Protocol for X-ray mutagenesis of plant material: seed

Background

Induced mutagenesis in plants dates back to the beginning of the 20th century. Physical mutagenic treatments have included gamma, X-ray and neutron irradiation. In the 1950s there was a global spread of gamma irradiators for plant mutagenesis, especially to create desired mutants for plant breeding. Protocols for gamma irradiation were optimised and many mutant plant varieties have been released. The plant mutant variety data base (http://mgvs.iaea.org/) However, gamma sources (usually the radioactive isotopes: Cobalt-60 and Cesium-137) have become security risks and strict international regulations are imposed on: 1) the shipment of gamma sources, 2) the production of gamma sources and 3) the refurbishment of old gamma irradiators (Mastrangelo et al., 2010). These restrictions now limit gamma irradiation for plant mutagenesis. The Plant Breeding and Genetics Laboratory (PBGL) of the FAO/IAEA has therefore embarked on a series of investigations aimed at optimizing X-rays for plant mutagenesis. Our initial studies have focused on developing procedures and adapting an existing commercially available X-ray machine, the RS-2400, which has been used extensively in the FAO/IAEA Insect Pest Control Laboratory to produce sterile male insects for SIT (Parker and Mehta, 2007; Mastrangela *et al*., 2010; Figure 1)

In order to obtain even sample irradiation, X-ray machines require rotation of the sample in the X-ray beam. In the RS-2400 X-ray irradiator samples are placed in canisters which orbit the X-ray source, in addition the canisters also rotate longitudinal along their axis (Figure 1).

The RS-2400 X-ray irradiator (produced by RAD Source Technologies Inc., USA) is a self – contained low-energy irradiator, which operates at 150 kV and 45mA to give a dose rate (to water) in a centre of the rice filled canister 14.1±0.7Gy/min (rice is used as a irradiation dummy as its density is close to that of other seeds such as rice, barley, wheat). The Specific dose rate (SDR) at that location is 0.0376 Gy/kJ^{-1} or 2.26Gy min^{-1} kW^{-1}. Samples are placed inside canisters (5 canisters in the RS-2400) which are suspended by cradles that revolve in a vertical plane around the fixed horizontal X-ray tube (Figure 2). A specific dose is achieved by setting up a control panel with the required amount of kWs to produce the required radiation absorbed dose. The RS-2400 is currently used in the sterile insect technique in 2 countries (Brazil and Costa Rica) and under installation in 2 others (Pakistan and Burkina) Faso) and is easily adapted for plant mutation induction. Here we describe a protocol for seed irradiation.

Adaptation of the RS 2400 irradiator - sample canister

Each sample canister of the RS 2400 is 178 mm in diameter by 167 mm in length, which gives a volume about 3.5 litres (Figure 3). To achieve more uniform dose by hardening the photon spectrum, 0.5 mm steel has been placed in all canister (Parker and Mehta, 2007). RS-2400 offers a possibility of 5 irradiator canisters for a total volume of 17.5 litres (Figure 2b).

The volume of the sample canisters of the RS 2400 is too large for seed samples of many crop species, e.g. the small grain cereals (rice, wheat, maize, etc.) in which a 1 litre volume may contain 15 thousand seed. Seed irradiation for mutagenesis typically involves sample sizes ranging from 3,000 – 50,000 seed. Additionally, it is important in X-ray irradiation that samples are packed tightly to minimize air space and to maintain near uniform field of X-rays through the entire sample, therefore a range of sample container sizes is required. Various containers may be used, e.g. 0.4 to 0.7 litre and these can be set inside the standard sample canisters of the RS 2400 using plexglass brackets (Figure 4).

1. Dose optimization:

The radiation dose is the radiation absorbed by the samples after the completion of the treatment. The standard 3.5 litre sample canister of the RS-2400 has been calibrated for dose uniformity using instant cooking rice to fill the canister because the density of pupae and of instant rice is quite similar, 0.46 and 0.44 g cm^{-3} respectively (Mehta and Parker, 2012). Uniformity is achieved when all (5) canisters are filled with instant cooking rice during the treatment. For seed irradiation, the seed samples need to be packed tightly in an appropriate sample container which is placed into a standard RS 2400 canister and the remaining empty space filled with instant rice (Figures 5, 6a and 6b).

