CHAPTER TWO
REVIEW OF LITERATURE

The present review includes the maize importance, tissue culture, growth regulators for callus induction and plant regeneration from maize embryo, role of tissue culture and modern genetics as Biolistic in transfer Anthranilate synthase gene, by particle bombardment to maize callus for tryptophan increasing

2.1. Maize importance as Genetics tool

Maize (also known as corn) is a domesticated cereal grain that has been grown as food and animal feed for tens of thousands of years. It is currently the most widely grown crop in the world, and is used not only for food/feed but also to produce ethanol, industrial starches and oils. Maize is now at the beginning of a new agricultural revolution, where the grains are used as factories to synthesize high-value molecules (**Shaista *et al.* 2011**).

Maize is one of the most important crops around the world because of its importance as food and feed in man life; thus, breeding technology in this crop has been the subject of intense efforts high-value products from transgenic maize using in several biotechnology approaches applied mainly in order to incorporate desiderable traits on several maize lines (**Valdez-Ortize *et al.* 2007)**

Maize (*Zea mays* L.) is the third most planted cereal crop after wheat and rice worldwide. Globally it is top ranking cereal in terms of productivity and has worldwide significance as human food, animal feed and fodder as well as source of large number of industrial products. It is used as a raw material for manufacture of large number of industrial products like corn starch and starch-based products, and in fermentation and distillation industries (**Wada *et al.* 2008**).

Maize grain is used as feedstock for many large volume industrial products (e.g. ethanol, biodiesel, poly-lactic acid, sweeteners) and has been demonstrated to be an effective expression system for functional proteins of rokaryotic (**Witcher *et al.* 1998; Streatfield *et al.* 2001; Chikwamba *et al.* 2002a; Bailey *et al.* 2004); viral (Streatfield *et al.* 2001) and** eukaryotic **(Hood *et al.* 1997; Zhong *et al.* 1999; Yang *et al.* 2002).**

Maize is one of the main food crops worldwide, with a global production of 794.05 million tons in 2009/2010 period (**FAO, 2009**). It is also an important monocot plant model in genetics, genomics and molecular biology studies (**Vega *et al.* 2008)**. The aim of plant biotechnology is to improve agronomical, medical and industrial applications of crops so as to provide better nutritional qualities for animal feed, healthier and more nutritionally enriched foods, specially chemical and biological compounds, and to improve the processing capabilities (**Shoemaker *et al.*, 2001**).

2.2. Tissue culture technique in Maize

The history of maize tissue culture can be traced back to the early 1930's, when **Lampe and Mills (1933)** cultured young endosperm tissue as well as embryos on a medium supplemented with potato extract but observed only limited proliferation. The first continuously growing long-term tissue cultures of maize, initiated from immature endosperm, were obtained by **Larue (1947, 1949),** followed by more detailed studies on the physiology, morphology and cytology of endosperm cultures by others (**Straus and Larue, 1954; Straus, 1954, 1960; Tabata and Motoyoshi, 1965; Shannon and Batey, 1973**).

Mature as well as immature embryos of maize were also cultured in order to determine their nutritional requirements for growth and development **(Larue, 1936, 1952; Haagen-Smit *et al.*, 1945; Green *et al.*, 1974)**.

None of these early studies were aimed at regenerating plants and none were recovered. Microprojectile bombardment system (MPS) proved to be a powerful technique for genetic modification of maize lines by delivering foreign DNA into scutellar tissues of immature embryos, reviewed by **O'Kennedy *et al.*, (2001)** and **O'Connor-Sa´nchez *et al.*, (2002).**

Tissue-culture techniques are part of a large group of strategies and technologies, ranging through molecular genetics, recombinant DNA studies, genome characterization, gene-transfer techniques, aseptic growth of cells, tissues, organs, and *in vitro* regeneration of plants that are considered to be plant biotechnologies.

The use of the term biotechnology has become widespread recently but, in its most restricted sense, it refers to the molecular techniques used to modify the genetic composition of a host plant, i.e. genetic engineering. In its broadest sense, biotechnology can be described as the use of living organisms or biological processes to produce substances or processes useful to mankind and, in this sense; it is far from new (**Zhong *et al.* 1995**).

Biotechnology can also be inserted into the industry and directly affect the economy of a sector. An outstanding example of this is that biotechnology may be applied to dramatically decrease costs in corn ethanol production and improve energy input requirements. There are numerous opportunities to improve important characteristics of the corn plant to decrease the cost of ethanol production **Kemble *et al.*, (2006)**.

More than 50 different plant species have already been genetically modified, either by vector-dependent (e.g. *Agrobacterium)* or vector-independent (e.g. biolistic, micro-injection and liposome) methods (**Sasson 1993 and Anon 1994**). In almost all cases, some type of tissue culture technology has been used to recover the modified cells or tissues. In fact, tissue-culture techniques have played a major role in the development of plant genetic engineering. For example, four of the seven papers listed by **Davis and Reznikov (1992)** as classic milestones in plant biotechnology used a range of protoplast, microspore, tissue and organ culture protocols.

Tissue culture will continue to play a key role in the genetic engineering process for the foreseeable future, especially in efficient gene transfer and transgenic plant recovery (**Hinchee *et al.* 1994**).

The most common reason for post-zygotic failure of wide hybridization is embryo abortion due to poor endosperm development. Embryo culture has been successful in overcoming this major barrier as well as solving the problems of low seed set, seed dormancy, slow seed germination, inducing embryo growth in the absence of a symbiotic partner, and the production of monoploids of barley (**Raghavan 1980, 1994; Yeung *et al.* 1981; Collins and Grosser 1984; Zenkteler 1990**).

Genetic improvement of cereals has been a major focus of plant breeding efforts during the past 50 year, resulting in remarkable increases in the yield and improvement in the quality of this important group of food crops. The authors provided that, modern plant biotechnology had novel means for crop improvement through the integration and expression of defined foreign genes into plant cells, which can then be grown *in vitro* to regenerate whole plants. The efficient regeneration of normal and fertile plants from single cells, a basic prerequisite for the production of genetically transformed plants, proved to be rather difficult for gramineous species because of their extreme recalcitrance to manipulation *in vitro.* **Zhang *et al.* (2002)**

The diverse uses of maize reflect its long history as a domesticated crop and its wide gene pool (**Gewin, 2003**). Interest in maize as a platform for high-value products reflects a number of unique advantages over other plants (**Ramessar *et al.* 2008a**). These include its GRAS (Generally Regarded As Safe) status, the established agricultural infrastructure, its well characterized genetic properties, its amenability to *in vitro* manipulation and gene transfer,

and its efficient biomass production resulting from the C4 photosynthetic pathway.

