

BIOTECHNOLOGICAL APPROACH IN WEED RESEARCH - AN OVER VIEW

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Abstract

Research on weeds has been reported extensively and in most cases traditional techniques have been used. But during the last two decades, emphasis has been given on the use of biotechnology in weed research. As a result, some advances have been made (Sharner et al, 1985; Mujeeb-Kazi & sitch, 1989; persely, 1990). Potential exist for further advances in weed research with modern biotechnologies. The present paper gives an over view on biotechnological approach in weed research.

1. Introduction

Broadly speaking biotechnology is any technology Where living organisms are made use of. Good examples, with a long history in Sri Lanka are making curd and toddy. Preparation of kassippu (illegally prepared alcoholic drink) in also a biotechnology. Although the term " Biotechnology" has been highlighted recently, it has always been there throughout the civilization of man. It is not new. We all know that man has a long history of marking beers, wine and cheese which are also biotechnologies. However, even at present, this term appears to cause some illusion in our country.

The term "Biotechnology" is most suitably defined as "Any technique that make use of living organisms or substances from these organisms to make or to modify a product, to improve plants or animals, or to develop microorganisms for specific uses" (Office of Technology Assessment, US Congress, 1989).

As mentioned, biotechnology is not new. What is new about this "modern biotechnology" that scientists talk today is the style of approach and methodologies available. At present biotechnology research has a great impact in the whole world and is similar to the impact that electronics made in 1960' s.

Internationally, considerable sums of money is being spent on the biotechnology research and development. It is very much hoped that through modern biotechnology, the existing problems in food and health would be tackled. Developing countries are naturally attracted to the potential application of

biotechnology research to provide food and energy and finally to improve the quality of life. The significant advance in food production must come from biotechnology, where scientist would see the new ways of tackling old problems. Therefore it is more important in developing countries than in developed countries where food production is in excess.

Biotechnology normally is used as a profit generating process requiring a certain amount of capital. This is why it is more popular in the private sector. However with proper management, the initial capital requirement for biotechnology projects can be minimized.

Biotechnology can be categorized into two:

- 1). Traditional biotechnology (fermentation techniques used in brewing, biological control of pests, conventional animal vaccine production etc). These are well established techniques used commercially.
- 2). Modern biotechnology: use of more recently available technologies.

Modern biotechnology

The below gives the " Modern biotechnologies" which can be used for the development of agriculture.

for the development of agriculture.

- * Recombinant DNA techniques and genetic engineering in plant and rhizobia.
- * Plant protoplast culture fusion
- * Plant cell and tissue culture technologies
- * Monoclonal antibody production
- * Diagnosis of plant and animal diseases.
- * Biological nitrogen fixation

The above biotechnologies are used have potential to solve the following problems in agriculture:

- * Plant propagation
- * Plant protection from pest and diseases
- * Production of drugs
- * Development of herbicide resistant plants
- * Increased protein production
- * Stress tolerance
- * To understand various biochemical pathways in the growth and development of plants.
- * Post-harvest protection
- * Requirement of N Fertilizer
- * Environmental pollution (Dubey, 1993).

Weed Biology

The term "weed" has been defined in many ways. An updated definition of a weed from the ecological stand point is now documented (Chandrasena, 1992). It is well known that weeds cause very significant losses to crop yield. They interfere and compete with the cultivated plant as the weed and the crop plant both have similar requirements for growth and development. This competition is intensified as available resources such as nutrients, light, space and even water are limiting in the environment where they grow. Unfortunately, weeds are tough enough to win this competition, resulting in great losses to the cultivated plants.

The problem of weeds can be overcome by two ways:

- 1). to minimize or totally remove the weeds from the cultivated land and intercrop with economically important plant species.
- 2). For the crop plants to become more hardy and increase tolerance so that they can compete with "weeds"

Man from a very long time has attempted to minimize or remove weeds from the cultivated land. "Herbicides" are a result of this attempt. By adding herbicides what the farmer wants is the weeds to be killed and crop plant to survive. Mostly the crop plants are also sensitive specially at higher doses. With continuous use of herbicides, level of herbicide application tends to increase as the weeds evolve to be more tolerant. As a result of using higher doses, crop plants too become sensitive. Scientists have tried to solve this problem by breeding for herbicide-resistant crop plants. Traditional method is to select herbicide resistant crops during conventional breeding. This is inefficient with respect to time, labour and land. The modern biotechnology: cell culture engineering technologies offer a rapid and a safe way to select resistant crops.

