Phenotyping for Resistance to *Striga Asiatica* in Rice and Maize Mutant Populations in Madagascar



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Abstract *Striga asiatica* is a major constraint to rain-fed rice (*Oryza sativa* L.) and maize (*Zea mays* L.) production in the Middle West and South regions of Madagascar. This work aims to establish a robust and efficient field and glasshouse screening protocol for identification of rice and maize mutants having resistance/tolerance to *Striga asiatica* by assessing the host plant phenotypic response under severe *S. asiatica* infestation in soil. Our screening strategy involved field and glasshouse experiments where soils were artificially infested with *S. asiatica* seeds under fertilizer-free growing conditions. Two maize (Plata and IRAT200), and two rice (B22 and F154), farmer-preferred *Striga* susceptible varieties, gamma irradiated at different doses were screened in a *Striga* endemic area of the Middle West and compared to their respective unmutagenized lines. Rice M3 to M5 progenies were also screened in *Striga* infested pots in a glasshouse. Developed protocols enabled the production of promising new *Striga* resistant/tolerant varieties in both maize and rice adapted to Malagasy agriculture.

Keywords *Striga asiatica* · Mutation breeding · Resistance · Maize · Upland rice · Madagascar

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Introduction

The large island nation of Madagascar has several agroecologies. The East is the wettest benefiting from trade winds that bring on annual average 4000 mm of rainfall. The North experiences seasonally heavy rainfall due to cyclones while the South and West are dependent on less frequent anti-cyclonic rains, with the Southwest receiving as little as 350 mm of annual rainfall and prone to devastating droughts (Rigden et al. 2022). *Striga asiatica* came to the island in the early 20th Century, likely with grain imports, and has now become a major cereal production constraint mainly in Middle West (Scott et al. 2020). Located between the Central Highlands and the West Coast at an elevation of 900–1300 m above sea level, this area of undulating plateaus called "*tanety*" is cut by lowlands and deeply eroded areas (Ripoche et al. 2019). The annual rainfall here is about 1100 mm, which is erratically distributed mainly during the hot season from November to April. The ferralitic soils of the *tanety* are low in organic matter and nutrients (especially N and P), acidic (average pH 5.3) and prone to erosion (Randrianjafizanaka et al. 2018). While the *tanety* have been used by subsistence farmers to grow upland rice and maize, traditional low input agricultural practices and *S. asiatica* often leaves them unfit for cereal production after a few years (Scott et al. 2021). Although conservation agricultural practices like no-till rice-maize rotations with perennial legume intercropping can, in the long run, lead to sustained cereal productivity in the *tanety* (Randrianjafizanaka et al. 2018), they are rarely permanently adopted by Malagasy farmers here (Razafimahatratra et al. 2021).

The most important staple cereal crop in Madagascar is by far rice, which is consumed daily with every meal in almost every Malagasy household. Rice accounts for approximately 46% of daily caloric intake of all Malagasies (Rigden et al. 2022), and more than half of that of rural people (Ozaki and Sakurai 2020). Following the stories of Burkina Faso (Chapter "Screening for Resistance to Striga Hermonthica in Mutagenized Sorghum and Upland Rice in Burkina Faso") and Sudan (Chapter "Mutation Breeding for Resistance to Striga Hermonthica in Sorghum and Rice for Sustainable Food Production in Sudan"), national rice production is insufficient to meet national need and therefore must be imported at an annual cost of $180 million in 2020 (TrendEconomy 2021). Rice is produced on the island mainly (> 90%) in wet irrigated coastal areas but upland rice, with government encouragement, is increasingly contributing to national production (Rakotoarisoa et al. 2019; Ozaki and Sakurai 2020). Unfortunately, this upland rice is vulnerable to *S. asiatica.* In uncontrolled conditions on the *tanety*, yield losses to *Striga* in upland rice average 73% (Rodenburg et al. 2020).

Maize is of lesser national importance than rice, but still the second most cultivated cereal in Madagascar with 225,000 t grown on 127,000 ha in 2020 (FAOSTAT 2022). It was initially used mainly as animal feed, but now accounts for nearly 5% of daily caloric intake (Rigden et al. 2022). Maize has become the third staple after rice and cassava and is particularly consumed in gruels by Malagasy children (Razafindratovo and Raveloarimalala 2021). Maize production has expanded an average of 2%

annually under a "National Maize Project" launched by the Malagasy government in 2004, though it is currently down from its peak in 2012 (Palchetti et al. 2021). Main production areas for Malagasy maize include the Middle West *tanety* where it is often rotated with upland rice and its yield loss to *S. asiatica*, where uncontrolled, averages 80% (Rodenburg et al. 2020).

