AnisaNaeem
ICDD MSc Scholar
Date Palm Diversity
Supervisors: Dr. Asif Ali Khan & Dr. Andreas Buerkert

Use Of Ecotilling for SNP Discovery and Barcoding in Date Palm

Objectives

The objectives of this project are
i) To assess Eco-tilling as a reliable and cost-effective method to detect SNP in Date Palm
ii) To test for its validity in diploid Species like Date Palm

Variation in nucleotide sequence

The ability of individuals within a species to adapt to different environments resides in their genetic diversity.

This diversity, can most commonly manifested as single nucleotide polymorphisms (SNPs).

Variation in nucleotide sequence

This variation in Nucleotide sequence is a major determinant of heritable phenotypic difference in plant genomes.

A number of different techniques for identifying SNPs have been developed.

TILLING

The critical approach and method we are using is based on TILLING (Targeting Induced Local Lesions IN Genomes).

TILLING is a technique for detecting DNA polymorphisms (induced mutations)using a mismatch-specific endonuclease .

ECOTILLING

The use of the TILLING technique to survey natural variation in genes is called ECOTILLING.

It looks for natural mutations rather than induced mutations.

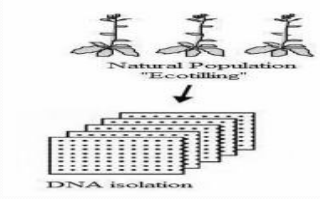
ECOTILLING

TILLING and ECOTILLING are closely related methods that are useful in the rapid detection of induced mutations by EMS & natural polymorphisms, respectively.

Steps in Ecotilling

•Collection& extraction of DNA from natural population.

•Pooling of samples.



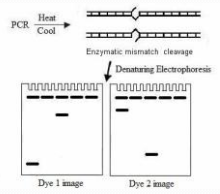
Steps in Ecotilling

- The target region is amplified by PCR with gene-specific primers that are end-labeled with fluorescent dyes.
- Following PCR, samples are denatured and annealed to form heteroduplexes that become the substrate for enzymatic mismatch cleavage.

Steps in Ecotilling

A "bubble" forms at the mismatch of the two DNA strands , which is then cleaved by single stranded nucleases(e.g, cell 1 enzyme).

Cleaved bands representing mutations or polymorphisms are visualized using gel electrophoresis.



USES Of Ecotilling

- ECOTILLING allows the rapid detection of variation in many individuals and is cost effective
- The technology is applicable to any organism including those that are heterozygous and polyploid.
- Screen many individuals at one locus
- Discover rare haplotypes or SNPs.
- Good in heterozygous populations

Barcoding

- **Barcoding** is a taxonomic method that uses a **DNA** short genetic marker in an organism's DNA to identify it as belonging to a particular species.
- Although barcodes are sometimes used in an effort to identify unknown species or assess whether species should be combined or separated

Barcoding

- A desirable locus for DNA barcoding should be standardized
- For land plants, matK chloroplast gene has been identified to distinguish between the majority of plant species on Earth.

Barcoding

- DNA sequences of the gene 'matK' differ among plant species, but are nearly identical in plants of the same species.
- So,matK gene can provide an easy way of distinguishing between different plants, even closely related species that may look the same to the human eye.

Barcoding

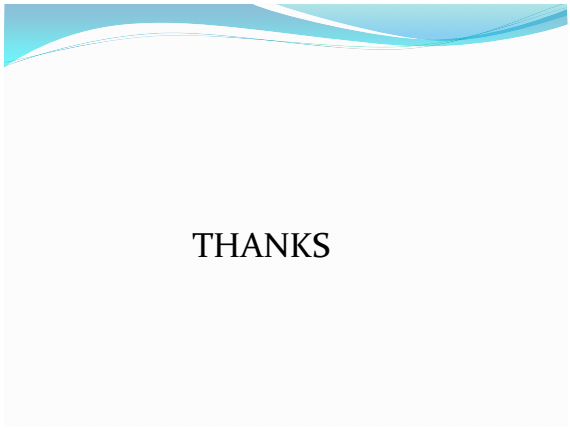
- Scientists found that when one plant species was closely related to another, differences were usually detected in the matK DNA

Mathodology

- Isolation of DNA.
- The amplification of DNA regions using standard polymerase chain reaction (PCR).
- Primers are designed against standard genes.
- The PCR products are sequenced.

Barcoding

DNA barcoding is a method of species identification and recognition using DNA sequence data.



THANKS