

Varietal Response of Cowpea (*Vigna unguiculata* (L.) Walp.) to *Striga gesnerioides* (Willd.) Vatke Race SG5 Infestation

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Abstract

The parasitic plant *Striga gesnerioides* (Willd.) Vatke is becoming a serious threat to cowpea production in Sub-Saharan Africa. At least 7 races of *S. gesnerioides* exhibiting specific virulence have been identified. The objectives of this study were to identify sources of resistance to *S. gesnerioides* race SG5 spreads in northern Cameroon and to study the inheritance of the resistance. Twelve accessions of cowpea, IT98K-205-8, 'IT98K-503-1', 'IT98K-1092-1', 'IT99K-216-1-1', 'IT98K-494-6', 'IT99K-529-4', 'IT99K-573-1-1', 'IT99K-573-2-1', 'IT00K-1207', 'IT03K-378-4', 'IT04K-227-4' and 'VYA' were assessed in field trials for resistance to *S. gesnerioides* race SG5 in Maroua, northern Cameroon and the genetics of the resistance from two sources was elucidated in artificially infested pots. Data on resistant and susceptible cowpea plants were analyzed using the chi-square test to ascertain the goodness of fit to different genetic ratios. Tests of resistance using field screening techniques showed that among these varieties, four were susceptible, four tolerant, two partially resistant while 'IT99K-573-1-1' and 'IT98K-205-8' appeared completely resistant to *Striga* race SG5. On susceptible varieties, the mean number of emerged *S. gesnerioides* plants 9 weeks after sowing was 1.8 to 2.4 per cowpea plant, while completely resistant lines carried none. The cowpea genotypes showed significant differences for percentage of infected plants, the decimation rates and *Striga* height. On susceptible genotypes, the seeds yield losses due to *Striga* infestation varied from 28.4 to 36.6% while for the fodder yield these losses ranged between 23.63 to 41.03%. The analysis of F₁ and F₂ populations resulting from crossings between resistant varieties and the susceptible tester 'VYA' demonstrated that resistance is controlled by two factor inheritance with duplicate dominant genes (*Rsg5a* and *Rsg5b*) for 'IT99K-573-1-1' and a single dominant gene (*Rsg5*) for 'IT98K-205-8'. These two varieties provide newly additional sources of resistance to *S. gesnerioides* and can be used in programmes of genetic improvement of this legume.

Keywords: *Vigna unguiculata*, *Striga gesnerioides*, race SG5, varietal screening, genetic resistance, genetic improvement

Introduction

Cowpea (*Vigna unguiculata*) whose global annual production stands at 7.6 million tonnes is one of the important food grain legumes in the tropics including Africa, which accounts for 64 percent of the world production (Elhers and Hall, 1997; Kamara *et al.*, 2008). In the semi-arid and arid regions of sub-Saharan Africa, it is a major source of dietary protein that nutritionally complements staple low-protein cereal and tuber crops. In addition to its nutritional value, cowpea is a valuable and dependable commodity that produces income for farmers and help to restore soil fertility for succeeding cereal crops growing in rotation with it. One of the major biological constraints to increase cowpea productivity in the smallholder farming sector is attack by parasitic weeds, *Striga gesnerioides* (Willd.) Vatke and *Alectra vogelii* Benth, particularly in the semi-arid regions of West and Central Africa (Hibberd *et al.*, 1996; Ehlers and Hall, 1997). *S. gesnerioides*, a member of *Scrophulariaceae* family, is a devastating ob-

ligate root hemiparasite that primarily parasitizes dicotyledonous species, including tobacco, sweet potato, cowpea and other legumes (Thalouarn and Fer, 1993). At present, the Sudano-Sahelian belt of Africa is more affected by *S. gesnerioides* but the parasite is fast spreading beyond this limit (Carsky *et al.*, 2003). Crop yield losses due to *S. gesnerioides* may be up to 70% depending on the extent of damage and level of infestation (Aggarwal and Ouedraogo, 1989; Alonge *et al.*, 2005). On susceptible cultivars, yield losses can reach 100% when *S. gesnerioides* population was over 10/plant (Kamara *et al.*, 2008). Omoigui *et al.* (2009) added that yield losses caused this witchweed in dry savanna of sub-Saharan Africa are estimated in millions of tons annually and the prevalence of *Striga* soils is steadily increasing.

Several control strategies have been developed including improved cultural practices, the use of chemical control and breeding using many sources of resistance (Berner *et al.*, 1995). Control strategies based on the use of herbicides

