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CO_2-assimilation and chlorophyll fluorescence as indirect selection criteria for host tolerance against *Striga*

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Abstract *Striga hermonthica* (Del.) Benth. is a parasitic weed on tropical cereals causing serious yield losses in Africa. The use of host crop varieties with improved resistance and tolerance against this parasite is a key component of an integrated control strategy. Breeding for tolerance is however seriously hampered by the absence of reliable and yet practical selection measures. The observation that the photosynthetic rate of tolerant genotypes is less sensitive to *Striga* infection was used as a starting point to search for suitable selection measures. In a greenhouse pot experiment the effect of *Striga* infection on the photosynthesis of four sorghum (*Sorghum bicolor* [L.] Moench) genotypes, differing in *Striga* tolerance level, was measured at three moments in time (26, 48 and 75 days after sowing). Genotypes were CK60-B, E36-1, Framida and Tiémarifing. Measurements involved CO_2-assimilation (*A*) and three chlorophyll fluorescence characteristics (electron transport rate through photosystem II [*ETR*], photochemical [*Pq*] and non-photochemical quenching [*NPq*]). *Striga* infection negatively affected *A*, *ETR* and *Pq*. Based on *A* and *Pq*, genotypes with superior levels of tolerance (Tiémarifing) could be discriminated from genotypes with superior level of resistance (Framida). Both *A* and *Pq* showed high heritabilities and consequently clear and predictable differences between genotypes. Using discriminative ability, heritability and cost effectiveness as main criteria, photochemical quenching (*Pq*) was concluded to possess the highest potential to serve as indirect selection measure for host plant tolerance to *Striga*. Screening should preferably be conducted at relatively high *Striga* infestation levels, between *Striga* emergence and host plant flowering.

Keywords Defence mechanisms · Parasitic weeds · Photochemical quenching · Photosynthesis · Selection measures · *Sorghum bicolor*

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Abbreviations

A	Leaf CO_2-assimilation rate (μmol CO_2 m^{-2} s^{-1})
ETR	The electron transport rate through photosystem II (μmol m^{-2} s^{-1})
f_{abs}	The absorbtivity of the leaf (–)
Fm'	The maximum fluorescence emission induced by a saturating light pulse in the light (–)
Fm	The maximum fluorescence emission induced by a saturating light pulse in the dark (–)
Fo'	The basic fluorescence in the light when all PSII centres are oxidized by a period of far-red light (–)
F_t	The steady-state fluorescence emission (–)
I	The light intensity (μmol photon m^{-2} s^{-1})
NPq	The level of non-photochemical quenching (–)
φ_2	The electron transport efficiency of PSII (–)
PAR	Photosynthetically active radiation (μmol photon m^{-2} s^{-1})
PS	Photosystem
Pq	The level of photochemical quenching (–)
R	Repeatability
RYL	Relative yield loss (%)
S^2	Within-group variance component
S_A^2	Among-group variance component
V_{EG}	General environmental variance
V_{ES}	Environmental variance due to temporary or localized environmental effects
V_G	Genotypic variance
V_P	Phenotypic variance

Introduction

Striga hermonthica (Del.) Benth. is an obligate hemiparasitic C_3 plant of the *Orobanchaceae* (formerly: *Scrophulariaceae*) family that parasitizes hosts of the *Poaceae* family. Among the C_4 hosts of *Striga* are crops such as pearl millet (*Pennisetum glaucum* [L.] R. Br. and *P. americanum* [L.] K. Schum), maize (*Zea mays* [L.]) and sorghum (*Sorghum bicolor* [L.] Moench) accounting for an estimated 25, 27 and 31% of the total area under cereal production in sub-Saharan Africa (FAOSTAT 2004). More than half of this area is estimated to be infested with *Striga* (Sauerborn 1991). Average yield losses due to *Striga* in West Africa are estimated to range between 10 and 31% and can reach 100% in severely infested fields (Sauerborn 1991). For these reasons, *Striga* is considered one of the major constraints to food production in semi-arid Africa (Bebawi and Farah 1981; Doggett 1982; Vasudeva Rao et al. 1989).

Striga parasitizes on the host root, subtracting host carbon assimilates (Rogers and Nelson 1962; Okonkwo 1966; Press et al. 1987a), water, nutrients (nitrate) and amino-acids (Pageau et al. 2003). However, the main part (80–84%) of the yield loss is caused by a pathogenic effect and its attendant decreased levels of cytokinins and giberellic acid (Drennan and El Hiweris 1979), and increased levels of abscisic acid (ABA) in the infected host plant (Drennan and El Hiweris 1979; Taylor et al. 1996; Ackroyd and Graves 1997; Frost et al. 1997). The latter cause reduced stomatal conductance, one of the reasons for reduced CO_2-assimilation of infected hosts (Prabhakara Setty and Hosmani 1981; Press and Stewart 1987; Press et al. 1987b; Graves et al. 1989; Gurney et al. 1995; Smith et al. 1995; Gurney et al. 1999). CO_2-assimilation of *Striga* infected host plants is further negatively affected through a reduced efficiency of the photosynthetic apparatus (Gurney et al. 2002).