The two biotechnologies that have been used and have the potential use in weed research are:

- i) Plant cell and tissue culture.
- ii) Recombinant DNA and Genetic Engineering of plants.

I). Plant cell and tissue culture:

In general, tissue culture is a technique where single cell, unorganized group of cells (e. g. callus) or organized cell masses (e. g. tissues and organs) of plant or animal origin, are grown on an artificial medium under aseptic conditions, in glass or plastic vessels.

The basic principle is the "totipotency" of the plant cell: "every living plant cell has the ability to divide, grow and develop into a complete plant" In plant tissue culture one tries to provide artificially, the chemical and physical environment required for optimum plant growth. The culture medium comprises the major and minor inorganic nutrients, vitamins, amino acids, sucrose as the carbon source and suitable combination of plant growth regulators like auxins, cytokinins, gibberellins etc (Dodds, 1982 Taji, 1992). The equipment needed are not very complicated. The initial input is high, but the equipment can be used for several decades, if properly maintained.

Plant cell and tissue culture techniques encountered are:

- a) Callus culture:- Callus is a mass of unorganized cells composed of both differentiated and non differentiated cells. Almost all parts of a plant can be induced to form a callus *in vitro*.
- b) Cell suspension culture:- single cells and cell aggregates are grown in liquid media with agitation.
- c) Protoplast culture:- culturing of cells without the cell wall.
- d) Organ culture :- plants organs such as roots, shoots embryos, anther, ovaries and ovules are cultured.
- e) Meristem tip culture (shoot tip culture):- also a type of organ culture where shoot tip (meristem together with a few leaf primordia) are cultured. Depending on the objective, the tip can be developed into a single plant or a large number of shoots (proliferation) (Thorpe, 1980).

Through plant cell and tissue culture technique, it is now possible to obtain a complete plant from a single cell.

The following are the applications of plant cell and tissue culture:

- * clonal propagation
- * disease elimination
- * germplasm exchange
- * gene transfer (through protoplast fusion)
- * variant selection (somaclonal selection)
- * induction and selection of useful mutants at the cellular level
- * production of haploids
- * secondary natural product formation
- * incorporation of desirable genes through molecular genetic engineering.

Applications which are relevant to weed research are:

- (1) clonal propagation
- (2) variant selection (somaclonal selection)
- (3) Induction and selection of useful mutants at the cellular level
- (4) Incorporation of desirable genes through molecular genetic engineering

(a) Clonal propagation of plants:

(to obtain a large number of homogeneous plants from a single elite plant)

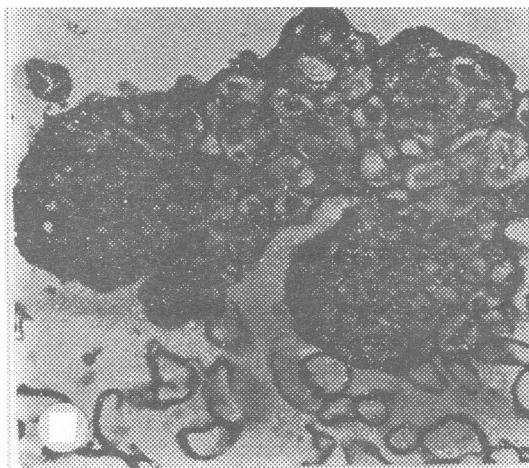
This can be achieved through a callus or meristem tip culture. A callus can be induced from all parts of the plant, but may depend on the plant species and the culture condition (George & Sherrington, 1988). In nature, callus is formed at the sites of wounds.

A callus can be regenerated into plants through:

- a) organogenesis (formation of shoots and roots) (Thorpe, 1980)
- b) somatic embryogenesis (development of embryos from somatic cells without nuclear fusion). These somatic embryos would then develop into plants (Fig 1&2) (Krikorian, 1983; Hiriburagama, 1996).



