MEMORIA DE LAS ACTIVIDADES DESARROLLADAS

PROYECTO DE INNOVACIÓN EDUCATIVA PARA GRUPOS DOCENTES
CURSO 2014/2015

DATOS IDENTIFICATIVOS

Título del proyecto

APLICACIÓN DE LA METODOLOGÍA CLIL EN LA ENSEÑANZA DE LA MEJORA VEGETAL Y FORESTAL

Código del proyecto

2014-12-5007

Coordinador del proyecto

Nombre y apellidos	**Grupo docente**	**Departamento**
Teresa Millán Valenzuela	128	Genética

Otros participantes (pertenecientes a un grupo docente)

Nombre y apellidos	**Grupo docente**	**Departamento**
Ana Mª Maldonado Alconada	131	Bioquímica y Biología Molecular
Adoración Cabrera Caballero	128	Genética
Mª Ángela Martín Cuevas	128	--

Otros participantes

Nombre y apellidos	
Eva Mª Madrid Herrero	Personal externo

MEMORIA DEL PROYECTO DE INNOVACIÓN EDUCATIVA PARA GRUPOS DOCENTES

1. Introducción

Este proyecto surge de la necesidad de introducir en sus aulas por un lado nuevos modelos de enseñanza-aprendizaje y por otro, la enseñanza términos técnicos en inglés. En el campo de la mejora genética agroforestal, al igual que en otros muchos, tener un conocimiento básico de términos técnicos en lengua inglesa es indispensable para desenvolverse profesionalmente y estar actualizado en las nuevas tecnologías. Por lo tanto, una enseñanza de calidad implica incluir en la docencia de esta materia términos técnicos específicos que serán esenciales para comprender textos escritos en inglés. La metodología CLIL permite por un lado, incluir en la enseñanza estos términos técnicos pero además ayuda a cambiar la metodología de la enseñanza de la asignatura, haciéndola más dinámica y participativa .

La acción se ha desarrollado tanto en una asignatura de grado y dos asignaturas de Máster. Los alumnos de grado estaban cursando la asignatura optativa "Biotecnología Agroforestal" de cuarto curos del grado de Ingeniería Agroalimentaria y del medio Rural. En el curso 2014-15 los alumnos matriculados fueron seis, dos de ellos del programa Erasmus, con un buen nivel de inglés. En el caso de los alumnos españoles el nivel era variado, desde básico a avanzado. También se realizó la experiencia con alumnos de Máster de las asignaturas "Recursos fitogenéticos: evaluación, conservación y utilización" (Máster en Producción, Protección y Mejora Vegetal) con 5 alumnos y "Biotecnología y cambio global" (Máster en Cambio Global: Recursos Naturales y Sostenibilidad) que la cursaron 4 alumnos españoles y un alumno Erasmus.

Nuestra experiencia previa en actividades de plurilinguismo ha consistido en:

- Participación en el proyecto de innovación coordinado por Pilar Martínez Ortigosa (PMO) del departamento de Arquitectura de Computadores y Electrónica (ACyE) de la Universidad de Almería titulado "Desarrollo de herramientas y actividades para la incorporación del plurilingüismo en el ceiA3"

- Participación en cursos de formación de profesorado en inglés para fines académicos de la UCO durante los cursos 2010-11 (*CLIL*) y 2014-15 (*Inglés aplicado a la docencia II*)

-Participación en el curso *Lecturing in English*, de 25 horas en la Universidad de Edimburgo

2. Objetivos

Por una lado, a partir de este proyecto hemos iniciado la preparación de material docente enfocada a adoptar la metodología CLIL en los próximos cursos. Hemos preparado un listado de términos técnicos que son básicos en la enseñanza de la Mejora

agroforestal además de un borrador de una clase de introducción sobre la utilización de la biotecnología en Mejora de plantas. Estos materiales servirán para facilitar nuevas actividades en el próximo curso.

También hemos realizado una actividad con alumnos de grado y postgrado para iniciarlos en vocabulario técnico. Para ello hemos elaborado un protocolo de prácticas, en concreto sobre el proceso de clonación, traduciéndolo en clase de forma que fuera comprensible para ellos y hemos elaborado un cuestionario para comprobar el grado de comprensión de la práctica.

3. **Descripción de la experiencia**

Para recopilar información de términos técnicos en inglés relacionados con nuestra materia hemos utilizado términos que se han definido en libros de texto y trabajos de revisión. También nos ha servido de gran ayuda el curso básico de Mejora en plantas editado por el proyecto internacional Generation Chalenge Programme (www.generationcp.org) del Consultative Group on International Agricultural Research (CGIAR) as. Este listado servirá para seleccionar vocabulario específico en futuras lecciones en inglés y organizar actividades orales o de escritura que promueva la utilización este vocabulario por parte de los alumnos. También se ha elaborado una breve lección de introducción a la biotecnología que incluye términos que son básicos para comprender la materia, con una serie de diapositivas y un texto que puede servir de base para la exposición de la misma.