are too expensive for low-input farming systems, whilst cultural practices offer mainly long term benefits. Since 1980, researches mainly conducted by the International Institute of Tropical Agriculture (IITA) and Semi-Arid Food Grain Research and Development (SAFGRAD) led to identification or creation of cultivars exhibiting varying degrees of resistance to *Striga* parasitism, i.e. 58-57, Suvita-2, B301 (Singh and Emebeche, 1980; Moore *et al.*, 1995). *S. gesnerioides* resistant cultivars offer an economically feasible and culturally sustainable technology for smallholder farmers since they do not require additional inputs (IITA, 1983; Singh and Emebeche, 1990; Atokple *et al.*, 1993; Berner *et al.*, 1995). Based on the differential resistance response of various cultivars, landraces, and breeding lines, at least seven distinct races of cowpea-parasitic *S. gesnerioides* have been identified within the cowpea-growing regions of West Africa (Lane *et al.*, 1996). According to Botanga and Timko (2005), race formation in cowpea-*S. gesnerioides* was largely a result of host-driving selection because the parasite is autogamous, with a floral biology that makes any possibility of outcrossing very low. These races were designated as follow: SG1 (Burkina Faso), SG2 (Mali), SG3 (Nigeria and Niger), SG4 and SG4z (Benin), SG5 (Cameroon) and SG6 (Senegal). The biotype SG5 closely related to the race SG1 from Burkina Faso is present in northern Cameroon (Lane *et al.*, 1996; Botanga and Timko, 2006). However, there are no high yielding *S. gesnerioides* resistant cultivars for resource-poor farmers in northern Cameroon (Moffi Ta'ama, 1995; Guissai, 2010). B301 (originating from Botswana) and 'IT82D-849' (a breeding line from IITA) are resistant to races 1, 2, and 3, whereas Gorom (from Burkina Faso) and 'IT81D-994' (another breeding line for IITA) are resistant to races 1, 2 and 4 (Lane *et al.*, 1993). In cowpea, resistance depends on *Striga* strains and results from one or a combination of several recognised mechanisms that influence the development of the parasite (Parker and Polniaszek, 1990; Müller *et al.*, 1990; Lane *et al.*, 1996; Touré *et al.*, 1997; Reiss and Bailey, 1998). In addition, it is noteworthy that low emergence of *Striga* under infestation is sufficient to confer field resistance to *S. gesnerioides*, independent of other resistance mechanisms (Reiss and Bailey, 1998).

The genetics of cowpea *Striga*-resistance varies according to the biotype of the parasite and varieties, and it is inherited mainly as a single, nuclear, dominant gene (Singh and Emebeche, 1990; Atokple *et al.*, 1993; Lane *et al.*, 1993; Moore *et al.*, 1995; Touré *et al.*, 1997; Carsky *et al.*, 2003). However, few works pointed out that the resistance is conferred by two independent dominant genes (Dubé, 1988) or a recessive single gene (Touré *et al.*, 1997). The inheritance of resistance to *S. gesnerioides* biotype SG5 spread in northern Cameroon remains little known contrary to races present in West Africa (Ouedraogo *et al.*, 2000).

The objectives of this study were to screen twelve selected cowpea cultivars for their resistance to *S. gesnerioides*

race SG5 and to determine the inheritance of resistance in order to include the genes in cowpea improvement programs.

Materials and methods

Field experiments took place in the research field of the Institute of Agricultural Research for Development (IRAD) Maroua Station, at Giring (09°30' N and 10° 32' E), 7km from Maroua, Northern Cameroon. The soil was heavily infested by *S. gesnerioides* race SG5 during 2008 rainy season (Guissai, 2010). Giring belongs to the Sudano-Sahelian belt with ferruginous vertisol soil type. The soil is sandy clay with 8.2 mg kg⁻¹ organic matter and pH 5.65 (Guissai, 2010). Annual average pluviometry ranges between 800 to 900 mm, with four months rainy season, from June to September.

The cowpea cultivars consisted of 11 improved cowpea varieties of the IITA, and of the main local check 'VYA', which is susceptible to *S. gesnerioides* race SG5 (Moffi Ta'ama, 1995; Guissai, 2010). The eleven IITA varieties recommended for testing among farmers in the Sudano-Sahelian zone were 'IT98K-205-8', 'IT98K-503-1', 'IT98K-1092-1', 'IT99K-216-4-4', 'IT98K-494-6', 'IT99K-529-4', 'IT99K-573-1-1', 'IT99K-573-2-1', 'IT00K-1207', 'IT03K-378-4' and 'IT04K-227-4'. They were indeterminate, semi-erect, and photosensitive and high yielding under *Striga* free conditions (Guissai, 2010). There was little information about their performance under *S. gesnerioides* infestation in northern Cameroon.

In field trials, the cowpea varieties were arranged in a randomized complete block designs with four replications. Sowing took place on June 28, 2009, at the beginning of the rainy season on a rectangular surface of 270 m² (27m length x 10m broad). Each plot consisted on four ridges 4m long. Two seeds of each variety were sown at an intra-row spacing of 0.25 m. A standard insecticide formulation, cypermethrin + dimethoate at the rate 30g + 250g a.i./L was applied at flowering stage to control flowers *Thrips*, pod sucking and *Maruca* insects' pests.

The days taken to first *Striga* emergence was recorded in each ridge. At 9 weeks after planting (WAP), emerged *S. gesnerioides* plants were counted on 20 selected cowpea plants from the two middle ridges. On these plants, the percentage of those infected by *Striga* as well as the ratio of decimated plants was also determined. *Striga* height was measured on 10 randomly selected parasite plants as the distance from the soil surface to the tip of the shoot. To evaluate the incidences of the parasite on the legume grain yield, the number of pods per plant and the weight of the seeds were measured on 10 cowpea plants infected by *Striga* and 10 uninfected cowpea plants in each plot. After the pods were separated, the leaves and stem were combined and weighed to estimate also fodder yield on dry matter basis. The data obtained on these parameters from *Striga*

infected cowpea plants (INF) were compared with that from uninfected cowpea plants (C), as shown below.