Fig 1. A somatic embryo. of leaf primordia



Use of meristem tip culture in clonal propagation is commercially used, both in developed and developing countries, to propagate elite cultivars (Fig 3). This technique allows fast and a safe way to produce similar plants in large numbers. (George & Sherrington, 1988)

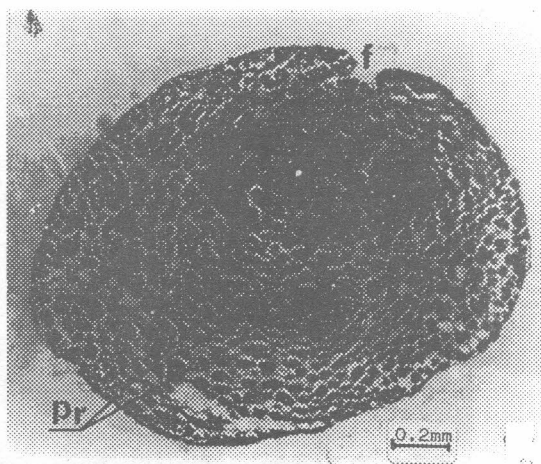


Fig 2. Some developmental stages in somatic embryos. a: early stage in the development b: a mature stage.

f: cotyledonary slit, s: shoot meristem, pr: root primordia

(b) Variant selection for desirable traits through cell culture:

The traditional method of selection is through the spontaneous mutations observed from plants grown in the field. This requires more time, labour and land for experimental plots. Using tissue culture techniques it is possible to select naturally occurring mutants among somatic cells. It is known that one in every 10,000 to 1 million cells in the plant body is a naturally occurring mutant. These mutant somatic cells of a plant body are difficult to observe and identify under natural conditions. But through cell culture, where cells are allowed to multiply and grow, mutants can be selected (Chaleff, 1986). Through this technique, mutants have been selected for tolerance to diseases, saline soils, drought, heat or cold and herbicide resistance (Mujeeb-Kazi & Sitch, 1989; persley, 1990).

Biotechnology involves culturing of cells of the particular plant and exposing the cells to different treatments. The variant selection can be increased through the use of physical and chemical mutagens. Those cells that survive can be isolated and regenerated into complete plants through organogenesis or somatic embryogenesis. A plant can then be propagated through meristem tip culture. (Filippetti & De Pace, 1986; Novak et al, 1990). Also, through anther and pollen culture, haploid cells can be obtained. It is reported that through this technique it is possible to reduce the time required for variation by one-third (Krikorian, 1987). In fact plant cell and tissue culture have contributed significantly to crop improvement of important crops and also offers high potential for the future.

Use of cell culture in weed research:

There are two objectives of using cell culture in weed research:

- a). To obtain herbicide-resistant plants
- b). To understand the actions of herbicides at molecular level
- a). To obtain herbicide-resistant plants**

Rather than finding more selective herbicides, it is much more appropriate to develop new crop varieties that are resistant to very effective herbicides. This is because, in a cell culture, there are millions of cells growing in it. Each living cell can be considered as a potential plant because of the totipotency phenomenon. Therefore, using cell culture technique, millions of plant cells can be screened.

Much work has been done with the use of cell culture to screen for herbicide-resistant plants. Some examples are:

1. Resistance to the herbicide class imidazolinones: using cell cultures of maize, cell lines resistant to this class of chemicals have been obtained (Shaner et al, 1985). Cell cultures of maize have been established and have been exposed to different concentrations of imidazolinones. The cells in the

culture are variable, many are susceptible but a few may have gained resistance. These cells have been isolated, multiplied to obtain cells that survive have been isolated, multiplied to obtain cell lines with resistance to this herbicide.

2. Picloram resistant tobacco plants (Chaleff, 1980)
3. Glyphosate tolerant crops (Tomes, 1990).

b). Action of herbicides at molecular level

1. It has been found that imidazolinones resistance is due to a change in acetohydroxy acid synthase enzyme (Shaner *et al*, 1985). Normally the herbicide would inhibit this enzyme and thus would cause metabolic irregularities resulting in the death of the plant.
2. Glyphosate and sulfometuron methyl which are successful herbicides act on the aromatic amino-acid biosynthesis pathway (Shikimate pathway). The target enzyme for glyphosate is now known to be EPSP synthase (5 enolpyruvyl-3 Phosphoshikimate). This EPSP synthase is a key enzyme of shikimate pathway (Chaleff, 1986).

Problems encountered in the above biotechnology are:

1. Whether the herbicide has the same effect on plant cells and tissues in culture, and also on tissues of the intact plant.
2. Level of resistance. This fact is important to consider the crop as an agronomically desirable variety.
3. Even though it is not difficult to establish cell cultures, regeneration of the cells into whole plants could be a very difficult step.

