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Induced Resistance to *Striga hermonthica* in Sorghum by Gamma Irradiation

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Abstract

Striga species affect the potential productivity of cereals in sub-Saharian Africa due to the lack of durable *Striga*-resistance in host crops. This study aimed at inducing the new source of resistance in sorghum using gamma irradiation. Dry seeds of three Sorghum varieties; Grinkan, ICV1049 and Sariaso14 were gamma-irradiated with 200 Gy, 300 Gy, 400 Gy and 500 Gy. Screening strategies involved a 2-year field and greenhouse experiments, where mutant Sorghum families, their parents and resistant control were artificially infected with *Striga hermonthica* seeds. Field screenings revealed induced genetic variability among them, forty families significantly reduced the number of emerged *Striga* plants or showed good Sorghum grain yield performance despite the infection by *S. hermonthica* ecotype from Burkina Faso. The induced putative resistant mutants were identified across the four applied gamma-irradiation doses. Greenhouse experiment confirmed *Striga* resistance in seven mutant Sorghum families leading to no emergence of Burkina's *S. hermonthica* ecotype along with high resistance index (RI) and low *Striga* damage score. Among them, two mutants SA38M5 and IC47M5 withstood *S. hermonthica* ecotype from Sudan. The induced mutants will be evaluated for the release to farmers for commercial production. Further studies are ongoing on confirmed mutants to highlight their *Striga* resistance mechanisms and explore the potential of pyramiding different mechanisms to produce durable resistance to *S. hermonthica* in sorghum.

Keywords

Sorghum, Induced Mutation, *Striga* Resistance

1. Introduction

Sorghum (*Sorghum bicolor* (L.) Moench), the fifth most important cereal crop grown in the world; is one of the world's leading cereal crops, providing food, feed, fiber, fuel, and chemical/biofuels feedstocks [1]. It is a staple food crop for millions of farmers in African semi-arid tropics [2]. In Burkina Faso, sorghum covers about 45% of total cultivated land and is the first cereal in terms of production and cultivated by more than 71% of farm households in rain-fed conditions [3]. However, sorghum production is highly affected by one of the major biotic constraints, *Striga hermonthica* occurring in almost all cultivated areas [4]. *S. hermonthica* remains the most feared weed by producers because it has a strong negative effect in descending order on the productivity of sorghum (*Sorghum bicolor* (L.) Moench), Pearl Millet (*Pennisetum glaucum* (L.) R.Br.), Maize (*Zea mays* L.) and upland Rice (*Orysa sativa* L.) in infested fields.

Several control measures were recommended such as agronomical techniques and chemical control [5]. However, none of these options individually proved fully effective and they were applied when at least 75% of damage occurred during the underground growth of *Striga* [6]. Integrated management strategies with host plant resistance should be one of the viable solutions [7] because the use of resistant genotype seeds does not require additional technique and farming inputs. Seven *Striga*-resistant Sorghum varieties with effective field resistance were reported, including SRN39, IS9830, Framida, 555, Dobbs, Serena and N13 [8]. Among them, 555, Framida, IS9830 and SRN39 were classified as low germination stimulators [8] to *Striga* while N13 has both mechanical barriers [9] as a post-germination *Striga* resistance mechanism [10] that affects *Striga* seedbank in the soil. Unfortunately, these resistant Sorghum varieties are generally landraces with low yielding and/or are not adapted to *Striga*-infested areas [7].

Consequently, there is a need to investigate other technologies as mutagenesis that may induce genetic variability in farmers' preferred varieties to integrate some emerged resistance to the parasite *Striga* in agronomically adapted varieties. The use of induced mutation has been widely accepted by breeders as a tool for crop improvement over spontaneous mutations that occur very slowly [11] at a very low rate. The mutation induction can be carried out using chemical or physical mutagens [12]. Among both strategies, physical mutagenesis is the most common [13] as more than 89% of the mutant varieties in the world were created with physical mutagens (gamma ray, X-ray, neutrons), of which 60% were generated using gamma rays [14]. A number of beneficial traits such as dwarfing, early flowering, high protein digestibility, and high lysine generated by mutation induction have been widely used in sorghum breeding [15].

This study aimed at creating genetic variability in farmers' preferred Sorghum varieties through induced mutation and selecting of mutants endowed with *Striga*-resistance to ensure sustainable grain Sorghum production in infested fields.

2. Material and Methods

2.1. Genetic Material

Farmer surveys were conducted in five administrative regions of which the geographical limits are 1) Boucle du Mouhoun (11˚41'40"N - 13˚43'42"N and 2˚2'5"W - 4˚4'7"W); 2) Hauts Bassins (10˚40'39"N - 11˚41'40"N and 3˚3'6"W - 5˚5'8"W); 3) Centre Ouest (11˚41'40"N - 12˚42'41"N and 2˚2'5"W - 3˚3'6"W); 4) Centre Est (10˚55'30"N - 11˚56'40"N and 00˚26'30"W - 00˚34'40"W) and; 5) Est (10˚40'39"N - 12˚42'41"N and 00˚0'3"W - 02˚1'59"E) of Burkina Faso, where Sorghum is widely grown. Seeds of twenty-six famers' preferred landraces and improved varieties of sorghum were collected. They were then screened for *Striga*-resistance in pots artificially *Striga*-infested [5]. No variety had an acceptable level of *Striga*-resistance (data not presented). Based on farmers' preference and varietal purity, three *Sorghum* varieties; Sariaso14, Grinkan and ICSV1049 with preferred white grains, agronomic and commercial values were chosen for mutagenesis induction and seeds were provided by the national breeding programme. Sorghum varieties ICV1049 and Grinkan have a cycle length of 120 days (from the sowing to the grain maturity) while that of Sariaso14 is 115 days. The varieties ICV1049 and Sariaso14 are grown in areas with annual rainfall of 600 - 900 mm compared to 800 - 1000 mm for Grinkan. Seeds of *Striga hermonthica* ecotype from Burkina Faso, harvested during September to October 2016 from farmers' Sorghum fields located in Kouaré village (Eastern region of Burkina Faso) with a germination capacity of 75% were used for artificial *Striga*-infestation of field and greenhouse experiments. The seeds of *Striga hermonthica* ecotype from Sudan with a germination capacity of 75% were only used for glasshouse screenings.