The use of crop varieties with improved levels of defence is an important component of an integrated control strategy against *Striga*. Resistant genotypes are less frequently infected, while tolerant genotypes suffer less damage once they have been exposed to similar levels of infection (Parker and Riches 1993). Plants are susceptible to the degree that they lack resistance and sensitive in so much as they lack tolerance. Complete resistance against *Striga* has not yet been found. Hence every host plant remains exposed to the risk of *Striga* infection and concomitant damage and yield losses. As every host genotype combines resistance with tolerance in various degrees, breeding against *Striga* should be directed to enhance the levels of those two defence mechanisms (Kim 1991; DeVries 2000; Kling et al. 2000; Haussmann et al. 2001a; Haussmann et al. 2001b; Pierce et al. 2003; Showemimo 2003; Rodenburg et al. 2006a). Lines with superior levels of resistance should be crossed with lines showing superior levels of tolerance to achieve maximum defence against *Striga*. However, finding superiorly tolerant candidates for these crosses is complicated. Due to

differences in resistance level, it is difficult to induce identical infection levels across genotypes that would enable identification of tolerance (Rodenburg et al. 2006a). A direct quantification of tolerance based on relative yield loss is thus hampered by interacting effects of resistance (Rodenburg et al. 2005). To overcome this problem screening for tolerance at more than one *Striga* infestation level was proposed (Rodenburg et al. 2006a). The additional requirement of inclusion of *Striga*-free control plots for expressing the yield loss on a relative basis, makes screening for tolerance expensive and laborious.

Hence there is a need for alternative selection tools that (1) clearly discriminate superior tolerance from other reaction types (2) enable a reliable phenotypic assessment of genetic tolerance, and (3) are low-cost and practical. Previous studies revealed that tolerant host plant genotypes are able to maintain high levels of CO_2-assimilation upon infection (Gurney et al. 1995; Gurney et al. 2002). Therefore, this study explored options for the use of CO_2-assimilation and related chlorophyll fluorescence characteristics as indirect selection traits in screening host plant genotypes for tolerance to *Striga*.

Materials and methods

Experimental set-up

A pot experiment was carried out in the tropical greenhouse of Wageningen University, The Netherlands in 2004. The experiment was laid out according to a split-plot design in five replications, with four sorghum (*Sorghum bicolor* [L.] Moench) genotypes at the plot level and two *Striga* infestation levels (0 [control] and 4.0 seeds cm^{-3}) at the split-plot level. Reaction types of the four sorghum genotypes to *Striga* varied from susceptible and sensitive (CK60-B and E36-1) to resistant and moderately tolerant (Framida), to moderately resistant and tolerant (Tiémarifing). Furthermore, genotypes differed in origin, race, grain colour and morphology (Table 1). *Striga hermonthica* seeds were collected in 1998 in Samanko (Mali) from *Striga* plants parasitizing sorghum. Seed viability was 60%.

Pots used for this experiment had a volume of 12 l and a diameter of 28 cm. Pot spacing in the plot was 0.3 m and between plots 0.5 m. A 3:1 quartz sand: arable soil mixture was used and *Striga* seeds were mixed through the upper 10–12 cm soil layer. After infesting the soil with *Striga* seeds, all pots, including the uninfested ones, were kept moist for 10 days to allow preconditioning of the *Striga* seeds. The sorghum seeds were pre-germinated for 36 h before they were sown at a rate of 3 seeds per pot in late April. Thinning to one plant per pot was done at 7 days after sowing (DAS). Just before sowing, fertilizer was applied in a single dose equivalent to 50 kg N, 42 kg P and 75 kg K per hectare (N–P–K; 12:10:18).

Day length was held constant at 12 h (08.00 am–20.00 pm). Additional light was provided by high-pressure sodium lamps (400W SON-T Agro-Philips lamps) when incoming radiation dropped below 910 µmol photon m^{-2} s^{-1} (photosynthetically active radiation: PAR). Day temperatures did not fall below 28°C. Mean night temperature was 24°C. Mean relative humidity was kept between 50 and 70% for the duration of the experiment. Pots received water every two days, to create non-water-limited conditions.

Measurements and observations

Leaf CO_2 assimilation rates (*A*) of sorghum were measured on the same plants at 26, 48, and 75 DAS. Measurements were always made halfway along the length of the youngest fully expanded leaf, excluding the flag leaf. CO_2-assimilation was measured with the LI-COR-6400-40 (LI-COR Bioscience, Lincoln, Nebraska USA). This system has an incorporated light source with a programmable light intensity and an integrated modulated chlorophyll fluorescence measurement system. After a dark adaptation period of 5 min, CO_2-assimilation and fluorescence responses were measured at 1600 (at 26 DAS) or 2000 (at 48 and 75 DAS) µmol m^{-2} s^{-1} (PAR; over the waveband 400–700 nm). During the measurements, leaf temperature ranged between 28 and 33°C (mean: 31°C), the inlet CO_2 concentration was 400 ppm and depletion never exceeded 24 ppm.