More specific for the production of high value recombinant proteins, maize seeds are excellent production vehicles because of their large size compared to other cereals, with 82% of the seed made up of endosperm (**Watson, 1987**). Furthermore, the mature maize seed is desiccated lacks active proteases and contains a rich mix of molecular chaperones and disulfide isomerases, helping to ensure correct protein folding, assembly and enhanced stability. Maize endosperm tissue can perform post-translational modifications (e.g. glycosylation) that occur in animal cells, thus ensuring that heterologous proteins expressed in seeds retain their biological activity. The stability of recombinant proteins produced in maize seeds is favored because the maize endosperm is a natural protein storage organ, thus proteins in dry maize seed remain stable for more than 6 years at ambient temperatures, and for months in cracked and flaked maize seeds stored at up to 10 °C.

Many researchers such as **Green and Phillips (1975); Armstrong and Green (1985); Hodges *et al.* (1986); Lee and Phillips (1987) and Shillito *et al.* (1989)** suggested that, the regeneration ability of any plant is influenced by different factors. They showed that the type of explant is considered one of the main factors that attracted the concern of many investigators. Also, immature embryos have been the most widely used explant in many cereals, including maize.

In the previous research on tissue culture in maize immature embryos **Armstrong and Green, (1985)** detected two different types of embryogenic callus i.e. Type I, a compact organized and slow-growing callus and Type II, a soft, friable and fast-growing one characterized by its high regeneration capacity. Those results showed that, production of Type II callus arises at low frequency and only for specific genotypes. Also, regenerability is influenced, to a great extent, by the media composition (**Vain *et al.* 1989a, b, Songstad *et al.* 1991, Bohorova *et al.* 1995 and Carvalho *et al.* 1997**).

Bohorova *et al.* (1995) discussed the effect of the genotype on somatic embryogenesis. They detect the genetic background or genotype is main factor of the explants which was found to influence the regeneration potentiality. These results also reports by **Tomes and Smith (1985)** and **Hodges *et al.* (1986)**, they indicated that the regeneration was genetically controlled by

nuclear genes in maize. Moreover, studies by **Willman *et al.* (1989)** suggested that at least one gene or a block of genes controlled the expression of somatic embryogenesis of maize tissue cultures.

So far almost all maize tissue culture and transformation involves the use of immature zygotic embryos as an explants source for regeneration (**Danson *et al.*, 2006; El-Itriby *et al.*, 2003**). However, immature embryos are seasonally available and have strictly limited suitable duration of culture, 14-19 DAP (**Odour *et al.* 2006).**

This imposes tedious routine tissue culture activities within the specified time frame and continuous planting for continuous supply of the immature embryos. In contrast, mature embryos are readily available throughout the year in large quantities. Furthermore despite few reports about the recalcitrance of tropical maize lines and mature embryos for tissue culture work (**Bohorova *et al.*, 1995; Hodages *et al.*,1986**), successful regeneration of temperate maize lines and other cereal food crops from mature embryos has been reported by different authors (**Akula *et al.*,1999; Green and Philips 1974; Ward and Jordan, 2001).**

Green and Phillips (1974) first reported that mature embryos of maize could be used to induce callus but no plantlets

Wang (1987) successfully regenerated plants from mature embryos of two maize inbreeds, B73 and Mo17, but the regeneration was genotype dependent and the frequency was only 4 to 5%. **Huang and Wei (2004)** reported regeneration of temperate maize lines from mature embryos at a frequency ranging from 19.85 to 32.4%.

Most recently **Al-Abed *et al.* (2006)** reported more efficient regeneration system for two hybrid and two inbred temperate maize lines using split mature seeds as an explant. Identification of genotypes that respond well to embryogenic callus induction and plant regeneration is a necessary initial step for their successful genetic transformation.

Immature embryos have been the most widely used explants for initiation of regenerable tissue cultures (**Armstrong and Green, 1985; Phillips *et al.*, 1988**). Immature embryos can initiate two types of callus cultures from their scutella surfaces: Type I and type II callus. Type I is compact and organogenic and easily obtained from immature embryos. On the other hand type II is friable and embryogenic and is initiated at a lower frequency than type I (1974) first

reported that mature embryos of maize could be used to induce callus but no plantlets

Carvalho *et al.* (1997) indicated that only a few tropical genotypes have been shown to be capable of initiating type II callus **(Oduor *et al.*, 2006; Carvalho *et al.*, 1997)**. Type II callus has been found to be more regenerable than type I **(Armstrong and Green, 1985).**

Maize plant regeneration can take place through two avenues, that is, organogenesis or somatic embryogenesis. Somatic embryogenesis is the most common avenue of plant regeneration **(Odour *et al.* 2006).** With the rapid development of tissue culture techniques, many types of explants, including gametic embryo and leaf tissue had been successfully regenerated into plants by tissue culture **(Aulinger *et al.* 2003, Huang and Wei 2004 and Ahamadabadi *et al.* 2007).** But at present, the most popular is still immature zyotic embryo in maize transformation, owing to simple inoculation operation and facile callus induction **(Binott *et al.* 2008).**

Thus, screening of genotypes for *in vitro* plant regeneration is always a very important research task. Regarding the optimization of media composition, there have been many elite media compositions in literatures **(Du *et al.*, 2007; Binott *et al.*, 2008; Zhang *et al.*, 2008),** but to be mentioned, any media containing specific reagents was only suitable for limited materials. Thus, to develop a new media for given materials is always necessary and significant.

Tissue and cell culture systems are vital to many areas of plant science and crop improvement, particularly in mutant selection and plant transformation (**Phillips, 2004**).

The ability to regenerate shoots from callus and cells is essential for establishing a successful plant culture system. However, because of restrictions in genotype and culture conditions, not all plant species or varieties can be regenerated easily (**Huang and Wei 2004**).

Furini and Jewell (1994) suggested that callus obtained from immature embryos in presence of dicamba developed into somatic embryos than the callus obtained with 2,4-D.

Huang and Wei (2004) mentioned the role of 2,4-D with MS media in inducing highly regenerable calli from mature embryos. Many published reports are available in maize suggesting successful regeneration from mature embryos,

split seeds **(Al-Abed *et al.* 2006)** other than use of immature embryos as explant **(Furini and Jewell 1994 and Bohorova *et al.*1995).**

Genotypes are reported to play an important role in callusing response in various crop plants including maize (**Aguado-Santacruz *et al.*2007). Bohorova *et al.* (1995)** suggested that N6 medium, which contained lower level of nitrogen than that of MS, showed better callus induction and maintenance.

Rooz (2002) reported good regeneration in calli derived from MS with 2,4-D and regeneration in RM1 (refer materials and methods).

Bohorova *et al.* (1995) studied genotype dependent regeneration response among tropical and sub-tropical maize lines. Genotype dependent regeneration response has also been reported by various authors **(Wenbin *et al.*2002; Aguado-Santacruz *et al.* 2007).** Genotypic differences in terms of regeneration response might be related to variations in endogenouse hormone levels **(Bhaskaran and Smith 1990).**

However, because profound differences exist in the potential of maize lines for *in vitro* culture **(Armstrong and Green,1985)** with only a small number of maize genotypes possessing regenerative capacity , it is important to analyze the response of particular maize materials to *in vitro* culture to define the specific growth conditions required for generating the totipotent material across which to exploit the potential tools of the *in vitro* technology, such as genetic transformation, somaclonal variants recovery, somatic hybridization, and molecular farming, among others.

