POPULATION SIZE IN MUTATION BREEDING, SELECTION OF PARENTS AND HANDLING OF M_1-M_3 GENERATIONS FOR SELECTION OF MUTANTS

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Population Size in Mutation Induction Studies

(After Brock 1977 & Hermelin - 1984)

Mutation is an event

Mutant is an individual

Before starting a mutation breeding programme, some assessment should be made of the plant populations that will have to be examined to obtain the desired mutation.

Mutation frequency determines the number of cell progenies that have to be examined to detect a particular mutant.

For single gene mutations, if we assume a mutation frequency or rate as μ and set a level of probability of occurrence of the mutation as P_1, then the number of treated cells that have to be examined, n, can be calculated from the formula

$$n = \frac{\log(1-p_1)}{\log(1-\mu)}$$

μ = Mutation rate or frequency

p = Probability to have at least one mutation.

Brock estimated the following mutation frequencies from various studies for different types of mutations (* & **)

Mutation rate* (u)	No.of cell progenies(n) p = 0.90	p = 0.99	Type of mutation**
$1 \times 10^{-2} = 0.01$	233	465	Chromosome changes and quantitatively inherited variation.
$1 \times 10^{-3} = 0.001$	2326	4652	Several recessive genes
$1 \times 10^{-4} = 0.0001$	26260	46520	Single recessive gene.
$1 \times 10^{-5} = 0.00001$	232600	465200	Single dominant gene.

The table provides an estimation of the required no. of cell progenies needed to obtain at least one mutation of the desired type.

Mutations are detected in the progeny of the treated cells and recessive mutations are not expressed until at least the second generation (M_2) in diploid organisms.

Hence the values of n given in the table represent the number of M_2 families that have to be examined.

It is important to know the number of initial cells taking part in the formation of the shoot meristem i.e. Genetically Effective Cell Number (G.E.C.N.). Species vary in this number; the number being 2 to 10.

Assuming GECN to be 5 in a species and mutagenic treatment to have 50% lethal effect (i.e. 50% plant survival at LD 50 dose), number of M_1 plants required to provide these M_2 families will be

$$= \frac{n}{5} \times 2$$

Caution

From a practical point of view, it is rarely possible to predict mutation frequencies accurately and these calculations are only useful guidelines. However, these figures do emphasize that considerably large populations are required even in the simplest cases. Examples in legume mutation breeding experiments show that such a large population may not be necessary to isolate desirable mutants. An M_1 population of 5000-10,000 and on M_2 population of around 15000 to 20,000 should be sufficient to yield the desired mutants. A large population will only be required if a recessive to dominant mutation is sought.

Selection of parents for mutation breeding

(Manual 1977)

Any mutation breeding program must have clearly defined and specific objectives. These may be:

(a) To improve one/more specific characters of a variety/line
(b) To induce a morphological marker (colour, awn, bract, leaf etc.) to establish an identity in a promising line.
(c) To induce male sterility or fertility restoration, making a line useful for hybrid variety production.
(d) To obtain simply inherited mutations within an adapted genotype for using in hybridization programs.

On the basis of these objectives, the parent variety should be either of the following:

1. A variety/cultivar in use but have one or more deficiencies, or
2. An advanced promising line about to be released, or,
3. An advanced promising line or introduced variety restricted from release by specific limitations, such as susceptibility to disease, lodging, pest, shattering or presence of a toxin, late or early maturity, tall or short height.

Preparation of seed:

The seed should be mature, clean, free from mixture, etc. It may be necessary to grow a pure line selection from the variety and subsequent multiplication of it for providing samples for irradiation.

Doses:

There should be two doses (minimum) and a control. The doses can be selected on the basis of the results of preliminary studies.

Handling of M_1-M_3 generations (Manual 1977)

Sowing of M_1 seed:

- Mutagen treatment renders M_1 seed meristem to be weak
- Physical and moisture condition of the M_1 field should be optimum for seedling growth & development of a plant.
- Nitrogen fertility should be normal to slightly sub-normal to limit excess tillaring/branching.

- Other nutrients should be at optimum levels.
- Spacing between rows and between seeds should be closer than normal to limit excess growth. Spacing should be such as to restrict development of not more than 3-4 tillers in cereals and 3-4 branches in legumes.
- Slightly early/late sowing may also suppress vigorous growth.

Harvest of M_1 plant:

The choice of harvest procedure depends on (i) the desired mutant phenotype and its expression, and (ii) the availability of resources (fund, labour, etc.).

1. Harvest of one seed/plant.

   Separate single seed bulks can be made if independent screening for different traits is required.

2. Harvest of one fruit/capsule/pod/branch per plant.

   M_2 popn. will be more in this method compared to No.1 above but the advantage is that more mutant individuals can be identified both within and between progenies.

3. Harvest of several fruits/capsules/pods per plant.

   Supposition: Each harvested fruit or branch etc. originates from a different cell (at the time of mutagen treatment)

   - M_1 popn. can be smaller compared to above two methods.
   - Useful for large plants, perennials etc. (example Winged Bean)
   - Equal No. of M_2 seeds/M_1 plant should be sown in the

in the M_2 generation.

- Spare seeds can be kept in store.

4. Bulk harvest of several sectors per M_1 plant.
   - The whole M_1 plant is harvested and a fixed number of seeds per plant is progeny=tested.
   - Not in use very much these days.

Growing M_2 population

1 - M_1 population bulk:

Bulk sowing of M_2 seeds collected as per single seed descent (SSd) method.

2 - Fruit/capsule/pod/branch bulk:
   - All seeds from each fruit collected from each M_1 plant may be bulked together and grown as bulk in M_2.
   - Seeds from each fruit/capsule/pod/branch may be sown separately.

3 - M_1 plant to row :
   - All or part of the seed from each M_1 plant sown in M_2 progeny rows.
   - Sample seed from each M_1 plant, put in one bag and a bulk M_2 population is grown.

Growing of M_3 population

Similar methods as in M_2 generation may be followed depending upon the type of mutation sought and resources (fund, labour) available.