AN INTRODUCTION TO GENETIC VARIABILITY MATERIAL
BASIS OF GENETIC ENGINEERING

To understand the basis of genetic engineering, one must have a thorough knowledge of the science of genetics. The objective of this lecture is to overview the subject before starting on genetic engineering.

Introduction to Genetics

Genetics is the science that deals with the heredity and variability of living organisms. The structure and the functioning of the most simplest cell is more complex than the most sophisticated machine produced by man so far. Structure of the nucleus of atoms is being studied and its energy used by man in his day to day life. Therefore, we have a more substantial and a complex problem in studying the structure and function of the gene. The communication gap between pure science and biology was broken only when the unit of measurement of heredity - the gene was discovered. It is only a few years back when we started to learn how to directionally change heredity, using methods of genetic engineering. With the perfection of methods of directional change of heredity and variability new vistas have open up for the control and construction of new forms of life.

A historical perspective of genetics

In 1990 Carl Correns in Germany (working with maize), Hugo de Vries in Holland (working with poppy) and Ernst Von Tschermak in Austria (working with pea) cited Mendels report of 1866 submitted to the Brunn Natural History Society.

They explained how their results with crosses of three different plants can also be explained by Mendelian laws. Although Mendel formulated the basic laws of heredity as far back as 1865, we mark 1900 as the beginning of genetic studies, because since then only research on genetics has continued uninterrupted. Bateson in 1906 named this science as Genetics.

Four phases of development of genetics since 1900 can be roughly identified:

Phase I (1900-1910) - Phase of acceptance of Mendels laws. Universal nature of the laws and that they are common to all living beings was accepted by Biologists. Two important findings of this periods are:

(a) Theory of mutations put forward by De Vries
(b) Theory of pure lines put forward by Johanson.

Phase II (1911-1953) - Chromosomal Theory of Inheritance was accepted. Wide use of Drosphila in genetic studies. Thomas Morgan wins the first Noble prize in the field of Genetics.

Cytogenetics develops as a branch of genetics with the sophistication of microscopy. First successful attempts to induce mutations - changes in the genes. Muller (1927) showed that by using x-rays the frequency of mutations can be increased. So a new branch of genetics - Radiation genetics started developing.

Sright, Holden, and Fisher laid the foundation for the mathematical methods to study the genetic processes in populations. The field now called population genetics has an enormous impact on the improvement of methods of plant and animal breeding. By 1944 it was shown that the carrier of genetic information is none other than Deoxy Ribo Nucleic Acid.

Phase III (1953-1970) - Watson and Crick presented the molecular model of DNA which had a tremendous impact on the studies of gene and its properties. 1961 - concept of the genetic code was formulated. By 1969 the scientists were able to artificially synthesise the gene.

Phase IV (1970-onwards) - This new phase started when the basis of genetic engineering was formulated by showing that a particular group of enzymes, later called restriction enzymes can cut the DNA molecule at certain points. This led to cloning of molecules of DNA and to new science which we now call genetic engineering, which has tremendous potential in industry, plant and animal breeding, microbiology and medicine.

Now it is possible to isolate the gene responsible for a particular enzyme and with the use of another set of enzymes called ligases to join to DNA molecules from other species. The methods of genetic engineering, however are not simple but requires expensive equipment and teams of devoted workers. Now hundreds of laboratories worldover are working on different aspects of genetic engineering and therefore we can safely claim that if the first half of this century belonged to the development of pure sciences, the second half belongs to the biological sciences where man is attempting to handle the most complicated structures of nature for its benefit.

Variability and Heredity

A gene can be identified only if there are at least two states to it, because it is the expression of the gene (Phenotype) that we study to interpret the genotype. In other words, a gene that does not mistake can not be identified.

"Mendelian Inheritance"

Mendel used hybridological analysis to derive his laws of inheritance. Using monohybrid cross (A cross involving one locus or gene) he derived the law of dominance and law of segregation (or law of purity of gametes). Using dihybrid cross (study of two pairs of contrasting characters simultaneously) he formulated the law of independent assortment. However, as we know, the law is valid only if the characters studied are located in different chromosomes.

Knowing only Mendelian principles, we can imagine already how new genotypes can be created by making a dyhybrid cross (9:3:3, - did we have originally the six genotypes in the middle?).

Gene Interactions - another Source of Variability

Natesons experiments with the lamb structure of chickens demonstrated that there can be deviations to the law of dominance but still can be explained by mendelian terms. The new character (the walnut appearing in F. when Rose and Pea comb genotypes are crossed) appearing in the first generation is due to gene interactions, which is another source of variation.

Gene interactions in broad sense can be divided into Intra-allelic (interaction of allelic genes) and Inter-allelic (interaction of non-allelic genes) interactions. As a result of Intra-allelic interactions we can observe:

(a) Incomplete dominance (1:2:1 ratio)
(b) Lethal genes (2:1 ratio)
(c) Multiple alleles

(blood groups in man, skin colour in rabbits, self incompatible genes in cross pollinated plant species), and

(d) Codominance (blood groups in man = A=B>O).