Percent (%) change in the parameter =

$$\frac{\text{INF}-\text{C}}{\text{C}} \times \frac{100}{1}$$

The level of resistance of each genotype was appreciated on the basis of degree of parasite infestation as well as the development and cowpea yield (Aggarwal and Ouedraogo, 1989; Thalouarn and Fer, 1993).

For the study of the inheritance of the resistance, two F_2 populations were developed and used in the study. These populations were generated by crossing the *Striga* completely resistant cultivars 'IT99K-573-1-1' and 'IT98K-205-8' with the *Striga* susceptible 'VYA'. The crossings were carried out with emasculation between 6h and 10h in the morning. First filial generation hybrids F_1 derived from these crosses were self-fertilized during the rainy season to generate F_2 populations segregating for resistance. Parental, F_1 and F_2 individuals from the two populations were grown in four liters pots (Stewart seedling pot 7) from December 2009 to January 2010. The pots were filled with a mixture of ground and conditioned seeds of *Striga*. About 0.03 g of preconditioned *S. gesnerioides* seeds and 2 g of mineral fertiliser (7% N; 14% P_2O_5 ; 7% K_2O) were thoroughly mixed with the top 5cm of soil. Seeds of *S. gesnerioides* were preconditioned in sands for 10 days prior to planting the cowpeas. Four cowpea seeds were subsequently planted per pot and thinning was done one week after crop emergence to leave two plants per pot. Plants were regularly watered as needed. The observations proceeded in February 2010. Plants were pulled from the pots and their roots examined for attached and pre-emergent *S. gesnerioides* seedlings. The roots were unearthed and cleaned delicately at the time of the last observation (85 days after planting) to check the presence of not emerged seedlings of *Striga*. The presence of *Striga* was recorded from each plant. Plants allowing parasite attachment, development and emergence were classified as susceptible while those free from infestation were deemed resistant (Singh and Emebeche, 1990).

All field data recorded were subjected to analysis of variance (ANOVA) using computer program STATGRAPHICS PLUS. Varietal means were compared using Least Significant Difference at 5% level of probability (LSD 5%). Pearson's correlation coefficient was used to test for a correlation between number of emerged *Striga* and the percentage of decimed plants and grain and fodder yield.

Chi-square analysis was used to determine the goodness-of-fit of observed to expected genetic ratios from F_2 data.

Results and discussion

The analysis of variance revealed that differences among the genotypes were significant for all tested characters ($P < 0.05$). Replications effects were not significant for all variables ($P > 0.05$). The details of *Striga* emergence and degree of parasite infestation as influenced by genotype are presented in Tab. 1. Days to *S. gesnerioides* emergence for the parent stocks ranged from 37 to 54 from cowpea planting. Cultivars 'IT03K-378-4' and 'IT98K-1092-1' significantly ($P < 0.05$) delayed the emergence of witchweed by about two weeks, compared to 'IT99K-216-4-4', 'IT04K-227-4', 'IT99K-529-4' and 'VYA' whose emergence appeared 5 weeks after cowpea planting. The mean witchweed count for the 10 parasitized cowpea varieties was 1.63 ± 0.38 *S. gesnerioides* seedlings cowpea⁻¹ 9WAP. The number of emerged *Striga* per plant (mean = 1.63) was globally fewer compared to that of others studies (Carsky et al., 2003; Kamara et al., 2008). No *Striga* emerged from 'IT98K-205-8' and 'IT99K-573-1-1' while cultivars 'VYA', 'IT99K-529-4' and 'IT99K-216-4-4' reached maximum count after 9 WAP (1.75-2.40 *Striga* cowpea⁻¹). 'IT98K-1092-1' and 'IT03K-378-4' supported significantly lower numbers of *S. gesnerioides*. On varieties 'IT03K-378-4' and 'IT98K-1092-1', the few emerged *Striga* were stunted and did not flower.

Major differences in expression of resistance have been observed in different varieties (Parker and Polniaszek, 1990; Mohamed et al., 2010). The biology of *S. gesnerioides* and the histology of infected cowpea plants has been extensively studied (Müller et al., 1992; Thalouarn and Fer, 1993; Reiss and Bailey, 1998; Botanga and Timko, 2005). Germination, haustorial induction, attachment to, and penetration of the host vascular system are all critical events in *Striga* life cycle. An array of molecules which differ in chemical structure and specific activity are produced by the host roots. Conditioned seeds of *S. gesnerioides* germinated when exposed to these root exudates. Following germination, a second host derived chemical signal from the root, known as the haustorium initiation factor, is required for the differentiation of radicle into the haustorium by which the *Striga* seedlings attach to and penetrate the host roots. Once, in contact with cowpea roots, the radicular apex develops numerous hairs, which attach to host roots. When the vascular connection is established between the host and the parasite, the development of the haustorium ceases, the *Striga* seedling enlarges, forming a thick mass of tissue called the tubercles. The haustorium permits the transfer of water and nutrients to the parasite. Resistant cowpea genotypes are freed from parasitic *Striga* and show delayed or less parasite emergence. They may produce no or little amounts of root exudates or may inhibit the haustorial formation and/or further growth. *Striga* penetration of host root tissues involves a combination of intrusive growth and enzymatic digestion (Godwa et al., 1999). Lane et al. (1993) observed that on