Both rice and maize production in the erosion and *Striga*-prone *tanety* are demonstratively improved with conservation agricultural practices (Randrianjafizanaka et al. 2018; Rodenburg et al. 2020). These gains were even more pronounced when *Striga* resistant rice (Randrianjafizanaka et al. 2018; Scott et al. 2021) and maize (Andriamialiharisoa 2019) varieties are incorporated into the conservation agricultural practice. *Striga* resistance or tolerance is currently available in certain NERICA and in one farmer discovered rice variety called "Jean Louis" (Rakotoarisoa et al. 2019). Currently no *Striga* resistant maize varieties are adapted to the Malagasy Middle West where *S. asiatica* is a major concern to farmers (Autfray et al. 2022). We undertook this mutagenesis project in collaboration with the IAEA in the hopes of introducing *Striga* resistance into popular rice and maize varieties adapted to the *tanety*.

The Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, Department of Nuclear Sciences and Applications, Plant Breeding and Genetics programme has supported the country's national crop improvement program helping to apply plant mutation breeding techniques to develop new cereal mutant lines since 1998. A Coordinated Research Programme CRP under close collaboration with the International Atomic Energy Agency (IAEA) was established in 2016 to support the *Striga* management in the country in order to develop new rice and maize mutant lines with enhanced *Striga* resistance. To realize gains from mutation breeding to cereal productivity in Madagascar, it is important to develop efficient methods for screening maize and rice after mutagenesis for both continued adaptation to local culture and select for any gained *Striga* resistance. The main objective of this document is to present established robust and efficient field and glasshouse screening protocols for identification of rice and maize mutants with resistance/tolerance to *Striga asiatica* under artificial *Striga* infestation.

Protocols

Collecting Striga asiatica *seeds.* In order to conduct field and pot experiments using artificial infestation sufficient to differentiate resistant maize and rice plants from susceptible ones, sufficient quantities of good quality *S. asiatica* seeds are needed. They must therefore be collected at the proper time and place and stored in a way that preserves their germinability. The life cycle of *S. asiatica* is similar to *S. hermonthica*, but the plants are smaller and flowers are scarlet rather than purple (Fig. 1). Seeds are best harvested from an area near where the screening takes place. Capsules (fruits) mature as the flowers die and fall off. Seed is mature when the capsules turn brown. Bundles of stems should be collected in tight plastic mesh bags just after most



Fig. 1 *Striga asiatica* plant development: **a** vegetative stage, **b** flowering stage, **c** post-flowering stage with green capsules, **d** mature stage where capsules are dried and seed is fully mature

capsules have turned brown (Fig. 2). In Middle West Madagascar where upland rice and maize are grown, this period generally occurs in late June. The bags are ideally hung in an open shelter with a roof and plenty of air circulation so that they can quickly dry. They should be kept in these bags, protected from excessive humidity and temperature until they are fully dry. Like *S. hermonthica*, seeds of *S. asiatica* have a dormancy period such that they will not germinate in the season in which they were produced. Therefore, *Striga* seed should be collected at least one year previous to their intended use.

Once the harvested *Striga* stems are fully dry, they may be removed from the mesh bag and threshed over a pan by rolling them between the palms of your hands or by picking the capsules from the dried stems (Fig. 3). Any seed left in the bag after removing the stems can be added to the collection pan. The capsules can be gently crushed with a mortar and pestle to dislodge any remaining seeds. The material in the pan is then passed through a series of sieves to separate the seeds from the capsules and other debris. Sieve size should begin at 2 mm, followed by 300 μm then 250 μm and finally 180 μm until only *Striga* seed remains. The seed can then be transferred to a storage container such as the magenta boxes shown in Fig. 3 which are then put into a second container (large enough to fit the container of seed) into the bottom of which activated silica gel desiccant is placed to keep the seed dry during storage for up to several years at room temperature.

Conditioning and germination test for Striga asiatica *seeds.* Prior to all experiments, the *Striga* seed batch germination rate should be checked. As with *S. hermonthica*, *S. asiatica* seeds that are at least one year old (after-ripened) need to be surface sterilized under aseptic conditions and allowed to condition for two weeks before testing their germination in the laboratory. Under a laminar flow hood, line the bottoms of three sterile 100 mm petri dish each with two sterile 90 mm Whatman No. 1 filter paper circles. Transfer an aliquot of 50 mg dried *S. asiatica* seed to a 2.5 mL Eppendorf tube and add 1 mL of 70% ethanol. Cap the tube and shake for 1 min. Under a laminar flow hood, remove the alcohol and add 20% (v/v) of household bleach (5.25% NaOCl) in water. Keep the seeds in this solution for 15 min, shaking every 5 min. Rinse the seeds five times with sterile distilled water (ddH_2O). Distribute the surface sterilized *Striga* seeds across the surface of the filter papers in the three petri dishes. Add 5 mL of ddH_2O to each to soak the filter papers. Seal



