

## Mutation induction and reverse-genetics for functional genomics and breeding

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### Abstract

Induced mutations have remained a powerful means of rapidly generating novel genetic diversity since their first application in the 1920s. Forward-genetic approaches using induced mutations have been applied for mutation breeding and functional genomics. This approach is advantageous because prior knowledge about genes is not required. There are many examples of success. Recently, increased resistance in wheat to black stem rust race *Ug99* has been developed through an IAEA funded multi-country project. Wheat seed were irradiated with varying dosages of gamma irradiation and subjected to phenotypic evaluation for disease resistance. The first two varieties are undergoing seed multiplication for farmer release in Kenya. The mutated gene(s) responsible for the improved disease resistance, however, remain unknown. The task to identify causative mutations in a large polyploid genome remains difficult. We are working to develop exome capture strategies for Illumina based sequencing to identify induced mutations in plant genomes. Pilot work has been conducted with sorghum and cassava. For each species, capture probes covering approximately 20 Mbps of exonic sequences have been designed. Gamma irradiated and EMS mutagenized populations have been developed to optimize mutation discovery using this approach. Such tools will serve several purposes. First, rapid evaluation of induced mutations in early generations will provide a snapshot of the density and spectrum of induced mutations. This will allow a decision to be made regarding the quality of the mutant population and enable calculations to determine the size of a population that needs to be screened to have a reasonable probability to recover desired alleles. Secondly, the ability to sequence all coding sequence of

a genome should facilitate the cloning of mutant alleles. Finally, exome sequencing strategies can be used for reverse-genetic approaches.

Since the first description of reverse-genetics using induced mutations (commonly referred to as Targeting Induced Local Lesions in Genomes, or TILLING) in the late 1990s, projects have been developed for more than 25 species. Reverse-genetics is an advantageous approach because it starts with the identification of mutations in genes hypothesized to have a specific function. Hypotheses can be based on homologies to genes in other species with a known role in a particular trait. Since only a handful of plants in a population will carry potentially interesting mutations, the number of plants that need to be characterized phenotypically is dramatically reduced. Our recent work has focused on optimizing mutation induction and dissolution of chimeric sectors using tissue culture approaches.

Our results show that the density and spectrum of induced mutations is similar to that of seed mutagenesis. We have also been developing and adapting low-cost methods for molecular characterization of plants. This includes collection and storage of leaf material at room temperature, extraction of DNA without toxic chemicals, and mutation discovery using self-extracted enzymes. The methods are broadly applicable. For example, we have recently adapted them to validate the production of doubled haploid plants in barley. These efforts to enhance the efficiency of mutation breeding are aimed at assisting developing countries in their progress towards sustainable food security.

### Keywords

Barley, sequencing, TILLING, *Ug99*, wheat

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