En cuanto a la experiencia con los alumnos, se realizó a partir de un protocolo en castellano, que ya había sido elaborado previamente en nuestro departamento, en el que se describe el proceso de transformación de un fragmento de ADN en una bacteria. Para comprobar el nivel de compresión tanto de los términos en inglés como el proceso de transformación se elaboró un cuestionario con preguntas muy concretas que los alumnos respondieron de forma individual.

4. **Materiales y métodos**

Para el desarrollo de la **actividad 1**, por un lado hemos elaborado las definiciones de términos técnicos basándonos en literatura especializada, adaptando los textos, en la medida de lo posible, al enfoque de nuestras asignaturas. Para preparar la presentación de la lección corta de introducción hemos utilizado diapositivas muy claras, con fotografías y esquemas. El texto que iría acompañando a la exposición se ha enviado a corregir a especialistas (servicio UCO idiomas).

En cuanto a la **actividad 2,** en primer lugar realizó una pre-actividad, para comprobar si los alumnos conocían el significado de algunos términos específicos de la materia, así como alguna otra dificultad en el vocabulario. Tuvimos que considerar que el nivel de conocimiento de idioma era muy variado entre los alumnos. Además cada profesor explicó el peso que tendría en la nota final de la asignatura. Una vez finalizada la práctica se repartieron las encuestas de evaluación que las contestaron cada alumno de forma individual. Las respuestas se analizaron en otra sesión para comprobar donde habían tenido fallos y discutir los términos que aún no quedaban clarificados.

5. **Resultados obtenidos y disponibilidad de uso**

Actividad 1: Hemos elaborado una presentación de introducción a la biotecnología en la Mejora vegetal que se adjuntan. Tanto la presentación como el texto de la lección, corregido por el servicio de UCO idiomas, están disponibles para cualquier docente que lo solicite (Anexo 1). También hemos elaborado un glosario de términos que incluye 64 definiciones técnicas (Anexo 2).

Actividad 2:

El texto en inglés de la práctica se presenta en el Anexo 3 y el test de evaluación en el Anexo 4. Se incluyen también algunas de las respuestas de los alumnos. En general los resultados fueron positivos ya que en esta práctica, al ser en otro idioma, nos detuvimos más que en otras prácticas. La experiencia nos permitirá elaborar una rúbrica detallada para ser más objetivos en el proceso de evaluación y valorar por un lado el grado de comprensión de la práctica y por otro el conocimiento del idioma.

6. Utilidad

Esta experiencia nos ha servido para iniciar en nuestro grupo docente actividades de plurilingüismo. La experiencia de nuestro grupo docente en actividades de plurilinguismo ha sido muy escasa, pero pensamos que el material que se ha elaborado puede ayudarnos a continuar con esta actividad en cursos posteriores y servir de base para elaborar materiales docentes más completos en nuestra materia. Para elaborar los materiales hemos tenido en cuenta estrategias de la metodología CLIL y así hacer más asequible la comprensión de los textos.

Por otro lado, la práctica realizada y el cuestionario nos ha servido para adquirir experiencia en la forma de exponer una técnica en otro idioma, siendo conscientes de los términos que necesitan explicarse y antes comenzar la clase y la necesidad de establecer una rúbrica que facilite la evaluación de estas actividades.

7. Observaciones y comentarios

El importe económico concedido se ha utilizado para corregir con un servicio de traducción el texto de la lección magistral, la traducción del protocolo y del test de evaluación. Hemos consumido un total de 138,67 €, algo menos de la mitad del presupuesto concedido (373,90 euros) ya que algunos de los miembros del equipo del proyecto tienen un nivel de inglés alto, y la complejidad de la corrección no ha sido elevada abaratando el coste.

En futuras experiencias tenemos que considerar que el nivel de inglés de los diferentes alumnos puede ser muy heterogéneo lo que puede dificultar la realización de actividades. No obstante pensamos que ha sido una experiencia beneficiosa, que en general ha gustado a los alumnos y puede ser de gran utilidad en su formación. También beneficia a la integración de alumnos del programa Erasmus con un bajo conocimiento de español.

8. Bibliografía.

Collard BCY, Jahufer MZZ, Brouwer JB, Pang ECK (2005) An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts *Euphytica* (2005) 142: 169–196

Eriksson G., I. Ekberg, D.Clapham. 2006. An introduction to Forest Genetics. ISBN 91-576-7190-7.Department of PlantBiology and ForestGenetics, SLU.