Fig. 2 Mature *Striga asiatica* plants collected and stored in a tight plastic mesh bag

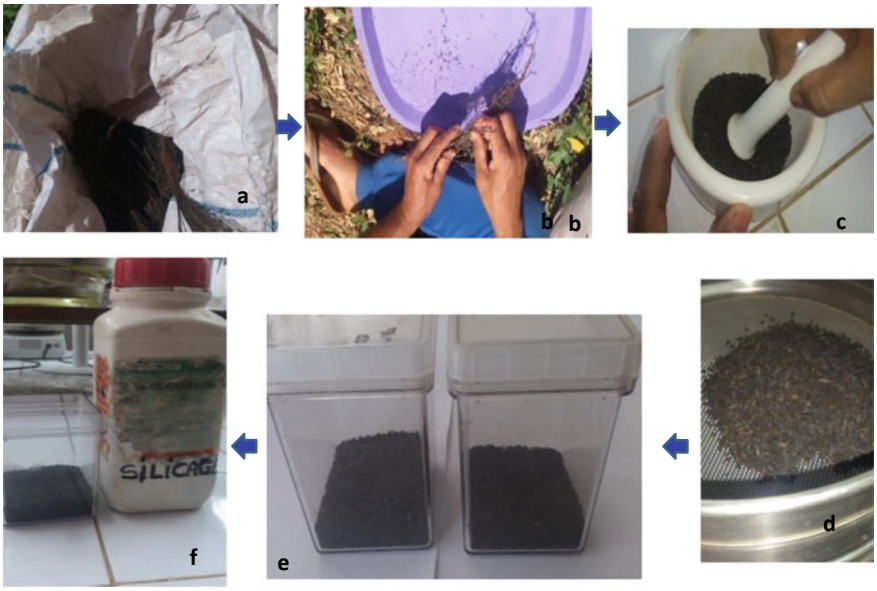


Fig. 3 Process of threshing and cleaning *Striga asiatica* seeds: **a** Dried *Striga* stems with capsules are removed from the tight plastic mesh bag in which they were collected, **b** seeds are gently threshed by hand over a shallow pan, **c** capsules are gently ground in a mortar and pestle to dislodge seed, **d** threshed material is sieved until only seed remains, **e** seeds are transferred to storage containers **f** the storage containers are placed in a desiccator over silica gel

the plates with parafilm and incubate at 30 °C for 14 days in a dark incubator to condition the *Striga* seed.

After the seeds have conditioned for two weeks, open the plates under a laminar flow hood and add 5 mL of 1 ppm GR24, a synthetic strigolactone that stimulates *Striga* germination. Seal the plates with parafilm. Incubate for 1–2 days at 30 °C in darkness. Count germinated *Striga* seeds under a dissecting microscope. Divide the number germinated by the total number of seeds in a viewing field in multiple fields across the three plates to determine germination rate of that batch of *S. asiatica* seed.

Preparing an inoculum for pot and field screening. If the *Striga* seed germination rate determined from the laboratory check of the batch is at least 50%, the batch is suitable for screening purposes in field or greenhouse. Before using it for such, mix it with an equal volume of fine dry sand and return to the desiccator until use (Fig. 4).

Plant material used and irradiation treatment. The upland rice varieties chosen for mutagenesis were B22, a line originally from Brazil but widely grown in Madagascar, and F154, a variety developed by FOFIFA, the Malagasy national agricultural research entity. The maize used were Plata and IRAT200, both yellow OPVs (Fig. 5). All chosen varieties are farmer-preferred (yield, growth habit, grain quality), but sensitive to *S. asiatica* and adapted to the Middle West *tanety* where *Striga* is particularly problematic.