2.2. Generating of Mutagenized Sorghum Populations and Selecting of Putative Mutants

Dry seeds of Sorghum varieties were irradiated at four selected doses: 200, 300, 400 and 500 Gy from the Center for the Application of Isotope and Radiation Technology, National Nuclear EnergyAgency (BATAN, in Jakarta, Indonesia). The irradiated seeds (M1) and controls (parental lines) were sown in the INERA's experimental field (Burkina Faso). Self-pollinated M1 panicles were harvested and planted as M2 panicle-to row. M2 plants were selected and advanced to M3/M4 families using pedigree selection method based on phenotypic variation and improved agronomic traits compared to that of the parent plants.

Two-year rain-fed field experiments were conducted on sandy-loam, tropical ferruginous soil at Kouaré research station (11˚95'03"N and 0˚30'58"E) located in

the Eastern Sudan-savannah area of Burkina Faso to select putative *Striga* resistant mutants. Six hundred ninety-nine (699-M3/M4) and 221 (M4/M5) mutant lines (Table 1) were phenotyped for their resistance to *Striga* in 2017 and in 2018, respectively. Each genotype (putative mutant or parent) was sown on a row of 8 m long. The distance between rows was 1m and the hills within a single row were spaced by 0.80 m (11 hills per row). Each planting hill was artificially infested with 5×10^3 *S. hermonthica* seeds [5]. The blocks and replications were spaced by 1 m. The experimental design was an alpha lattice design with three replications.

Sorghum was planted on 15 July in 2017 and on 12 July in 2018 and harvested on 18 and 20 November, respectively. Sorghum seedlings were thinned at 14 days after the sowing to leave one plant per hill. Mineral fertilizers, NPK (12-24-12) and urea ($(CO)_2NH_2$) with 46% N were applied on 21 days after the planting (DAP) and 45 DAP, respectively. Two hoeings were carried out 21 and 35 DAP and the weeds, except *Striga* plants were manually pulled out during the rest of Sorghum cycle. The self-pollination by bagging of sorghum plants was carried out at heading time. Rainfall recorded during the Sorghum growth period in 2017 and in 2018 was 440.8 mm and 525.6 mm in 24 rain events for both years.

Field screening aimed at identifying Sorghum mutant lines which delayed *Striga* emergence and/or reduced the emerged *Striga* number along with high yielding. Five quantitative traits were therefore used to phenotype sorghum accessions. From each planting hill within a single row (family), number of *Striga* plants emerged 70 and 100 DAP were recorded in 2017; in addition to days to

Table 1. Number of sorghum mutant lines screened to *Striga hermonthica* in rain-fed fields, in 2017 and 2018.

		Gamma irradiation dose				
Sorghum varieties	**Mutant lines**	**200 Gy**	**300 Gy**	**400 Gy**	**500 Gy**	**Total**
Cropping season 2017						
Grinkan	M3 line	37	41	10	16	104
ICSV1049	M4 line	12	5	7	0	24
Sariaso14	M4 line	95	117	116	243	571
Total		**144**	**163**	**133**	**259**	**699**
Cropping season 2018						
Grinkan	M4 line	24	21	4	5	54
ICSV1049	M5 line	8	4	6	0	18
Sariaso14	M4 line*	0	0	5	6	11
	M5 line	48	30	10	50	138
Total		**80**	**55**	**25**	**61**	**221**

*: lines led to delayed emergence of high number of *Striga* plants or high mortality of *Striga* seedlings in 2017.

the first *Striga* emergence (DFSE), days to grain Sorghum maturity (DSMa) and grain sorghum weight per panicle (GrWP) in 2018. From infested hills, *Striga*-infected Sorghum plant rates (SISPR) at 70 DAP (SISPR70) and 100 DAP (SISPR100) were derived.

2.3. Verification of *Striga*-Resistance in Sorghum Mutants under Pot Screening in Greenhouse Conditions

Pot experiments were performed to verify the *Striga*-resistance of forty mutant lines (M4-M6) selected in field conditions. Among them, 22, 5, 7 and 6 lines were generated from irradiations of 200, 300, 400 and 500 Gy respectively (Table 2). They were compared to a *Striga*-resistant reference control (Sorghum variety Framida) and their parents in the greenhouse of the Plant Breeding and Genetic Laboratory (PBGL) of the Joint FAO/IAEA Division, Seibersdorf, Austria. Greenhouse conditions included the temperature of 25˚C - 28˚C, 60% relative humidity and 16/8 h (light/dark) photoperiod during July-October of the year.

Each genotype was planted in two sets of plastic pots (11 cm diameter × 12 cm height): no *Striga*-infested versus *Striga* infested mixture of 900 g of soil-sand (1 v/1v) with 0.5 mg of *Striga* seeds/pot. The bottom of the pot was covered by filter paper to avoid run-off of *Striga* seeds during watering. For *Striga* seed conditioning, the pots were watered every 3 days for 10 days and then two Sorghum seeds were sown per pot. Sorghum seedlings were thinned to one plant at 14 DAP. The experimental design was completely randomized design with four replications (pots) for each accession. The pots were watered every three days without any additional treatment. Data were collected in individual pot 95, 118 and 140 DAP in both sets. Sorghum plant height was measured in both sets while emerged *Striga* plant number and height and, *Striga* damage score as the rate burned leaves in infested set and reduction in plant growth relative to the negative (non-infested) control-5 of [16] were recorded. From these variables, *Striga* resistance index [17] and reduction percentage of Sorghum plant growth [18] were derived as follows:

Table 2. Putative Sorghum mutant lines screened to *Striga hermonthica* in greenhouse conditions.