the resistant line B301, the roots stimulate germination of *Striga* seeds and permit attachment, but haustorial formation and growth are inhibited. In cowpea, Botanga and Timko (2005) evaluated the host-parasite interactions and the germination stimulation by roots exudates from different cowpea varieties. They concluded that the differences in the level of germination were highly significant ($P = 0.01$) with cowpea-*S. gesnerioides* race SG5 from Cameroon having the highest germination. Developmental processes occurring post germination and attachment were likely important in limiting successful host-parasite interactions. Botanga and Timko (2005) mentioned that incompatibility appeared to be the result of the failure of the parasite to establish proper vascular connection (xylem-xylem linkage) with the host. It is possible that in the absence of a specific gene-for-gene resistance response, a default non-host resistance or basal resistance is mounted, leading to cessation of further invasion and arrest of parasite development. Godwa et al. (1999) pointed out that the response to *Striga* varies among genotypes suggesting that differences exist in the ability of these plants to recognize the pathogen and to activate defence response cascades.

According to Kamara et al. (2008), the magnitude of infestation is higher where the soil is sandy with poor fertility and low rainfall. Fertilisation and higher rainfall may reduce the severity of infestation. High level of fertilizers particularly nitrogen reduces or delays *Striga* germination or development (Alonge et al., 2005). There was a positive and highly significant ($P < 0.01$) correlation between the number of emerged *Striga* seedlings per cowpea plant and the number of decimated cowpea plant ($r = 0.93$, $n = 10$). The total number of parasite might be difficult to estimate from the number of emerged *Striga* because many subterranean *Striga* plants attached to the host failed to emerge (Aggarwal and Ouedraogo, 1989; Ajeibe et al., 2008). On cowpea, symptoms of infection are interveinal chlorosis, general stunting, smaller leaves and straw-colored necrotic

spots on the lamina. Reiss and Bailey (1998) and Alonge et al. (2005) noted similar observations on infected cowpea. Approximately half of the *Striga* life cycle is underground, during which time much of the damage to crop occur, in part through the diversion of host resources to the parasite. It has been estimated that the loss of carbon from the host to the parasite is more important than reduced photosynthetic capacity of the host imposed by the parasite (Hibberd et al., 1996). The death of young cowpea seedlings can be explained by complete desiccation leaves due to the transfer of water to parasitic *Striga*. The varieties which show low *Striga* height would be those which allocate small amount of water and nutrients to the parasite. The damage done to the susceptible crop host could not be explained only by nutrient diversion. Botanga and Timko (2005) reported that the exchange of chemicals is bidirectional and that the parasite possibly releases some toxins that inhibit the growth and the development of the host. This study confirms the findings of Kamara et al. (2008) and Carsky et al. (2003) that there were differences in the growth of *Striga* on cowpea varieties. To date, little is known about the actual host defense that discourage parasitic growth and establishment (Mohamed et al., 2010)

By 9WAP, the percentage of yield losses for the number of pods per plant and grain yield was evaluated by comparing the parameters on cowpea parasitized by *Striga* and uninfected plants (Tab. 2). Changes in fodder yield were also recorded (Tab. 3). Infestation with *S. gesnerioides* induced a highly significantly yield loss for cultivars 'IT99K-216-4-4', 'IT04K-227-4', 'IT99K-529-4' and 'VYA'. Cultivars 'IT98K-205-8', 'IT99K-573-1-1', 'IT98K-1092-1' were the genotypes that did not have a significant yield reduction between infected and uninfected plants. The impact of *S. gesnerioides* infestation on cowpea grain production and fodder yield varied significantly with varieties (Tab. 2 and 3). Moffi Ta'ma (1995) observed that in northern Cameroon, the grain yields of cowpea varieties grown by

Tab. 1. Mean *S. gesnerioides* counts, days to *Striga* emergence, percentage of infected plants and ratio of decimated plants of ten cowpea cultivars at Giring Research Station, 1999 rainy season

Cowpea cultivar	Days to <i>Striga</i> emergence	<i>Striga</i> counts per plant	Percentage of infected plants	Percentage of dead plants	<i>Striga</i> height (cm)
'IT98K-503-1'	48.50 ^b	1.60 ^{cc}	68.75 ^{ab}	22.50 ^c	10.86 ^e
'IT98K-1092-1'	54.25 ^a	0.70 ^g	21.25 ^d	15.00 ^{dc}	9.25 ^f
'IT99K-216-4-4'	51.00 ^b	2.00 ^{abc}	68.75 ^{abc}	34.38 ^b	13.21 ^{abc}
'IT99K-494-6'	42.50 ^{dc}	1.25 ^{cf}	60.00 ^c	17.50 ^d	11.39 ^{dc}
'IT99K-529-4'	37.00 ^f	2.15 ^{ab}	70.00 ^{ab}	40.00 ^a	12.44 ^{bcd}
'IT99K-573-2-1'	37.25 ^f	1.85 ^{bc}	66.25 ^{abc}	16.25 ^d	12.09 ^{cdc}
'IT00K-1207'	43.00 ^{cd}	1.65 ^{cde}	62.50 ^{bc}	23.13 ^c	13.47 ^{ab}
'IT03K-378-4'	54.00 ^a	0.90 ^{fg}	22.50 ^d	11.25 ^c	8.49 ^f
'IT04K-227-4'	45.75 ^{bc}	1.75 ^{bc}	67.50 ^{abc}	33.75 ^b	12.08 ^{cdc}
'VYA'	39.75 ^{ef}	2.40 ^a	73.75 ^a	43.75 ^a	13.90 ^a
Mean	45.30	1.63	58.12	25.72	11.72
LSD (0.05)	2.80	0.42	9.25	4.55	1.27

