

EMS mutagenesis of Bd21-3

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adapted from Caldwell et. al. A structured mutant population for forward and reverse genetics in Barley (*Hordeum vulgare* L.), *The Plant Journal* (2004) 40, 143–150

Calculations for 7,200 M₁ population.

7,200 M₁ plants is the target

want 20 live plants/6 inch pot

assuming 80% germ will need to plant 25 seeds/6 inch pot so will need 8,600 seeds total

this equals 43.2g of seed

to allow for losses in washing use 45g seed split into two flasks (22.5g / flask)

(Note, do NOT dehusk the seeds. We tried this and it decreased germination rates substantially.)

treat with 0.6% = 50mM EMS (I selected this concentration based on a dose response curve. Different sources of EMS may perform differently, so I recommend you test a few concentrations before making a large population.)

Include flask of 0 EMS control

Use 400 ml EMS soln= 200 ml/flask + 1.2 ml EMS

1) 9:30 am start soaking seeds in water. Put 22.5g in each of 2 2.8 L flasks (the kind you grow bacteria in) add 300 ml water to each flask and soak for 4 hours. Change water every hour.

2) 1:30 pm drain and add 200 ml water to each flask then add 1.2 ml EMS to each flask and mix thoroughly. soak in 0.6% EMS for ~17 hr w gentle shaking. Note keep flasks in a secondary container in case of breakage. We place the flasks in an autoclave tub securely taped to a rotary shaker. **KEEP IN FUME HOOD**

3) 6:30 am After 17 hrs wash 2X with 300ml per flask of 100mM sodium thiosulphate for 10 min each **IN FUME HOOD**

4) wash 8x with 300ml water. Shake for 10 min each wash. **KEEP IN FUME HOOD**

5) Put seeds on damp paper towels (5 layers in large petri plates add water until paper towels are saturated and pour off excess) and place in the cold for about 14 days. If they germinate earlier then plant earlier. Plant about 25 seeds in an 6 inch pot. Grow in a growth chamber with 20hr day and 4 hr dark light cycle.

NOTE If you want to grow them in a greenhouse you will need more vernalization to make them flower. After treatment with EMS the seeds germinate slower than untreated seeds and seem to need longer vernalization to induce flowering under shorter day conditions. We put them in the greenhouse at the height of summer after 18-21 days in the cold and they took a long time to set seed and got very large. Untreated seeds planted

at the same time flowered and set seed quickly. We also tried longer vernalization of about 6 weeks before moving to the greenhouse and had very poor germination. This is why we now grow them in a growth chamber after EMS treatment.

6) count number of seeds that germinate (our germination was 80-90%)

7) count number of plants that set seed

8) score albino phenotype in M_1 and M_2 (our M_1 s had about 15% sectors and about 2% of our M_2 s were albino)

Note, I have not tried this protocol with Bd21. People have mentioned to me that Bd21 had poor germination after EMS treatment, but I do not know the specifics of how they did their EMS treatment.

Safety concerns

EMS is a powerful mutagen (that is why we use it for this in the first place)! It is probably the most dangerous thing you will handle in this lab. Wear double gloves, lab coat, and safety glasses, and always handle it behind the fume hood. Solutions are decontaminated either by disposal onto solid sodium thiosulfate or 2.5 X vol of 0.5 M NaOH. Let them stand overnight, then dispose down the drain in the hood with 30 min of rinsing afterwards.