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Review

Biolistic maize transformation: Improving and simplifying the protocol efficiency

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Two decades have passed since the first maize transformation protocol. 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. 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.

Key words: Biolistic, transient expression, reporter genes, plant genetic improvement.

INTRODUCTION

Maize (*Zea mays*) 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, specialty chemical and biological compounds, and to improve the processing capabilities (Shoemaker et al., 2001). Two specific examples are Syngenta's corn amylase and phytase varieties with embedded enzymes for improved ethanol production and increased use of phytate in animal feed (Kemble et al., 2006). 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.

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

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Abbreviations: HPGG, High pressure gene gun; **LPGG,** Low pressure gene gun; **2,4-D,** 2,4-dichlorophenoxyacetic acid; **N6,** Chu's medium (Chu et al., 1975).

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 explant regeneration, the plant adaptation to *ex vitro* conditions and the seed production capacity (Zhang et al., 2007).

A powerful tool to optimize the genetic transformation conditions is the use of reporter genes. The most widely used reporter genes nowadays are the *gusA* gene, which encodes the protein ß-glucuronidase, and the *gfp* gene, which encodes the Green Fluorescent Protein (GFP). The *gusA* gene has been widely used in plants (Jefferson et al., 1987; Finer et al., 1992; Weeks et al., 1993; Ishimaru et al., 1999). The activity of the protein ß-glucuronidase can be detected in transformed tissues by histochemical analysis. However, this assay is destructive and not suitable as a reporter for gene expression in live samples. On the other hand, the *gfp* gene, from the jellyfish *Aequorea victoria* has been expressed in many organisms (Haseloff et al., 1997). At the cellular level, *gfp* is being used as an *in vivo* reporter to assess the frequency of transient and stable transformation because it does not involve a destructive assay. The use of the *gfp* gene in plant transformation is experimentally increased, either from the cellular level to the final level to obtain fertile plants (Leffel et al., 1997).

The aim of this work was to review the critical stages of the production of fertile transgenic maize plants mediated by microprojectile bombardment.

TISSUE CULTURE AND REGENERATION: A CRITICAL STEP FOR GENETIC TRANSFORMATION OF MAIZE

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).

The development of robust plant regeneration protocols from single cells capable of being transformed at high frequency, combining speed, output, genotype independence, and host genome stability, has been long recognized as the basis of plant biotechnology (Sairam et al., 2009). In order to meet this purpose, many different explants, such as shoot apices, leaf and stem segments, hypocotyls, epicotyls, immature embryos, and mature intact seeds, have been tested for their regeneration capacity (Ball et al., 1980; Glimelius et al., 1984; Wang et al., 1987; Mc Nicol et al., 1989; Halámková et al., 2004; Shan et al., 2009). In cereals, especially in maize, immature embryos of 1 to 2 mm size have been found to be the optimal explant for induction of embryogenic calluses (Fernandes et al., 2008).

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 (weather, available area and economic resources) 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.

The transformation and regeneration process involves several steps. First, immature embryos are aseptically isolated from Hi-II seeds, 10 to 12 days after pollination. The seeds are sterilized in a sodium hypochlorite 2% solution with two drops of surfactant Tween-20, for 20 min with soft stirring, and are then washed five times with sterile distilled water in laminar flow. Embryos are cultured in the dark at 27 ± 1°C in N6-1 medium (Chu et al., 1975) (Table 1). The next stage begins when type II

Table 1. Culture media for maize transformation protocols.

Medium	Composition
N6	Chu N6 medium (1975)
N6-1	N6 medium containing 2,4 D 2 mg/L
N6-Osmotic	N6 medium containing manitol 0.4 M and sorbitol 0.4 M
N6-Proline	N6 medium containing proline 1.45 g/L
Selection I	N6 medium containing phosphinothricin 3 mg/L , 2,4-D 2 mg/L, inositol 100 mg/L, nicotinamide 0.5 mg/L, pyridoxine 0.5 mg./l, thiamide 0.5 mg/L, glycine 2 mg/L and AgNO3 0.85 mg/L
Selection II	N6 medium containing phosphinothricin 6 mg/L, 2,4-D 2 mg/L, inositol 100 mg./L, nicotinamide 0.5 mg/L, pyridoxine 0.5 mg/L, thiamide 0.5 mg/L, glycine 2 mg/L and AgNO3 0.85 mg/L
Selection III	N6 medium containing phosphinothricin 9 mg/L, 2,4-D 2 mg/L, inositol 100 mg./L, nicotinamide 0.5 mg/L, pyridoxine 0.5 mg/L, thiamide 0.5 mg/L, glycine 2 mg./L and AgNO3 0.85 mg/l
Regeneration I	Murashige y Skoog MS medium (1962) containing inositol 100 mg/L, nicotinamide 0.5 mg./l, pyridoxin 0.5 mg/L, thiamide 0.5 mg/L, glycine 2 mg/L, ANA 0.25 mg/L and 6 mg/L phosphinothricin
Regeneration II	MS medium containing inositol 100 mg/L, nicotinamide 0.5 mg/l, pyridoxin 0.5 mg/L, thiamide 0.5 mg/L and 3 mg/L phosphinothricin

