

Mutation Breeding for Resistance to *Striga Hermonthica* in Sorghum and Rice for Sustainable Food Production in Sudan



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Abstract *Striga* is the most limiting biotic constraint to sorghum and rice production in much of Sudan. This chapter describes protocols used to screen for *Striga* resistance among gamma irradiated elite sorghum and rice cultivars adapted to Sudanese agroecologies. The original cultivars show high yield potential but are *Striga* susceptible. The physical mutagenesis attempted to create genetic variants among these Sudanese cultivars with improved *Striga* resistance. Methods for collecting, cleaning, storing and testing germination of *S. hermonthica* seeds suitable for resistance screening are also described.

Keywords *Striga hermonthica* · Sorghum · Upland rice · Mutation breeding · Resistance

Introduction

In Sudan, sorghum is grown on over six million hectares, 30% of which are infested with *S. hermonthica* (Samejima et al. 2018). Although it has the most land devoted to sorghum production, a 17.3% global share, its global production share is only 7.8% (Pingali et al. 2020). Despite its versatility and economic importance in the livelihoods of millions of subsistence farmers, average sorghum productivity in Sudan is only about 700 kg/ha, less than half the global average (Pingali et al. 2020). This yield shortfall is mainly due to the use of traditional low yielding varieties, limited fertilizer use, low erratic rainfall and *Striga* infestation (Samejima et al. 2018). Increased use of higher yielding sorghum varieties with *Striga* resistance among subsistence farmers could substantially alleviate these dramatic productivity shortfalls.

Rice has become a staple food in many countries of Africa and constitutes a major part of the diet, particularly in urban areas. In Sudan rice is the fourth major

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food source, after sorghum, millet and wheat (Elhassan 2017). Although rice is mainly cultivated under irrigation in areas along the White Nile, the use of upland varieties expands the potential rice growing areas to 300,000 ha (Elhassan 2017). As in many African countries, rice demand exceeds production in Sudan requiring net import to meet demand at a huge cost in hard currency. Sudan consumed a total of 90,000 tonnes of rice in 2020 but produced only 35,000 tonnes (FAO 2021). The ability to grow upland rice could meet Sudan's domestic needs. As with Sudan's other major cereals, upland rice production is constrained by *S. hermonthica* (Samejima et al. 2018). Protecting rice yields from losses through cultivation of *Striga* resistant varieties could further ensure sufficient production to meet Sudan's domestic needs.

Although some *Striga* resistance and tolerance is known among both rice and sorghum cultivars (Dafaallah et al. 2019; Samejima et al. 2018), broadening genetic diversity underlying these traits through mutation breeding offers further and more durable protection. Introduction of resistance into otherwise agronomically promising but *Striga* susceptible cultivars could have a very positive impact on Sudan's cereal productivity. The protocols described herein are derived from an attempt to introduce *Striga* resistance through gamma irradiation into several improved Sudanese sorghum and rice cultivars with susceptibility to the parasitic weed.

Protocols

***Striga* seed harvesting.** *Striga* seed is best harvested in late October in rain fed areas of Sudan after the capsules turn brown in color. *Striga hermonthica* flowers progressively from the bottom to top of the floral head (Fig. 1). When collecting seeds, it is best to cut only the upper part (floral heads) of the *Striga* plants (Fig. 2). This will lessen the amount of trash to be screened out later. A mature floral head is one on which all florets have completed flowering, with no visible flowers or only the upper flowers remaining (Fig. 3). Harvest only the mature heads with mature capsules (Fig. 4).

Although a single *S. hermonthica* plant may have multiple flowering branches and a single plant may produce several thousand seeds, the seeds are quite small (Fig. 4) so several plants must cut and collected to get an appreciable volume of seed suitable for pot and field studies. The harvested mature florets should be spread on a 4 × 4 m white polyethylene sheet to dry. Mix the *Striga* plants daily to avoid rotting and to ensure even drying. After 10–14 days of drying, tap the floral heads gently on the plastic sheet to release the seeds from the dried capsules.