2) Recombinant DNA technology and genetic engineering in weed research

The code (message) for characteristic features of a plant are stored in the DNA as genes. Expression of a single or multiple gene gives a character to the plant (the environment may modify the expression).

It is now possible to take the genetic material (DNA) out of the cell, manipulate them and reinsert to the cell. This highly increases the ability of cells to produce what is desired. This phenomenon is the basic concept in genetic engineering, which is a modern biotechnology.

The basic principle of Genetic Engineering is that the genetic material (a gene) can be transferred from a cell of a one species to another and make the recipient cell to express the gene. The recipient cell will now have a piece of new DNA inserted in its own

DNA. This DNA of the recipient cell is now called a "recombinant DNA". The recipient cell is said to be "transformed" by the new genetic information (Fig 3). Plant arising from the recipient cell is a "transgenic plant" (Watson *et al.* 1983). There are 3 key points in this technology. They are:

- 1). Identification and isolation of suitable genes to transfer
- 2). Delivery systems to introduce the desired gene into the recipient cell.
- 3). Expression of the new genetic information in the recipient cell (Old & Primrose, 1981).

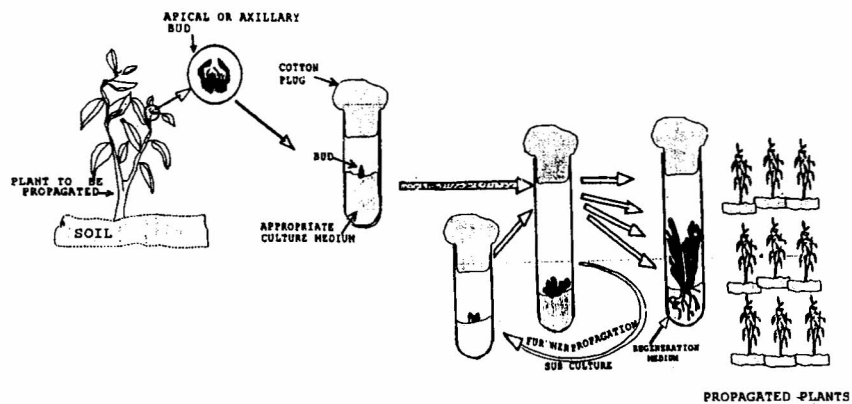


Fig 3. Micropropagation through meristem tip culture

This area of regulation of genes has important application in the crop improvement and protection, and also to understand fundamental biochemical processes.

Gene transfer is done by 3 main ways:

- 1) Use of *Agrobacterium tumefaciens*
- 2) Use of plant viruses (e.g. Caulimovirus Gemini virus, RNA viruses) (Potrykus *et al*, 1986)
- 3) Direct DNA transfer:
 - protoplast transformation
 - Microinjection
 - Particle bombardment (gene gun)

In the first two types of transformation systems a vector is used. This vector would initially take up the gene and then subsequently transfer to the plant cell. The vector should be able to enter to the plant cell. grow and multiply there. Scientists have made use of this natural plant genetic engineers to insert desirable genes into crop plants. The first vector used was *Agrobacterium tumefaciens* which in nature cause crown gall tumour in tobacco and in many other dicotyledons. This technology is much higher but successful out comes would be much more profitable.

For the genetic engineering technology to be successfully applied for plant improvements the transformed cell should be developed into a complete plant and the introduced gene/s should be expressed' Therefore, plant cell and tissue culture technology is important for genetic engineering to be successful'

However this biotechnology is still at preliminary stage even though it has a great potential in near future. The lag of this technology is due to: 1) lack of useful genes (limited availability of identified genes). 2). inability to develop a complete plant from a single cell for the particular crop.

In the approach of genetic engineering in weed research, the objective is to genetically modify the crop in two ways:

1. For herbicide resistance
2. To incorporate weed characteristics (to be able to compete with weeds).

1. For herbicide resistance:

Resistance can be achieved through the following means:

- a). To alter the target gene/enzyme.
- b). To induce the crop to overproduce the herbicide target enzyme.
- c). To introduce a herbicide detoxification gene (enzyme to detoxify the herbicide) (Shaner *et al*, 1985)

Scientists have been successful in achieving the above 3 types.