highly embryogenic calluses are obtained. It is necessary to subculture the calluses in fresh N6-1 medium every two weeks in order to obtain suitable material for embryogenesis and transformation (Fromm et al., 1994; Armstrong et al.,1994; Frame et al., 2000). It has been reported that, in bombardment assays, it is more suitable to use selected embryogenic calluses subcultured for 10 days in N6-1 medium (Décima et al., 2010). Four hours before bombardment assays, calluses are placed in N6-osmotic medium (Table 1). Finally, the calluses remain in the N6-osmotic medium for 16 h after the bombardment and then placed in N6-1 medium for 10 days, before starting the selection process.

MAIZE GENETIC TRANSFORMATION WITH MICROPROJECTILE BOMBARDMENT

Although, the first attempt of DNA transfer to plants was made in the 1960s, the lack of selectable markers and molecular tools to confirm transgene integration and expression made the outcome of such experiments unclear (Stroun et al., 1967). A breakthrough came in the 1970s with the elucidation of the mechanism of crown gall formation by *Agrobacterium tumefaciens* (Larebeke et al., 1974). Nowadays, it is possible to introduce and express DNA stably in nearly 150 different plant species (Twyman et al., 2002).

In general, plant transformation systems are based on the introduction of DNA into totipotent plant cells, followed by the regeneration of such cells into whole fertile plants. Essential requirements for plant transformation are: (i) an efficient method to introduce DNA into plant cells, (ii) adequate regulation sequences, (iii) availability of cells or tissues that can easily regenerate a whole plant and (iv) an efficient scheme for the selection of transformed cells (Birch et al., 1997; Komari et al., 1998; Hansen 1999). Several techniques for plant transformation have been developed through the last decades. The procedure of Agrobacterium-mediated transformation has been developed and refined since its discovery in the 1970s and is currently a widely used strategy for gene transfer to plants. Even so, success in genetic transformation of monocotyledonous plants has been difficult to achieve, and often limited to transient gene expression, because of the lack of suitable regenerative systems and incapability of Agrobacterium to infect cereal tissues (Komari et al., 1998). In order to resolve this problem, a number of alternative plant transformation methods have been developed to facilitate gene transfer to these recalcitrant species. These methods can be grouped under the term "physical methods" and include the transformation of protoplasts using polyethylene glycol (PEG) or electroporation, microinjection and particle bombardment.

The development of methodologies for gene delivery into plant tissues by particle bombardment has, in fact, revolutionized the field of plant transformation (Klein et al., 1987). The concept of accelerating DNA-coated particles into cells and tissues has evolved from being a novelty to becoming an established tool in plant molecular biology. Microprojectile bombardment is based on a device of small gold or tungsten particles used at a speed of approximately 400 MS^{-1}. The particles are coated with DNA and can penetrate plant cells without killing them. This system has shown that particle

Table 2. Bombardment conditions that have given best results.

Optimal parameter	Gene gun device	
	HPGG	LPGG
Pressure	1100 Psi	101,5 Psi
Size particle	1 µm	1 µm
Distance	6 cm	9 cm (gusA)/ 3cm (gfp)
DNA amount	2 µg	2 µg

bombardment is an efficient method for stable integrative transformation (Klein et al., 1988; Birch et al., 1997; Dai et al., 2001). The rather erratic low-pressure device has been refined to a system based on a high-pressure discharge of helium (Ye et al., 1990).