After “threshing” all of the *Striga*, screen the material on the sheeting by passing it through sieves of 250 and 150 openings. Sieving helps remove most of the non-seed plant debris in the seed lot and makes subsequent infestations with the seed more accurate. In addition, the seed will store better and be less susceptible to fungal spoilage once this debris is removed. Most of the *Striga* seed will be collected on the 150 µm screen (Berner et al. 1997; Figs. 5 and 6).



Fig. 1 Flowering *Striga hermonthica* plants



Fig. 2 Mature *S. hermonthica* plants with seed capsules

Cleaning Striga seeds. The *Striga* seeds can be separated by weight from further impurities and cleaned in a 500 mL beaker containing 100 mL 70% ethanol, 50 mL 20% bleach (1% NaOCl) and 300 mL tap water. Add 50 g of the sieved *Striga* seeds into the beaker containing the liquid and stir it gently with a spoon for three minutes. Let the seeds rest in this solution for 20 min. Heavier particles, including viable



Fig. 3 A *Striga* floret nearly completed flowering



Fig. 4 Mature *Striga* capsules shedding seed

seeds, precipitate to the bottom of the beaker, while the lighter particles (empty seed coats, immature or bug eaten seeds, bits of capsules) will float on the surface of the liquid (Figs. 7 and 8).



Fig. 5 Threshed unsieved *S. hermonthica* seeds



Fig. 6 *Striga* seeds after sieving



Fig. 7 Washing sieved *Striga* seeds



Fig. 8 Viable *Striga* seed settles to the bottom

Skim the debris (upper) layer off with a spoon then carefully pour off the cleaning solution into a waste container leaving the *Striga* seeds in the bottom of the beaker. Use a Pasteur pipet fitted with a bulb to suck away as much of the remaining liquid from the seeds as possible. Pressing the end of the pipet flat against the bottom of the beaker while the bulb is compressed and then slowly releasing the bulb will suck



Fig. 9 Washed *Striga* seeds spread on a blotter to dry

up the liquid without the seed. Add 50 mL tap water to wash the remaining seeds, stir together gently and let the seeds resettle for at least 10 min. Pour away the wash water to waste and suck away remaining liquid as before. Repeat the washes several times until the liquid above the settled seeds looks clear. Used distilled water for the last wash. After sucking away the remaining liquid in the final wash with the pipet, invert the beaker over a tray lined with white blotter paper. Tap the bottom of the beaker to expel the seeds onto the blotter paper. Use the spoon remove any seeds still in the beaker and spread the washed seeds evenly in a thin layer across the blotter. Allow them to air dry at room temperature overnight (Fig. 9).

Striga seed storage. Once the seeds are fully dry, they will not clump together and can be carefully poured or scooped from the paper into waterproof glass, plastic or metal jars or vials. The clean dried *Striga* seeds can be stored for years at room temperature in these storage containers as long as they remain fully dry (Fig. 10). Putting the containers in a desiccator helps to keep them dry. The stored seeds should be checked periodically for fungal and/or insect damage, especially in the first days and weeks after washing. If such damage is evident, resieve and rewash the seeds as before. Check the germination rate after a year of storage as described below and recheck annually or before each time they are used. They should have a germination rate of at least 30%.

Checking the Striga seed germination. *Striga* seed has an after-ripening requirement of several months to a year after they are collected. Be sure they have been properly cleaned and stored for at least 10 months before testing their germinability. Surface sterilize the *Striga* seeds by placing a small scoop of dried seed in a 25 mL flask. Add 10 mL 70% ethanol, move to a laminar flow hood and agitate the seeds



Fig. 10 Example containers for *Striga* seed storage

in the liquid with a sterile Pasteur pipet fitted with a bulb for 5 min. Carefully pour off the ethanol solution into a waste container after the seeds have settled. Use the Pasteur pipet to suck away as much of the remaining liquid from the seeds as possible. Pressing the end of the pipet flat against the bottom of the flask while the bulb is compressed and then slowly releasing the bulb will suck up the liquid without the seed. Add 10 mL sterile distilled water and agitate for 5 min with the pipet to wash the seeds. Remove the wash water from the flask as with the ethanol. Add 10 mL 20% bleach (1.05% NaOCl)/0.2% Tween[®] 20 (Sigma-Aldrich) to the flask of seeds and agitate with the pipet for 5 min. Remove the liquid as before. Wash three times with 10 mL sterile distilled water as before. Leave the flask open under the laminar flow hood after removing the final wash liquid to air dry overnight.