Koebner R, Fulton T (2015) Plant Breeding, concepts and methods course. Generation Challenge Programme. https://www.integratedbreeding.net/185/training/e-learning/plant-breeding-concepts-and-methods-course

Koh HJ, Kwon SY, Thomson M (Eds.) 2015. Current Technologies in Plant Molecular Breeding A Guide Book of Plant Molecular Breeding for Researchers. Springer ISBN 978-94-017-9996-6 (eBook)

Pavón V (2011) Planning CLIL lessons in Higher Education- Bilingual programme Training course-

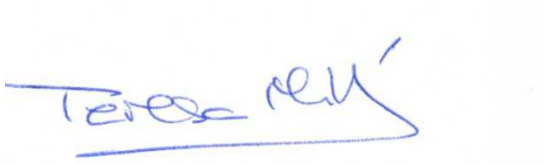
9. Relación de evidencias que se anexan a la memoria

- Anexo 1: Presentation of Mini Lesson "Biotechnology in Plant Breedign" and text

-Anexo 2: Glossary

-Anexo 3: Transformation of bacteria with plasmidic DNA protocol

.Anexo 4: Evaluation test for laboratory practice



Fdo. Teresa Millán Valenzuela

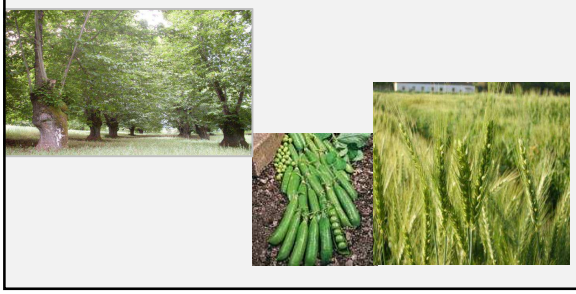
Córdoba, 29 de septiembre de 2015

Sra. Vicerrectora de Estudios de Postgrado y Formación Continua

ANEXO 1

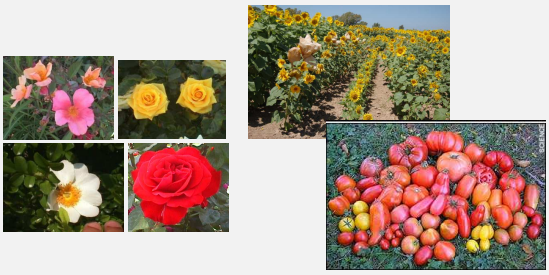
LESSON: PRESENTATION AND TEXT

Biotechnolgy in plant breeding

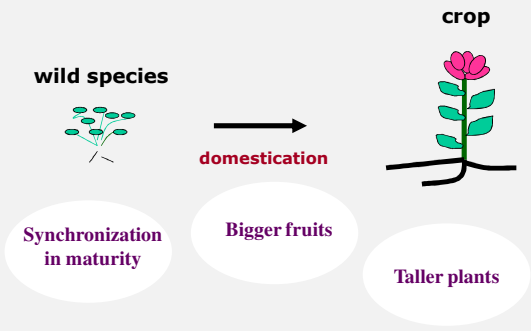


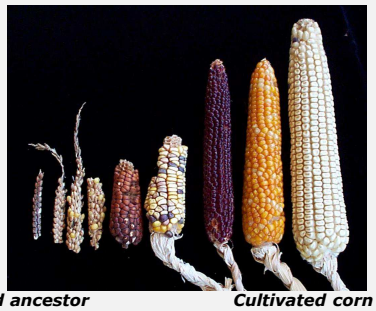
- **Plant Breeding**
  - Definition
  - Early stages: domestication
  - Scientific period
- **Biotechnology**
  - Definition
  - Biotechnology as a tool
  - Organisms Genetically modified (OGM)
- **Conclusions**

The application of science to the development of new varieties according to different uses that will benefit to man.



When man started to do plant breeding?





Wild ancestor *Cultivated corn*

Genetics

1699: plants have SEX

1717: first cross (carnation)

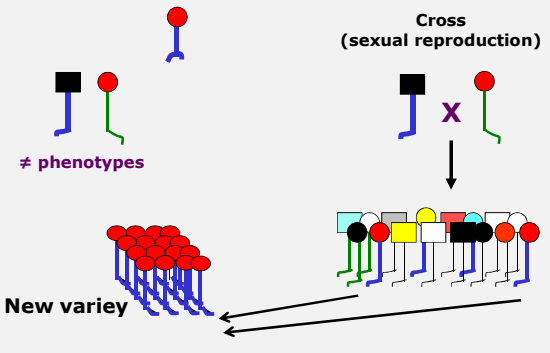
1900: Mendel laws of inheritance

⬇

Scientific Plant Breeding

¿Why using plant breeding?

- **To know spacial an temporal distribution in populations**
- **- To study of taxonomic and phylogenetic relationship among different taxons**
- **Determination of matting systems**
- **Marker assisted breeding (MAB) / selection (MAS)**



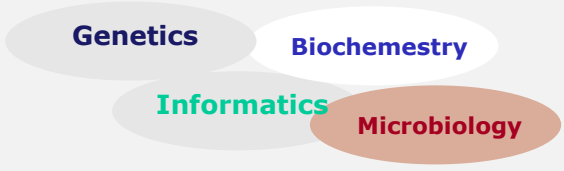
Phenotype = genotype + environmental conditions

Chickpea hybridization

- Plant Breeding
  - Definition
  - Early stages: domestication
  - Scientific period
- **Biotechnology**
  - Definition
  - Biotechnology as a tool
  - Genetically modified organisms (GMO)
- Conclusions

Biotechnology

Any **technological** application that uses **living organisms**, or **derivatives**, to make or modify products for a specific use