Chlorophyll fluorescence measurements were used to derive the electron transport rate through photosystem II (PSII) (*ETR*), as well as the level of photochemical (*Pq*) and non-photochemical quenching (*NPq*). For the derivation of *ETR*, first the

Table 1 Characterization of the four sorghum genotypes used in the experiment

	CK60-B	E36-1	Framida	Tiémarifing
Origin	USA/N.E Africa	Ethiopia	S. Africa	Mali
Morphology	Short	Medium	Medium	Long
Race	Kafir	Caudatum	Caudatum	Guinea
Photoperiodicity	Insensitive	Insensitive	Insensitive	Sensitive
Cycle length (days)	100–110	120–130	120–130	Depends on day lengths
Grain colour	White	Cream	Red	White
Resistance	Very low	Very low	High	Medium
Tolerance	Very low	Low	Medium	High

Sources: (El Hiweris 1987; Olivier et al. 1991; Arnaud et al. 1996; Rodenburg et al. 2005; Rodenburg et al. 2006a; Rodenburg et al. 2006b)

electron transport efficiency of PSII (φ_2) was calculated as:

$$\phi_2 = 1 - Ft/Fm' \quad (1)$$

where Ft is the steady-state fluorescence emission, and Fm' is the maximum fluorescence emission induced by a saturating light pulse in the light (Genty et al. 1989). ETR was then calculated as:

$$ETR = \Phi_2 \rho f_{abs} I \quad (2)$$

where ρ is the factor to account for the partitioning of energy between the two photosystems (PSI and PSII), f_{abs} is the absorbtivity of the leaf and I is the light intensity (PAR) (Genty et al. 1989). Parameter ρ was set to 0.5, which is a common value (Maxwell and Johnson 2000; Rascher et al. 2000), and assumes that at any light level the excitation energy is equally distributed between PSI and PSII. The absorbtivity was set to 0.8, which indicates that of the incoming photosynthetically active radiation 80% is absorbed by the leaf (Goudriaan and Laar 1994).

Photochemical quenching of fluorescence (Pq) was computed as:

$$Pq = (Fm' - Ft)/(Fm' - Fo') \quad (3)$$

where Fo' is the basic fluorescence in the light when all PSII centres are oxidized by a period of far-red light (Schreiber 1986). Finally, non-photochemical quenching (NPq) was computed as:

$$NPq = (Fm - Fm')/Fm' \quad (4)$$

where Fm is the maximum fluorescence emission induced by a saturating light pulse in the dark (Genty et al. 1989).

Above ground *Striga* counts were done at each CO_2-assimilation measurement time. Sorghum total grain weight of each individual plant at harvest time was assessed after drying (48 h at 70°C) and threshing of the panicles.

Statistical analysis

All data were subjected to analysis of variance followed by a comparison of means with the least significant difference (LSD) using the Genstat (8th Edition) statistical software package. Pearson correlation tests were done with the SPSS (version 10.0) statistical software package.

A quantitative genetics framework was used for approximating the heritability of each parameter in the data set. To do so, the genotype repeatability was calculated for each parameter. Repeatability is normally measured for individuals, not genotypes, and is then defined as the proportion of phenotypic variance of a parameter that is explained by individual:

$$R = (V_G + V_{EG})/V_P \quad (5)$$

where V_G is the genotypic variance, V_{EG} the general environmental variance (variance due to environmental factors with irreversible effects on the phenotype), and V_P the total phenotypic variance (Falconer and Mackay 1996). When calculated for genotypes instead of individuals, repeatability thus approximates heritability (h^2):

$$h^2 = V_G/V_P \quad (6)$$

because V_{EG} is effectively excluded from the equation. Repeatability can readily be calculated as:

$$R = s_A^2/(s^2 + s_A^2) \quad (7)$$

where s_A^2 is the among-groups and s^2 the within-group variance component (Lessells and Boag 1987). Variance components for these calculations were calculated from the mean squares derived from one-way ANOVAs conducted with SPSS (version 10.0), and standard errors were calculated following Becker (1984).

Results

Striga infection and sorghum yield loss

Table 2 presents observations on *Striga* infection and sorghum kernel yield. Mean *Striga* emergence on CK60-B was significantly ($P < 0.01$) earlier than on Framida and Tiémarifing. Furthermore mean *Striga* emergence on Tiémarifing was significantly ($P = 0.002$) later than on E36-1. Differences in *Striga* emergence between CK60-B and E36-1, on the one hand, and Framida and Tiémarifing, on the other, were already found at 48 DAS ($P < 0.001$) and were still present at 75 DAS ($P < 0.01$). *Striga* infection significantly ($P < 0.001$) reduced sorghum total grain dry weight of all genotypes (Table 2). Yield loss of CK60-B was most severe (100%), followed by E36-1 (93%), Framida (67%) and Tiémarifing (46%).