Conditioning the surface sterilized *Striga* seeds for a week in a 100 mm petri dish following these steps. Place a 90 mm Whatman #1 filter paper disc in the petri dish. Add 1 mL of sterile distilled water to the dish to wet the filter paper. Place twenty 15 mm Whatman #1 filter paper discs onto the larger wetted paper in the petri dish. Sprinkle 20–40 dried surface sterilized *Striga* seeds to each small disc with the aid of a small metal spatula previously wiped with 70% ethanol. Add an additional 1 mL water with a pipet onto the bottom paper, taking care not to wash the *Striga* seeds off the small discs. Close the petri dish and seal with parafilm. Place petri dish with seeds in a dark incubator set at 30 °C for one week.

After one week of conditioning, open the plate containing the *Striga* seeds under a laminar flow hood. Apply a total of 1 mL by pipet of a 10 ppm strigolactone germination stimulant (strigol or GR24) onto the 90 mm bottom paper in the petri dish taking care not to wash any *Striga* seeds from the 15 mm discs. Try to move the

pipet tip to different exposed areas of the bottom paper surrounding the smaller discs as the solution is delivered so that the germination stimulant solution is distributed evenly across the plate. Close the petri dish and seal with parafilm. Place the petri dish with seeds in a dark incubator set at 30 °C for 48 h. After the two days of exposure to striogolactones, count the germinated and total number of seeds on each of the 20 discs seeds under a stereomicroscope. Add the total number of germinated seeds from all 20 discs and divide by the total number of seeds (germinated and ungerminated) on all discs to determine the germination rate of the seedlot. If it is at least 30%, it may be used for infestations of pots or planting hills as described below.

Striga seed inoculum preparation for infesting pots or planting hills. Because *Striga* seeds are so small, soil infestations are most easily accomplished if the *Striga* seeds are mixed with a carrier material to increase volume. Sand is a good carrier, but it should be sieved so that only particles of the same size as *Striga* seed are used. A 250 or 150 µm sieve should be used for sieving the sand. To prepare uniform artificial *Striga* seed inoculum for infesting pots or planting hills, evenly mix 1 g of cleaned stored *S. hermonthica* seeds determined to have acceptable germinability with 2 kg of sieved sand. Add one full cap from a 2 L plastic bottle (approximately 10 cc) of *Striga* inoculum per planting hill of 5 cm deep and 5 cm wide. This should contain approximately 2000–2500 germinable *Striga* seeds.

Striga hermonthica infests all the major cereals grown in Sudan (sorghum, millet, rice and even wheat). There does seem to be, however, *Striga* strains specifically adapted to each host species (Dafaallah 2020). Therefore, resistance screening for sorghum should be done with a *Striga* seeds collected from a sorghum field and for rice with those collected from rice plots.

Field protocol followed for Striga resistance screening of mutagenized sorghum. Seeds of four Sudanese farmer-preferred cultivars with good yield potential were gamma irradiated with a ⁶⁰Cobalt source at the IAEA PBGL as described in Chapter “Physical Mutagenesis in Cereal Crops”. Three doses, 100, 200 and 300 Gy, were chosen based on the radio-sensitivity experiments performed at PBGL. The mutant seed lots (M1) population was sent to Sudan and immediately grown in the field to generate the M2 seeds. About 1000 M2 plant seeds from each of the three doses of the four farmer preferred cultivars were screened under *Striga* infestation in rainfed field plots. Equivalent plots of unmutagenized seed were used as controls.