Means followed by the same letter(s) in each vertical column are not significantly different ($P = 0.05$) using LSD

Tab. 2. Mean pods per plant, grain yield (kg ha⁻¹) and fodder yield (kg ha⁻¹) cowpea response for infection by *Striga gesnerioides* at Giring Research Station, 1999 rainy season

Cultivar	Pods per plant			Grain yield (kg ha ⁻¹)		
	INF	C	L (%)	INF	C	L (%)
IT98K-205-8	7.88 ^{cc}	7.88 ^{bc}	+0.00 ^{ab}	871.10 ^{bc}	871.10 ^{bcd}	0.00 ^a
IT98K-503-1'	7.06 ^{efg}	7.92 ^{bc}	-10.85 ^c	770.00 ^{cd}	852.11 ^{bcd}	-9.63 ^b
IT98K-1092-1'	8.55 ^{bc}	8.70 ^{ab}	-1.72 ^{bc}	904.23 ^{bc}	934.77 ^{bcd}	-3.26 ^a
IT99K-216-4-4'	6.02 ^{gh}	8.39 ^{bc}	-28.25 ^{fg}	678.92 ^{de}	955.40 ^{bc}	-28.94 ^c
IT99K-494-6'	8.22 ^{bc}	8.74 ^b	-5.95 ^d	887.48 ^{bc}	912.09 ^{bc}	-2.70 ^a
IT99K-529-4'	5.60 ^h	7.99 ^{bc}	-29.91 ^{gh}	480.51 ^f	755.93 ^{de}	-36.43 ^e
IT99K-573-1-1	9.42 ^{ab}	9.42 ^{ab}	+0.00 ^{ab}	1042.75 ^{ab}	1042.75 ^{ab}	0.00 ^a
IT99K-573-2-1'	7.46 ^{ef}	8.00 ^{bc}	-6.75 ^d	726.65 ^{cde}	804.17 ^{cde}	-9.64 ^b
IT00K-1207'	6.56 ^{efgh}	6.92 ^c	-5.20 ^{cd}	641.24 ^{def}	697.00 ^e	-8.00 ^b
IT03K-378-4'	10.66 ^a	10.41 ^a	+2.40 ^a	1191.10 ^a	1208.50 ^a	-1.44 ^a
IT04K-227-4'	6.36 ^{gh}	8.53 ^b	-25.43 ^f	528.54 ^f	766.32 ^{cde}	-31.02 ^{cd}
'VYA'	5.54 ^h	8.18 ^{bc}	-32.28 ^h	579.24 ^{ef}	904.72 ^{bcd}	-35.97 ^{de}
Mean	7.44	8.43	-11.74	775.15	892.07	-13.91
LSD (0.05)	1.32	1.55	3.90	178.10	196.30	4.00

INF, Cowpea plants infected with *Striga*; C, Uninfected cowpea plants; L, Percentage of yield loss. Means followed by the same letter(s) in each vertical column are not significantly different (P= 0.05) using LSD

smallholders were between 300 to 1200 kg ha⁻¹ while the fodder yields were between 600 to 2000 kg ha⁻¹. There was a negative and highly significant (P = 0.01) correlation between the number of emerged *Striga* seedlings per cowpea plant and the number of pods per plant (r = -0.88, n = 10), and the seed yield loss (r = -0.91, n = 10), and the haulms yield (r = -0.78, n = 10). Number of pods, and grain and fodder yield were negatively correlated with number of emerged *Striga* suggesting that the *Striga* infestation was responsible for yield reduction. Varieties that supported a

Tab. 3. Mean fodder yield (kg ha⁻¹) cowpea response for infection by *Striga gesnerioides* at Giring Research Station, 1999 rainy season

Cultivar	Fodder yield (kg ha ⁻¹)		
	INF	C	L (%)
IT98K-205-8'	2000.50 ^b	2000.50 ^b	0.00 ^a
IT98K-503-1'	2266.00 ^a	2499.35 ^a	-9.34 ^c
IT98K-1092-1'	1107.44 ^c	1178.13 ^{de}	-6.00 ^{bc}
IT99K-216-4-4'	839.35 ^{ef}	1176.23 ^{de}	-28.64 ^f
IT99K-494-6'	811.76 ^{ef}	839.70 ^c	-3.33 ^{ab}
IT99K-529-4'	686.57 ^f	1164.24 ^{de}	-41.03 ^g
IT99K-573-1-1'	1119.98 ^{de}	1119.98 ^{de}	0.00 ^a
IT99K-573-2-1'	1636.28 ^{bc}	1887.36 ^b	-13.31 ^d
IT00K-1207'	863.55 ^{ef}	967.04 ^c	-10.70 ^{cd}
IT03K-378-4'	1490.25 ^{cd}	1478.98 ^{cd}	+0.76 ^a
IT04K-227-4'	681.60 ^{ef}	892.54 ^c	-23.63 ^c
'VYA'	740.92 ^f	1233.20 ^{cde}	-39.92 ^g
Mean	1187.01	1369.77	-14.59
LSD (0.05)	372.66	394.25	4.94