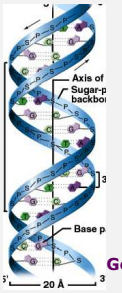


Today we know the whole genome sequence of many cultivated and forest species

Arabidopsis thaliana

December 2000

Species	Published in
Rice	2002
Black cottonwood	2006
Grape	2007
Banana	2012
Melon	2012
Tomato	2012
Chickpea	2013
Oak	2015

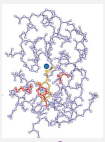


DNA

Deoxyribonucleic acid

the genetic code is universal

Gene



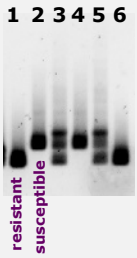
Proteins

Phenotype

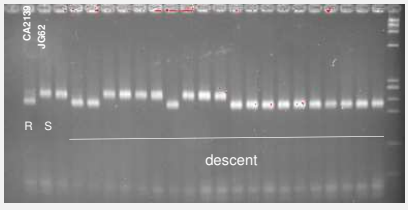
Biotechnology as a tool in plant breeding

Molecular markers

resistant susceptible

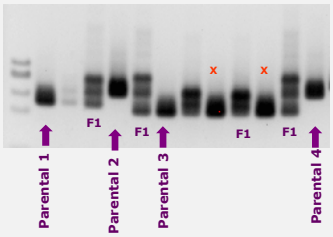


Identification of genotypes associated to a particular trait in a segregant population



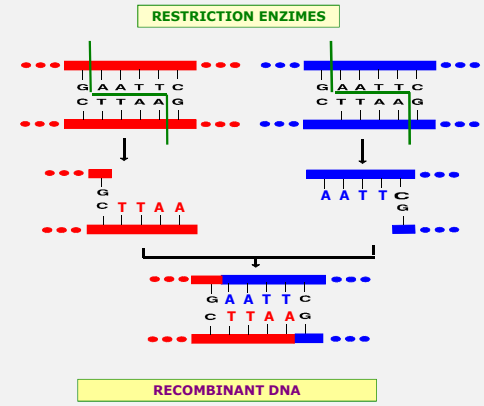
Susceptible x Resistant

Checking crosses by molecular markers

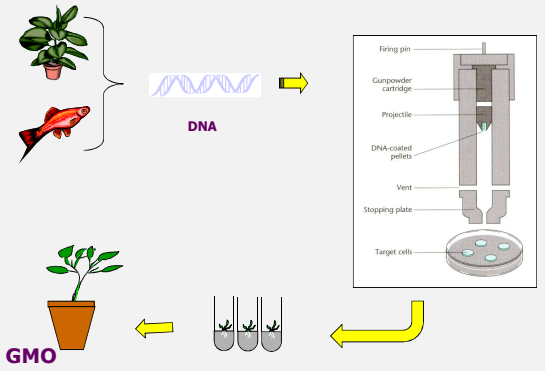


Transgenics or Genetically Modified Organisms

(GMO)



Genetically Modified Organism (GMO)



Varieties produced by biotechnology

✓**Resistance**

- virus, bacteria and fungus
- insects
- Herbicides

Corn Bt

✓**Industrial and Nutritive quality**

- Golden rice
- Wheat suitable for celiac

Objections to the transgenics

Not natural

May damage environment

Only benefit multinational

May cause allergies

But...

A decade of EU-funded GMO research (2001 - 2010) support them

Projects dealing with the development of new products and processes based on GMO technology fully integrate safety assessments in their conception, experimentation and development

https://ec.europa.eu/research/biosociety/pdf/a_decade_of_eu-funded_gmo_research.pdf

Conclusions

-Plant breeding started with domestication and suffered modifications since they were cultivated.

-The knowledge of genetic inheritance of different characteristics in plants permit to do a conscius plant breeding

-Biotechnology is a very usefull tool to support breeding and facilitate the obtention of new varieties.

Do you agree with the use of biotechnology in plant breeding?

Write a sentence with argument either **for** or **against**

Biotechnolgy in Plant Breedign. Text

First, I'd like to introduce you to the ideaof plant breeding, and show how it has developed over time. Then I will briefly explain the concept of biotechnology (based on DNA recombinant technology) and show why it can be regarded as a useful tool in plant breeding programs. Finally, I shall make a few closing remarks.

Here you can see the definition of plant breeding as "The application of science to the development of new varieties. A variety is a different form of a cultivated plant.

At the bottom left of the slide, you can see varieties of roses which differ from each other in colour or in number of petals; in the middle of the slide, different kinds of bread made with flour from different wheats. On the right, tomatoes of various shapes.

This whole huge range of varieties of cultivated plants has been obtained by plant breeding.

Plant breeding started 10,000 years ago, when Man first became a farmer. In choosing some wild species as crops, these gradually changed in form compared to wild species, simply because man unconsciously selected certain traits. For example: taller plants are easier to gather, and bigger fruits tend to ripen simultaneously.

This slide shows how different a cultivated plant can be compared to its wild ancestor (or the wild species from which it developed).