CO_2-assimilation rate

CO_2-assimilation rates (A in µmol m^{-2} s^{-1}) were measured at 26, 48 and 75 days after sowing on infected and uninfected plants (Fig. 1). The coefficients of variation (cv) of A (8.3% [26 DAS], 17.2% [48 DAS] and 31.0% [75 DAS]) show that variation of measurements increased with time. The response of the various genotypes to *Striga* infection differed considerably. Significant ($P < 0.01$) reductions in CO_2-assimilation rates of infected CK60-B and E36-1 were observed between 26 and 48 DAS whereas CO_2-assimilation rate of infected Framida was significantly ($P < 0.01$) reduced at 48 DAS (Fig. 1). Tiémarifing was the only genotype without a significant (all $P > 0.3$) reduction in CO_2-assimilation rate due to *Striga* infection at any observation date. At 75 DAS, CO_2-assimilation rates (A in µmol m^{-2} s^{-1}) of control plants were clearly reduced while none of the genotypes showed significant differences between control and *Striga*-infected plants (all $P > 0.05$). The observed *Striga*-induced reductions in CO_2-assimilation measured at 26 and 48 DAS correlated significantly ($P < 0.01$) with reductions in total grain dry weight ($r = 0.6$, N = 20; for both dates; not shown). No such correlation was found with the CO_2-assimilation rates measured at 75 DAS.

Chlorophyll fluorescence

The three parameters (photochemical quenching [Pq], non-photochemical quenching [NPq] and electron transport rate [ETR in µmol m^{-2} s^{-1}]) that were calculated based on the chlorophyll fluorescence measured at 26 and 48 DAS, for both *Striga* infected and uninfected sorghum plants, correlated significantly ($P < 0.01$) with CO_2-assimilation rate (Table 3). Relative reductions in CO_2-assimilation

Table 2 Average first *Striga* emergence time (DAS), average above ground *Striga* numbers per pot at time of CO_2-assimilation measurements and average sorghum total grain dry weight (DW: g) per plant for control and *Striga* infected plants and relative yield loss (*RYL*: %) of the four sorghum genotypes

	Emergence time (DAS)	*Striga* numbers			Sorghum total grain DW (g)		*RYL* (%)
		26 DAS	48 DAS	75 DAS	Control	*Striga*	
CK60-B	33.8a[a]	–	4.2a	22.8a	40.6a	0.0e	100
E36-1	40.0ab	–	4.3a	23.3a	23.6c	1.6e	93
Framida	49.0bc	–	1.3b	7.5b	30.5b	10.2d	67
Tiémarifing	56.2c	–	0.2b	7.5b	26.4bc	14.3d	46
S.E.D.	5.19		0.84	4.71	2.31		
P	0.002		<0.001	0.005	<0.001		

[a] values in the same column followed by a different letter differ significantly

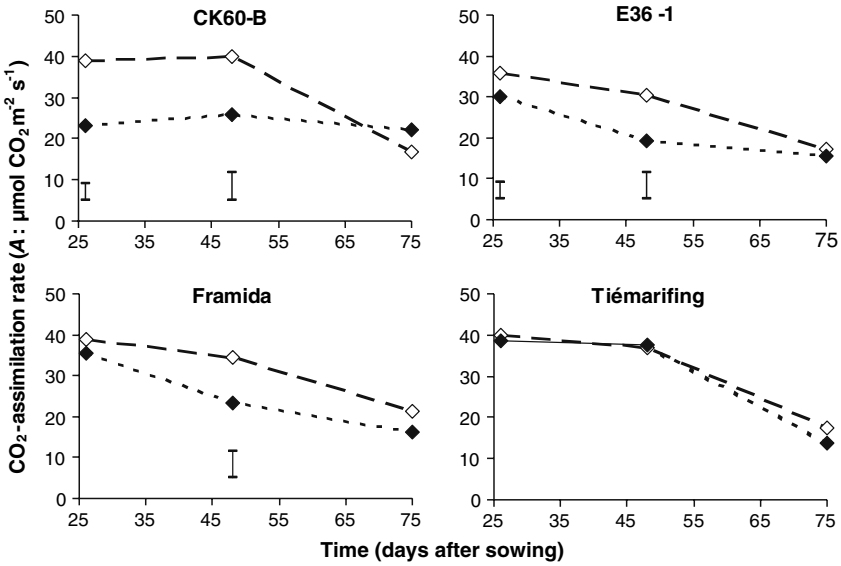

Fig. 1 CO_2-assimilation rate (*A;* µmol CO_2 m^{-2} s^{-1}) over time (days after sowing) for four sorghum genotypes (CK60-B, E36-1, Framida and Tiémarifing). *Striga* infestation levels (seeds cm^{-3}): 0.0 (open diamonds) and 4.0 (closed diamonds). Light intensity (400–700 nm): 1600 (26 DAS) and 2000 (48 and 75 DAS) µmol photon m^{-2} s^{-1}. Bars represent LSD ($P < 0.05$) values based on split-plot (genotype × *Striga*) ANOVA: 4.131 (26 DAS), 6.683 (48 DAS). SEM: 1.420 (26 DAS), 2.306 (48 DAS) and 2.497 (75 DAS)