		Gamma irradiation dose of dry Sorghum seeds				
Sorghum varieties	Mutant lines	200	300 Gy	400 Gy	500 Gy	**Total**
Grinkan	M4 lines	2	0	1	0	**3**
	M5 lines	6	4	2	3	**15**
ICSV 1049	M5 lines	3	0	2	0	**5**
	M6 lines	3	0	0	0	**3**
Sariaso 14	M5 lines	4	1	1	3	**9**
	M6 lines	4	0	1	0	**5**
	Total	**22**	**5**	**7**	**6**	**40**

$$\text{Resistance index}\left(RI\right)=\frac{\text{Height of infested sorghum plant}}{\text{Height of uninfested sorghum plant}}$$

Sorghum plant growth reduction $\left(GR\ \%\right)=\frac{X-Y}{X}*100$; where X is *Striga*-free plant height (average of 4 plants) and Y is *Striga*-infested plant height.

The numbers of *Striga* plants emerged 95 DAP were not significantly different to that counted at 118 and 140 DAP. Therefore, only the average number of *Striga* plants emerged 95 DAP were presented.

2.4. Response of Putative Resistant Sorghum Mutants to Sudan's *Striga hermonthica* Ecotype

Five putative mutants (SA38M5, IC83M5, IC47M5, GK715M4, GK629M4) identified in the field and verified in glasshouse were tested for their reaction to *Striga hermonthica* ecotype from Sudan. The experimental design and artificial *Striga*-infection of each genotype (mutants and parents) were as described above for pot-experiment in the glasshouse of the PBGL.

2.5. Statistical Analysis

Statistical analyses were carried out using Statistical Analysis 1 System (SAS, 9.1, 2 Institute, Cary, NC) and Rx64 3. 5.2. ANOVA was performed and means were separated using Newman Keuls Multiple Range test and differences were considered significant at 5% threshold (SAS, 9.1, 2 Institute, Cary, NC). The software Rx64 3. 5.2 was used to cluster Sorghum mutant families and establish Pearson correlation between *Striga* resistance parameters. The trend curve of plant height means of sorghum mutant lines at 95, 118 and 140 DAP under *Striga* infection versus no infection was also performed with Rx64 3. 5.2. Hierarchical clustering of mutant sorghum lines according to their *Striga* resistance was also performed (Rx64 3. 5.2) by treating each of three variables namely *Striga* resistance index, emerged *Striga* plants and *Striga* damage as a separate cluster. It repeatedly identified the two clusters that were closest together, and then merged the two most similar clusters. This iterative process continued until all the clusters are merged together.

3. Results

3.1. Screening of Mutant Populations for Resistance to *Striga*-Infection under Field Conditions

Within the mutagenized Sorghum populations (699) screened to *Striga* during the cropping season 2017, 144, 163, 133 and 259 mutant lines were generated from seeds irradiated with gamma rays at 200, 300, 400 and 500 Gy, respectively. In the first round of screening in 2017, Sorghum plants with a *Striga*-infestation level ranging from 0 to less than or equal to five emerged *Striga* plants per sorghum planting hill were selected for a second round of screening in similar conditions. These observations resulted in the selecting of a total of 221 *Striga* puta-

tive resistant mutant lines of which, 80, 55, 25 and 61 mutant lines were generated from irradiations of 200 Gy, 300 Gy, 400 Gy and 500 Gy respectively. Analysis of variance for the *Striga* resistance traits observed in 2018, ranked Sorghum mutants into clusters. Coefficient of variation values indicated a moderate variation (20% - 30%) between clusters for days to the first *Striga* emergence (DFSE) counted in Sariaso14 and ICSV1049 derived mutant lines. Conversely, there was a large variation (38% - 85%) between clusters for the *Striga*-infected Sorghum plants at 70 DAP (SISPR70) and at 100 DAP (SISPR100) for all mutant lines. A significant difference between the different clusters ($P < 0.0001$) is observed for each trait, which reveals genetic diversity between the individual Sorghum plants that make up these clusters. Sariaso14 mutant lines were classified into three clusters for the traits SISPR70, SISPR100 and DFSE. At 70 DAP, 92 sensitive lines were discriminated with 26% - 69% infected plants and 32 out of 149 lines were not infected by *Striga* compared to nine non-attacked lines at 100 DAP to the end of Sorghum growth phase. Grinkan mutant lines were subdivided into four clusters, 14 lines were *Striga*-free at 70 DAP against 10 lines at 100 DAP to Sorghum harvest time. ICSV1049 mutants were also ranked into four clusters. Seven and five mutant lines with the lowest rate of *Striga*-infection were recorded at 70 DAP (0% - 8%) and 100 DAP (0% - 17%), respectively. Only two mutant lines were not parasitized until Sorghum harvest time (Table 3).

Table 3. Frequency of Sorghum lines segregating for *Striga hermonthica* resistance observed in rain-fed field conditions.