In the seventeen century, breeding became more scientific, thanks to the discovery of plant sex and Mendelian laws of inheritance. It was at this point that genetics started to be regarded a science; since then, genetics has provided the basis for plant breeding

We move on now to the definition of Biotechnology, its use as a plant breeding tool and also the development of GMOs.

Biotechnology refers to any technological application that uses living organisms, or derivatives, to make or modify products for a specific use.

This slide shows the DNA molecule, which carries the genetic information inside the cell. In other words, it contains all the genetic information in living organisms, thus controlling inheritance; it is used to support classical breeding methods to obtain crops with new characteristics. The genetic code is known to be universal, in other words it is the same for bacteria, fungi, animalsand plants. We know the letters of this language, but we do not know always the word formed by the letters. However, our knowledge of the genetic code has increasedrapidly, and today we can identify a segment of DNA that codifies a product, for example a toxin in a bacteria. Thislittle segment of DNA can be transferred another organism, for example, a plant.

One example of the use of DNA analysis in plant breeding is the development of "Molecular markers". A marker is just a signal in the DNA that helps us to select a particular plant. For example, we have a big problem with some fungi that affect

chickpea crops; however, some varieties are resistant, in other words they possess a gene which renders the plant resistant. Susceptible plants do not possess this gene. If we analyse the DNA, for example,of different varieties grown in Spain or in NorthAfrica, we can determine which varieties will be resistant to these fungi, and which will be susceptible to them.

Another important application of biotechnology in plant breeding is the development of GeneticallyModified Organisms (GMO). The steps required for this technique are: 1) extraction of a particular gene form the source organism; 2) cloning of the gene (generation of multiple copies in a vector); 3) transfer of this vector into the target plant (by a process known as *Transformation*) using Agrobacterium-mediated or particle-bombardment techniques; and 4) confirmation of the success and stability of the process.

Conclusions

-Plant breeding started with domestication; plants then underwent modifications due to cultivation.

-Knowledge of the genetic inheritance of different traits in plants enables deliberate plant breeding

-Biotechnology is a very useful tool to support breeding and facilitate the development of new varieties.

ANEXO 2

Adaptability[4]**:** the ability of a population to respond genetically or phenotypically to a changed environmental conditions. The amplitude of a trait of a genotype studied in at least two different environments is called phenotypic plasticity.

Allele[1*]**:** alternate forms of a genetic locus. For example, at a locus determining flower colour, an individual might have the allele for white flower, purple, etc.

Clone[4]**:**genetically identical individuals multiplied by vegetative propagation: grafts, cuttings, root suckers, somatic embryos.

Co-dominance: Here both alleles are expressed. Many proteins and DNA markers are like this and both parental alleles can be detected separately and equally

Crop[1]**:** a plant such a grain, vegetable or fruit grown in large amounts on a farm.

De novo assembly[3] : Sequencing without reference genes

Domestication[1]: the process by which plants are genetically modified by selection over time by humans for traits that are more desirable or advantageous for humans

Ecotilling[4]: A high-throughput method to identify allelic variants of a given DNA sequence from germplasm collections. It allows the detection of SNPs and indels and can be used for homozygous and heterozygous organisms.

Emasculation[1*]**:** The removal of anthers from a flower before the pollen is released.

***Ex situ g*ene conservation**[4]**:** in forestry it generally stands for storage or cultivation gene resource population.

Full dominance: where, In the heterozygote, only one allele (the dominant one) is expressed, while the other allele (the recessive one) is silent.

Gamete[1]**:** The haploid cell produced by meiosis. The male gamete is the pollen grain, while the female gamete is the egg cell

Gene flow[4]**:**individuals from one population participate in the procreation of a new generation in the recipient population and that the donor and recipient populations have different allele frequencies. Forplants, which are mostly stationary there are gene flows via pollen, seed or fruit dispersal.

Gene[1]**:** the unit of heredity, transmitted from generation to generation during reproduction. Each gene consists of a sequence of nucleotides, occupying a specific position along a chromosome. Most genes encodes a specific functional product

Genetic drift[4]**:** is a random process that leads to allele fixation independent of the fitness contribution of the fixedallele.

Genome[1]**:** all the genetic material of an organism

Genome-wide association studies (GWAS)[1]**:** Association mapping identifies marker/trait correlations, and is especially useful for quantitative traits, but uses large sets of diverse germplasm rather than specifically developed populations. Genome-wide association mapping scans the entire genome for complex trait associations.

Genotype x Environment (GxE)[1]**:** the interaction of a plant's genotype with the environment in which it is grown that contributes to its performance

Genotype[1]**:** the inherited genetic constitution of an organism

Genotyping by sequencing (GBS)[1*]**:** allows a breeder to use genomic selection without developing any prior molecular tools such as markers and maps

Genotyping[1]**:** the process of identifying the genetic make-up of an organism, by using molecular markers, DNA sequencing, etc.

