Selectable Marker Genes:

One of the constraints in plant transformation technique is the obtaining of transformed plants with less frequency. Thus, it is indispensable to select transformed cells under cultural conditions itself. Selection is based on the deployment of some selectable marker genes, which accompany genes of interest in expression vectors.

Plant cells that are highly sensitive to various toxic chemicals are included in culture medium. Thus, only transformed cells are able to survive and regenerate into plants due to the expression of toxic resistant selectable marker genes. In contrast, non-transformed cells in absence of marker genes get killed by toxic chemicals supplemented in the culture medium.

Some of the toxic substances included in the toxic media are antibiotics, herbicides and antimetabolic compounds. Utility of selectable marker genes such as antibiotic resistance genes, herbicide tolerance genes and several antimetabolite genes are in common practice.

Antibiotic Resistance Genes as Markers:

Antibiotic resistance genes used as selectable markers are basically microbial in origin. E.coli bacterium contributes many of the antibiotic genes for selection in plants. Antibiotics are toxic to plants by inhibiting protein synthesis particularly in the cell organelle like chloroplast. Following are some of antibiotic resistance genes used to select transformed plants.

Neomycin Phosphotransferase:

Popularly known as NPT II is the most widely chosen antibiotic resistance gene in plant transformation strategy. It confers resistance to the antibiotic kanamycin. Neomycin phspho-transferase is a derivative of Tn5 transposon.

Expression of NPT II results in the degradation of variety of antibiotics including kanamycin, neomycin and puromycin. In transformation strategy, the concentration of kanamycin is adjusted between 0.1 and 0.5 percent to facilitate screening efficiency. Higy level resistance to kanamycin was noticed in cereal members is one of the significances of NPT.

Spectinomycin Phosphotransferase (spt):

Transposon like Tn5 contributes to antibiotic resistant gene spt. Similarly, aminoglucoside adenyl transferase (ada) is also being used to confer resistance to both streptomycin and spectinomycin. Transformed cells are able to differentiate based on its colour, i.e., transformed cells are visible in green colour in contrast to bleached non transformed cells.

Hygromycin Phosphotransferase:

Usage of hygromycin phosphotransferase gene (hpt) is the direct result of the development of alternative selective selectable marker in plant transformation. Hygromycin phosphotransferase is more powerful than kanamycin and it can kill non transformed cells by blocking protein synthesis during selection process.

Gentamycin Acetyl Transferase:

Expression of amino glucoside N-acetyl transferase in transformed cells is able to degrade gentamycin antibiotic when included in the medium. Non transformed cells are highly sensitive to the entry of this antibiotic in the cells.

Herbicide Resistance Marker Genes:

Enolpyruvyl Shikimate Phosphate Synthase (EPSP):

In plants, the enzyme enolpyruvyl shikimate phosphate synthase is indispensable for biosynthesis of aromatic aminoacids like tyrosine, tryptophan, etc. Several potential herbicides of various actions are employed to destruct weeds. Of which glyphosate, a potential herbicide, is widely used to kill weed plants by inhibiting the enzyme EPSP synthase, and thereby blocking aromatic aminoacid production.

In transformation selection technique, using herbicide resistant marker gene is based on the introduction of mutant EPSP synthase gene. Expression of mutant EPSP synthase gene in transformed tissues is able to survive glyphosate herbicide included in the culture medium. In contrast, non-transformed cells get killed by inhibition of EPSP synthase enzyme by herbicide.

Bar Gene:

Bar gene encodes phosphinothricin acetyl transferase, which inactivates herbicides like biolophos. This is based on damaging of non-transformed tissues by including biolophos herbicide in the culture medium. Biolophos can kill plant cells by inactivating glutamine synthase, which is required to regulate nitrogen metabolism by delivering NH_4 into aminoacid production.

Accumulation of NH_4 is highly toxic to the cell. In the selection process, expression of biolophos resistant bar gene encodes an enzyme which can able to degrade and survived by avoiding accumulation of NH_4 compound, whereas non transformed cells accumulate NH_4 due to the inactivation of glutamine synthase by biolophos.