Sorghum mutant lines	Phenotype description	Sorghum lines segregating				P. values	CV (%)
		Mutant line number and frequency of *Striga*-infected Sorghum plants (%)					
Sariaso14	SISPR70	92 (25 - 69)	25 (6 - 24)	32 (0.00)	-	P < 0.0001	57.75
	SISPR100	132 (20 - 86)	8 (5 - 17)	9 (0.00)	-	P < 0.0001	44.52
	DFSE	130 (46 - 82)	10 (22 - 44)	9 (0.00)	-	P < 0.0001	20.34
Grinkan	SISPR70	30 (16 - 47)	8 (7 - 15)	2 (3 - 4)	14 (0.00)	P < 0.0001	85.19
	SISPR100	37 (20 - 71)	7 (5 - 19)	10 (0.00)	-	P < 0.0001	62.95
	DFSE	44 (26 - 80)	10 (0.00)	-	-	P < 0.0001	46.85
ICSV1049	SISPR70	1 (68.33)	5 (33 - 50)	5 (17 - 23)	7(0 - 8)	P < 0.0001	38.85
	SISPR100	5 (38 - 66)	8 (19 - 35)	5 (0 - 17)	-	P < 0.0001	37.85
	DFSE	16 (39 - 79)	2 (0.00)	-	-	P < 0.0001	29.58
		Mutant line number and frequency of cycle duration and grain Sorghum weight					
Sariaso14	DSMa	18 (111 - 119)	118 (101 - 110)	13 (92 - 100)	-	<0.0001	2.81
	GrWP	80 (38 - 97)	13 (35 - 37)	31 (26 - 34)	25 (7 - 24)	<0.0001	39.95
Grinkan	DSMa	6 (117 - 120)	43 (111 - 116)	3 (105 - 109)	2 (94 - 97)	<0.0001	2.78
	GrWP	5 (79 - 104)	27 (48 - 78)	17 (29 - 45)	5 (13 - 25)	<0.0001	36.25
ICSV1049	DSMa	11 (114 - 118)	7 (106 - 112)	-	-	<0.0001	1.78
	GrWP	1 (111)	3 (76 - 87)	5 (54 - 75)	9 (21 - 51)	<0.0001	15.31

SISPR70: *Striga*-infected Sorghum plant rate 70 DAP; SISPR100: *Striga*-infected Sorghum plant rate 100 DAP; DFSE: Days to first *Striga* emergence: DSMa; days to Sorghum maturity: GrWP: grain sorghum weight (g) per panicle.

Regarding both variables of DSMa and GrWP, coefficient of variation values revealed high variation between clusters only for Sorghum grain/panicle for Sariaso14 mutants (40%) and Grinkan mutants (36%). ANOVA showed significant differences ($P < 0.0001$) between clusters of mutants within the same Sorghum variety (Table 1). The 120-day cycle length of ICSV1049 and Grinkan varieties was significantly reduced with seven ICSV1049 mutants (106 - 112 days), three Grinkan mutants (105 - 109 days) and two Grinkan mutants (94 - 97 days) while the cycle length of Sariaso14 (115 days) was highly reduced with 13 mutants (92 - 100 days). Grain Sorghum weights per panicle of mutants were ranked into four clusters. The most yielding mutants were 80 lines of Sariaso14 mutants (97 g grain/panicle), 5 lines of Grinkan mutants (79 - 104 g grain/panicle) and one ICSV1049 mutant (Table 3).

3.2. Response of Sorghum Mutant Lines to *Striga* Infection under Glasshouse Conditions

No *Striga* emergence occurred in pots planted with the known resistant Framida and eight mutant lines (GK715M4, GK220M5, GK225M5, IC83M5, IC47M5, IC17M6, SA38M5 and SA188M6) (Table 4). These mutant lines displayed weaker *Striga* damage ($P < 0.0001$) with significantly high resistance index ($P < 0.0001$) compared to the susceptible parent varieties. The reaction of sorghum plants to all *Striga* plants attached to their root system (emerged or buried in the soil) showed that four mutants; SA38M5, GK715M4, IC47M5 and IC83M5 scored *Striga* damage of less than 40%. Count of *Striga* plant number at 95 DAP with 14 mutant lines is not significantly different from that of the known resistant control Framida. The average height of *Striga* plants varied between 0.2 cm and 28 cm. The highest *Striga* plant height was recorded in the pot of the parent Sariaso14 ($P < 0.0001$), which is similar to that measured in pots of the parent ICSV1049 and 17 of the mutant lines (Table 4).

Table 4. Response of mutant Sorghum lines to the infection of *Striga hermonthica* ecotype from Burkina Faso 95 DAP under greenhouse conditions at PBGL, Seibersdorf.

Sorghum Genotypes	Gy Dose	Number of emerged *Striga* plants	*Striga* plant height (cm)	*Striga* damage score (%)	*Striga* resistance Index
Framida (Control)	0	0.00 ± 0.0 b	0.00 ± 0.0 b	61.29 ±5.08 cde	0.92 ± 0.01 a
Sariaso14 (Parent)	0	2.25 ± 0.2 a	28 ± 6.5 a	75 ± 1.77 ab	0.60 ± 0.1 cde
SA21M5	200	3.00 ± 0.4 a	19.13 ± 3.8 a	60.75 ± 0.88 cde	0.49 ± 0.02 e
SA22M5	200	2.25 ±1.1 a	11.25 ± 6.6 ab	62.75 ± 0.9 cd	0.58 ± 0.01 de
SA251M5	400	1.5 ± 0.5 a	9.25 ± 3.2 ab	50.5 ± 1.43 ef	0.88 ± 0.01 a
SA399M5	500	2.00 ± 2 a	15.75 ± 5.8 ab	43 ± 2.46 f	0.79 ± 0.04 abc
SA38M5	200	0.00 ±0.0 b	0.00 ± 0.0 b	34.37 ± 3.5 g	0.86 ± 0.02 a
SA458M5	500	2.25 ±1.4 a	13 ± 2.8 ab	59 ± 2.39 de	0.61 ± 0.02 cde
SA585M5	500	1.00 ± 1 a	3 ± 3 b	47 ± 1.48 f	0.82 ± 0.05 ab