Since the early tissue culture studies in maize were first reported by **Green and Philips (1975)**, immature zygotic embryo have become the explant of choice in cereals **(EI-ltriby *et al.* 2003, Ward and Jordan, 2001 and Oudor *et al.* 2006).**

Auxins, especially 2, 4-D in the range of 1-3 mg/l are essential for the formation of embryogenic callus from cereal embryos **(Bi *et al.*, 2007, Danson *et al.* 2006, El-ltriby *et al.* 2003 and Odour *et al.* 2006).**

Media composition is one of the most important factors affecting maize tissue culture **(Frame *et al.* 2006 and Binott *et al.* 2008).** Somatic embryogenesis from immature embryos in temperate maize tissue cultures occurs from scutellum cells **(Lu *et al.*1982).**

Hanaiya *et al.*, (2003) developed a regeneration system for elite Egyptian maize inbred lines using immature embryos as explants. They reported that, this

system proved to be highly genotype-dependent. Their results indicated that, line Gz 643 was identified as the best line, revealing the highest regeneration frequency (42.2%). Addition of L-proline and silver nitrate to culture media greatly enhanced the formation of embryogenic type II callus and the regenerability of some of the tested lines. Transform ation of the scutellar tissue of immature embryos from inbred line Gz 643 was performed with the particle delivery system using a single plasmid carrying both the GUS and Bar genes (pAB-6) or by co-transformation with two plasmids, pAct1-F (GUS) and pTW-a (Bar). They evaluated different transformation parameters, i.e. osmotic treatment, acceleration pressure, and number of shots. Their results clearly showed that osmotic treatment of 0.25M sorbitol and 0.25M of mannitol along with the use of either acceleration pressure 1300 psi and one shot per plate (for co-transformation with pAB-6) or 1100 psi and two shots per plate (for transformation with pAct1-F and pTW-a) gave the best results, as expressed by the number of blue spots in the b-glucuronidase (GUS) assay.

2.3. Tryptophan amino acid

In plants, the tryptophan (Trp) biosynthetic pathway provides not only the amino acid Trp for protein synthesis, but also a wide array of important secondary metabolites, including the growth regulator IAA (**Wright *et al.* (1991) and Normanly *et al.* (1993**), antimicrobial phytoalexins **Tsuji, *et al.* (1993)**, and other indolic molecules that influence plant/microbe and plant/animal interactions. Since these secondary metabolites are not produced in microorganisms, the control of Trp gene expression in plants is likely to be more complex than that in microorganisms. *Anthranilate synthase* (AS) catalyzes the conversion of chorismate into anthranilate, the first reaction leading from the common aromatic amino acid (shikimic acid) pathway towards the biosynthesis of Trp in both microorganisms and plants, and is feedba ck inhibited by the end product, Trp.

Tryptophan (Trp) is an essential amino acid for animal growth, and cereal crops such as wheat, maize and rice exhibits relatively low content of Trp in seed proteins. In higher plants, the Trp biosynthetic pathway provides an amino acid for protein synthesis as well as precursors for secondary metabolites such as the phytohormone indole-3-acetic acid and phytoalexins as reported by **Normanly, *et al.* (1995), Radwanski and Last (1995) and Tsuji *et al.* (1993)**.

Tryptophan is utilized in microorganisms and in plants as a substrate for protein biosynthesis; however, in some plants it also has an important role as a precursor of secondary metabolites such as the endogenous auxin indoleacetic acid and other molecules which help to protect the plant against pathogens and herbivores. Synthesis of tryptophan from chorismate involves five reactions and the organization of genes encoding for the necessary enzymes varies considerably among prokaryotes. While *Escherichia coli* contains five genes in a single operon (**Yanofsky, *et al* (1981**), *Bacillus subtilis* has six in a single operon **Henner, *et al.* (1984)** and Acinobacter calcoaceticus contains seven genes in three un linked clusters **Sawula, and Crawford, (1972).**

Anthranilate synthase (EC 4.1.3.27) catalyses the first committed step in the sequence of reactions which lead to the biosynthesis of tryptophan from chorismate. In almost all microbial species, *anthranilate synthase* (AS) is an oligomer of no identical subunits designated AS α- subunit (ASI or component I) and AS β-subunit (ASII or component II). In some organisms the subunits are

a associated to give an αβdimer and in others an $\alpha_2\beta_2$ tetramer **Kawamura, *et al.* (1978)**

The ASα subunit is encoded by the genes trpE in prokaryote (bacteria and blue-green algae) and, TRP2 and ASA1/ASA2 in eukaryote (fungi and plants, respectively), but since theβ subunit of the AS enzyme complex sometimes contains other enzymes of tryptophan bio-synthesis, other genes, besides trpG (prokaryote) and TRP3 and ASB (eukaryote), can encode the multifunctional subunit **H/itter, *etal* (1986)**

In plants although the enzyme has two subunits, the enzyme complex does not contain other functionalities. The genes for these subunits have been isolated from *Arabidopsis thaliana* **Niyogi *et al.* (1992) and Niyogi, *et al.* (1993)**. AS iso enzymes have been detected in cell cultures of tobacco and potato, whereas in microorganisms only the cyanobacterium

