EMS MUTAGENESIS OF ARABIDOPSIS

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* While there is a commerical supplier (Lehle Seeds, 6531 N. Camino Katrina, Tucson, AZ 85718 U.S.A. Tel: +1-602-544 0733; FAX: +1-602-624 7476) of mutagenised Arabidopsis seeds there are considerable advantages to making them yourself. The preferred mutagen is Ethyl Methanesulphonate (EMS) because it is a powerful mutagen, but causes little lethality. EMS is a volatile, colourless liquid and all manipulations involving it should be carried out in an 'Atmos' bag in a fume hood (Atmos bags and EMS can be obtained from Sigma. EMS is listed as Methanesulfonic acid Ethyl Ester). The technique is straight-forward and is outlined below. The mutagenesis procedure is adapted from chapter 6 of Bibliographica Genetica XXI 1969 by George Redei. This chapter includes detailed information on the use of EMS and other mutagenic treatments in Arabidopsis.

1. MUTAGENESIS
   (i) Pre-imbibe a known number of seeds o/n in a 0.1% solution of potassium chloride. (N.B. 250 Landsberg erecta seeds weigh approximately 5 mg.)
   (ii) Soak the seeds for 3-5 hours in a solution of 0.1M sodium phosphate, pH 5, 5% Dimethyl Sulphoxide, and 50-100mM EMS. We have found that concentrations of EMS in this range give a high frequency of mutation in the M1; the use of 200mM EMS results in little lethality, but M1 plants are sterile. Typically we mutagenise 5,000 seeds in 20 ml of the above solution.
   * Steps (ii)-(iv) must be carried out in an 'Atmos' bag in a fume hood which can make manipulating the seed cumbersome. We use adapted 10 ml syringes to hold the seeds throughout the entire procedure. These are made by cutting off the front end of the syringe and attaching a circle of fine nylon mesh in its place. The seeds are placed in the syringe and the plunger can then be used to draw the appropriate solutions through the nylon mesh onto the seeds.
   (iii) Wash the seeds twice in 100mM sodium thiosulphate for 15 min. Sodium thiosulphate destroys the EMS. All waste solutions must be discarded into a beaker containing solid sodium thiosulphate.
   (iv) Wash the seeds twice in distilled water for 15 min.
   (v) Open the 'Atmos' bag, pour the sodium thiosulphate inactivated waste into the sink and wash down with plenty of water. Leave all beakers, etc, used in the procedure under running water for half an hour.
   (vi) Transfer the seeds to 3MM (Whatman) paper and allow them to dry out o/n.
   (vii) Dilute seed into dry sand to make their distribution easier. Plant at a density of 1cm-2.
* This generation of plants is called the M1 generation. An indication that the mutagenesis has been successful is the appearance of chlorophyll deficient sectors at low frequency among the M1 plants (1 in 100-1000 plants).

* Under our conditions these plants mature in 6-8 weeks where upon the M2 generation can be harvested. Recessive and dominant mutations can be recovered from this generation. In a successful mutagenesis 0.5-3% of M1 plants segregate chlorophyll deficient plants in their M2 progeny.

2. SEED HARVESTING
* There are several strategies for harvesting the M2 seed. The most appropriate method will depend on the nature of the mutant screen you want to carry out. The two extremes of the spectrum are:
  (i) to keep the seed harvested from each M1 plant separate and generate a collection of M2 families.
  (ii) to pool all the seed harvested from the M1 plants to give a bulked M2 population. (Lehle seeds supplies a bulked M2.).

* Although harvesting and screening each M2 family separately is labour intensive there are two main advantages to this approach. Firstly, it offers the possibility for recovering mutations which are infertile when homozygous via the heterozygous siblings of the mutant plants. Secondly, this strategy guarantees the independence of all mutants isolated. This ensures that mutations isolated from the M2 collection that are subsequently found to be allelic are known to be different alleles at the same locus rather than 2 isolates of the same mutational event.

* If the mutants you are looking for are unlikely to be infertile or if the screen you are carrying out would be much more difficult to do with individual families then these advantages may be outweighed and a compromise strategy of screening groups of M2 families together might be preferable.

* It is impossible to predict in advance the frequency at which a new mutant class will be observed in the M2. Similarly, it is impossible to assess how large an M2 population should be in order to be sure of finding one or more mutants. In practice such screens are usually limited by the effort involved. If a mutant class is rare, increasing the size of M1 may increase the probability of recovering it more than simply screening a greater number of M2 plants from the same mutagenesis. For example, if a particular trait cannot be found in a M2 population twenty times the size of M1 it probably cannot be recovered from the