Table 3 Phenotypic correlation coefficients (*r*) between CO_2-assimilation rate (*A*) and electron transport (*ETR*), photochemical (*Pq*) and non-photochemical (*NPq*) quenching (N = 40) and between relative reduction in CO_2-assimilation rate (*ΔA*) and *ETR*, *Pq* and *NPq* (N = 20) at 26 and 48 days after sowing (DAS)

Time	Parameters		*ETR*	*Pq*	*NPq*
26 DAS	A	*r*	0.94**	0.91**	–0.86**
	ΔA	*r*	–0.91**	–0.85**	0.89**
48 DAS	A	*r*	0.95**	0.93**	–0.70**
	ΔA	*r*	–0.86**	–0.84**	0.51*

** $P < 0.01$; * $P < 0.05$

rates correlated significantly with *ETR*, *Pq* ($P < 0.01$; both dates) and *NPq* ($P < 0.01$ at 26 DAS and $P < 0.05$ at 48 DAS) measured on *Striga* infected plants.

CO_2-assimilation rates (*A*) and the three chlorophyll fluorescence parameters (*ETR*, *Pq* and *NPq*) are presented in Fig. 2. The figure shows measurements at 26 DAS at light intensities of 1600 µmol PAR m^{-2} s^{-1} and at 48 DAS at 2000 µmol PAR m^{-2} s^{-1}. At both measurement dates (26 and 48 DAS), coefficients of variation of *NPq* (3.1 and 2.7%) and *Pq* (4.2 and 11.4%) were smaller than those of *A* (8.3 and 17.2%) and *ETR* (7.1 and 15.1%), measured at the same time on the same plants (not shown).

At 26 DAS, *A*, *ETR* and *Pq* showed significant *Striga* infection × genotype effects. Neither parameter distinguished between the moderately tolerant genotype Framida and the superiorly tolerant genotype Tiémarifing. Neither of those two genotypes showed significant negative *Striga* effects on any of the three parameters. Significant genotype differences between control plants were observed for *A* and *Pq* but not for *ETR*.

At 48 DAS, only *A* and *Pq* showed significant *Striga* × genotype effects. With both parameters it was possible to distinguish between the sensitive (CK60-B and E36-1) or moderately tolerant (Framida) genotypes and the superior tolerant genotype (Tiémarifing). Significant genotype differences between control plants were observed for *A* but not for *Pq*.

Repeatabilities

Table 4 shows the results of genotype effects on all parameters (*A*, *Pq*, *NPq* and *ETR*), separately for control plants and plants exposed to *Striga* infestation