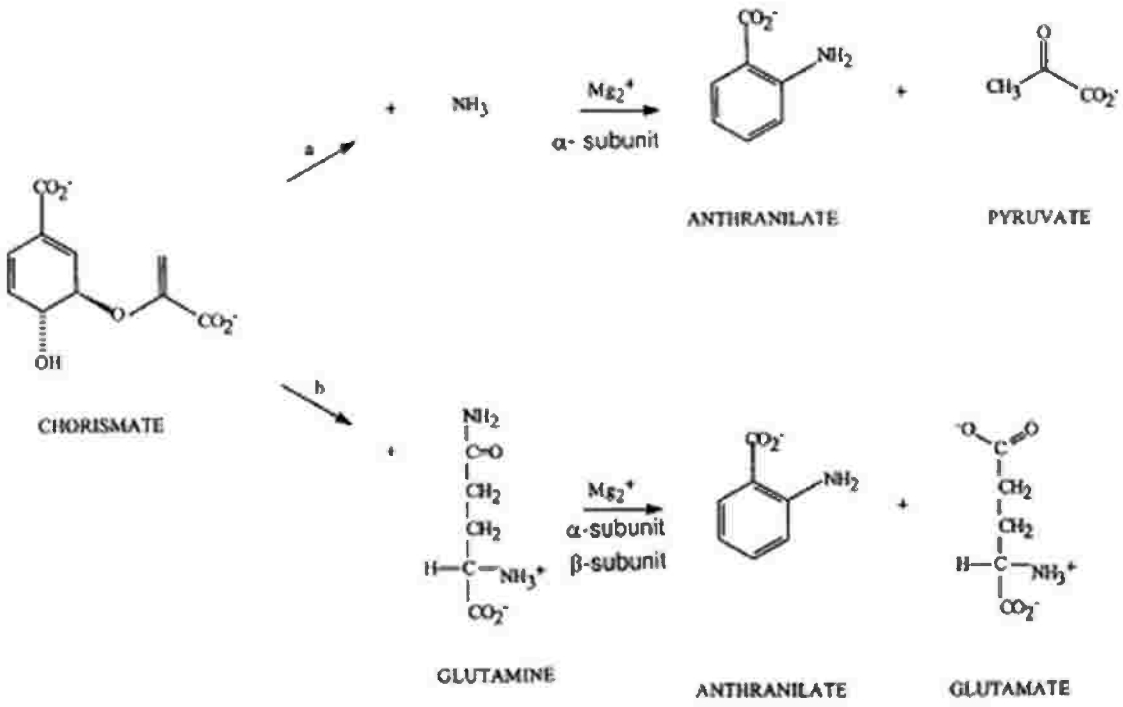

Figure (1) formation of *anthranilate* from chorismate

2.4. Molecular genetics and Particle bombardment

In the 1980s, *Agrobacterium*-mediated gene transfer emerged as the most straightforward way to generate transgenic dicotyledonous plants, but few monocots were amenable to this process. Efforts to transform maize therefore began with physical delivery systems targeting protoplasts **Fromm *et al.*, (1985) and Klein *et al.*, (1988).**

The first transgenic maize plants produced by protoplast transformation were sterile **Rhodes *et al.*, (1988)** but fertile transgenic plants were eventually generated by bombarding suspension cell cultures from inbred line A188 with DNA-coated metal particles **Gordon-Kamm *et al.*, (1990).**

In 1996, fertile transgenic plants from the same inbred line were produced by *Agrobacterium-mediated* transformation using a strain carrying a "super binary" vector system **Ishida *et al.*, (1996).**

Additional methods that have been used to generate transgenic maizeplants include PEG-mediated protoplast transformation **Golovkin *et al.*, (1993**), electroporation of immature embryos or embryonic callus cultures **D'Halluin *et al.*, (1992**) and the transformation of embryogenic cell suspensions **Frame *et al.*, (1994**) or callus cultures **Petolino *et al.*, (2000**) using silicon carbide whiskers-mediated transformation and particle bombardment remain the most widely used methods for transformation.

In humans, eight of the twenty standard amino acids are described as essential because they cannot be synthesized and must be obtained from the diet. However, most staple food crops are deficient for certain essential amino acids, with the levels of lysine and tryptophan being particularly low in maize (**Galili and Höfgen, 2002; Huang *et al.*, 2006**).

Thus tryptophan and lysine deficiency are prevalent in developing countries where maize is the sole or predominant source of food. The naturally occurring opaque-2 mutant has higher lysine and tryptophan levels than wild type maize but has a soft, chalky kernel which is unsatisfactory for cooking (**Mertz *et al.*, 1964**).

The higher content of essential amino acids in the mutant results from a reduced level of certain storage proteins (e.g. α- and β-zeins) allowing other proteins to replace them and increase the level of lysine and tryptophan. Breeding programs have resulted in the development of Quality Protein Maize

(QPM) which benefits from the higher lysine and tryptophan levels of the opaque-2 mutant but combines this with a hard kernel with superior cooking qualities (**Prasanna *et al.*, 2001**). Further investigation has shown that although the QPM has lower levels of α- and β-zeins compared to wild type maize, it has higher levels of the γ-zein.

Zarkadas *et al.* (2000) showed that high lysine QPM may provide up to 73% of human protein requirements, compared to 28–50% for common maize. The laborious breeding program that gave rise to QPM can be replicated much more rapidly with RNAi.

Segal *et al.* (2003) transformed maize with an RNAi construct resulting in a phenocopy of the opaque-2 phenotype. Transgenic seeds contained less α-zein than normal (and lower levels of leucine, alanine and glutamine) but higher levels of lysine. Recently, **Huang *et al.* (2006)** obtained similar results by transforming maize with constructs expressing chimeric double-stranded RNA.

Two transgenic strategies have been reported in the literature to increase the content of essential amino acids in maize. The first involves increasing the free amino acid pools through manipulation of the corresponding biosynthetic pathways, although as a sole approach this can result in the amino acids leaching during food processing and cooking.

The second is to enhance the level of proteins containing essential amino acids either by modifying the sequence of seed storage protein genes to incorporate more lysine and tryptophan codons, or by importing heterologous or completely synthetic proteins containing tryptophan and lysine and expressing such proteins in the seeds. Although leaching is not a problem with this strategy, high lysine/tryptophan proteins will not accumulate if the amino acid pools are depleted, so the best overall approach is to enhance the free amino acid pools and express proteins with high essential amino acid contents **Wang, *et al.* (2008)**.

More and more attention has been paid on study of maize tissue culture and great improvement has been made in the recent years. Green and Philips firstly reported callus induction from maize immature embryos and successfully obtained diploid regeneration plant. Afterwards, rapid progress has been made on maize tissue culture. Nowadays, maize immature embryos are used as explants more and more in maize tissue culture relative to other parts of maize, **Niu, *et al.* (2009), and Liang, *et al.* (2010)**.

However, it has strong restriction of geography condition, growth period and development season when immature embryos used as explants for getting experiment materials. Immature embryos must be utilized during 9-12 days after pollination. Comparative to this, mature embryos are satisfactory explants for adapting maize genetic transformation because of that maize seeds are easily stored in large normal and can be conveniently obtained without time and quantity restriction.

A breakthrough is being on in the study of maize tissue culture with mature embryos used as explants. More and more papers reported tissue culture success of maize mature embryos. So the recent progress of maize tissue culture with mature embryos used as explants is reviewed in this review.

In the year of **2007, Xiang *et al.*** investigated some factors affecting maize mature embryos regeneration used maize elite in-bred line 178 as materials. The results showed that medium including 4. 0 mg/L 2, 4- D was capable to producing primary callus. Among different concentrations of phytohormone, 2. 0 mg/L 2, 4-D in combination of 0. 2 mg/L BA and 10 mg/L silver nitrate produced the best results, which promoted the formation of embryogenic callus in the subculture medium. And 0.5 mg/L 6-BA had significantly increased the frequency of plant regeneration in the regeneration medium. The results showed maize elite inbred lines 178 had been successfully regenerated and the frequencies were 78 %.

Xiang *et al.* (2007) considered that an efficient maize regeneration system was developed using mature embryos. Using this system, the plantlets were regenerated from maize elite inbred lines 178, providing a powerful basis for genetic transformation of maize. Afterwards, **Wang *et al.* (2008)** established an efficient transgenic acceptor system and developed some new methods on maize (*Zea mays* L.) tissue culture, plant regeneration and genetic transformation with embryogenic callus initiated from mature embryos of three elite inbred lines CML295, CML304, and 18-599R.