Germplasm[1]the collection of a set of genetic resources for an organism, which can consist of a seed collection, nursery, or other types

Hand pollination or artificial hybridization[1]**:** Man pollinate with a particular polen the female plant. Date palm is a good example. Date palms are single sex (monoecious); so to maximize fruit set, female trees (which normally rely on the wind for pollination) are commonly pollinated by hand

Haploid[1]: having a single set of chromosomes, for example as in a gamete

Haplotype[1]**:** A combination of genetic variants (usually SNPs) which are inherited as a unit, because they are present along a stretch of DNA so short that the chance of a recombination event

Heterosis[1]: the phenomena where the progeny of 2 inbred lines performs better than: either of the parents (best-parent heterosis) or the mean of the parents (mid-parental heterosis). The molecular basis of heterosis is still unknown

Heterozygous[1]**:** contains different alleles at a particular locus

Homozygous[1]**:** contains identical alleles at a particular locus

Hybridize[1]**:** to cross-pollinate, to produce hybrids

***In situ g*ene conservation**[4]**:** in forestry it generally stands for a naturally regenerated

Inbred lines[1]: lines that have been selfed to the point of homozygosity

Inbreeding depression[1]: the loss of vigor when some crops have reduced heterozygosity due to enforced self-pollination

Incomplete Dominance[1*]**:** This refers to the (common) situation when two alleles are neither dominant nor recessive, but blend to produce an intermediate phenotype i.e. red flower x white flower produces a pink flowered hybrid

Introgression[1]**:** movement of a gene or locus from one species into another by hybridization

Landrace[1]**:** typical end product of farmer selection, tend to be genetically heterogeneous, but phenotypically homogeneous.

Linkage disequilibrium[1]: the non-random association of alleles at two or more loci, i.e. they occur together more often than would be expected by chance

Linkage map (=Genetic map) [2*]**:** indicate the position and relative genetic distances between markers along chromosomes, which is analogous to signs or landmarks along a highway.

Linkage, genetic linkage[1*]**:** when two chromosomal regions are located physically near each other such that there is a high likelihood they will be inherited together .

Mapping[1*]: the representation of the location of genes or DNA segments along different chromosomes (linkage groups). In *genetic* mapping, this is done by analyzing patterns of inheritance in segregating populations (measured in recombinational units, commonly centimorgans). In *physical* mapping, this describes the actual location of a sequence in a particular genomic region (measured in bp)

Marker-Assisted Breeding (MAB)[1*]**:** combines the use of molecular markers with plant breeding techniques.

Marker-Assisted Selection[2]**:** a phenotype is selected on the genotype of a marker

Molecular marker[2*]: Genetic markers represent genetic differences between individual organisms or species. Generally, they do not represent the target genes themselves but act as 'signs' or 'and whose inheritance can be followed

Mutation[1]: an abrupt change in the genotype of an organism that is not the result of recombination

Pedigree[1]: line of descent, lineage, ancestry

Phenotype[1]: the visible appearance of an organism. The phenotype reflects the combined action of the genotype and the environment where the individual exists

Plant Breeding[1*]: the intentional development of new forms or varieties of plants by crossing, hybridization, and selection of offspring for desirable characteristics, also defined as "The art and science of changing plant form and/or performance for the benefit of humankind"

Plant Genetic Resources[4]: any genetic material of plant origin of actual orpotential value for conservation and breeding purposes.

Plastid (=cytoplasmic) inheritance[1*]**:**. Plastids are rarely present in the pollen, and so are maternally inherited

Plastid[1]: Organelles within the plant cell cytoplasm which contain their own DNA, and replicate independently of the nucleus. Green tissue cells include both *chloroplasts* (which are largely resposible for photosynthesis) and *mitochondria* (which is the major site of energy production in the cell).

Polygenic: When applied to a trait, this term implies that many genes are involved in its determination. When only a few genes are involved, the trait is said to be under *oligogenic*control, and when only one gene is involved, the trait is under *monogenic* control. Most quantitatively inherited traits are under polygenic control

Polyploidy[1]: a state in which multiple copies of a complete genome are present. The polyploid series is haploid (1 copy), diploid (2 copies), triploid (3 copies), tetraploid (4 copies), pentaploid (5 copies), hexaploid (6 copies) etc.

Pure line variety[1]: A variety which has been multiplied by a succession of self-fertilizations from a single seed (or sometimes from the progeny of a single plant). Pure line varieties are expected to be essentially fully homozygous and so their phenotype should be highly homogeneous

Qualitative inheritance[1*]: trait controlled not one or few genes following Mendel Laws

Quantitative inheritance[1*]: trait controlled not by one gene, but by several or many, all acting together. The result of the simultaneous action of many genes is a continuous (often normal) distribution. Most of breeding involves handling this sort of inheritance.

Quantitative trait loci (QTL)[1]: regions of the chromosome associated with the inheritance of polygenic traits.

Recombination[1]: the formation among the offspring of a mating of genetic combinations not present in either parent, achieved via the physical exchange of genetic material during meiosis

Resequencing[3*]: Sequencing with reference genome or genes

Restriction Endonucleases[3]: Restriction endonucleases (or restriction enzymes) are enzymes that recognize a specifi c DNA sequence (restriction sequence) and cut the DNA there.