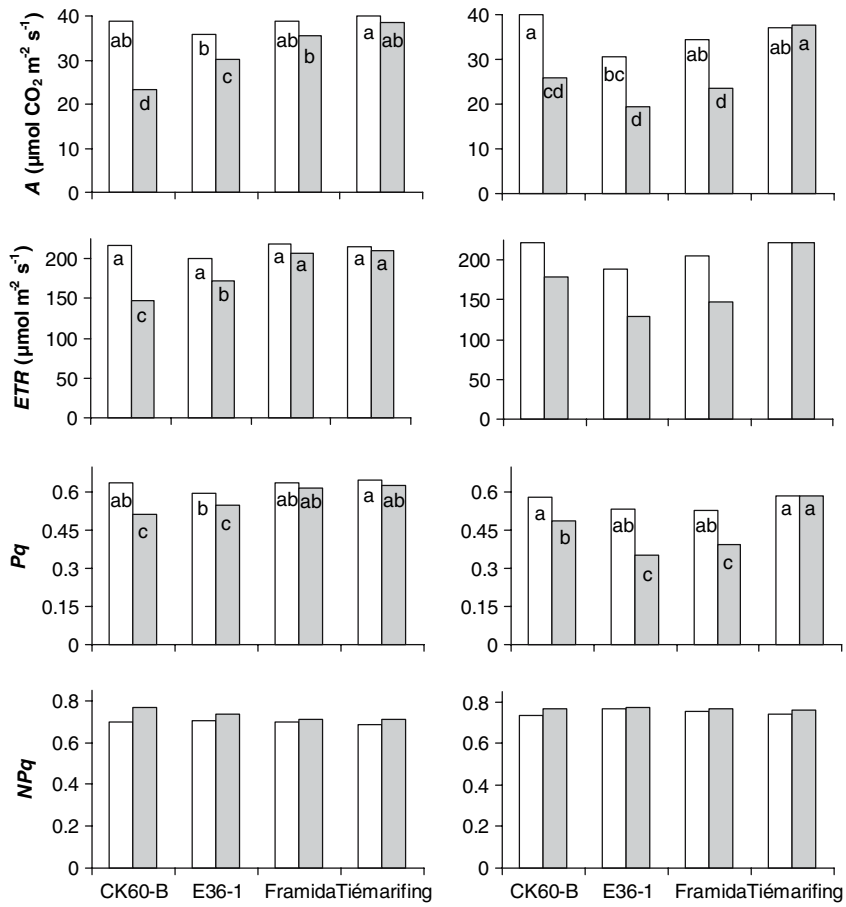

Fig. 2 CO_2-assimilation (*A*: µmol CO_2 m^{-2} s^{-1}), photochemical quenching (*Pq*), non-photochemical quenching (*NPq*) and electron transport rate (*ETR*: µmol m^{-2} s^{-1}) of control (open bars) and *Striga*-infected plants (shaded bars) of four sorghum genotypes (CK60-B, E36-1, Framida and Tiémarifing) at 26 (left) and 48 (right) DAS. Bars with different letters (a–d) are significantly different ($P < 0.05$) according to split-plot (genotype × *Striga*) ANOVA. LSD and SEM values: 4.13 and 1.420 (*A* 26 DAS), 6.68 and 2.306 (*A* 48 DAS); 21.09 and 7.23 (*ETR* 26 DAS) 33.49 and 11.56 (*ETR* 48 DAS); 0.0409 and 0.01397 (*Pq* 26 DAS), 0.0693 and 0.0239 (*Pq* 48 DAS); 0.0239 and 0.00821 (*NPq* 26 DAS), 0.0262 and 0.00902 (*NPq* 48 DAS)

(4 seeds cm^{-3}) at 26 and at 48 days after sowing of the host crop. For all parameters and at both measurement times (except *NPq* at 48 DAS) repeatabilities of uninfected plants (control) were distinctly lower (ranging from 0.058 to 0.450) than those of infected plants (ranging from 0.600 to 0.849), implying that *Striga* infestation induced the expression of genotypic variation. No systematic differences between repeatabilities of different measurement times could be observed. Repeatabilities of *Pq* and *ETR* measured on infected plants were consistently high (>0.7) at both measurement times indicating that those measures provide a clear distinction between genotypes.

Discussion

The objective of this study was to find an indirect selection measure to identify superior tolerant genotypes to be used in breeding programs for durable defence against *Striga hermonthica*. A suitable selection measure should: (1) clearly discriminate superior tolerance from other reaction types (2) enable a consistent and reliable prediction of the genetic variation and (3) be low-cost and practical. Table 5 provides an overview of the suitability of the four physiological parameters as indirect selection tools, using these three main criteria.

The first criterion (discriminative ability) is tested by looking at the F-probabilities derived from split-plot (*Striga* × genotype) ANOVA and by verifying whether the parameters discriminate between the highly resistant but moderately tolerant Framida and the moderately resistant but superior tolerant Tiémarifing. The second criterion (consistency and heritability) is tested through comparison of coefficients of variation (*cv* in %) and the repeatabilities (*R*) as a proxy of heritability of a trait. Assessment of

Table 4 Results of a one-way ANOVA (N = 20; four genotypes and five replicates) and calculations of repeatibilities[a] (R) and phenotypic variance (V_P) for all parameters (A, ETR, NPq and Pq) at both observation times (26 and 48 DAS) with (4 seeds cm^{-3}) and without (0 seeds cm^{-3}) exposure to *Striga*

Measure/time		*Striga*	MS-between	MS-within	F(3,16[b])	P	R	SE[c]	V_P^d
A	26	0	16.990	9.330	1.821	0.184	0.170	0.249	10.539
		4	225.902	9.651	23.408	0.000	0.849	0.107	0.001
	48	0	81.731	38.454	2.125	0.137	0.220	0.258	43.796
		4	305.802	38.521	7.939	0.002	0.634	0.211	0.003
ETR	26	0	371.489	181.972	2.041	0.149	0.207	0.256	0.001
		4	4370.415	307.906	14.194	0.000	0.767	0.153	942.929
	48	0	1247.254	885.868	1.408	0.277	0.093	0.231	0.000
		4	8218.093	663.952	12.378	0.000	0.740	0.167	1856.711
NPq	26	0	2.057 E^{-4}	1.652 E^{-4}	1.245	0.326	0.058	0.220	45.287
		4	0.004	0.001	7.002	0.003	0.600	0.223	0.003
	48	0	0.001	4.775 E^{-4}	2.485	0.098	0.271	0.263	80.723
		4	1.590 E^{-4}	3.101 E^{-4}	0.513	0.679	0[e]	0.132	0.012
Pq	26	0	0.002	0.001	4.278	0.021	0.450	0.257	0.000
		4	0.015	0.001	10.873	0.000	0.712	0.180	211.896
	48	0	0.005	0.003	1.628	0.222	0.136	0.242	0.001
		4	0.053	0.004	13.931	0.000	0.764	0.155	949.354

[a] Repeatability calculated following (Lessells and Boag 1987)

[b] Degrees of freedom between genotypes (3) and within genotypes (16)

[c] Standard errors are calculated following (Becker 1984)

[d] Phenotypic variance providing information on the total variance in the data set

[e] R had a negative value due to estimation errors. In this case repeatability should considered to be 0

Table 5 Suitability (ranging from completely unsuitable [–] to very suitable [++]) of different physiological parameters as indirect selection measure for tolerance against *Striga* according to three criteria: (1) discriminative ability (F-probability [P: *Striga* × genotype ANOVA] and identification (ID) of superior tolerance [Yes or No]), (2) consistency (coefficient of variation [cv:%]) and upper limit of heritability (repeatability [R]) and (3) practicability and costs of measurement (costs of measurement system [High or Low] and necessity of *Striga*-free controls [Yes or No]). Only the first two measurement times (T) were included: 26 and 48 days after sowing