Bromoxynil Nitrilase (bxn):

Expression of bacterial based marker gene bxn in plant tissue can alter the structure of herbicide bromoxynil. This potential herbicide is able to kill cells (non-transformed) by interfering photosynthetic machinery. Therefore, selection is based on the herbicide resistant enzyme in transformed cells, able to survive and continue to grow in presence of bromoxynil in the culture medium.

D-aminoacid Oxidase (DAAO):

This marker gene, encoding D-aminoacid oxidase can be used for either positive or negative selection of transformed cell or tissue. Positive or negative selection is based on the type of aminoacid used.

Plants have restricted capacity for D-aminoacid metabolism. DAAO catalyzes the oxidative deamination of a range of D-aminoacids. Selection is actually based on differences in the toxicity of different D-aminoacids and their metabolites for plants, for example, D-alanine and D-serine are toxic to plants, but are metabolized by DAAO into non-toxic products, whereas D-isoleucine and D-valine are less toxic but they are degraded by DAAO enzyme into highly toxic products like keto acids, 3-methyl-2-oxopentanoate and 3-methyl-2-oxobutanoate.

Hence, both positive and negative selection can be accomplished by deploying DAAO gene derived from yeast into the plant as effective marker gene. The use of antibiotic and herbicide resistant marker gene is a matter of concern. Therefore, DAAO has potential to provide safe marker genes in selection strategy of transformed plants.

Reporter Genes:

Popularly known as scorable genes which makes plant selection process easier due to their simple assays. Various reporter genes can be employed by assessing promoter activity or scored indicators of transformation.

β-glucoronidase:

The most widely used reporter gene currently in plant transformation is glucoronidase commonly known as GUS. Expression of GUS gene can be analyzed by immersing the tissue in a solution containing the substrate known as X-glue (5-bromo-4-chloro-3-indolyl-β-D-glucoronic acid). Incubation period is upto 24 h. GUS activity is restricted to localized region in the tissue and it can be visualized as blue spots.

Clarity of GUS visualization can be achieved by treating the tissue with 70-100% alcohol. Transferring into alcohol removes chlorophyll pigments. Green coloured chlorophyll pigments over mask blue spots within the tissue. One of the serious constraints associated with GUS assay is the tissue death. Quantification of GUS activity is also possible with fluorometric assay using 4-methyl umbelliferyl β-D, glucoronide (MUG).

Chloramphenicol Acetyl Transferase (CAT):

Screening with CAT requires labeling of substrates. Chloramphenicol acetyl transferase was the first bacterial gene to be expressed as reporter gene in bacteria. CAT is very sensitive and requires radioactive assay. Screening is done using labeled chloramphenicol and acetylCoA, and these get converted into acetyl chloramphenicol. The enzymatic product can be detected by autoradiography.

Opine Synthase:

The gene for opine synthase is present in Ti plasmid. Both octopine synthase and nopaline synthase genes are employed as reporter genes. Screening is based on the synthesis and accumulation of opine class of aminoacids in transformed tissues.

Their enzyme assay is performed by isolating the protein from the transformed tissue and then provided precursors like arginine, pyruvate and NADH for octopine synthase and ketoglutarate, arginine and NADH for nopaline synthase. Accumulation of opine is detected by various methods.

Luciferase:

The fire fly based luciferase gene is used as non-toxic reporter gene. Selection of non-toxic luciferase gene has advantage over other destructive screening methods. The transformed tissue is treated with luciferin (substrate) solution in presence of ATP. Oxidation of luciferin results in the emission of light which indicated transformed status of the cell.

This can be documented by using either X-ray film or specialized light-sensitive cameras. In addition to fire fly luciferase, some bacterial luciferase has also been used. They catalyze oxidation of long-chain fatty aldehydes resulting in rapid emission of light in transformed cells.

Green Fluorescent Proteins (GFP):

Green fluorescent protein gene is widely used as one of the most popular reporter gene in the present status of plant transformation technology. This gene is derived from jelly fish (Acquarea victoria). The selection is based on the bright luminescent nature of GFP proteins in transformed tissues. GFP protein is made up of 238 aminoacids of which three aminoacids like serine, tyrosine and glycine impart chromophore character to the protein.

Several modifications have been done to GFP to improvise or enhance its performance during screening, for example, implication of site directed mutagenesis results in the replacement of threonine for serine at position 65 in the GFP chromophore. This engineered strategy exhibits elevated fluorescent signal making assay more efficient.