Chapter 1
Introduction

Abstract A range of molecular methods can be employed for the characterization of natural and induced nucleotide variation in plants. These facilitate a better understanding of gene function and allow a reduction in the time needed to breed new mutant varieties. Molecular biology, however, can be difficult to master, and while efficient, many protocols rely on expensive pre-made kits. The FAO/IAEA Plant Breeding and Genetics Laboratory (PBGL) has developed a series of low-cost and easy to use approaches for the molecular characterization of mutant plant materials. The protocols are designed specifically to avoid complicated procedures, expensive equipment, and the use of hazardous chemicals. Furthermore, these protocols have been validated by research fellows from many developing countries.

1.1 Background

The extraction of high quality and quantity genomic DNA from tissues is at the heart of many molecular assays. Indeed, with the routine use of molecular markers and more recently the application of next generation sequencing approaches to characterize plant variation, the recovery of DNA can be considered a fundamental objective of the plant scientist, and is often a bottleneck in genotyping. The basic steps of DNA extraction are: (1) proper collection and storage of plant tissues, (2) lysis of plant cells, (3) solubilization of lipids and proteins with detergents, (4) separation of DNA from other molecules, (5) purification of the separated DNA, and (6) suspension in an appropriate buffer. Isolation of DNA dates to the late 1800s with the work of Friedrich Miescher and colleagues who first discovered the presence of DNA in cells long before it was established that DNA was the genetic material (Dahm 2005).

B.J. Till et al., *Low-Cost Methods for Molecular Characterization of Mutant Plants*,
DOI 10.1007/978-3-319-16259-1_1

1.2 Methods Used to Isolate Genomic DNA from Plant Tissues

The advent of recombinant DNA technologies and DNA sequencing technologies in the 1970s marked the beginning of a rapid expansion of molecular biology analyses in plants that continues to this day. In parallel, DNA isolation procedures tailored to the unique aspects of plant cells have evolved. A range of DNA extraction methods have been described; however, some are more commonly used by plant biologists. One of the most enduring methods for plant DNA extraction employs a lysis buffer the main component of which is cetyltrimethylammonium bromide (CTAB), which solubilizes membranes and complexes with the DNA. The so-called CTAB method, first described in 1980, employs an organic phase separation using a chloroform-isoamyl alcohol extraction, and alcohol precipitation to isolate DNA from proteins and other materials (Murray and Thompson 1980). The method remains popular in part due to the fact that all components can be self-prepared, and thus the per-sample cost remains low. Wide and prolonged usage of the method also validates the approach for many different molecular assays. However, manual phase separation means that human error can introduce unwanted cross-contamination of organic compounds that may result in an inhibition of downstream enzymatic assays. Further, chloroform is a toxic organic compound and proper ventilation and waste disposal measures are needed.

An alternative to the CTAB method is the use of high concentrations of potassium acetate and the detergent sodium dodecyl sulfate (SDS) (Dellaporta et al. 1983). Proteins and polysaccharides are precipitated and removed from the soluble DNA. This approach is advantageous to the CTAB method in that organic phase separation is avoided. An additional filtration step may be required to remove cell wall debris and other insoluble materials from soluble DNA, limiting the throughput of the method.

In recent decades, commercial kits for the rapid extraction of DNA from plant tissues have been routinely used by many laboratories. Commercial kits have proven to be very reliable in producing high yields of highly purified DNA and so have become the standard when performing sensitive molecular assays. Many such kits utilize the binding of DNA to silica in the presence of chaotropic salts. In the presence of high concentrations of chaotropic salt, the interaction of water with the DNA backbone is disrupted and charged phosphate on the DNA can form a cationic bridge with silica, while other components remain in solution. Silica is either used in a solid phase as with spin columns, or in a slurry form for batch chromatography. Washing the DNA-bound silica in the presence of a high percentage of alcohol removes excess salt. The subsequent addition of an aqueous solvent (water or buffer) drives the hydration of the DNA and its subsequent release from silica. The now soluble DNA can be separated from silica through a quick centrifugation step. The method is rapid, taking less than 1 h, and is scalable such that a 96-well plate format is commonly employed to increase sample throughput. While highly advantageous over other methods, such kits remain expensive when

compared to home-made ones such as the CTAB and Dellaporta protocols. Therefore, the methods described here were developed to provide the ease and quality of silica-based DNA extraction at a fraction of the cost while using basic laboratory equipment.

1.3 Methods for the Discovery and Characterization of Induced and Natural Nucleotide Variation in Plant Genomes

Nucleotide variation is the major source of the phenotypic diversity that is exploited by plant breeders. Variation can be either natural or induced. In the late 1990s, a reverse-genetic strategy was developed whereby induced mutations were used in combination with novel methods for the discovery of nucleotide variation (McCallum et al. 2000). Known as Targeting Induced Local Lesions IN Genomes (TILLING), this approach allows for the recovery of multiple new alleles in any gene in the genome, provided the correct balance of population size and mutation density can be achieved (Colbert et al. 2001; Till et al. 2003). Efficient techniques for the discovery of Single Nucleotide Polymorphisms (SNP) and small insertion/deletions (indels) were developed utilizing single-strand-specific nucleases that can be easily prepared through extractions of plants such as celery, or mung beans (Till et al. 2004). TILLING has been applied to over 20 plant and animal species, and similar approaches have been used to characterize naturally occurring nucleotide variation, known as Ecotilling (Comai et al. 2004; Jankowicz-Cieslak et al. 2011). While TILLING and Ecotilling have been primarily used in seed crops, the methods work well in vegetatively (clonally) propagated and polyploid species such as banana and cassava (Jankowicz-Cieslak et al. 2012; Till et al. 2010). The PBGL has developed low-cost methods for the extraction of enzymes from a variety of plant materials, including easily obtainable weedy plants. The laboratory has also adapted low-cost agarose gel-based TILLING and Ecotilling assays.

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