INF, Cowpea plants infected with *Striga*; C, Uninfected cowpea plants; L, Percentage of fodder yield loss. Means followed by the same letter(s) in each vertical column are not significantly different (P= 0.05) using LSD

higher number of *Striga* also had a lower number of pods, grain and fodder yield than the *Striga*-resistant genotypes.

Striga-resistant cowpea varieties have relative good growth and probable less export of assimilate to the parasite would have ensured adequate biomass accumulation and grain development. Similar reports were published by Hibberd *et al.* (1996) and Alonge *et al.* (2005). According to Hibberd *et al.* (1996), final biomass accumulation by cowpea infected with *S. gesnerioides* was significantly lower than that by uninfected plants. The additional sink created by *Striga* when parasitizing cowpea plants replaces the sink normally present within the host (pods). In contrast with this was the varying degree of grain yield reduction in heavily susceptible or tolerant varieties. These varieties with reduced vegetative growth, probably suffered from reduced leaf area, photosynthetic capacity and consequently limited flowering, podding and seed development, due to water shortage resulting from the characteristically high transpiration rate of the parasite.

Infested plants failed to make any significant investment of dry matter in pods. Alonge *et al.* (2005) pointed out that on susceptible cowpea, *Striga* infestation induced grain yield losses by 78.9-86.2%. According to Singh and Emebeche (1990), the extent of yield reduction depends upon the time and level of infection. The decrease in grain yield could have been partly due to the reduction in the root nodulation and root growth by the parasite. According to Alonge *et al.* (2005), even though few attached *Striga* on resistant varieties, in most cases, it is likely that the seeds of these parasites contain toxins which leaked into the soil and hindered the root growth. This could result in inadequate nitrogen and nutrient absorption for vegetative growth and consequently reduced grain yield.

Aggarwal *et al.* (1989); Singh and Emebeche (1990) also mentioned reduced growth and vigour in susceptible cowpea genotypes with reduced leaf area and stunted plants. The reduction of fodder yield will lead to serious shortages because cowpea fodder is very important as livestock feed in northern Cameroon.

Considering the categorization suggested by Thauouarn and Fer (1993); Ajeigbe *et al.* (2008), cowpea cultivars without *Striga* and showing a normal productivity under infestation are regarded as resistant, while those with many *Striga* and a considerable yield reduction are known as susceptible. Tolerance implies the ability of the host crop to produce acceptable yield in the presence of heavily *Striga* infestation. Thus, improved lines 'IT98K-205-8' and 'IT99K-573-1-1' are completely resistant (immune) to *S. gesnerioides* race SG5 while 'IT99K-216-4-4',

'IT00K-1207', 'IT99K-494-6' and 'IT99K-573-2-1' were tolerant, considering the high number of witchweed count (1.25 – 1.85 *Striga* cowpea⁻¹) and moderate yield reduction (-2.70 to -9.64% for grain and -3.33 to -13.31% for haulms) (Fig. 1). In northern Nigeria where *S. gesnerioides* race SG3 is widely distributed, Ajeigbe *et al.* (2008) observed during two years of field evaluation under natural infestation that 'IT98K-205-8' and four other cultivars namely 'IT98K-205-10', 'IT98K-452-1', 'IT98K-428-3' and 'IT98K-503-1' were completely resistant to *Striga* and thus supported no *Striga* emergence. The cowpea genotype 'IT98K-205-8' might combine resistance to *S. gesnerioides* biotypes SG3 and SG5. Cultivars 'IT03K-378-4' and 'IT98K-1092-1' supported the lowest *S. gesnerioides* counts, delayed emergence of the parasite for about two weeks while maintaining the normal productivity. These cultivars were therefore considered as partially resistant.