Tissue slice showed that the structure of callus formed from mature embryos was the same as those from immature embryos, being the type II embryogenic callus. The regeneration frequencies of the calli from mature embryos of CML295, CML304, and 18-599R were 68.6%, 75.4%, and 84.8%, respectively. The transgenic rates of embryogenic callus from mature embryos of the three inbred lines were similar to those from immature embryos. Wang's

conclusion is that using embryogenic callus from mature embryos as transgenic acceptors is efficient and available.

King *et al.* (1978) reported an important requirement for new approaches towards plant somatic cell genetics is the successful handling of cell cultures in vitro, the basic sources of cell suspension are callus cultures composed of dedifferentiated, UN organized and actively dividing cells. On the other hand, **Aitchison *et al.* (1977), and Noguchi *et al.* (1977)** reported, there are numerous reports of different types of variation which occurs during callus culture. From tissue of the same origin it is possible to obtain separate callus lines differing in morphology, pig- mentation or growth rate. Thus, attempts were undertaken to derive uniform and friable lines from maize callus.

Regeneration of plants from tissue cultures initiated from immature embryos and other explants of cereal species was first described during the 1960's and 1970's (**Vasil, 1986**). These included plant regeneration from cultured immature embryos and mesocotyl tissue of maize (**Green and Phillips, 1975; Harms *et al.*, 1976**).

In all instances-including maize (Springer *et al.*, 1979) plant regeneration was found to take place by the *de novo* formation of shoot meristems or by microtillering caused by the derepression of shoot primordia already present in the explants (**Dunstan *et al.*, 1979; Nakano and Meda, 1979**).

The cultures often were short-lived and could not be maintained for more than a few subculture cycles, the number of plants produced was small, and the regenerative ability was limited to specific genotypes (often to a single genotype, such as the inbred A188 in maize) and lost rapidly.

Plants regenerated from well-defined and stringently maintained embryogenic cultures of maize are predominantly normal with 2n=20 chromosomes. Nevertheless, instances of genetic variation in the regenerated plants have been reported **Edallo *et al.*, (1981); Mccoy and Phillips, (1982); Beckert *et al.*, (1983); Benzion *et al.*, (1986); Lee and phillips, (1987**). These are likely the result of mixed cultures that contain sectors of embryogenic as well as non embryogenic cells, and non-embryogenic multicellular origin of some the regenerants as evidenced by their chimeric nature. Furthermore, in many cases the observed variability is physiological and transient in nature as it disappears with time or with hormonal treatments, and is not genetic as it is completely eliminated after a sexual cycle.

Larkin and Scowcroft (1981) proposed the concept of somaclonal variation, which stated that plants derived from tissue cultures, including those of maize, exhibit novel and useful genetic variation that could be used for the genetic improvement of plants. After scores of studies over nearly two decades it became clear that much of the observed variation was neither novel nor useful. To date, no important new maize germplasm has been developed as a consequence of somaclonal variation, and this is no more an active field of research. Among the biotechnology tools, those related to transferring DNA have received special attention, leading to several strategies such as Biolistic or *Agrobacterium tumefaciens* **Gordon-Kamm W. J *et al.* (1990).**

Particle bombardment and *Agrobacterium* mediated transformation are two popular methods currently used for producing transgenic cereals the application of *Agrobacterium* mediated transformation to monocotyledonous species, including rice and maize, has been recently reported **Shouh *et al.* (2004)**. The main characteristics of the *Agrobacterium* system in these species are the high frequency of transformation; proper integration of the foreign gene into the host genome and low copy number of the gene inserted, resulting in most cases in a correct expression of the transgene itself.

There has been a rapid increase within the last twenty years in the number of researchers involved in the aseptic culture of plant cells, tissues, and organs; that is, "plant tissue culture". The reasons are twofold: (1) important technical developments have allowed for greater reproducibility of experimental results, and (2) numerous investigations have demonstrated the utility of these techniques in the study of many biological questions especially in genetics, physiology, and development **Chaleff and Carlson (1974); Day (1977); Murashige (1978); Smith (1974); Sprague *et al.* (1980) and Zenk(1978)**.

Genetic breeding has been decisive and essential to transform maize a major food crop worldwide. Biotechnology plays an important role in plant genetic breeding, particularly for the introduction of novel traits in order to improve agronomic performance, medical and industrial applications and food quality. In recent years, the development of efficient plant regeneration systems in cereal crops and the field of biotechnology have opened up new opportunities for genetic transformation of crop plants **Nickell and Torrey (1969)**.

Some monocot plants were initially considered difficult for genetic engineering, primarily due to their recalcitrance to *in vitro* regeneration and their resistance to *Agrobacterium.* Continuous efforts and studies of different

tissues for regeneration potential, development of various DNA delivery methods, and optimization of gene expression cassettes have led to the development of reliable transformation protocols for major cereals, including maize. Consequently, this research group has focused its attention on maize transformation mediated by microprojectile bombardment as a device of DNA delivery into maize cells. This method offers a rapid and simple way of introgression of candidate genes into cells. However, there are some points that still need to be studied and improved in order to achieve appropriate transformation efficiency to optimize the processing conditions to obtain fertile plants **Cecilia *et al.* (2010)**

About 20 years have elapsed since the initiation of the first experiments which led to the production of fertile transgenic maize plants. The commercial application of agricultural biotechnology in corn has primarily focused on the development of input traits that can provide attributes beneficial to the grower. However, in order to obtain high-quality and high-frequency transformation systems, transformation techniques need to be optimized frequently. Particle bombardment offers a rapid method for DNA delivery into plant cells **Rasco-Gaunt *et al.*, (1999)**.

Particle acceleration can be achieved through High Pressure Gene Gun (HPGG) or Low (LPGG) helium pressure gene guns **Li *et al.*, (2003); Fadeev *et al.*, (2005)**. Several factors affect the transformation efficiency of gene guns **Décima *et al.*, (2010)**, and establishing changes in the transformation protocols is critical in order to obtain an adequate technique that fits with the lab resources and difficulties.

The plant transformation technique using the helium pressure gun involves inert particles such as gold or tungsten coated with DNA. The particles go through the plant cell membranes to reach the nucleus and then integrated into the plant genomic DNA. The transformation efficiency depends on several variables: the explant genotype, the helium pressure, the particle size, the *in vitro* culture capacity and explants regeneration, the plant adaptation to *ex vitro* conditions and the seed production capacity **Zhang *et al.*, (2007)**.

Immature embryos have been extensively used for maize regeneration and transformation **Green and Phillips, (1975)**. This ability of immature embryos to produce embryogenic calluses makes them the most suitable primary explants for genetic transformation of maize **Gordon-Kamm, (2002)**. Nevertheless, the chance of using this kind of explants depends on the

availability of embryos from plants growing in the greenhouse or the field. In certain working conditions this could be a critical point.