Fig. 4 *Striga asiatica* seed inoculum, 50:50 mix of cleaned *Striga* seeds with fine dry sand

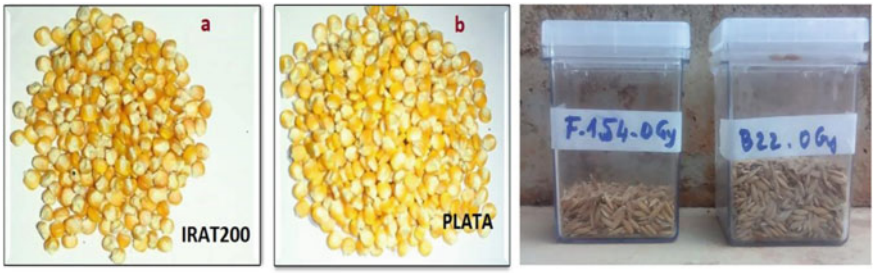


Fig. 5 Varieties mutagenized by gamma irradiation and derived populations screened in Madagascar. From left to right, yellow maize IRAT200 and PLATA and upland rice F154 and B22

In order to increase genetic variability within these rice and maize genotypes, 1000 seeds of each were gamma irradiated at various doses at the Plant Breeding and Genetics Laboratory of the Joint Food and Agricultural Organization of the United Nations International Atomic Energy Agency (FAO/IAEA) Division of Nuclear Techniques in Food and Agriculture at Seibersdorf, Austria using a ^{60}Cobalt source.

(van Harten 1998; FAO/IAEA 2018). Radiosensitivity tests of each variety was done prior to all experiments to determine the appropriate dosages (Chapter "Physical Mutagenesis in Cereal Crops"; van Harten 1998; Razafinirina 2011; Rakotoarisoa et al. 2017; FAO/IAEA 2018). Effective mutagenic but non-lethal doses chosen were 100, 200, 300 Gy for maize Plata and B22 rice varieties, and 100 and 200 Gy for maize IRAT200 and F154 rice varieties. Mutant populations derived from these mutagenized materials were screened and advanced in Madagascar first in the field and subsequently in greenhouse pots.

Field screening. Screening of early generations after mutagenesis (M2/M3) was done at the Station of Kianjasoa, Regional Research Center of the Middle West FOFIFA. The station is located 190 km from Antananarivo, the capital of Madagascar, in the district of Tsiroanomandidy of the Bongolava region. Coordinates are: 49°22′62.1″ East longitude, 19°03′20.3″ South latitude at 899 m above sea level. Both maize and upland rice production are constrained by *S. asiatica* in this region due to erratic rainfall and poor soils prone to erosion. Organic matter (1.7%), phosphorous (< 2.5 ppm) and nitrogen 0.25%) in the plots were all suboptimal. Screening was performed without nutrient inputs, typical of traditional farming in the region that favors *Striga* infestation (Andriamialiharisoa 2019; Rakotoarisoa et al. 2019). Soil was ploughed in advance by a tractor and hand weeded where necessary just before plots were marked and planting holes dug by hand hoe (Fig. 6).

The experiments were planted at the corresponding regular seasons for maize and rice in the region into 8 cm deep holes spaced 25 cm apart for rice and 70 cm for maize (see details below) into which 0.05 g of the sand/*Striga* inoculum (50:50, v/v,



Fig. 6 Field preparation, **a** ploughing the land, **b** hand-weeding, **c** setting plots, **d** digging planting holes

approximately 5000 weed seeds) was added to ensure uniform and heavy infestation. This was covered by 3 cm of soil before sowing the cereal. Each crop seed was covered by 3 cm of additional soil (Fig. 7). Five meter row plots were used for sowing M2/M3 rice seeds and 10 m for the maize seeds per variety, per initial irradiation dose, replicated in three plots for each rice entry and four plots per tested maize variety (Fig. 7). Plots were labeled with the name of the variety and the dose of irradiation. The same plot layout in the same area of the field was used in the subsequent (M3) generation screening with additional infestation to ensure high *Striga* pressure.

Harvest and maintenance of M2/M3 seeds. Assuming that the first generation screened in the field was at the M2 generation, certain individuals may be selected based on apparent improved performance (e.g., fewer emerged *Striga*, less host plant damage) over their unmutagenized counterparts (0 Gy). These selected individuals need to be advanced to the M3 for further testing. This requires self-pollination. For rice, no special techniques are needed since self-pollination occurs within each spikelet before anthers emerge. However, to ensure self-pollination, a small bag may be put over the panicle before anthers emerge. This bag should be left on until harvest. In maize, which is a naturally cross-pollinated species, self-pollination requires some effort. As the uppermost ear begins to emerge, usually when tassels have fully emerged and expanded, but before anthers emerge, cover the ear shoot with



Fig. 7 Field screening of maize and rice for *S. asiatica* resistance: **a** artificial infesting planting holes with *Striga* inoculum, **b** sowing of cereal seeds, **c** labeling of planted plots (photo taken at maturity in maize plots), **d** labeling of the holes sown with rice or maize seeds of M2 or M3 families in the same row

a coin envelope or shoot bag before silks emerge. Leave the shoot covered for several days until silks emerge (usually when the first anthers are visible on the tassel). When dew dries from leaves in the morning, use a larger envelope or pollinating bag to collect pollen (gently bend into the pollinating bag with opening facing up and shake tassel inside the bag to release pollen). Briefly uncover shoot on the same plant and immediately invert bag of collected pollen over the silks. Cover the ear with the pollinating bag and secure by folding bottom edges around the stalk and stapling. Leave bag until harvest.