Sow the sorghum seeds into 10 m long row, with holes spacing of 20 cm within row and 80 cm between rows. Add one cap from a 2 L plastic bottle (≈ 10 cc) of *Striga* seed inoculum in each hole. Thin sorghum seedlings to one plant per hole ten days after sowing. Apply N fertilizer (urea) equivalent to 18 kg/ha around the plants four weeks after sowing. This delayed and suboptimal urea fertilizer rate should maintain sorghum growth but add sufficient nitrogen stress to favor *Striga* infection. Weed the plots of all but *Striga* by hand at 15, 30, 45, and 60 days after sowing. Count the number of emerged *Striga* around each sorghum plant beginning at two weeks after the appearance of the first *Striga* in the plots and repeat these counts three times at two week intervals.

Field protocol followed for Striga resistance screening of mutagenized rice. Seeds of the three upland rice cultivars, released for their commercial productivity in Sudan, were gamma irradiated with a $^{60}\text{Cobalt}$ source in doses of 100, 200 and 300 Gy at the IAEA PBGL in Austria as described in Chapter “[Physical Mutagenesis in Cereal Crops](#)”. The irradiated M1 seeds and non-irradiated seeds (control) were sent back to Sudan for field testing. Approximately 600 irradiated seeds from each dose of each cultivar were sown individually in the field in order to raise M1 plants. Separate control plots consisting of 600 seeds of non-irradiated seeds of each cultivar were sown near for comparison of survival rates. Fitness of M1 plants were determined by comparing days to heading, days to maturity and grain yield to controls.

The *S. hermonthica* field screening experiment was conducted during the rainy season of 2017 (July–October) at the research farm of the White Nile research station at Kosti, Sudan. No supplemental irrigation was applied to the plots. M2 seeds of the three upland rice cultivars at the three different doses were sown along with seed from their unmutagenized parents in *Striga* sick plots to estimate whether mutagenesis improved *Striga* resistance (measured by number of emerged parasites per plot) in individual M2 plants relative to controls. Seeds were sown in a row trail, with spacing of 20×20 cm between rows and three seedlings per hill. Each experimental plot consisted of 20 rows. *Striga* inoculation was done as previously described for sorghum (10 cc *Striga*/sand mixture in each planting hole). Weeds other than *Striga* were removed by hand 15, 30, 45, and 60 days after sowing.

Individual M2 sorghum or rice plants around which no *Striga* emerged were advanced to M3 by self-pollination. Of the approximately 1000 M2 sorghum and 5400 M2 rice plant seeds from the three irradiation doses (100, 200 and 300 Gy) of farmer preferred cultivars field screened for *Striga* resistance, 46 sorghum and 62 rice lineages of promising putative *Striga* resistant mutants were selected. M3 seed from individual M2 selections were retested with *Striga* infestation in pots in a greenhouse.

Sorghum pot experiment. Fill each plastic pot (round, 20 cm diameter) with 2 kg of a mixture of field soil and sand (3:1, v/v). Dig a 5×5 cm hole in the middle of each pot and add to this one plastic bottle cap (10 cc) of *Striga* inoculum as described previously. Move pots to a greenhouse and water to keep the soil mixture damp for 7–10 days to condition the *Striga* seed. Re-excavate the 5×5 cm hole in the middle of each pot sow three sorghum seeds. Plant four pots of each M3 lineage. Continue to water as needed to keep soil visibly moist. Thin sorghum to one plant per pot at two weeks after sowing. Remove any weeds other than *Striga* by hand at 15, 30, 45, and 60 days after sowing. Count the number of emerged *Striga* plants at 45, 60 and 90 days after sowing.