2. Determining the Relative Biological Effectiveness of X-rays

A prerequisite in developing an X-ray irradiation protocol for seed treatment is to determine sample radio-sensitivity and thereby optimum dose for mutagenesis. These studies are described in detail in Bado *et al.* (2012); an outline is given here. The effectiveness of X-ray irradiation was assessed through the Relative Biological Effectiveness (RBE) by measuring growth of M1 seedlings. The RBE for a given test irradiation was calculated as the gamma radiation dose required to produce the same biological effect as a standard x-ray radiation treatment. Seedling height or hypocotyl length as a percentage of control seedlings (from untreated seed, M_0) were plotted against the absorbed dose and growth reduction of 30% and 50% (GR30 and GR50, respectively) were estimated base on the linear regression analysis. Tests were carried out on a range of seed samples of barley, lupin, sorghum and wheat (Table 1).

Table 1: X-ray irradiation doses giving growth reductions of GR30 and GR50 in M1 seedlings of barley, lupin, sorghum and wheat. The RBE with respect to equivalent gamma ray treatments is also given.

Crop	Variety	Gamma ray dose in greys		X-ray dose in greys		1/RBE	
		GR30	GR50	GR30	GR50	GR30	GR50
Barley	Rum	249.8	400.3	187.2	347.5	0.75	0.87
	ASCAD 176	121.4	281.4	191.2	338.0	1.57	1.20
Wheat	Hourani	222.3	314.1	35.5	146.8	0.16	0.47
	ASCAD65	244.0	350.6	195.7	281.1	0.80	0.80
	Um Quis	249.1	352.7	88.36	187.82	0.35	0.53
Sorghum	Koden	246.8	406.4	226.3	349.5	0.92	0.86
Lupin	LG-15	401	826	499	991	1.24	1.20
	LG-46	586	1037	473	909	0.81	0.88
	LG-92	622	1129	628	1047	1.01	0.93
	LAE-1	451	897	423	873	0.94	0.97
	AU 11257-19/1	430	786	468	806	1.09	1.03

These studies indicate that lower X-ray doses are required compare to gamma doses to produce the same biological effect. This was also reported in sterile insect work (Mastrangelo et al., 2010).

3. Protocol

Seeds sample preparation

Step 1

Prior to irradiation the seeds are kept at least for 3 days, in a desiccator with 60% glycerol for moisture equilibration to 12-15% (Figure 7).

Step 2

Seed are packed into appropriately size sample containers to minimize air space (Figures 4). Different seed samples may be placed in paper bags before placing into the sample container to avoid sample contamination or mixing (Figure 8). For small samples, small containers or Petri dishes (size depend of the adaptor groove pre-defined) may be used and the samples are immobilized by packing with tissue paper (Figure 9)

Step 3

The packed sample container is fitted with brackets and fixed into position inside a standard RS 2400 canister. The void volume is filled with instant cooking rice. (Figures 10 and 11).

Step 4

Canisters are placed into the irradiator (Figure 12) and the required irradiation dose is given

Post treatment activities

Post treatment activities are the same as for other physical and chemical induced mutagenesis (including gamma ray) (Kodym and Afza 2003 and Mba et al., 2010).

Radiosensitivity checks

Grow up M1 to produce M2 seed

Screen for mutations, evaluation

Entry into breeding programmes

Examples

Country	Crop	Variety	Dose used (Gy)	Purpose (target trait)	Mutants detected	Current generation of population/varietal development
Jordan	Barley	Rum	200	Smooth awns	Plant heading time , maturity, grain yield and any change in softness of awns	M2
		ASCAD 176	150	Increased production under drought condition	Plant heading time, maturity and grain yield	M2
	Wheat	Hourani	200	Increased production	Plant heading time, maturity and grain yield	M2
		ASCAD65	250	Increased production and plant height	Plant heading time, maturity and grain yield	M2
		Um Quis	200	Improved smut disease tolerance	Plant heading time maturity, grain yield and tolerance to smut	M2
Eritrea	Sorghum	Koden	200 and 400	High yielding , striga resistance and drought tolerance	Diverse phenotypes mutants (seeds, seedling, panicle structure, early tillering)	M4
Poland	Lupin	LG-15	250, 500, 750, 1,000 and 1,500	Radiation test and induce genetic variability in the species and line	Changes in plant architecture, type of growth (from indeterminate to determinate)	M2
		LG-46	250, 500, 750, 1,000 and 1,500	Radiation test and induce genetic variability in the species and line	Changes in plant architecture, type of growth (from indeterminate to determinate)	M2
		LG-92	250, 500, 750, 1,000 and 1,500	Radiation test and induce genetic variability in the species and line	Changes in plant architecture, type of growth (from indeterminate to determinate)	M2
		LAE-1	250, 500, 750, 1,000 and 1,500	Radiation test and induce genetic variability in the species and line	Restricted-branching and any change in growth habit, early maturity, better yield potential, potential to regenerate androgenic plants in *in vitro* culture	M2
		AU 11257-19/1	250, 500, 750, 1,000 and 1,500	Radiation test and induce genetic variability in the species and line	Restricted-branching and any change in growth habit, early maturity, better yield potential, potential to regenerate androgenic plants in *in vitro* culture	M2
Senegal	Jatropha	Bacary Sarre	100, 200, 300, 400, 500	Radiation test, high yielding, good quality and oil content	Currently under screnning	M4
Jamaica	Ginger	Jamaica Native	8 and 10	Development of rhizome rot resistance	Chlorotic abnormalities (10 Gy)	M1V4
		China Blue	8	Development of rhizome rot resistance	Plantlets recently subcultured to M1V4	M1V4
		Jamaica Yellow	8	Development of rhizome rot resistance	Plantlets recently subcultured to M1V4	M1V4
Kenya	Artemisia	Artemisia spp	150	Good quality and quantity biochemical (artemisin)for malaria management	Currently under developpment	M2
	Barley	Barley	250	Increased yield and adaptation to harsh environments	Currently under development	M2