When selected plants are mature, cut rice panicles or maize ears and dry in paper envelopes within mesh bags labeled with plot number, plant number, variety, original radiation dose. Thresh or shell when harvested panicles or ears are fully dry and store seeds in labeled envelopes in plastic bags in a refrigerator (4–8 °C) until use.

Details of the screening plot plantings. Sow up to 20 M3 rice seeds from each selected M2 plant into a single 5 m row following the field layout and inoculation method described above with each planting hole spaced 25 cm apart. Plant one seed per hole. Leave 25 cm between rows such that spacing is 25 × 25 cm between plants. It is convenient to organize the M3 family rows by variety and radiation dose. Do the same for maize, but use 10 m long rows, with holes spacing of 70 cm within row and 70 cm between rows planting 14 M3 seeds from each selected M2. Repeat the same plantings in three replicate plots for rice and four for maize of each M3 family that was advanced from the selected M2 plants. You should therefore have up to 60 M3 plants from each selected M2 mutant and 56 M3 plants from each maize mutant to evaluate. Plant the corresponding control (parent variety-0 Gy) in two rows in the middle of each replicate plot as a check for phenotype comparison. After sowing, label each family row with the variety and original radiation dose as well as the number of the M3 family (Fig. 7).

Aside from clearing weeds before sowing (Fig. 6), hand weeding should be practiced during the crop vegetative stage at 30, 45 then 60 days after sowing (DAS), taking care not to damage *Striga* plants in latter days. A hoe may be shallowly used at the 30-day weeding since *Striga* is unlikely to emerge by then (Fig. 8). During the crop reproductive phase, weeds on plots should no longer be removed. *Striga* emergence counts and host sensitivity parameters (see below) are taken at flowering for both rice and maize. Selection of potential *Striga* resistant/tolerant mutant lineages to advance to further trials is based on comparisons with the unmutagenized (0 Gy) original parent line within each plot. Those lineages with apparently fewer *Striga* and/or fewer symptoms of *Striga* infection (less leaf firing, larger ears and grain) than the unmutagenized check are tested in subsequent field or pot trials.

Screening advanced rice mutant lineages in pots. Once the number of selected *Striga* resistant mutants from field screening is reduced to a manageable number, further screening and verification of resistance/tolerance can proceed in greenhouse pots. This way, more detailed observations are possible including comparisons to the original unmutagenized line with limited impact of environmental influences encountered in field trials. We used a well-vented greenhouse during the normal cropping season to evaluate advanced mutant rice lineages (M4–M6) in both rice varieties (B22 and F154). Temperature remained in the range of 28–38 °C in the

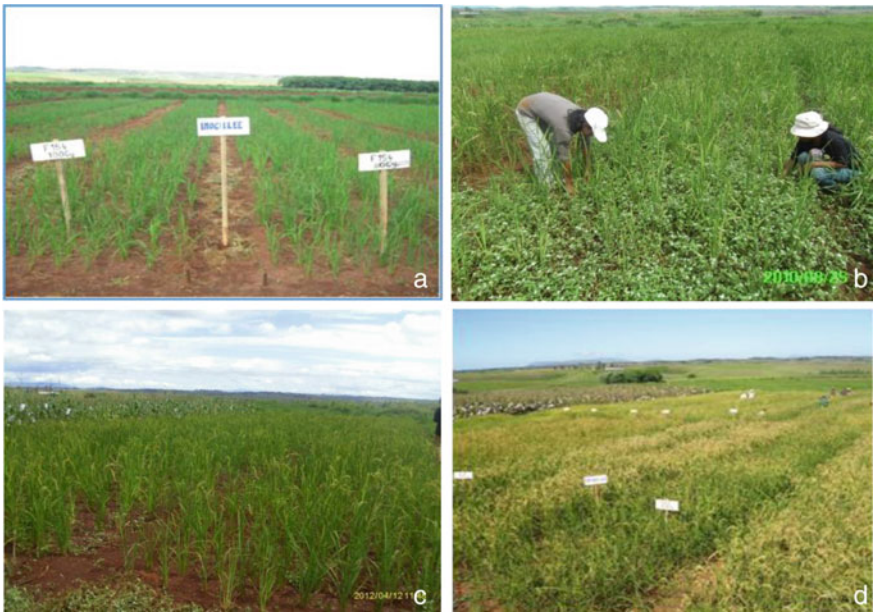


Fig. 8 Rice plots at **a** vegetative phase, **b** during hand weeding, **c** after weeding, **d** seed set

greenhouse during the pot trials. We did not find pot screening useful for maize as plants did not grow well in the greenhouse. Screening rice for *S. asiatica* resistance can be performed as follows.