Continued

SA7M5	200	1.25 ±1.2 a	1.25 ± 1.2 b	46.75 ± 1.14 f	0.78 ± 0.04 abcd
SA43M6	200	1 ± 0.0 a	7.1 ± 2.1 b	68.5 ± 2.54 bcd	0.66 ± 0.05 bcde
SA53M6	200	1 ± 0.0 a	27 ± 11.3 a	81.8 ± 4.92 a	0.66 ± 0.02 bcde
SA109M6	200	1.7 ± 0.8 a	1.7 ±1.5 b	64.2 ± 1.54 cd	0.65 ± 0.02 bcde
SA188M6	300	0.00 ± 0.0 b	0.00 ± 0.0 b	48.3 ± 4.05 f	0.89 ± 0.02 a
SA311M6	200	1.2 ± 0.6 a	8.1 ± 5.8 ab	72.2 ± 2.6 bc	0.53 ± 0.07 e
SA316M6	400	0.75 ± 0.0 ab	1.2 ± 0.5 b	60.7 ± 0.9 cde	0.77 ± 0.02 abcd
Grinkan (Parent)	0	1 ± 0.2 a	7.7 ± 4.4 b	84 ± 2.9 a	0.75 ± 0.03 ab
GK629M4?	200	2.00 ± 0.29 a	7.75 ± 2.8 ab	64.75 ± 5.38 bc	0.87 ± 0.01 ab
GK657M4	200	1.75 ± 1.03 a	10.25 ± 5.95 ab	46.75 ± 1.81 d	0.71 ± 0.02 ab
GK715M4	400	0.00 ± 0.0 b	0.00 ± 0.0 b	31 ± 3.11 e	0.92 ± 0.01 a
GK206M5	500	0.5 ± 0.2 ab	12.7 ±10.8 ab	73.9 ± 3.78 abc	0.72 ± 0.02 ab
GK220M5	400	0.00 ± 0.0 b	0.00 ± 0.0 b	62.1 ±1.70 bc	0.90 ± 0.0 a
GK231M5	300	1 ± 0.5 a	0.7 ± 0.0 b	77.7 ± 3.32 ab	0.76 ± 0.04 ab
GK209M5	400	0.2 ± 0.2 ab	0.2 ± 0.2 b	72.4 ±1.64 abc	0.7 ± 0.05 ab
GK255M5	300	0.2 ± 0.2 ab	6.6 ± 6.6 b	73.3 ±1.55 abc	0.79 ± 0.04 ab
GK226M5	200	0.7 ± 0.4 ab	0.5± 0.2 b	62.9 ± 2.16 bc	0.75 ± 0.02 ab
GK239M5	200	0.2 ± 0.2 ab	12.7±12.7 ab	75.8 ± 2.36 abc	0.82 ± 0.05 ab
GK251M5	300	0.7 ± 0.1 ab	0.00 ± 0.0 b	65 ± 2.56 bc	0.86 ± 0.06 ab
GK225M5	200	0.00 ± 0.0 b	0.00 ± 0.0 b	48 ±5.40 d	0.92 ± 0.01 a
GK256M5	300	0.7 ± 0.4 ab	2.8 ±1.6 b	70 ± 2.00 abc	0.82 ± 0.02 ab
GK320M5	200	0.2 ± 0.2 ab	6.7 ± 6.7 ab	81.4 ± 2.6 a	0.54 ±0.1 c
GK259M5	200	0.2 ± 0.2 ab	6.7 ± 6.7 ab	75.8 ± 3.62 abc	0.76 ± 0.03 ab
GK254M5	200	0.5 ± 0.2 ab	12.7 ± 10.8 ab	75 ± 5.07 abc	0.81 ± 0.03 ab
GK318M5	500	1.5 ± 0.5 a	10 ±1 ab	81.2 ± 1.20 a	0.66± 0.04 bc
GK321M5	500	0.5 ± 0.2 ab	2.2 ± 1.9 b	71.4 ± 1.29 abc	0.78± 0.06 ab
ICSV1049 (Parent)	0	2 ± 0.0 a	5.3 ± 2.1 ab	75.6 ±1.12 a	0.78 ±0.07 ab
IC10P1M6	200	0.7 ± 0.4 ab	2.6 ± 1.5 b	77.7 ± 2.90 a	0.78 ± 0.05 ab
IC10P5M6	200	2.2 ± 0.6 a	2.7 ± 1.3 b	79.3 ±3.57 a	0.76 ± 0.04 ab
IC17M6	200	0.00 ± 0.0 b	0.00 ± 0.0 b	51.5 ±1.39 c	0.91 ± 0.01 a
IC134M5	200	1.5 ± 1.1 a	4.25 ± 2.8 b	44 ± 1.53 cd	0.79 ± 0.06 a
IC47M5	400	0.00 ± 0.0 b	0.00 ± 0.0 b	36.75 ±1.18 d	0.93 ± 0.01 a
IC59M5	200	2.5 ± 0.2 a	9.00 ± 2.8 ab	63.5 ± 2.41 b	0.51 ± 0.10 b
IC74M5	400	2.00 ± 0.9 a	12.5 ± 6.2 ab	62.5 ± 3.2 b	0.5± 0.08 b
IC83M5	200	0.00 ± 0.0 b	0.00 ± 0.0 b	39.75 ±1.07 d	0.95 ± 0.03 a
CV%		38.92	49.91	9.02	15.28

Values are means ± standard error. Means with the same letter are not statistically different.

Striga damage was positively correlated to the number (r = 0.1, P = 0.49) and the height (r = 0.31, P < 0.04) of emerged *Striga* plants, showing that *Striga*

damage was more significant when *Striga* number and/or plant height increase (Figure 1). Positive significant correlation between *Striga* number and plant height ($r = 0.55$, $P < 0.0001$) was also revealed. These three *Striga* variables evolved in the same direction while resistance index (RI) evolved in the opposite. *Striga* resistance index was negatively and significantly correlated to damage (%) ($r = -044$, $P = 0.002$), emerged plant number ($r = -0.64$, $P < 0.0001$) and plant height ($r = -0.58$, $P < 0.0001$) of *Striga*. Resistance index therefore decreases when the other three *Striga* variables increase (Figure 1).