Calluses from maize cultures are mainly classified into three types: non-embryogenic, type I embryogenic, and type II embryogenic. Non-embryogenic calluses are watery, usually turn brown and lose their ability to regenerate; this makes them unsuitable for propagation or transformation. Type I embryogenic calluses are usually compact and white, and plants can be regenerated directly by organogenesis. Unfortunately, the usefulness of this type of calluses is diminished as it cannot be maintained for long periods of time **Sairam *et al.*, (2008**). Type II embryogenic calluses are friable, soft and yellowish **Green *et al.*, (1983**), and are the best option for transformation assays.

Genetic transformation of cereals including maize depends largely on the ability of transformed tissues to proliferate in selection medium and subsequently regenerate plants from the transformed and selected cells **Sahrawat *et al.*, (2003**). In fact, the totipotency of plant cells (that is, the ability of a single cell to divide and produce a whole plant) is the basis for the success of most plant transformation systems.

The theoretical framework and experimental basis of modern plant biotechnology was derived from concepts of cellular totipotency. The concept of totipotency is inherent in the cell theory of **Schleiden (1838) and Schwann (1839),** which recognize the cell as the primary unit of all living organisms **Vasil *et al.*, (2008**).

Sanford (2000) reported that, particle bombardment is a commonly used method for genetic transformation of plants and other organisms. Millions of DNA-coated metal particles are shot at target cells or tissues using a biolistic device or gene gun. The DNA elutes off the particles that lodge inside the cells, and a portion may be stably incorporated in the host chromosomes. Particle bombardment employs high-velocity micro projectiles to deliver substances into cells and tissues.

For genetic transformation, DNA is coated onto the surface of micron-sized tungsten or gold particles by precipitation with calcium chloride and spermidine. Once inside the cells, the DNA elutes off the particles. If the foreign DNA reaches the nucleus, then transient expression will likely result and the transgene may be stably incorporated into host chromosomes

Although the protein content of maize is relatively high (9% on average), its quality is poor due to an imbalance in three essential amino acids, in which the contents of tryptophan and lysine are low, whereas that of leucine is high. This amino acid balance ratio is not enough to satisfy the FAO requirements for human nutrition, especially for children (**Hamaker and Rahmanifar 1994**).

DNA transfer via particle bombardment is currently the most widely used method for wheat transformation, and the stable expression of transgenes has now been achieved by several groups **Vasil *et al.* (1992); Weeks *et al.* (1993); Becker *et al.* (1994); Nehra *et al.* (1994); Zhou *et al.* (1995); Altpeter *et al.* (1996); Ortiz *et al.* (1996); Barro *et al.* (1997)**. However, widespread application of the technology is still limited by relatively low and erratic stable transformation efficiencies and by the general use of tissue culture-responsive but agronomically less desirable "model" genotypes such as 'Bobwhite' and 'Florida'.

At the beginning of the 1960s **Mertz *et al.* (1964)** reported that, low levels of zein, and high levels of lysine and tryptophan, were discovered in opaque-2 maize which, on the other hand, displayed some undesirable agronomic traits, such as chalky soft kernel, low yields, lower resistance to fungi and insects, and longer drying time. The superior nutritional qualities of opaque-2 maize motivated maize researchers to overcome these problems. These efforts resulted in the discovery of modifiers of the opaque-2 maize trait, which conferred to it a normal, hard, and vitreous endosperm. The conversion of opaque-2 maize to dent- and flint-type maize gave rise to quality protein maize (QPM), which is, today, practically interchangeable with common maize in cultivation and kernel characteristics.

One of the major problems with all biolistic devices developed so far is the use of high pressure, which causes severe damage to target cells. They also suffer from several other disadvantages: (1) low transformation efficiency, (2) need to use large amounts of deoxyribonucleic acid (DNA), (3)laborious to use, (4) high noise level, (5) large size and design makes it less portable, (6) and the high cost of the basic instrument and disposable supplies needed for operation. The low-pressure Bio Ware gene gun has been used for genetic transformation of animal cells and human tumor cell lines only **Chang *et al.* (2008); Cheng *et al.* (2005)a, b;Tu *et al.* (2007); Lin *et al.* (2008)**

Zerihun *et al.* (2008) reported that, use of immature zygotic embryos as an explant for maize regeneration has been hampered by the strictly limited

suitable duration of immature embryos for culture. In contrast, mature zygotic embryos harvested from dry seeds are ubiquitous. The authors indicated that, however, generally mature embryos and especially tropical maize genotypes have been considered as the most recalcitrant for tissue culture work. Consequently tropical maize regeneration from mature embryos has not been reported so far. Here, they report successful regeneration of one inbred and one open pollinated tropical maize line from mature zygotic embryos using split seed technique. The results indicated that, the maximum average callus induction recorded using LS basal salts and B5 vitamins supplemented with 3 mg l-1 2,4-D alone was 90% and 52.5% when same level of 2,4-D was combined with Kinetin. A maximum of 75.6% Type II and 62.3% Type I callus was produced after maintaining calli on media composed of LS basal salts and B5 vitamins supplemented with 2 mg l-1 of 2,4-D. also the showed the frequency of regenerable calli induced was 21.14% for CIMMYT maize line 216 and 16.51% for Katumani. The number of shoots regenerated per callus induced on single split seed.

So far almost all maize tissue culture and transformation involves the use of immature zygotic embryos as an explant source for regeneration (**Armstrong and Green, 1985; Carvalho *et al.*, 1997; Danson *et al.*, 2006; Duncan *et al.*, 1985; El-Itriby *et al.*, 2003; Pareddy and Petolino, 1990; Shohael *et al.*, 2003**). However, immature embryos are seasonally available and have strictly limited suitable duration of culture, 14-19 DAP (**Odour *et al.*, 2006**).

This imposes tedious routine tissue culture activities within the specified time frame and continuous planting for continuous supply of the immature embryos. In contrast, mature embryos are readily available throughout the year in large quantities. Furthermore, despite few reports about the recalcitrance of tropical maize lines and mature embryos for tissue culture work (**Bohorova *et al.*, 1995; Hodges *et al.*, 1986**), successful regeneration of temperate maize lines and other cereal food crops from mature embryos has been reported by different authors (**Akula *et al.*, 1999; Green and Phillips, 1974; Ozgen *et al.*, 1998; Rueb *et al.*, 1994; Wang, 1987; Ward and Jordan, 2001**).

Green *et al.* (1974) first reported that mature embryos of maize could be used to induce callus but no plantlets were regenerated. **Wang (1987)** successfully regenerated plants from mature embryos of two maize inbreeds, B73 and Mo17, but the regeneration was genotype dependent and the frequency was only 4 to 5%.