In a 10 L plastic pot with drainage holes, put a thin sail (filter) in the bottom of each pot to prevent *Striga* seeds from coming out through the bottom holes (Fig. 9). Fill pots 2/3 to the top with local soil mixed with equal parts thin sand. Artificially infest pots by spreading 0.5 g of the *S. asiatica*/sand (50:50) inoculum described earlier in this chapter on the soil placed in the pot. This should put about 5000 germinable *Striga* seeds in each pot. Cover this *Striga* layer with enough of the same sand/soil mixture used in the bottom to raise the surface 4 cm. Add another *Striga*/sand layer as before and then add another 4 cm of the sand/soil mixture. Plant two rice seeds per pot and cover with an additional 3–4 cm of the sand/soil mixture and water until saturated. Continue watering to saturation two times a week for two weeks to ensure the rice seeds germinate and *Striga* seeds are conditioned. Thereafter, water once weekly until rice plants flower then stop watering to encourage *Striga* infection. Do not add fertilizer to pots, again to encourage *Striga* infection. This harsh selective pressure will help distinguish resistant from susceptible plants. Pull any weeds in pots besides *Striga* at 15, 30 and 45 days after sowing rice. Count the number of emerged *Striga* in each pot at these same intervals as well symptoms of infected rice plants (see below). Compare these measures on selected mutant lineages with those of infested and uninfested pots of unmutagenized counterparts. Arrange pots in a complete randomized design. Pots and soil may be reused in subsequent trials.

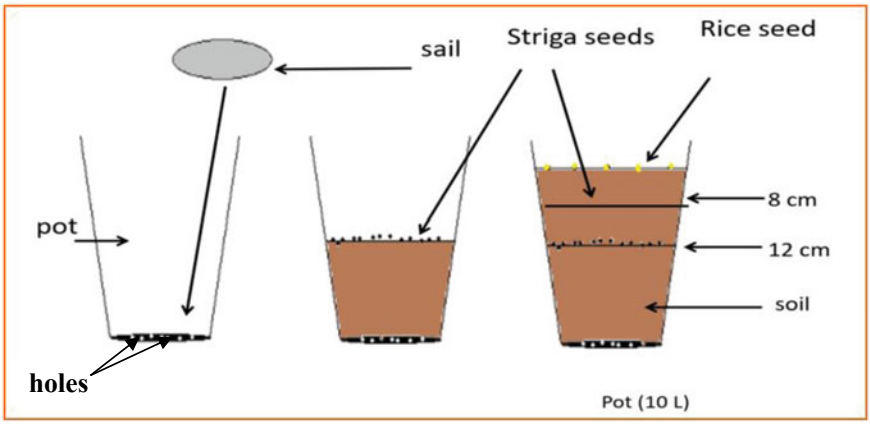


Fig. 9 Pot preparation for glasshouse screening of mutant rice lineages for resistance to *Striga asiatica*

General guidelines for screening maize and rice mutant lineages for S. asiatica *resistance and tolerance.* A general assumption in this mutagenesis breeding project is that gained *S. asiatica* resistance will be expressed by fewer emerged *Striga* around mutant maize and rice plants relative to their unmutagenized counterparts (0 Gy). Also, because there is less *Striga* infection around such plants, symptoms in resistant mutants will be less severe than those of *Striga* infected controls. All of the original rice and maize varieties chosen for mutagenesis are particularly sensitive to *Striga* infection which causes leaf chlorosis and firing (senescence), reduced shoot growth and fertility through reduced pollen production (smaller tassels in maize, smaller panicles in rice with fewer anthers) and reduced grain yield (smaller rice panicles and smaller or deformed maize ears). Since such symptoms, particularly reduced fecundity, can result from gamma irradiation itself, it is important to distinguish heritably reduced sensitivity to *Striga* from general reduced fitness due to mutation in genes controlling normal growth and reproduction. Mutants of the latter type are generally eliminated in early generation screenings. The goal of mutagenesis must be to improve *Striga* resistance and/or tolerance at no cost to overall plant productivity or adaptation to their target environment. Therefore, mutant lineages selected for advancement must show improved performance under *Striga* pressure relative to the original unmutagenized line from which they were derived with equal or better performance to the original line in uninfested conditions. It is therefore important to compare selected mutants with potential gained *Striga* resistance in both *Striga* infested and non-infested conditions at some point during the screening process in their target environment.