As a result Inter-allelic interactions an enormous number of segregation ratios can be created and some of which are:

(a) Complementory factors (9:7; 9:3:3:1 with changed "dominance" patterns; 9:6:1) - these are genes that act together to produce an effect that neither can produce separately.

(b) Epistasis -One pair of genes marks the expression of one or more pairs of non allelic genes.

Dominance is when A>a or B>b, i.e. interaction within same locus. Epistasis is when A>B or a>B i.e. interaction between different loci.

Dominant Epistasis can give 12:3:1 or 13:3 ratio. The latter is also called inhirbitony factor Recessive Epistasis can give ratios such as 9:3:4

(c) Duplicate factors and Polymeric genes

When more than one pair of allelic genes determine a single character. Ratios can be 1:4:6:4:1 with two loci interacting; 1:6:15:20:15:6:1 are obtained with polymeric genes. When dominant genes are added up in a genotype, their effect also gets added up i.e. they have an additive effect.

These factors have immense importance in plant and animal breeding as most of them economically important characters such as seed yield of a rice plant, milk yield of a cow are governed by polymeric genes. In working with such characters breeders work for transgressive segregants, those individuals which exceed (or lower than, in case of such characters seed colour of cereals, some legumes) both parents.

Pleiotropic effects - One allelic pair may have more than one effect on the organism. These are pleiotropic genes. (id id locus in sesame causes indehiscent capsule which is useful but brings about semisterility of pollen resulting in low yield.)

Crossing over can create still more variability

There are more genes than the diaploid number of chromosomes of an individual. The genes are arranged on the chrosmosomes. Therefore Mendels laws and some ratios obtained in gene interactions can be applied only to genes located in different chromosomes. However nature has provided provision to obtain additional variability by recombination of genes located in the same chromosome. Mechanism of meiosis provides the answer. At the diplotene phase of meiosis division I chiasmata are formed chromosome segments are exchanged between adjacent chromatids belonging to two different but homologous chromosomes. Imagine a situation such as following.

As a result of a single crossing over we get two new genotypes AB and ab in the gamates in addition to the parental genotypes Ab and aB. This can be extrapolated to multiple crossing over situations when a multitude of additional genotypes can appear as a result.

The advantage of crossing over is not only in creation of additional genetic variability but also it helps geneticists to locate the genes in chromosomes, identify the order of arrangement along the chromosome and finally develop the genetic maps of individual species. Such maps have been now developed for pea, maize, drosophila and one of the biggest projects that scientists have undertaken in recent years is to map the human genome! However, in the latter case they can not simply rely on crossing over frequencies but have to use other more advanced techniques.

Chromosome aberrations - Yet another source of variability

The number and structure of chromosomes can change within the same species. Such variations include:

(a) Presence of multiple sets of chromosomes called polyploidy
(b) The gain or loss of individual chromosome from a set called aneuploidy and
(c) The gain or loss of individual genes or chromosome segments. All these are examples of chromosome abberations. Thus a chromosome abberation is change in the structure or number of chromosomes.

The structural changes can be:

(a) Deletions
(b) Duplications
(c) Inversions
(d) Translocations. In organisms with deletions and duplications there will be a change in the genetic constitution. Those having inversions and translocations, have only a change in the sequence of arrangement of genes on the chromosomes. Still the resulting phenotype can be different. This is due to position effects of genes, a phenomenon whereby the degree of expression of a given gene is modified by the change in its position in the genome.

The changes in the number of chromosomes in a species can result in:

(a) Polyploidy - increase in number as a multiple of basic (n) number of chromosomes, greater than diploid

(b) <u>Aronploidy</u> - gain or loss of one or more chromosomes, resulting in irregular numbers.

Polyploidy has played a great role in the evolution of plant kingdom, including many of our food crops like banana (triploid-3n), wheat (hexaploid) potato (tetraploid), sweet potato (hexaploid) and so on. These changes are visible not only by phenotype but also cytologically.

<u>Gene mutations as a source of variability</u>

These are submicroscopic changes within the find structure of a gene locus (cistron). Even a change in a single base in DNA molecule e.g. substitution of gvanine for thiamine base is a mutation.

Now scientists have learnt to induce such mutations by artificial methods using ionising radiation or chemicals. Radiation can cause chromosomal aberrations as well.

Use of chemicals has resulted in polyploidization of diploids, some of which have been proved to be useful for man. From the foregoing it is obvious that genetic variability created by these methods is responsible for the diversity of plant and animal kingdom we observe today. By classical methods we can cross individuals within a species or between very closely related species. Methods of genetic engineering have broadened the possibilities of recombination leading to creation of novel organisms with novel properties.