The delay of witchweed emergence on resistant genotypes might partly explain why they do not significantly lose their yield under *Striga* infestation (Kamara *et al.*, 2008). *Striga* appeared taller on susceptible genotypes than resistant ones. The mechanism of resistance of completely resistant lines 'IT98K-205-8' and 'IT99K-573-1-1' might consist to prevent the germination of *Striga* seeds or the penetration of host roots by parasite radicles. Several host resistance mechanisms have been proposed in the literature including low germination stimulant, low production of the haustorial initiation factor, avoidance mechanisms, presence of physical barriers, and antibiosis (Mohamed *et al.*, 2010). According to Moore *et al.* (1995) the best characterized of these mechanisms in cowpea is low stimulant production by resistant genotypes and consequently, an absence of the germination of *Striga* seeds. In partially resistant cowpea 'IT03K-378-4' and 'IT98K-1092-1', the mechanism of resistance may consist of limiting the growth of the parasite. Atople *et al.* (1993), Lane *et al.* (1993) reported varying mechanisms of resistance to *Striga* in different cowpea varieties. Careful observation has provided evidence for at least two different mechanisms of resistance to *Striga* parasitism in cowpea (Li *et al.*, 2009; Li and Timko, 2009). One mechanism resembles the hypersensitive response (HR) observed in other plant-pathogen interactions and suggests the presence of a specific R gene-mediated response mechanism. The second type of resistance response involves arrested development of the parasite tubercle following attachment and attempted penetration of the host root cortex. Mohamed *et al.* (2010) mentioned four separate mechanisms of *Striga asiatica* resistance in sorghum: 1) low production of *Striga* seed germination stimulants; 2) evidence of germination inhibitors; 3) low production of signal required for haustorial initiation and 4) a hypersensitive response characterized by a distinct necrotic area on the host root at the attachment site that discourage parasitic establishment.

Inheritance studies of the resistance for the two populations were presented in Tab. 4 and 5. All F₁ plants derived

Tab. 4. Segregation ratio of F₂ plants derived from a cross of *Striga*-susceptible cowpea 'VYA' x *Striga*-resistant genotype 'IT98K-205-8'

Generation	Observed		Expected		χ^2 (3R:1S)	P-value
	R	S	R	S		
'VYA' (n = 8) ^a	0	8	0	8		
IT98K-205-8 (n = 8)	8	0	8	0		
F ₁ (n = 10)	10	0	10	0		
F ₂ (n = 136)	97	39	102	34	0.98	0.30 – 0.50

R, Resistant to *Striga* SG5; S, Susceptible to *Striga* SG5; P, Probability

^aNumber in parenthesis indicates number of tested plants.

'IT04K-227-4', 'IT99K-529-4' and 'VYA' were susceptible to this parasite strain. Their magnitudes of grain yield loss (-28.94 to -36.43%) and fodder yield loss (-23.63 to -41.03%) under *S. gesnerioides* infestation support this classification. In addition, cultivars 'IT98K-503-1',



Fig. 1. Cowpea cv. 'IT99K-494-6' infested by *Striga gesnerioides* in field at Giring Research Station, 1999 rainy season

Tab. 5. Segregation ratio of F₂ plants derived from a cross of *Striga*-susceptible cowpea 'VYA' x *Striga*-resistant genotype 'IT99K-573-1-1'

Generation	Observed		Expected		χ^2 (15R:1S)	P-value
	R	S	R	S		
'VYA' (n = 8) ^a	0	8	0	8		
'IT99K-573-1-1' (n = 8)	8	0	8	0		
F ₁ (n = 10)	10	0	10	0		
F ₂ (n = 144)	131	13	135	9	1.12	0.20 – 0.30

R, Resistant to *Striga* SG5; S, Susceptible to *Striga* SG5; P, Probability

^aNumber in parenthesis indicates number of tested plants

from the two combinations were totally resistant to *Striga*. Monogenic dominant inheritance has been demonstrated in the progeny of the cross between 'IT98K-205-8' and susceptible line 'VYA' (Tab. 4) The F₂ segregated in the ratio 3R:1S. We designated this gene *Rsg5*. This resistance is easy to transfer into elites cultivars.

Segregation patterns indicate two factors inheritance for resistance to *S. gesnerioides* in 'IT99K-573-1'. Results on genetic analysis (Tab. 5) showed that in 'IT99K-573-1-1', resistance to SG5 may be conferred by duplicate dominant genes (the resistance is expressed if a dominant gene is present at either locus or at both loci). The F₂ segregated in the ratio 15R:1S. We designated these genes *Rsg5a* and *Rsg5b*. Resistance may involve the interplay of several loci. High level of resistance in IT99K-573-1 could be explained due to the presence of two resistance gene. This suggests a breeding strategy of pyramiding suitable resistance genes for enhancing *Striga*-resistance in cowpea.

Initial inheritance studies indicated the nature of resistance to *S. gesnerioides* race SG1, race SG2, race SG3 and race SG4 in some cowpea genotypes to be monogenic dominant (IITA, 1983; Singh and Emebeche, 1990; Atokple et al., 1993; Moore et al., 1995; Touré et al., 1995). Dubé (1988) mentioned that in genotype HTR the resistance to *S. gesnerioides* from Burkina Faso may be controlled by two genes. The genetic basis of resistance to *S. gesnerioides* depends on varieties. Tests of allelism conducted by Atokple et al. (1995) indicated that the resistance presents in B301, 'IT81D-994' and Gorom is conferred by distinct genes designated *Rsg1*, *Rsg2* and *Rsg3* respectively. In Mali, Moore et al. (1995) observed that two cowpea landraces, APL-5 and 87-2 were completely resistant to *S. gesnerioides* from Cameroon. Touré et al. (1997) observed that the inheritance of resistance to *S. gesnerioides* biotype from Maradi (Niger) would be monogenic and recessive in line 'IT82D-849'. Cowpea is considered to have been domesticated in Africa and likely to have co-evolved with *Striga*. Thus, it may have many of the requisite genes for resistance. Resistance against *S. gesnerioides* is often difficult to assess due to numerous confounding factors in the field, including parasite variability and unpredictable environmental influences.