Because all the original rice (B22 and F154) and maize (IRAT200 and Plata) varieties are particularly sensitive and susceptible to *S. asiatica*, with multiple parasites eventually emerging around host plants with fairly severe symptoms (prematurely dying leaves and low yield) under *Striga* infestation, selection for *Striga* resistance/

tolerance began in early generations (M2 and M3). Verification was then done on advanced progeny in the selected lineages (M4 and M5). Under the severe selection pressure described earlier for field screening (*Striga* inoculum added to each planting hole and no fertilizer applied to plots), seeds were only harvested from plants of mutant lineages having fewer emerged *Striga* and less affected leaf area and more grain than unmutagenized counterparts for advancement to subsequent trials (field or pot) to verify their gained resistance and tolerance. In these verification trials, specific phenotypic characters were observed and recorded including the *S. asiatica* plants number emerging above the soil per plant, counted at host flowering, the survival rate per genotype (number of surviving plants divided by the total number of germinated seeds multiplied by 100), the maximum plant height, the number of rice tillers and fertile panicles or ear numbers produced per maize plant, the 100 (for maize) or 1000 (for rice) seed weights, the number of leaves showing premature senescence expressed as a percentage of total number of leaves per plant and for maize, the ear shape. Figures 10 and 11 show examples of observed phenotypes in maize trials and Fig. 12 shows rice examples.

From these detailed observations, advanced mutant lineages can be classified according to their performance under *Striga* infestation. We used modifications of those classes described by others (Kim 1991; Sinebo and Drennan 2001) without clear distinction between resistance and tolerance characters since the latter under



Fig. 10 Examples of maize performance under *S. asiatica* infestation. **a** Susceptible maize plant with most leaves affected, **b** a highly susceptible maize plant with premature shoot death, **c** a highly resistant maize



Fig. 11 Various maize ear types observed under *S. asiatica* infestation to classify resistance. **a** Ears from a moderately resistant mutant having small ears with empty rows and kernels arranged in disorder, **b** ear from a resistant mutant with < ¼ affected with slight blackening and curving at tip, **c** ear from a highly resistant mutant, large with full, well-arranged kernels. **d** Multiple ears from an advanced resistant mutant line, IRAT200Gy-L9-2-1-X22, harvested from uninfested plots in yield trials after selection

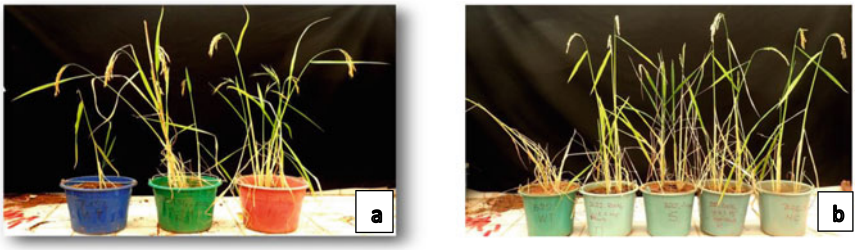


Fig. 12 Examples of rice performance in greenhouse pots. **a** original F154 rice line (left) in *Striga* infested pot, resistant F154 mutant (center) in *Striga* infested pot and original F154 rice line (right) in uninfested pot. **b** From left to right, original B22 rice line, a moderately resistant B22 mutant, a susceptible B22 mutant, a resistant B22 mutant in pots infested with *Striga asiatica.* The fifth pot contains the original B22 without *Striga.* All mutant lineages are at the M6

the high infestation conditions employed was largely a function of the former. Table 1 gives a summary of this classification system.

Resistance among the selected mutant lineages in this mutation breeding exercise may involve genes contributing to both tolerance and resistance to *S. asiatica.* Distinguishing alleles resulting specifically in tolerance would require detailed comparisons of the same mutants grown with and without *Striga* (see Chapter "Screening for Resistance to Striga Hermonthica in Mutagenized Sorghum and Upland Rice in Burkina Faso"). The end result, however, was that selected maize (Table 2) and rice (Table 3) mutants show improved yield under *Striga* infestations over their original varieties. Generally, these mutants appear to be resistant to *S. asiatica*, in that they supported fewer parasites than their unmutagenized counterparts. Several of the M5 selections are being propagated for evaluation in replicated field trials for possible new variety release in Madagascar.

