	S	–	–	–	60	Tvu-11979	S	–	–	–
16	Tuv-1886	S	–	–	–	61	Tvu-12029	S	–	–	–
17	Tvu-2723	S	–	–	–	62	Tvu-12139	S	–	–	–
18	Tvu-3282	S	–	–	–	63	Tvu-12431	S	–	–	–
19	Tvu-3552	S	–	–	–	64	Tvu-12705	S	–	–	–
20	Tvu-3739	S	–	–	–	65	Tvu-12746	S	–	–	–
21	Tvu-4557	S	–	–	–	66	Tvu-12786	S	–	–	–
22	Tvu-4622	S	–	–	–	67	Tvu-12848	S	–	–	–
23	Tvu-6855	S	–	–	–	68	Tvu-13017	S	–	–	–
24	Tvu-7127	S	–	–	–	69	Tvu-13249	S	–	–	–
25	Tvu-7226	S	–	–	–	70	Tvu-13746	S	–	–	–
26	Tvu-7290	S	–	–	–	71	Tvu-13958	S	–	–	–
27	Tvu-7331	S	–	–	–	72	Tvu-13965	S	–	–	–
28	Tvu-7697	S	–	–	–	73	Tvu-13998	S	–	–	–
29	Tvu-7798	S	–	–	–	74	Tvu-14136	S	–	–	–
30	Tvu-8464	S	–	–	–	75	Tvu-1272	R	+	+	+
31	Tvu-8671	S	–	–	–	76	Tvu-14719	S	–	–	–
32	Tvu-9143	S	–	–	–	77	Tvu-15565	S	–	–	–
33	Tvu-9232	S	–	–	–	78	Tvu-15742	S	–	–	–
34	Tvu-9265	S	–	–	–	79	Tvu-15805	S	–	–	–
35	Tvu-9285	S	–	–	–	80	Tvu-15892	S	–	–	–
36	Tvu-9343	R	–	–	–	81	Tvu-15973	S	–	–	–
37	Tvu-9391	S	–	–	–	82	Tvu-15995	S	–	–	–
38	Tvu-9474	S	–	–	–	83	Tvu-16225	S	–	–	–
39	Tvu-9516	S	–	–	–	84	Tvu-16253	S	–	–	–
40	Tvu-9621	S	–	–	–	85	Yvu-16278	S	–	–	–
41	Tvu-9651	S	–	–	–	86	Tvu-16504	S	–	–	–
42	Tvu-9676	S	–	–	–	87	Tvu-16505	S	–	–	–

Table 3 continued

S. no.	Accession	Phenotypic score Phenotypic	Genotypic score			S. no.	Accession	Phenotypic score Phenotypic	Presence of marker^{+}		
			SSR1	Mahse2	C42-B				SSR1	Mahse2	C42-B
43	Tvu-9732	S	–	–	–	88	Tvu-16514	R	+	+	+
44	Tvu-9820	S	–	–	–	89	Tvu-16574	S	–	–	–
45	Tvu-9829	S	–	–	–	90	B301	R	+	+	+
						91	TVx 3236	S	–	–	–

R, resistance; S, susceptible; +, presence of marker; –, absence of marker

Table 4 Segregation ratios of F_2 progenies derived from crosses between the newly identified sources of resistance and the known *Striga* resistant B301

Population	Generation	Total no. of plants	Number of plants		Genetic ratio	χ^2-value	Pr > ChiSq
			Resistant	Susceptible			
B301	Parent 1	20	20	0			
TVu 1272	Parent 2	20	20	0			
TVu 1272 × B301	F_1	16	16	0			
TVu 16514 × B301	F_1	20	20	0			
TVu 1272 × B301	F_2	100	92	8	15:1	0.71	7.8
TVu 16514 × B301	F_2	100	100	0	1		

R resistance, *S* susceptible

at 70 days after plant. The lack of significant difference observed at 70 days after planting among the cowpea accessions could have resulted from the early death of some of the highly susceptible *Striga* plants at this growth stages. One of the accessions that were identified as resistant in pot experiment was confirmed susceptible by the markers applied. The result was further confirmed in field trials as the cowpea accession supported many *Striga* shoots plant. This result stresses the effectiveness of DNA marker in selecting genotypes for resistance to *Striga*. In this present study, we showed the effectiveness of using DNA marker in identifying cowpea genotype for *Striga* resistance. This suggests that selection of genotype for *Striga* resistant cannot be based solely on phenotypic data. This finding agrees with Omoigui et al. (2015) who found SSR and SCAR markers to be effective in discriminating between *Striga* resistance and susceptible in cowpea.

The allelic test of resistance to *S. gesnerioides* derived from the cross between B301 × TVu 1272 identified one new independent gene responsible for the resistance in TVu 1272 cowpea accessions. This gene appeared to be different from that previously identified in B301. Considering the gene-for-gene hypothesis by Flor (1955), the differential reactions found in TVu 1272 and B301 when inoculated with the same *Striga* seeds suggested that the two cowpea genotype carried different resistant genes. Therefore, because the three resistance genes previously identified in B301, IT82D-849, and Suvita-2 were designated as *Rsg1*, *Rsg2* and *Rsg3* (Singh et al. 1997) respectively, the newly identified gene from TVu 1272 will also be designated *Rsg3* but with different resistance (R) gene. The resistance characteristics of both B301 and TVu 1272 are similarly defined as immune resistance. In other words, the immune resistance reacted hypersensitively to *Striga* infection, whereby no *Striga* symptoms occurred with very little root tissue necrosis and localized cell death surrounding the inoculation wound, similar to that reported on the root of B301 (Singh et al. 1997).

Studies by Omoigui et al. (2012) speculated the presence of different race of *Striga* in north east Nigeria. Therefore, the newly identified sources of *Striga* resistance that have a new gene reported in this study should be suitable for broadening the genetic base of resistance to *S. gesnerioides* in Nigeria. The

cowpea accession TVu 1272, an IITA germplasm collection from Uganda will provide additional sources of resistance to *Striga.* Therefore, TVu 1272 is recommended as potential donor parent for staking *S. gesnerioides* genes into adapted, farmers preferred cultivars in order to broaden the genetic base of cowpea for *Striga* resistance in West and Central Africa.

Conclusion

The application of molecular markers in this study has revealed that phenotypic data alone is not always comprehensible in identifying genotypes for resistance to *S. gesnerioides*. This inconsistency should incline to use markers in conjunction with phenotypic data for selection of genotypes. The study identified two cowpea accessions TVu 1272 and TVu 16514 as potential new sources of resistance to *S. gesnerioides*. Resistance to *S. gesnerioides* in both accessions was inherited in a dominant fashion. The mechanisms of resistance in the two cowpea accessions (TVu 1272 and TVu 16514) were found to be hypersensitive response as previously reported for B301. However, allelism test revealed that resistance gene in TVu 1272 is non-allelic with the gene that confers resistance in B301.

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