The trend curve of the height of *Striga*-infected Sorghum plants was compared to that of non-infected plants (Figure 2). The average of non-infected plant height was 67.87 cm, 71.06 cm and 78.89 cm against 51.22 cm, 55.56 cm and 56.4 cm for *Striga*-infected plant height at 95, 118 and 140 DAP, respectively. The trend curves showed that non-infested Sorghum plants continued to grow in height (Figure 2(A)) while infested plants reached their maximum height (Figure 2(B)) at 140 DAP. *Striga*-infection therefore reduced Sorghum plant height about 24.5%, 21.81% and 28.5% at 95, 118 and 140 DAP, respectively. Figure 3 shows that the biomass of the parent Sariaso14 was highly reduced by *Striga* attack compared to the mutant line SA38M5.

3.3. Hierarchical Clustering of Sorghum Mutant Lines According to Their *Striga* Resistance

The hierarchical clustering reveals that the three descriptive variables, *Striga* resistance index, emerged plants and damage, significantly discriminated three clusters among the screened Sorghum genotypes. The first cluster involved 14 mutant families which, did not induce *Striga* emergence leading to high resistance index and weak *Striga* damage. The second cluster consisted of 21 mutant lines and two parents (ICSV1049 and Grinkan) that showed high resistance index

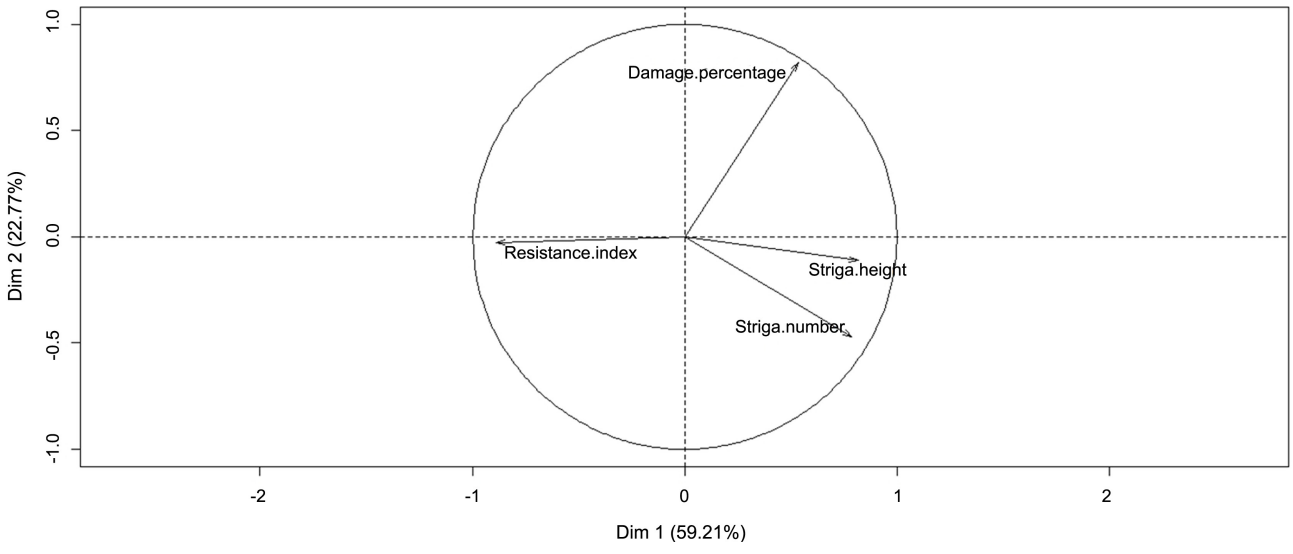

Figure 1. Distribution of *Striga* variables in Plan 1 - 2 revealed from principal component analysis with 40 sorghum mutant lines screened under pot conditions.

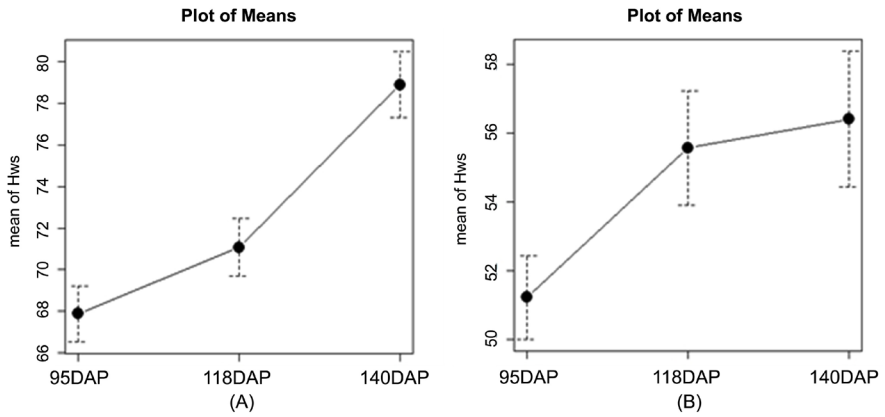

Figure 2. Reducing effect of *Striga*-infection on sorghum plant height. (A) Trend of sorghum height under non-infested pots; (B) Trend of sorghum height under infested pots.