Our results suggest that the resistance of all the two sources would be easy to transfer to promising breeding lines. A better understanding of the factors influencing the resistance of the host, or conversely the virulence of the parasite could lead to great improvements in the reliability of resistant varieties. Digenic resistance is more long-term than the monogenic breeding. Race specific type of resistance should be avoided to minimize epidemic caused by a single gene in future. In view of the fact that the strain diversity exists in *Striga*, it's desirable to have genetically diverse sources of resistance so that suitable resistance can be bred into new improved cowpea varieties. This will enable the development of cowpea plant resistance to a broad range of *Striga* strains, even in the wake of new strains. A decade, more or less, is needed to breed a superior improved line using traditional selection and hybridization strategies to combine resistance to numerous *Striga* races and other desirable traits. However, studies at the molecular level should be undertaken as a priority. Genomic libraries of *S. gesnerioides* and cowpea should be constructed using in vitro translation, cDNA synthesis, hybridization and cloning techniques studies of resistance gene should be initiated.

Plant disease resistance genes encode proteins grouped into distinct categories based upon their structural similarity and likely mechanistic role in the activation of defense responses. Pathogen recognition triggers a signal transduction cascade leading to the production of endogenous chemical compounds capable of activating both localized and systemic responses. Salicylic acid and its methyl esters, jasmonic acid and ethylene are signaling molecules involved in the pathogenesis-related gene expression. Initial studies of host and non-host plant interactions with *Striga* revealed considerable variation in both the extent and rapidity of defense responses, from failure to recognize the invading pathogen to rapid and multifaceted responses. Li et al. (2009) on the molecular genetics of race specific resistance to *S. gesnerioides*, suggested that phytohormone PR5 expression may be a useful marker of *Striga* infection and that the salicylic acid signaling appeared to play a key role in the cowpea-*Striga* interactions. Li and Timko (2009) observed on multirace-resistance cowpea cultivar 301 that a gene-for-gene resistance mechanism is operating in these unique plant-plant associations. Race-specific resistance of cowpea to *Striga* involves a coiled-coil nucleotide binding site leucine-rich repeat domain resistance protein encoded by the RSG3-301 gene. Knockdown of RSG3-301 expression by virus-induced gene silencing in the multirace-resistant cowpea cultivar B301 results in the failure of RSG3-301-silenced plants to mount a hypersensitive response and promotes parasite necrosis when challenged with *Striga* race SG3, whereas the resistance response to races SG2 and SG5 is unaltered. According to Godwa et al. (1999) in parasitism by *S. asiatica*, a 917bp cDNA encoding a 221 amino acid protein with significant homology to proteins encoded by disease resistance from

other plant species, including N, RPP5, L6 and M was used to isolate a host nuclear gene designated NRSA-1. This resistance gene is involved in the activation or regulation of downstream defense responses. NRSA-1 expression is induced by jasmonic acid and their transcripts accumulate to high level in the host roots near the site of *Striga* invasion.

The current focus in applied breeding is leveraging biotechnological tools to develop more and better markers linked to resistance traits and the establishment of protocols that will allow marker-assisted selection (MAS) to be readily employed. The use of MAS makes easier the selection of plant traits and reduces the time needed to develop new varieties. Races SG1 and SG2 specific resistance genes have been identified and located in one of the two linkage group of the current cowpea map (Ouedraogo *et al.*, 2000; Li *et al.*, 2009). Molecular markers associated with races SG1 and SG3 resistance genes have been identified, and several sequence-confirmed amplified regions (SCARs) have been developed for use in marker-assisted selection and breeding strategies for rapid cowpea improvement (Li *et al.*, 2009). According to Omoigui *et al.* (2009) the application of MAS using FTA (Flinders Technology Associations, matrix cards developed by Whatman for the rapid collection, purification and analysis of genomic DNA) technology for sampling, storage and retrieval of plant total genomic DNA has the potential to increase efficiency of molecular characterization of cowpea lines for their resistance to *Striga*, and speed-up the process of crop delivery to farmers. With well-defined race specific markers, it should be possible to breed cultivars with resistance to all currently races of *S. gesnerioides*. Although the ability to transform cowpea is actually limited, the integration of genetic engineering into traditional breeding programs is another issue that needs to be considered.

Conclusions

Given the importance of cowpea as a food security crop in northern Cameroon, the continuous production of susceptible varieties to *Striga* like 'VYA', lead to the reduction of yield which may pose a serious food security threat to the area. This study revealed that cowpea improved lines 'IT99K-573-1-1' and 'IT98K-205-8' which are completely resistant to *S. gesnerioides* race SG5 under infestation in field and pot. Results on genetic analysis demonstrated that in 'IT99K-573-1-1' resistance to SG5 may be conferred by duplicate dominant genes without cumulative effects while in 'IT98K-205-8', it is controlled by one dominant gene. These genes could be exploited in breeding for *S. gesnerioides* race SG5 resistance in cowpea. With these new sources, efforts should therefore be made to develop *Striga*-resistant cowpea varieties that meet end-user preferences.

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