Huang and Wei (2004) reported regeneration of temperate maize lines from mature embryos at a frequency ranging from 19.85 to 32.4%. Most recently **Al-Abed *et al.* (2006)** reported more efficient regeneration system for two hybrid and two inbred temperate maize lines using split mature seeds as an explant. Here, they report regeneration of one tropical maize inbred line and one open pollinated variety from mature embryos for the first time using split seed technique.

Miroslava *et al.* (2011) reported that somatic embryogenesis is the process by which somatic cells, under induction conditions, generate embryogenic cells, which go through a series of morphological and biochemical changes that result in the formation of a somatic embryo. These characteristics have designated somatic embryogenesis into a model system for the study of morphological, physiological, molecular and biochemical events occurring during the onset and development of embryogenesis in higher plants. In their experiments somatic embryogenesis and plant regeneration was achieved from immature embryos of two maize (*Zea mays* L.) lines A18 and A19.

There results showed that, callus was initiated on N6 medium supplemented with 1 mg.dm-3 2,4-D, N6 salts, 2 % sucrose, 25 mmol.dm-3 proline, 100 mg.dm-3 casein hydrolysate, N6 vitamins, 10 mg.dm-3 silver nitrate, 3g gelrite. Also, results indicated that, induction of primary callus ranged between 0 and 93%. Generally, three types of callus were formed: embryogenic, non-embryogenic and organogenic callus. The embryogenic callus was formed within two weeks of culture in callus maintenance medium. Induction of embryogenic callus ranged between 0 and 5%. Somatic embryos were matured on N6 medium supplemented with 6% sucrose and 1 mg.dm-3 NAA. After transfer of embryogenic calli on regeneration medium containing MS medium supplemented with 2% sucrose, somatic embryos started to form plantlets. Callus initiation and plant regeneration were genotype dependent. Regenerated plants were transferred on the surface of solidified MS medium supplemented with myo-inositol.

Somatic embryogenesis is developmental process by which somatic cells undergo restructuring to generate embryogenic cells. These cells then go through a series of morphological and biochemical changes that result in the formation of somatic or non-zygotic embryo capable of regenerating plants. Somatic embryogenesis represents a unique developmental pathway that includes a number of characteristic events: dedifferentiation of cells, activation

of cell division and reprogramming of their physiology, metabolism and gene expression patterns, reviewed by (**Zimmerman, 1993; Schmidt *et al.*, 1997; Komamine *et al.*, 2005**).

Somatic embryogenesis forms the basis of cellular totipotency that is unique to higher plants. Differing from its zygotic counterpart, somatic embryos are easily traceable, culture conditions can by monitored and lack of material is not a limiting factor for experimentation (**Kawara and Komamine, 1995**). Somatic embryogenesis plays an important role in clonal propagation. When integrated with conventional breeding programs and molecular and cell biological techniques, somatic embryogenesis provides a valuable tool to enhance the genetic improvement of commercial crop species (**Stasolla and Yeung, 2003**).

Plant regeneration through tissue culture of maize was first reported by **Green and Philips (1975)** utilizing immature embryos as the explants. Since the successful plant regeneration has been reported from callus initiating from different tissue sources (**Ting *et al.*, 1981; Rhodes *et al.*, 1986; Conger *et al.*, 1987**).

Maize immature embryos were most widely used as initial explant for maize regeneration (**Lu *et al.*, 1982; Lu and Vasil, 1983; Vasil *et al.*, 1984**).

However various conditions for somatic embryo induction and regeneration were tested and used, the ability to regenerate embryo derived from callus cultures has been reported to be dependent on the maize genotype used (**Lee and Phillips, 1987; Obert *et al.* 2009**). Hormones are the most likely candidates in the regulation of developmental switches.

Auxins and cytokinins are the main growth regulators in plants involved in the regulation of cell division and differentiation. The influences of exogenously applied auxins, preferentially 2,4-dichlorophenoxyacetic acid (2,4-D), on the induction of somatic embryogenesis are well documented (**Dudits *et al.*, 1995**).

Auxins promote, mainly in combination with cytokinins, the growth of calli, cell suspensions and organs, and also regulate the morphogenic processes. At the cellular level, auxins control basic processes such as cell division and cell elongation. Since they are capable of initiating cell division they are involved in the formation of somatic embryos.

These characteristics have made somatic embryogenesis a model for the study of morphological, physiological, molecular and biochemical events that occur during the onset and development of embryogenesis in higher plants. It also has potentially rich biotechnological applications such as artificial seed, micropropagation, transgenic plants, etc. (**Dudits *et al.*, 1995**).

Philippe *et al.* (1993) summarized the effects of osmotic conditioning on both transient expression and stable transformation by introducing plasmid DNAs via particle bombardment into embryogenic suspension culture cells of *Zea mays* (A188 x B73). Placement of cells on an osmoticum- containing medium (0.2 M sorbitol and 0.2 M mannitol) 4 h prior to and 16 h after bombardment resulted in a statistically significant 2.7-fold increase in transient B-glucuronidase expression. Under these conditions, an average of approximately 9,000 blue foci were obtained from 100 p.l packed cell volume of bombarded embryogenic tissue. Osmotic conditioning of the target cells resulted in a 6.8-fold increase in recovery of stably transformed maize clones. Transformed fertile plants and progeny were obtained from several transformed cell lines. The authors indicted that the basis of osmotic enhancement of transient expression and stable transformation resulted from plasmolysis of the cells which may have reduced cell damage by preventing extrusion of the protoplasm from bombarded cells.

Particle bombardment is valuable for both gene expression (**Ludwig *et al.* 1990**) and stable transformation research (**Christou *et al.* 1988**). The basis of particle bombardment is the acceleration of small DNA-coated particles toward cells resulting in the penetration of the protoplasm by the particles and subsequent expression of the introduced DNA. With certain plants, particle bombardment is currently the most efficient method for introduction of foreign DNA.

Although there have been many reports on optimization of physical bombardment parameters (**Klein *et al.* 1988**) and modification to the actual bombardment device (**Williams *et al.* 1991; Sautter *et al.* 1991; Finer *et al.* 1992**), limited data has been reported on cell preparation methods to make the target tissue more receptive to particle gun-mediated transformation.

Benefits from culture venting (**Russell *et al.* 1992**), cell filtration (**Finer *et al.* 1992**), and the use of cells in the proper phase of growth (**Armaleo *et al.* 1990**) or at the proper density (**Finer *et al.* 1992**) have been reported for different species using the particle gun.

Another factor affecting the efficiency of particle gun-mediated transformation is osmotic treatment of target tissues. A 7- to 10-fold enhancement in stable transformation of microorganisms (**Armaleo *et al.* 1990; Shark *et al.* 1991**) and nonembryogenic plant cells (**Russell *et al.* 1992**) was reported following culture on media containing mannitol and sorbitol.

Particle gun-mediated transformation of *Zea mays* has been reported by several laboratories (**Fromm *et al.* 1990; Gordon-Kamm *et at.* 1990; Waiters *et al.* 1992**) and is currently the most efficient technique for production of fertile, transgenic maize plants. The authors described the effect of osmotic treatment on transient expression and stable transformation of embryogenic maize cells and the recovery of fertile transgenic maize plants.