Segregating plant population[2]: a population derived from sexual reproduction whose parents will differ for one or more traits of interest.

Segregation[1*]: The process whereby alleles are separated from one another as a result of meiosis. Segregation can only occur with respect to genes which are in the heterozygous state in the parental plant

SNP[1*]: an abbreviation for “single nucleotide polymorphism”, referred to a change of a base in a particular position when are compared different genotypes. Pronounced "snip" A SNP which distinguishes two sequences can be used as a genetic marker

Trait[1]: A recognizable, measurable character in a plant, which is under some genetic control

Transcriptome analysisis[3]: All expressed genes in specifi c tissues or at certain times can be compiled into reference datasets by mRNA sequencing (= transcriptomics or RNAseq)

Transgenic or OGM (organism genetically modified)[1]: an organism containing genetic material from another organism transferred by genetic engineering

References.

[1]Koebner R, Fulton T (2015) Plant Breeding, concepts and methods course. Generation Challenge Programme. https://www.integratedbreeding.net/185/training/e-learning/plant-breeding-concepts-and-methods-course

[2]Collard BCY, Jahufer MZZ, Brouwer JB, Pang ECK (2005) An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts *Euphytica* (2005) 142: 169–196

[3]Koh HJ, Kwon SY, Thomson M (Eds.) 2015. Current Technologies in Plant Molecular Breeding A Guide Book of Plant Molecular Breeding for Researchers. Springer ISBN 978-94-017-9996-6 (eBook)

[4]Eriksson G., I. Ekberg, D.Clapham. 2006. An introduction to Forest Genetics. ISBN 91-576-7190-7.Department of PlantBiology and ForestGenetics, SLU.

* Definiciones adaptadas por nuestro grupo docente

ANEXO 3

TRANSFORMATION OF BACTERIA WITH PLASMID DNA.

INTRODUCTION

Transformation is the process by which an inserted substance changes the genetic characteristics of a cell or organism. We can take advantage of this process to introducea foreign DNA molecule into a host cell.

In*Escherichia coli*this process does not occurnaturally, and must be induced in the laboratory. To facilitate thisoperation,*E. coli* cells are treated with calcium chloride($CaCl_2$) at low temperatures (close to 4º C).This treatment causes tiny holes to open, thus making it possible for foreign DNA to enter the cell. Cells able to take up foreign DNA are referred to as **Competent Cells**.

A **plasmid** is a small DNA molecule within a cell,which is physically separated from chromosomal DNA and can replicate independently. The plasmid vector we will be using in this experiment is pGEM® T. This vector takes advantage of certain properties of TAQ polymerase to add an adenine nucleotide at the 3' end. The vector is therefore open and has afree 3'-T. This single 3'-T overhang at the insertion site greatly improves the efficiency of ligation of PCR products into the plasmid.The vector additionally contains a multiple cloning site within the the α-peptide cloning region of the enzyme β–galactosidase (Figure 1). Insertional inactivation of the α-peptide allows recombinant clones to be directly identified by color screening on indicator plates (white/blue).The pGEM® T vector also contains sequences matching theForward and Reverse primers derived from M13. These are used for the sequencing of the DNA fragment cloned in that vector.

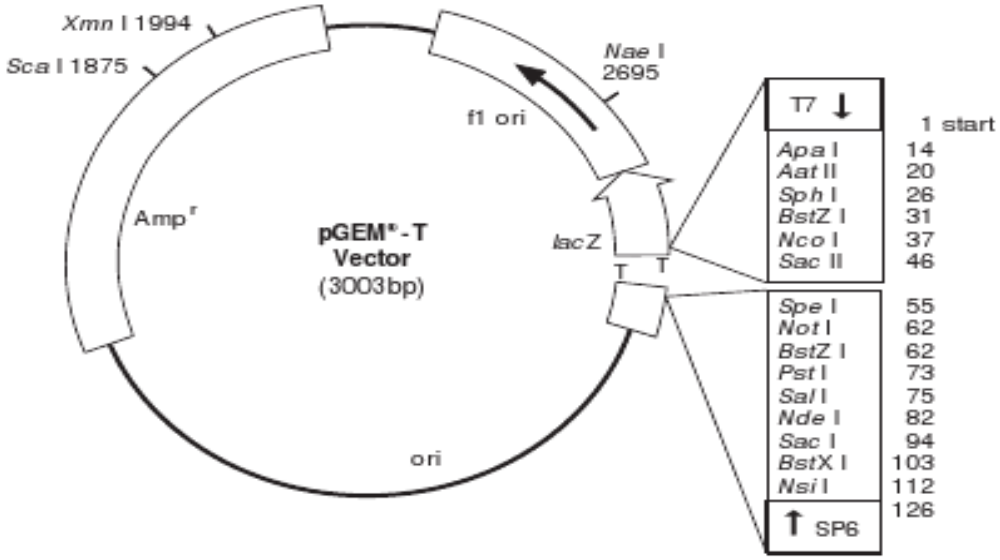


Figure 1. Diagram of the plasmid vector pGEM-T, showing location of: the replication origin (ori), the gene for ampicillin resistance (Amp^r), the replication origin of

bacteriophage F1 (f1 (-) ori), the promotor of the gene *lacZ* (P lac), the gene l*acZ*coding for β–galactosidaseand the multiple cloning site(MCS).