Conclusion

The protocols described in this chapter were successfully used to select *Striga* resistant mutants from gamma irradiated maize and upland rice varieties adapted to the *tanety* of Middle West Madagascar where *S. asiatica* is a serious production constraint. Introducing *Striga* resistance through mutagenesis in cultivars adapted to these conditions can help to sustain maize and expand rice production in the country, especially if used in combination with conservation agricultural practices that reduce erosion and improve soil fertility in this fragile agro-ecology. Mutation breeding with selection based on the protocols described has resulted in 19 maize varieties in two farmer-preferred backgrounds that are superior to the original varieties in terms of resistance and/or tolerance to the local strain of *S. asiatica.* Similarly, 34 *Striga* resistant/tolerant upland rice varieties in two popular genetic backgrounds were identified and advanced through a combination of field and greenhouse screenings among the progeny of mutagenized seed. Further characterization in both laboratory assays (Chapters "An Agar-Based Method for Determining Mechanisms of Striga Resistance in Sorghum"–"Striga Germination Stimulant Analysis") and multi-location field trials of the selected *S. asiatica* resistant maize and rice varieties continues with much promise for improved cereal production in Madagascar. Similar efforts are described in Chapter "Screening for Resistance to Striga Hermonthica in Mutagenized Sorghum and Upland Rice in Burkina Faso" for sorghum and rice in Burkina Faso and in Chapter "Mutation Breeding for Resistance to Striga Hermonthica in Sorghum and Rice for Sustainable Food Production in Sudan" for sorghum and rice in Sudan.

Table 1 Adapted method for assessing the resistance of tested maize and rice genotypes under severe *Striga asiatica* infestation

Plant[a] reaction to *Striga*	Score	No. emerged *Striga* / plant	(%) Affected leaves / plant	(%) infected ears or panicles / genotype	Ear or panicle symptomatic description	Symptoms at harvest	Plant growth
HR	0	0	0–10	0–10	Healthy, normal-sized maize ear or rice panicle	Full maize ears or rice panicles	Normal plant height and growth rate
R	1	0–2	11–20	11–20	< ¼ of the maize ear or rice panicle is affected but generally with normal size	Little curvature with slight blackening and reduced kernels at the upper quarter of the maize cob, rice panicles mostly complete	Few visible leaf symptoms, near normal height and growth
MR	2	2–4	21–40	21–40	¼–½ of the maize ear is affected and generally small ear and tassel size, rice panicles may show some sterility	Curvature, blackening and reduced kernels at the upper half of the maize cob with some empty rows and disordered arrangement, rice panicles with slightly reduced grain numbers	Slight but noticeable stunting

(continued)

Table 1 (continued)

Plant[a] reaction to *Striga*	Score	No. emerged *Striga* / plant	(%) Affected leaves / plant	(%) infected ears or panicles / genotype	Ear or panicle symptomatic description	Symptoms at harvest	Plant growth
MS	3	4–6	41–60	41–60	Up to 3/4 of the maize ear affected with size reduction, noticable sterility of rice panicle	Curvature of the maize cob with blackening and incomplete or empty rows arranged in disorder, reduced ear and tassel size, rice panicle fertility rate slightly higher than sterility	Moderate stunting
S	4	6–8	61–80	61–80	Maize tassel small with reduced pollen, ears very small or number reduced. Rice panicle shows high sterility, fewer tillers	Maize ears very stunted and often slightly curved often with blackening and incomplete, disordered and/or empty rows, rice panicles reduced or nearly empty	Plants short and growth retarded
HS	5	> 8	> 80	> 80	Little or no pollen production	No useful maize ears or rice panicles formed	Stunted, or growth stops with premature death

[a] *h*—highly resistant, *R*—resistant, *S*—susceptible and *HS*—highly susceptible

Table 2 Total number of maize plants selected and advanced for further testing at each generation after mutagenesis

Variety-original radiation dose	No. selected plants at **M2**	No. selected plants at **M3**	No. selected plants at **M4**	No. selected plants at **M5**
Irat200–100 Gy	630	250	65	18
Irat200–200 Gy	670	204	70	22
Plata–100 Gy	640	260	68	40
Plata–200 Gy	587	245	85	42
Plata–300 Gy	610	238	115	45

Table 3 Total number of rice plants selected and advanced for further testing at each generation after mutagenesis

Variety-original radiation dose	No. selected plants at **M2**	No. selected plants at **M3**	No. selected plants at **M4**	No. selected plants at **M5**
F154–100 Gy	500	180	15	8
F154–200 Gy	500	171	8	5
B22–100 Gy	500	195	15	9
B22–200 Gy	500	188	14	8
B22–300 Gy	500	145	20	5

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