

Appendix 1. Protocol for EMS Mutagenesis Application in Rice

Materials: 10.000 seeds approx., 25.000 ml pure water, 50 ml EMS, Container, Fumehood, Gasmask, protective clothes, class, fitotron or plant chamber.

1. Presoaking (12 hours): Rice seed should be a maximum germination capability of at least 90%. Make sure that the germination percentage of the seeds is sufficient before the application. Seeds are sterilized with a bleach solution (30% commercial bleach + 0.02% Triton X-100) for 15 min, washed 3 times with pure water. The seeds 10.000 seeds put in the flask or preferable corded tulle pouch. Seeds are placed in 10-liter pure water in a lidded container. Seeds are incubated for 12 hours in the water at 20-25 °C. Seeds are a taken-off from water and excessive water leach a few minutes. The water can be poured from the container to the sink.

2. EMS Application (6 hours): It should be very careful in terms of health in this stage because EMS is a cancerogenic chemical. When EMS is mixed with water, it releases carcinogenic vapors, which should not be inhaled. At this stage, protective clothing, glasses, gloves, and gas mask should be used. Mixing and incubation should be done under the fumehood condition. Firstly, 10-liter pure water is poured into a container and 50 ml EMS is gently added to the water. It might be better to use a pipet when preparing a chemical mixture instead of a direct pour of EMS. Then slightly mix the chemical, preferably it might be used mechanical shaker low speed constantly. The solution of 0.5% EMS is ready for mutagenesis application. Seeds are placed in 10 L container including 0.5% EMS solution. It can be used a corded tulle pouch (seed bag) handled with rope to avoid direct contact with the chemical. The solution container lid should be cover. The seeds are incubated 6 hours in the EMS solution at 20-25 °C. Make sure the fumehood works and it takes out the polluted air. Place a warning letter stating that it is working with the hazardous chemical in the laboratory.

3. Final Washing (6 Hours): The washing process is done to remove the chemical mutagen from the seed surface. Seeds, corded tulle pouch, were taken off by hold the help of corded and it is washed with pure water 5 times, 5 minutes. Afterward, the seeds are post washed with running tap water for 6 hours. Used EMS solution and 5 times washed wastewater is collected a storage container to send chemical waste department.

4. Drying: The seeds are placed on blotter paper then lightly pressed and dried by allowing them to leak. This process can be repeated several times. The mutated seeds are ready for planting and can be sown directly. If it is planted later, it should be dried up to 12-14% moisture in order not to lose its germination ability. Preferably, it can be dried by incubating in a drying oven at 38 ° C for 72 hours. Seeds left at room temperature after drying can maintain their germination ability for 4-5 weeks.

Note. All procedure at the room temperature (20-25 °C).