Parameter	T	*1*		*2*		3		Suitability
		P	ID	cv	R^a	Costs	Controls	
A	26	<0.001	N	8.3	0.85	H	Y	–
	48	0.034	Y	17.2	0.63	H	Y	+
ETR	26	<0.001	N	7.1	0.77	L	N	+/–
	48	0.117	_[b]	15.1	0.74	L	–	+/–
NPq	26	0.057	_	3.1	0.60	L	–	–
	48	0.489	_	2.7	–	L	–	–
Pq	26	<0.001	N	4.2	0.71	L	Y	+/–
	48	0.021	Y	11.4	0.76	L	N	++

[a] Only repeatabilities of parameters measured at infected plants are used here

[b] No conclusive statement due to absence of significant effects ($P > 0.05$)

the third criterion is based on purchasing costs of necessary equipment and the need for inclusion of *Striga*-free control plants in the screening trial.

CO2 assimilation

The significant correlations between the reduction in CO_2-assimilation measured at 26 and 48 DAS and the reduction in total grain dry weight at harvest suggests that CO_2-assimilation is an important indicator of the performance of *Striga*-infected sorghum plants. The ability to maintain high rates of CO_2-assimilation as mechanism to endure parasite infection was earlier reported by Gurney et al. (2002). They also suggested that CO_2-assimilation could be used as a screening measure for tolerance. The usefulness of this measure for identifying tolerance against biotic stresses was earlier demonstrated by Scharen and Krupinsky (1969) working with *Septoria nodorum* in wheat.

The CO_2-assimilation measurements in this experiment showed much higher repeatabilities for plants exposed to *Striga* than for the uninfected control plants. Because repeatability values are proxies for heritability, these results imply that the genotypic variation in CO_2-assimilation rate between genotypes was mainly expressed under *Striga* infested conditions. This in turn indicates that through CO_2-assimilation measurements differences in tolerance between the genotypes are identifiable.

CO_2-assimilation rates showed significant *Striga* × genotype effects at 26 and 48 DAS. The observations on CO_2-assimilation rate correspond to the earlier classification of sorghum genotypes with respect to tolerance to *Striga*, which was based on relative yield loss due to *Striga* and above ground *Striga* numbers (Rodenburg et al. 2006a). The current study showed that time of measurement is also important. Only at 48 days after sowing, did the measurement of CO_2-assimilation distinguish between the moderately tolerant Framida and the superiorly tolerant Tiémarifing. This study also showed a number of shortcomings of CO_2-assimilation measurements as selection tool for tolerance. The measurements had relatively high coefficients of variation (*cv*: 8.3–17.2%). Furthermore, and in spite of these high *cv*'s, rates of CO_2-assimilation of control plants of different genotypes were significantly different at both measurement times. This indicates that measurements on control plants are indispensable, as comparison of reductions in CO_2-assimilation rates between lines should be based on a relative measure. Other constraints to the use of CO_2-assimilation rate as screening measure are the relatively long time needed per measurement and the high costs of CO_2-assimilation measurement systems.

Chlorophyll fluorescence

The current study further investigated whether parameters derived from chlorophyll fluorescence measurements (electron transport, photochemical and non-photochemical quenching) would serve any better as indirect selection measures. The regulation of CO_2-assimilation in response to stress involves the protection of the photosynthetic apparatus. Photochemical and non-photochemical quenching are two essential elements of this photoprotection (Ort and Baker 2002). Photochemical quenching is proportional to the energy transfer to the functional CO_2-assimilation reaction centres. Non-photochemical quenching (*NPq*) refers to the process of dissipation of the excess excitation energy in the PSII antennae as heat, whereby down-regulation of PSII electron transport efficiency is triggered. As the capacity for photochemistry of leaves declines under stress conditions, both photochemical and non-photochemical quenching are potentially suitable measures for stress severity (Schreiber 1986) or stress tolerance (Harbinson 1995). This was demonstrated by studies on cold tolerance in maize (Schapendonk et al. 1989a; Fracheboud et al. 1999) and drought tolerance in wheat (Havaux and Lannoye 1985), barley (Nogues et al. 1994; Olsovska et al. 2000) and potato (Schapendonk et al. 1989b; Schapendonk et al. 1992). Some of the *Striga* effects show remarkable resemblance with drought stress effects (e.g. White and Wilson 1965; Björkman and Powles 1984), which opens the way for rapid selection of *Striga* tolerant genotypes, using fluorescence analyses. One obvious advantage of chlorophyll fluorescence based selection is that the equipment required for these measurements cost only about 10% of that of the gas exchange equipment necessary for a screening based on CO_2-assimilation rates.