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Gamma vs X-ray comparison (http://radsource.com/applications/sterile_insect_technique_sit_sir).

Figure 1: X-ray irradiator RS-2400, sample canisters are loaded and unloaded from the top,

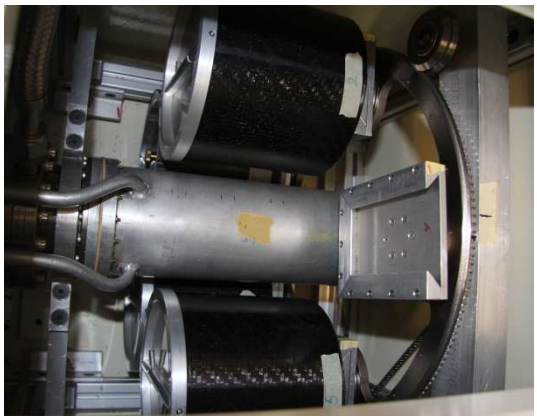

Figure 2a: X-ray tube (centre) with orbiting and rotating sample canisters. Figure 2b: Arrangement of sample canisters (5) around the X-ray tube

Figure 3: Sample canister and the cover, the interior of the canister is lined with a steel film to harden the X-ray beam.

Figure 4: Brackets of varying sizes to fit different sample containers (0.7 litres, 0.4 litres and Petri dishes), brackets are cut from 5 mm PMMA

Figure 5: Seed sample packed inside a contained and fixed in place using tissue. The container is fitted with brackets.

Figure 6a RS 2400 standard sample canister (left, 3.5 litre volume) and a sample container with adaptors (right, 0.4litre volume).

Figure 6b: Seed samples packed inside a sample container with adaptors to set inside the RS 2400 standard canister.

Figure 7: Desiccation treatment of seed to standardise moisture content to 60% glycerol

Photograph 8: Different seed samples in paper bag sealed in small container ready for different dose treatment.

Photograph 9: Small seed samples may be packed into Petri dishes using tissue paper.

Figure 10: The prepared sample container is placed inside a standard RS 2400 canister where it is fixed in position by adaptor brackets. The space between the sample container and the canister is filled with instant cooking rice.

Photograph 11: Canister of samples with spare volume filled with by instant rice and cover canister by side.

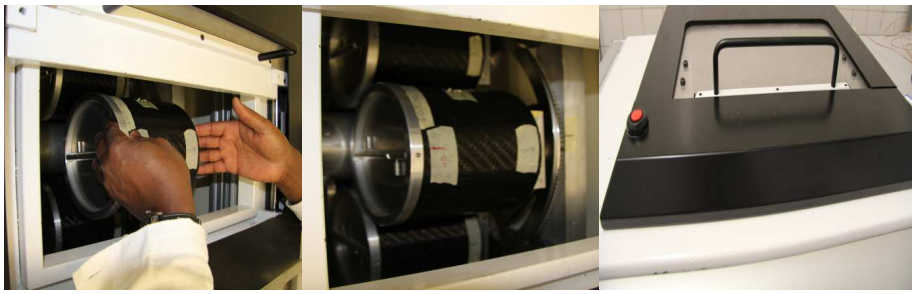

Figure 12: Placement of canister in Irradiator and closing the chamber.