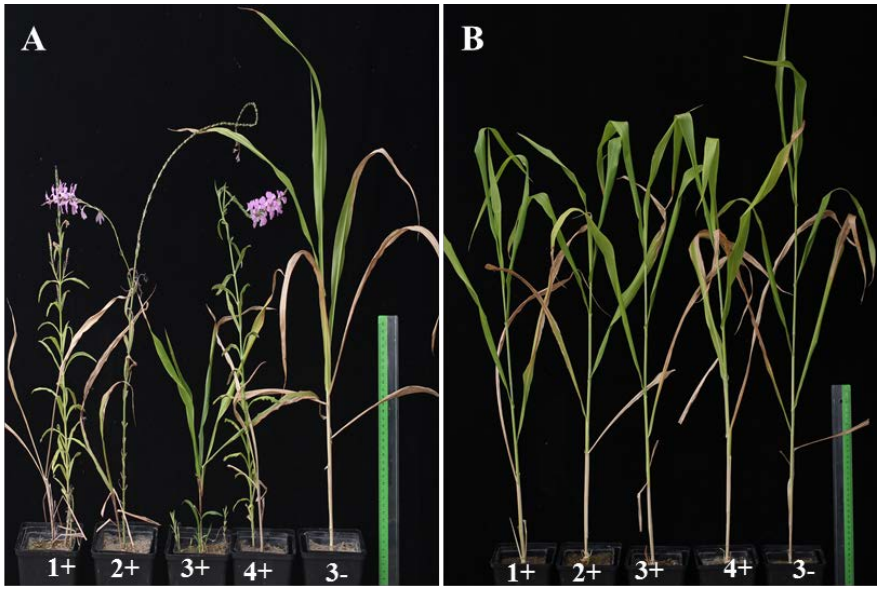

Figure 3. Plant vigour of Sariaso14 parent (A) and a mutant, SA38M5 (B) in *Striga*-infected pots (+) versus in non-infected pots (−), 95 days after planting (DAP).

and high *Striga* damage and the third cluster gathered six mutant lines and the parent Sariaso14 which displayed low resistance index and high damage rate (**Figure 4**).

3.4. Response of Five Sorghum Mutants Resistant to the Burkina *Striga* Ecotype to Another African Ecotype from Sudan

ANOVA showed significant differences between Sorghum genotypes for the number of emerged *Striga* plants ($P < 0.006$), percentage of *Striga* damage on Sorghum plants ($P < 0.0001$) and *Striga* resistance index ($P < 0.0001$) when infected by *S. hermonthica* ecotype from Sudan (**Table 5**). No *Striga* emergence was recorded at 95 DAP with three Sorghum mutants; SA38M5, GK715M4 and IC47M5 and the resistant control (Framida). *Striga* resistance index was highest

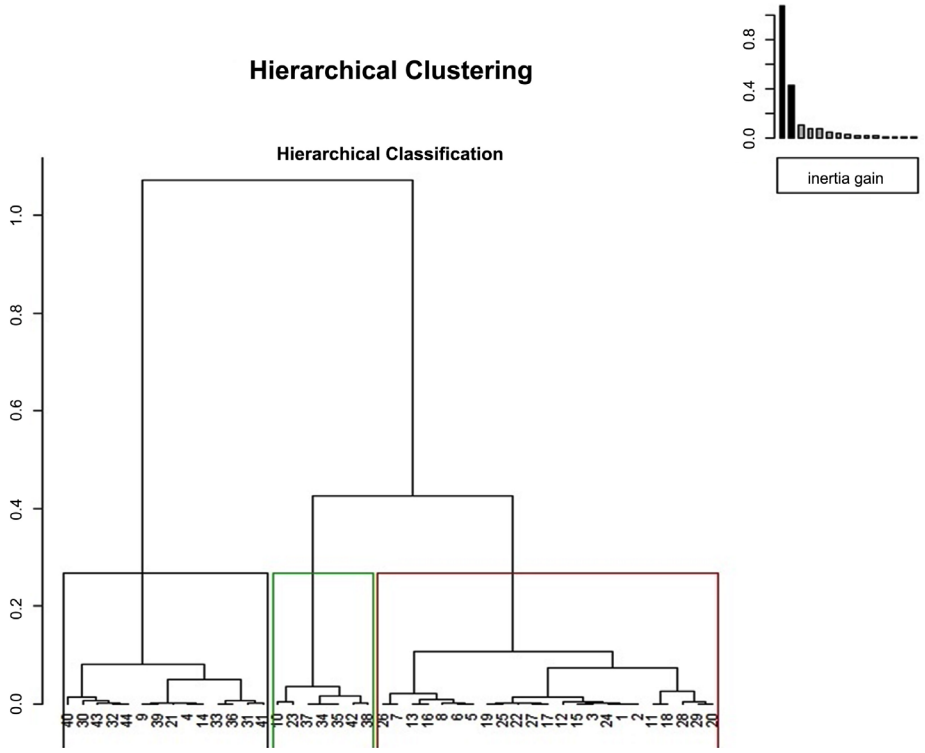

Figure 4. Hierarchical clustering of Sorghum mutant lines according to the resistance index, damage and emerged plant number of *Striga hermonthica*.

Table 5. Response of mutant sorghum lines to the infection of Sudan's *Striga hermonthica* ectype, 95 DAP in greenhouse conditions at PBGL, Seibersdorf.

		Striga hermonthica ecotype from Sudan		
Sorghum Genotypes	Dose Gy	Number of emerged *Striga* plants	Percentage of *Striga* damage	*Striga* resistance index
Framida (Parent)	0	0.00 ± 0.0 b	61.3 ± 5 bc	0.92 ± 0.01 c
Sariaso14 (Parent)	0	1.3 ± 0.3 a	75 ± 1.8 a	0.58 ± 0.06 c
SA38M5	200	0.00 ± 0.0 b	40 ± 1.2 d	0.91 ± 0.01 a
Grinkan (Parent)	0	0.6 ± 0.3 ab	73.6 ± 0.6 a	0.66 ± 0.02 bc
GK629M4	200	1 ± 0.4 ab	52.7 ± 1.7 c	0.63 ± 0.08 c
GK715M4	400	0.00 ± 0.0 b	49 ± 2 c	0.92 ± 0.02 a
ICSV1049	0	1 ± 0.5 ab	67.5± 1.6 b	0.63 ± 0.02 c
IC83M5	200	1 ± 0.4 ab	53± 3 c	0.78 ± 0.04 ab
IC47M5	400	0.00 ± 0. b	43 ± 1.7 d	0.92 ± 0.01 a
CV%		107.3	8.98	10.16

Values are means ± standard error. Means with the same letter are not statistically different in the same column.

with the plants of these four Sorghum genotypes. However, plant syndrome rating which reflects the damage caused to the Sorghum plant in reaction to *Striga* infection revealed only SA38M5 and IC47M5 are considered resistant (Table 5).