All three chlorophyll fluorescence parameters (*ETR*, *Pq* and *NPq*) of *Striga* infected plants showed

a strong correlation with CO_2-assimilation rates or the relative reduction in CO_2-assimilation rate due to *Striga* infection. In addition, all parameters were characterized by a relatively small *cv*. Clear differences in suitability of the various fluorescence parameters for use as screening measure are however present (Table 5). The most straightforward approach to estimate photosynthetic capacity from fluorescence analysis is provided by *ETR*, which is based on the efficiency of electron transport through PSII (φ_2) and the absorbed light intensity. Parameter φ_2 is based on *Ft* and *Fm′*, which are both measured in the light. However, based on *ETR*, CK60-B and E36-1 could only be identified as sensitive genotypes at 26 DAS, whereas the sensitivity of Framida could not be distinguished from that of Tiémarifing. No *Striga* effects were observed on non-photochemical quenching (*NPq*) at any observation time. Hence, *NPq* is not considered a very suitable screening measure for tolerance.

Photochemical quenching (*Pq*) changed significantly upon *Striga* infection in less tolerant genotypes in the early growth stages. Tiémarifing was the only genotype with virtual unchanged *Pq* values due to *Striga* infection throughout the experiment. Hence, as with CO_2-assimilation (*A*) measurements, *Pq* is able to discriminate genotypes with superior levels of tolerance (Tiémarifing) from genotypes with defence mainly relying on a superior level of resistance (Framida). Both *A* and *Pq* showed reasonably high repeatabilities for *Striga* infected plants at both measurement times indicating high heritabilities and consequently clear and predictable differences between genotypes. However, an additional requirement is that the genotypic variation of control plants should be as small as possible. Hence parameters or measurement times with relatively high repeatability values for control plants, such as *Pq* at 26 DAS ($R = 0.45$), are less suitable.

From the above analysis it appears that both *A* and *Pq* measured at 48 DAS provide suitable information for the selection of superior tolerant genotypes. For use as a selection measure, *Pq* has an additional advantage over CO_2-assimilation in that its control values (measurements at *Striga*-free plants) at this time (48 DAS) lack significant genotype effects. Hence, unlike *A* measurements, the identification of superior tolerance based on measurements of *Pq* would not require the inclusion of infected control plants. This would greatly simplify the design of a screening trial and reduce the costs of screening.

One of the major constraints for *Pq* measurements as a selection tool seems to be the requirement of far-red light to determine *Fo′*. Provision of far-red light is often not available on standard equipment for measuring chlorophyll fluorescence. However, such a technical constraint could be overcome. Alternatively, a method is available which estimates *Fo′* through a simple equation involving the minimum fluorescence yield in the dark-adapted state (*Fo*), the maximum fluorescence yield in the dark-adapted state (*Fm*), and the maximum fluorescence yield in the light-adapted state (*Fm′*) (Oxborough and Baker 1997). Disadvantage of this alternative is the requirement to conduct fluorescence measurements in both light and dark-adapted conditions.

Resistance as confounding factor

Earlier studies where chlorophyll fluorescence measurements were used as selection measure for tolerance (e.g. Havaux and Lannoye 1985; Nogues et al. 1994; Olsovska et al. 2000; Fracheboud et al. 1999; Olsovska et al. 2000) dealt with abiotic stresses such as cold or drought. *Striga* is a biotic stress and this implies some additional difficulties for screening. Uniform parasite infection or removal of resistance effects as a confounding factor is one of the first requirements for measuring tolerance (Schafer 1971), and typically this aspect was identified as the main constraint for developing a simple screening procedure based on actual yield data (Rodenburg et al. 2006a). Differences in *Striga* effects on CO_2-assimilation and chlorophyll fluorescence between genotypes as found in this study may partly result from differences in resistance, since significant differences in above ground *Striga* numbers were observed between Tiémarifing and Framida on the one hand and CK60-B and E36-1 on the other. Completely cancelling out differences in resistance seems impossible. The most practical solution would be to use very high infestation levels such as used in this study (around four viable *Striga* seeds cm^{-3} in the upper 10–12 cm, which is equivalent to 400,000 seeds m^{-2} in the field). This prevents misidentification of more resistant genotypes, such as Framida in this study, as tolerant, due to the fact that

they simply do not have enough infections to damage the host plant sufficiently. Use of high levels of *Striga* infestation in screening trials for tolerance against *Striga* has been recommended by Kim (1991).

Tolerance screening tool

In *Striga*-prone environments crop varieties with broad-based genetic defences are essential components of an integrated control strategy. For resource-poor farmers who cannot afford the failure of a cropping season, it is important that their crop combines superior levels of resistance with superior levels of tolerance to achieve maximum defence against *Striga* and safeguard yields. Good sources of tolerance and resistance (though not complete) have been identified in sorghum. This study suggests a useful tool for plant breeders to find superior tolerance and recommends combining this with superior resistant material.

Photochemical quenching (*Pq*) was identified as a suitable selection tool for host plant tolerance to *Striga.* Screening should be carried out at high infestation levels. Most reliable results will be obtained after *Striga* emergence above ground but well before host plant flowering. This selection protocol should be validated with a wider range of economically important host crops, including the C_3 plant rice (*Oryza sativa* [L.] and *O. glaberrima* [Steud.]), on more locations and with a larger sample of genotypes enabling the calculation of genetic correlations between the indirect selection criterion and the direct trait.

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