A regeneration system was developed for elite Egyptian maize inbred lines using immature embryos as explants by **Hanaiya *et al.* (2003)**. This system proved to be highly genotype-dependent. Line Gz 643 was identified as the best line, revealing the highest regeneration frequency (42.2%). Addition of L-proline and silver nitrate to culture media greatly enhanced the formation of embryogenic type II callus and the regenerability of some of the tested lines. Transformation of the scutellar tissue of immature embryos from inbred line Gz 643 was performed with the particle delivery system using a single plasmid carrying both the GUS and Bar genes (pAB-6) or by co-transformation with two plasmids, pAct1-F (GUS) and pTW-a (Bar).

Different transformation parameters were evaluated, i.e. osmotic treatment, acceleration pressure, and number of shots. Osmotic treatment (0.25M sorbitol þ 0.25M mannitol) along with the use of either acceleration pressure 1300 psi and one shot per plate (for co-transformation with pAB-6) or 1100 psi and two shots per plate (for transformation with pAct1-F and pTW-a) gave the best results, as expressed by the number of blue spots in the b-glucuronidase (GUS) assay **Hanaiya *et al.* (2003)**.

Stable transformation was confirmed in Ro transformed plants by means of histochemical GUS assay and herbicide application. PCR and Southern blot analysis proved the integration of the full-length genes in some of the transgenics

The genetic improvement of cereals has been a major focus of plant breeding efforts during the past 50 yr, resulting in remarkable increases in the yield and improvement in the quality of this important group of food crops. However, modern plant biotechnology has provided novel means for crop

improvement through the integration and expression of defined foreign genes into plant cells, which can then be grown *in vitro* to regenerate whole plants. The efficient regeneration of normal and fertile plants from single cells, a basic prerequisite for the production of genetically transformed plants, proved to be rather difficult for gramineous species because of their extreme recalcitrance to manipulation in vitro (**Zhang *et al.*, 2002**).

The regeneration ability of any plant is influenced by different factors. The type of explant is considered one of the main factors that attracted the concern of many investigators. Immature embryos have been the most widely used explant in many cereals, including maize (**Green and Phillips, 1975; Armstrong and Green, 1985; Hodges *et al.*, 1986; Lee and Phillips, 1987; Shillito *et al.*, 1989**).

Another important factor is the genetic background or the genotype of the explants which was found to influence the regeneration potentiality. **Bohorova *et al.* (1995)** discussed the effect of the genotype on somatic embryogenesis.

Tomes and Smith (1985) and Hodges *et al.* (1986) indicated that the regeneration was genetically controlled by nuclear genes in maize. Moreover, studies by **Willman *et al.* (1989)** suggested that at least one gene or a block of genes controlled the expression of somatic embryogenesis of maize tissue cultures. The transfer of defined genes is theoretically the most straightforward method for improvement of crop plants.

The microprojectile bombardment system proved to be a powerful technique for genetic modification of maize lines by delivering foreign DNA into scutellar tissues of immature embryos (**Koziel *et al.*, 1993; Wan *et al.*, 1995; Songstad *et al.*, 1996; Zhang *et al.*, 1996; Brettschneider *et al.*, 1997; Bohorova *et al.*, 1999; Frame *et al.*, 2000; O'Kennedy *et al.*, 2001; O'Connor-Sa´nchez *et al.*, 2002).**

Particle bombardment, or biolistics, is a commonly used method for genetic transformation of plants and other organisms. Millions of DNA-coated metal particles are shot at target cells or tissues using a biolistic device or gene gun. The DNA elutes off the particles that lodge inside the cells, and a portion may be stably incorporated in the host chromosomes.

In a typical experiment, transient gene expression averaged nearly 8000 "hits" per bombarded plate. Five months after bombardment, there were nearly five putative transgenic embryos per bombarded plate. About half of the

embryos were regenerated into confirmed transgenic plants. The basic bombardment procedures described are applicable to a wide range of plant genotypes, especially those for which embryogenic cell cultures are available. All users of particle bombardment technology will find numerous useful tips to maximize the success of transformation. (**Brettschneider *et al.*, 1997; Bohorova *et al.*, 1999; Frame *et al.*, 2000; O'Kennedy *et al.*, 2001; O'Connor-Sa´nchez *et al.*, 2002).**

Chien-Yuan *et al.* (2007) have successfully used the low-pressure BioWare gene gun, developed for gene transfer in animal cells, for plant tissues. The BioWare device is easy to manipulate. Just 50 psi helium pressure was sufficient to transfer foreign genes into the aleurone layer and embryo of maize without causing tissue damage in the impact area.

As shown by expression signals from invasive histochemical b-glucuronidase (GUS) activity, the foreign reporter gene expressed well in bombarded tissues. This successful GUS-transient expression extends the application of this low-pressure gene gun from animal cells to plant tissues The stable integration of foreign genes into plant genome represents one of the most significant developments in plant biology and crop improvement.

The first transgenic tobacco plant was produced by using *Agrobacterium tumefaciens* (**Horsch *et al.* 1985**). Several years later, the high-velocity microprojectile, or gene-gun technology, was invented by John Sanford and his colleagues (**Klein *et al.* 1987; Sanford 2000**). Both methods have been used extensively for genetic transformation of plants, leading to the commercialization of biotech crops.

The biolistics system can be used with intact tissues and is not restricted to any particular plant group. It has revolutionized plant genetics by producing most of the world's biotech crops and has also been used extensively in transient expression assays for studying gene regulation and function (**Christou 1992; Kao *et al.* 1996; Sambrook and Russel 2001; Vasil 2003**).

A number of modifications of the original biolisitcs device have been developed to improve its performance (**Gray and Finer 1993; Kikkert 1993; McCabe and Christou 1993; Oard 1993; Sautter 1993; Vain *et al.* 1993**). One of the major problems with all biolistic devices developed so far is the use of high pressure, which causes severe damage to target cells. They also suffer from several other disadvantages: (1) low transformation efficiency, (2) need to use large amounts of deoxyribonucleic acid (DNA), (3) laborious to use, (4)

high noise level, (5) large size and design makes it less portable, (6) and the high cost of the basic instrument and disposable supplies needed for operation.

The low-pressure BioWare gene gun has been used for genetic transformation of animal cells and human tumor cell lines only (**Chang *et al.* 2008; Cheng *et al.* 2005a, b; Tu *et al.* 2007; Lin *et al.* 2008**). By gene delivery and expression in the aleurone layer and embryos of maize (*Zea mays* L.) they show for the first time that it can be equally effective in plant transformation. Authors found the BioWare device to be safe and flexible to use, as well as light and handy. They used it obtain 18 stable transgenic plants of the orchids Mormodes lawrence and *Zygopetalum mackayi*, transformed with three different plasmid constructs (manuscript in preparation).