4. Discussion

Field screenings highlighted a strong genetic heterogeneity between screened mutant lines. The phenotypic types showed significant variation for the five qualitative traits. Mutant lines were therefore ranked into three or four clusters with respects to each of the measured variables. The large difference between the minima and maxima for all quantitative traits showed that there is a great diversity within the clusters discriminated. The high coefficients of variation (CV > 30%) revealed high genetic variability for the traits *Striga*-infected Sorghum plant rate 70 and 100 DAP (SISPR70 and SISPR100) within the mutant lines and grain sorghum weight (g) per panicle (GrWP) for Sariaso14 and Grinkan mutants. On the other hand, the low coefficients of variation indicate low genetic variability for the trait days to Sorghum maturity (DSMa). Sorghum mutant lines selected from field experiment as putative *Striga*-resistant were generated from the four doses of gamma irradiation (200, 300, 400 and 500 Gy). These results suggest that the development of *Striga*-resistant Sorghum mutant lines is not influenced by the irradiation dose of gamma rays. They also suggest that induced mutation using gamma irradiation is a powerful tool for creating genetic variability in order to exploit newly emerging traits to improve the agronomic characteristics of crops [19].

The differences in *Striga* emergence delay observed with the mutant lines showed that some mutant lines were endowed with some potential genetic that influences the time of *Striga* seed germination whereas others did not allow it. No *Striga* emergence in host plot was explained as the inhibition of germ tube exo-enzymes by host root exudates and the synthesis of phyto-alexins that would block the emission of *Striga* germ tube [20]. Late *Striga* emergence may be due to a hypersensitive host response that delays the development of parasite in the soil [21].

With regards to *Striga* infection on Sorghum growth, seven mutants (SA38M5, SA188M6, GK715M4, GK225M5, IC47M5, IC83M5 and IC17M6) recorded low damage scores (4 - 5) coupled with a high resistance index. They can therefore be considered as resistant mutant lines according to [20] who qualified *Striga*-resistance as the capacity for the host plant to prevent *Striga* attachment and seedling development and well-yielding compared to the sensitive plant. On the other hand, high resistance index was observed with four mutants GK220M5, GK251M5, GK629M4 and SA251M5 of which biomass was affected by *Striga* attack. These last four mutant lines growing better than others *Striga*-infected lines could be considered as tolerant. [22] defined *Striga* tolerance as the capacity of host plant to maintain biomass and grain yield compared to the sensitive plant under the same level of *Striga*-infection. The screening of five mutants to *Striga hermonthica* ecotype of Sudan showed that three mutants SA38M5, GK715M4 and IC47M5 did not allow for the emergence of *Striga* plants. These mutants could be recommended as resistant Sorghum to the parasite in Burkina Faso and Sudan. No *Striga* emergence doesn't mean no *Striga* attacks. Indeed, these three

mutants showed severe attack symptoms although no *Striga* plants emerged in pot. This could be explained by the fact that a large number of *Striga* seedlings are attached to their roots and then cause significant damages to the host. These mutants are therefore not completely immune to the two *Striga* ecotypes but are endowed with form of mechanism of resistance that allows them to escape from the parasite. The chlorosis or burnt leaves observed on *Striga*-infected mutant plants have been reported by [23] who emphasized that sensitive *Striga* infected sorghum displays disease and symptoms including severe stunting, leaf chlorosis, necrosis and desiccation which lead ultimately to pre-mature wilting. The reducing effect of *Striga*-infection on mutant plant height about 22% - 28% (95 - 140 DAP) may be due to the attachment of *Striga* seedlings on the host root system that results in the reducing of host plant height by taking the substantial amount of nutrients from the host plant [24]. [25] further explained that *Striga* infection on sorghum significantly affects its photosynthesis which reduces the host crop growth. *Striga* attack actively influences host transcription to foster parasitism by either up-regulating host genes associated with nutrient supply or by down-regulating defence-related genes [26]. Resistance trait reduces the number of successful attachments and, the reproductive output of the parasite accordingly [27]. However, the deleterious effects of *Striga* parasitism on resistant cultivar growth, morphology and yield are complex and are not always related [28].

The positive correlation between damage, emerged plant number and plant height of *Striga* revealed that these three variables may be measured simultaneously for the selection of Sorghum mutants for *Striga*-resistance. The strong negative correlation between *Striga* resistance index and the three parameters including *Striga* damage, emerged plant number and plant height indicated that the higher the resistance index, the lower *Striga* number, plant height and damage. This correlation indicated that the selection of Sorghum mutants with a higher resistance index results in reduced *Striga* number, plant height and damage.

Averaged over results recorded in field and greenhouse experiments, three mutant Sorghum lines SA38M5, IC47M5 and GK715M4 are the most promising to withstand the obligate root parasite *S. hermonthica*. Multi-site and multi-season field tests are needed to highlight the stability of the traits *Striga*-resistance and yielding of sorghum mutants in terms of reduced *Striga* number and yield components to multivariate agro-ecological conditions because of the eventual existence of local *Striga* strains. Mutation breeding enabled to generate genes of interest and identify *Striga* resistance sources that may be exploited through conventional plant breeding programs. However, further studies including microscopic bioassays and histological analysis should be done to understand the resistance mechanisms of those mutants. Genotyping studies would allow marker assisted breeding with the prospect to pyramid resistance genes into the most framers' preferred Sorghum variety from each agro-ecological area for more sustainable *Striga* resistance.

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Conflicts of Interest

The authors have no conflict of interest related to the work described in this manuscript.

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