The cloning process can be divided into three steps:

1. **Insertingforeign DNA into the host cell**

First, the foreign DNA is ligated into the plasmid. The vector-insert mixture is then shaken with a suspension of competent cells. Due to the permeability of the bacterial cell membrane, plasmids are able to enter the bacterial cells in an inefficient random manner.

2. **Selecting cells that have taken up a DNA molecule**

Decant the bacteria-plasmid mixture onto a plate containing agar, nutrients and ampicillin. Only cells transformed by a plasmid conferring ampicillin resistance will be able to grow and multiply in the presence of the antibiotic. Viable plasmid-containing bacterial cells will multiply to produce a colony of millions of genetically-identical cells. The colonies show up on the agar plate as spots about one millimeter in diameter, and are easy enough to see.

3. **Distinguishing insert-containing recombinant molecules from vectorsnot containing inserts.**

If prepared under proper conditions, most plasmids will contain an insert. Some, however, will slip through without one. The medium on which the transformer ampicillin-resistant bacteria grow contains, in addition to nutrient and ampicillin, a chemical compound known as X-Gal. This compound serves as a substrate for the reaction catalyzed by the intactβ–galactosidase enzyme (the protein encoded by the *LacZ* gene), and one product of the reaction is a new chemical with a blue pigment (IPTG). Cells containing vectors without inserts turn blue because they carry the original intact β–galactosidase gene; cells containing plasmids with insert remain colorless, because the interrupted *LacZ* gene does not allow production of functional β–galactosidase enzyme. Each time a plasmid-containing bacterial cell divides, it replicates its own chromosome as well as the plasmid established in the cell.

METHODOLOGY

PROTOCOL 1.

Transformation of competent cells with plasmid DNA

1.Mark the tube with your ID number

2. Add35µl (50-100 ng) of competent cells to the ligation tube (containing 5 µl). Mix slowly and carefully (competent cells are very fragile). Place the Eppendorf tube in ice for 5 minutes.

3. Label the plate (LA + ampicillin)with your ID number and date.

4. Open the plate in front of the burner and carefully dispense 40 µl of ligation + bacteria. Distribute it very gently.

5. Place the plate in an incubator at 37 °C overnight.

The following day, check whether colonies have grown.

PROTOCOL 2.

Selecting cells that have taken up a DNA molecule

1. For each colony prepare a 0.5ml tube and add 25 µl of sterile water.

2. Near the burner, select 3 isolated white colonies and one blue colony on the plate.Collect the selected colonies by just grazing them with a yellow tip. Place the tip with the colony in the tube containing water.

3. Prepare the reaction mixture for the PCR:

	Conc/tube	**µl/tube**
Colony+water	-	2.5
Buffer (10x)	1x	1
$MgCl_2$ (50mM)	2mM	0.4
dNTPs (10mM)	0.8mM	0.8
Rev M13 (10µM)	0.2 µM	0.2
Fw M13 (10µM)	0.2 µM	0.2
Taq (5U/µl)	0.25U	0.05
Water	Up to 10 µl	4.85

4.Program the PCR machine as follows:

1 cycle at 94ºC 10 minutes (to break the cell wall)

25 cycles {
- 94ºC 30 sec (to denaturalize the plasmid)
- 56ºC 30 sec (for primers and target sequences)
- 72ºC X sec (for complementary chain synthesis; the elongation time will depend on the fragment size)

Keep at 15ºC

5. Analyze 5µl of the PCR product in 2% agarose gel and select insert-containing colonies.

ANEXO 4

LABORATORY PRACTICE EVALUATION TEST

Name and Surname:

Subject:

Course/Master's Degree:

1. What needs to be done to *E. coli* cells in order to insert foreign DNA into them?

A) $CaCl_2$treatment

B) $CaCl_2$, high temperature and IPTG

C) $CaCl_2$ and cold temperature

2. Which of these is a property of the plasmid?

A) It has to be inserted into the bacterial chromosome for replication

B) It can replicate independently

C) Only one plasmid molecule is transformedin each bacterial cell

3. When you transform the cells you are interested in:

A) Blue colonies

B) White colonies

C) The color is irrelevant

4. True or False:

A) Some vectors can be opened if they have a free 3'-G

B) Directional cloning is possible using the multiple cloning site

C) All cells transformed by a plasmid are able to use IPTG

D) The cells not transformed are Amp^s

E) When the *LacZ*gene is interrupted by the insert the colonies are white in color

5. Describe in two sentences the value of transforming an insert or a gene into a plasmid.

6. Explain the results obtained in the following gel, bearing in mind that a negative colony amplifies a product around 400bp and the molecular weight of the cloned insert was 1300 bp