Some examples are:

- a) Isolating a resistance gene for the herbicide . This had been from the bacterium *Salmonella typhimurium* (Comai *et al*, 1985). Glyphosates which are a group of systemic herbicides inhibit the target enzyme (EPSP synthase) in many crops. The gene isolated from *Salmonella* spp. code for an enzyme which is less susceptible for inhibition by glyphosates. This gene has been incorporated into tobacco. Attempts are being made to incorporate into other crops as well.
- b) In *Petunia*, biotechnologists have isolated a mutant gene which codes for enhanced level of the target enzyme. This gene could confer resistance to a certain level of herbicide.
- c) Potatoes resistant to Basta[®]. A bacterial gene when transferred to the potato could produce a new enzyme, an acetyl transferase, which transforms Phosphinotricin (the active ingredient of Basta[®]). The plants would become non toxic to Basta. This technology looks idea, but there are major constraints. One is that genes which are responsible for herbicide tolerance must be identified. The genes could be either in the nuclear DNA or in the DNA of the chloroplast of in any other organella!!

2. To incorporate weed characters:

Through incorporating various stress tolerance, the crop plant would hopefully be able to compete with the weed thus minimizing the use of herbicides. Unlike herbicide resistance, not much attention has been given in this area of research. The main reason is most biotechnology research is done by the private sector and they would like to promote profit generating research. However, one should not forget the potential of this biotechnology especially to developing countries. Also, this approach is more environmental friendly.

So far successful research on genetic engineering to incorporate herbicide resistant gene/s to plants have done on tobacco, tomato, carrot, and potatoes. Herbicide resistant plants of tomato and potatoes (for Basta[®]) are expected to be available in the market in near future. Development of herbicide resistance would hopefully lead a reduction in the use of herbicides. This would also broaden the rate and time of application.

Danger!

But one should not forget the potential dangers: where more herbicides will be used (Haupti *et al*, 1985). With the availability of herbicides resistant crops, farmers will be able to use more herbicides more often on more crops. This would intensify environmental and health hazards. Proper planning to avoid such situations is required.

Finally one can imagine that the rate biotechnology research is being conducted, there is a great chance that in very near future, farmers will be able to buy a herbicide and the plants specifically resistant to it!

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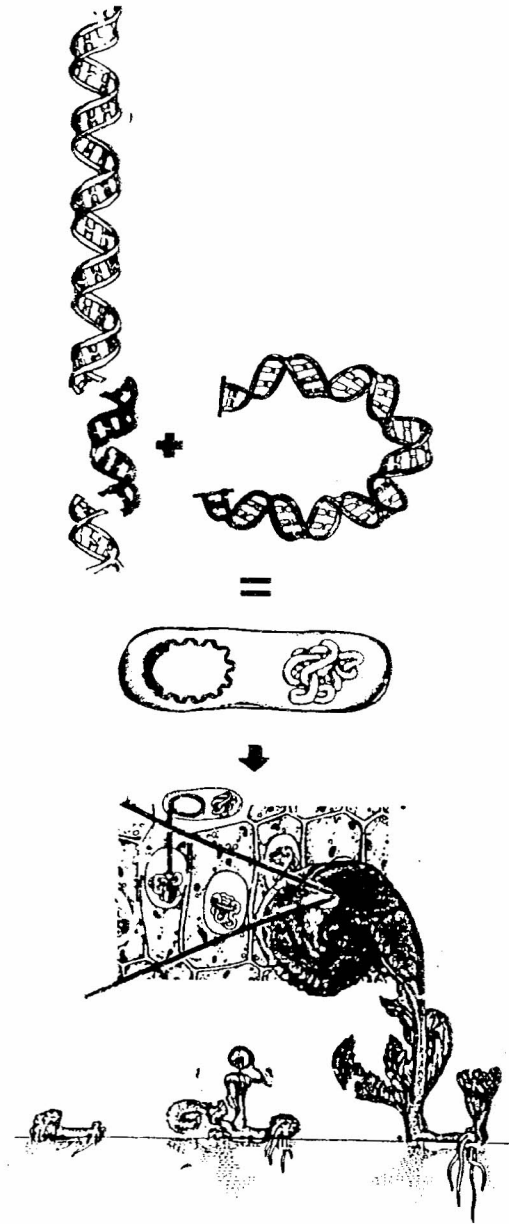


Fig 4 A picture illustrating the entire process of gene transformation (genetic engineering technology)