Rice pot experiment. M3 seed of the rice mutant candidates were screened in the greenhouse similarly to sorghum, except that plastic bags of similar volume (2 kg) holding the same 3:1 soil/sand medium were substituted for rigid pots. Six of these bags were prepared per mutant lineage, four infested with the *Striga* seed inoculums and two without as an addition control to the unmutagenized counterpart of each tested lineage. Comparing host plant fitness parameters (height, weight, verdancy, grain yield) in infested pots with those growing without *Striga*, can give an indication

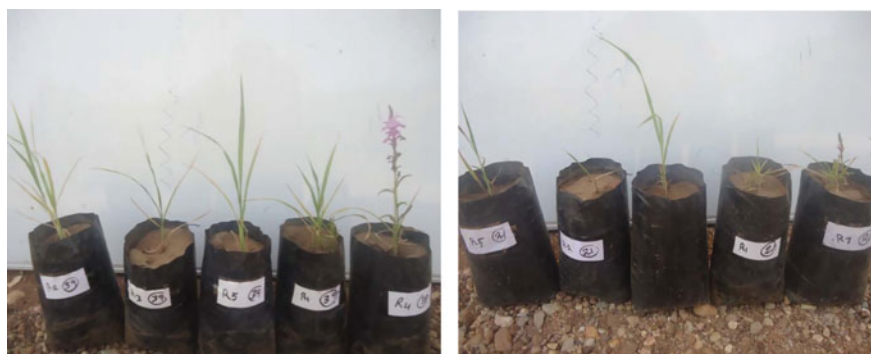


Fig. 11 Two rice M3 lineages from independent M2s selected from *Striga* free plants in infested field plots. Individual M3 plants (first four bags in each series) from both lineages remained *Striga* free in infested pot trials in contrast to their respective unmutagenized parent lines (fifth bag at right of each series)

of *Striga* tolerance. As with sorghum, sown M3 seeds were thinned to one rice plant per pack two weeks after sowing. Plants were watered at two day intervals throughout the experiment. *Striga* emergence counts were taken at 45, 60 and 90 days after sowing.

Quantification of gained *Striga* resistance. The *Striga* resistance of M3 potted plants advance from M2 field selections was quantified by plotting the average emergence count of the four plants per lineage over the three counting dates (45, 60 and 90 days). The area under these *Striga* progress curves were then compared to those of the respective unmutagenized progenitor lines (Gowda et al. 2021). Those showing an area less than half that of the original line were considered to have potentially gained *Striga* resistance through mutagenesis. Gained *Striga* tolerance over uninfested controls was not quantified. Of the 46 sorghum and 62 rice M2s advanced to pot studies, ten sorghum and 22 rice M3 lineages were selected as the best putative mutants for further characterization in laboratory assays, pots and field evaluations. Two of the rice selections in which no *Striga* emerged in the pot study are pictured in Fig. 11.

Conclusion

Striga hermonthica is a major contributor to low cereal productivity in Sudan, including sorghum and rice. Mutagenesis derived cultivars with good yield potential and *Striga* resistance can improve rice and sorghum production and contribute to national food security. From the protocols described in this chapter, ten putative *Striga* resistant mutants of sorghum and 22 of rice were identified in lineages from independent gamma irradiated events. These were selected from field screens of M2

populations and confirmed in derived M3 pot screens. Further agronomic characterization of these mutants to determine yield under *Striga* infestation at multiple locations in the country will follow before varietal release. Additionally, laboratory characterization of resistance mechanisms as those described in Chapters “An Agar-Based Method for Determining Mechanisms of *Striga* Resistance in Sorghum”, “Histological Analysis of *Striga* Infected Plants” and “*Striga* Germination Stimulant Analysis”, and underlying genetic changes in these mutants as in Chapter “Identification of Closely Related Polymorphisms with *Striga* Resistance Using Next Generation Sequencing” will help determine the uniqueness and utility of these mutations. Alternative screening protocols for gained *Striga* resistance through physical mutagenesis in rice and sorghum in Burkina Faso are described Chapter “Screening for Resistance to *Striga Hermonthica* in Mutagenized Sorghum and Upland Rice in Burkina Faso” and for maize and rice in Madagascar in Chapter “Phenotyping for Resistance to *Striga Asiatica* in Rice and Maize Mutant Populations In Madagascar”.

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