Integration patterns, inheritance and expression of transgenes in plants upon direct DNA delivery-mediated transformation have been reported by many research groups (Goto et al., 1993; Flavell et al., 1994; Hiei et al., 1994; Elmayan et al., 1996; Chen et al., 1998; De Neve et al., 1997; Kononov et al., 1997; Dean et al., 1998; Kohli et al., 1998). The direct DNA delivery systems tend to result in a multiple-copy integration of the transgenes at a single locus and a rearrangement of the transgenes (Finnegan et al., 1994; Flavell et al., 1994; Pawlowski et al., 1996; Kohli et al., 1998; Dai et al., 2001), while most of the Agrobacterium-mediated transgenic plants result in a lower copy number of transgenes and a more predictable pattern of integration (Smith and Hood, 1995). However, there is not a clear correlation between transgene expression and transgene copy number (Dean et al., 1988; Hobbs et al., 1993). While single copies of transgenes may tend to be more stably expressed than multiple gene copies or scrambled inserts, there are additional factors that influence transgene expression (Iglesias et al., 1997). Transgenic plants exhibiting classical Mendelian inheritance ratios have been obtained using both types of transformation methods (Pawlowski et al., 1996; Hiei et al., 1997). Transgenic plants with non-Mendelian patterns of inheritance have also been obtained with both transformation methods (Spencer et al. 1992; Goto et al. 1993; Peng et al. 1995). However, there are several events obtained using biolistic procedures approved by different regulatory agencies (Center for Environmental Risk Assessment, http://cera-gmc.org/).

Several research groups have concluded that microprojectile bombardment is the most suitable maize transformation method (Finer et al., 1992; Frame et al., 2000; Aulinger et al., 2003). In this context, the group has attempted to improve the transformation protocols trying to increase the maize transformation efficiency and regeneration (Décima et al., 2010). In this regard, two details that are critical in the protocol were identified: (i) the use of only embryogenic calluses cultured for ten days after the last subculture, in N6-1 medium and (ii) the use of different conditions to obtain better results for each device. Different bombardment conditions and DNA amounts for HPGG and LPGG were evaluated by the transient expression of two gene markers, *gusA* and *gfp*, in order to obtain the best results for both devices (Table 2).

Although, these conditions are the optimal ones for transient transformation, the parameters for stable transformation are quite different: 600 Psi and 0.6 µm particles in a HPGG device (Finer et al., 1992). With these parameters several events for each bombarded plate were obtained (Table 3).

VISUALIZABLE MARKER GENES AND APPROPRIATE SELECTIVE METHOD

It is essential to consider that in all cell-transformation processes only a small proportion of cells will be transformed. These cells rather than non-transformed cells must be induced to proliferate. This can be achieved by introducing a selectable marker gene and regenerating plants under the appropriate selective regime (Twyman et al., 2002).

With any of the physical or biological methods performed for DNA delivery into target tissues of maize, the choice of markers, reporter and promoters sequences greatly influences the final outcome. In addition, better selections of transformed cells minimize the risk of escapes and increase the chance to recover the transformed cells and to subsequently obtain transgenic plants (Sahrawat et al., 2003). The initial steps in transgenic transformation involve delivery of a reporter gene cassette into plant cells followed by analysis of the expression of the delivered gene. The results of this kind of events can be detected by assaying the expression of a reporter gene introduced into plant cell cultures or intact tissues. The reporter genes cause a visible effect, either directly or indirectly, due to their activity in transformed cells. Analysis of reporter gene expression does not require the integration of the transgene into the host genome and is commonly used to test promoter and gene functions (Patnaik et al., 2001).

On the other hand, most of the selectable marker genes that have been used in maize transformation give resistance to antibiotics or herbicides (Spencer et al.,

Table 3. Events per bombarded Petri plate.

Assay	Number of Petri plate bombarded	Event
E105	5	1
E205	4	0
E305	8	0
E405	8	1
E505	9	18
E605	6	20
E705	6	7
E109	5	15
E209	5	18
E309	5	29
E409	5	8
E110	5	11
E59	1	2
Average	5.54	10
Events/bombardment	1.81	

Figure 1. Small sterile plastic ring around the transformed calluses during the detection of β-glucuronidase activity.

1990; Walters et al., 1992).

Issues related to public acceptance of antibiotic- or herbicide-resistant genes have guided the tendency to replace them with other less controversial gene markers. Examples of this are the phosphomannose isomerase gene (PMI) (Wright et al., 2001) and the maize R gene. The latter stimulates endogenous anthocyanin accumulation in the vacuoles of plant tissues and is useful as scorable marker in mature and differentiated cells. Due to their high sensitivity and easiness of visualization, R genes have been used in plant cell transformation by different groups (Kloti et al., 1993; Dhir et al., 1994; McCormac et al., 1998; Chawla et al., 1999).

The most widely used scorable marker in maize transformation is the *gusA* gene (codifying for β-glucuronidase) (Jefferson et al., 1987; Finer et al., 1992; Ishida et al., 1996; Dai et al., 2001). The β-glucuronidase activity can be detected in transformed tissues with a simple histochemical analysis. However, one of the main limitations of the *gusA* reporter gene system is its destructive nature. This makes the *gusA* gene marker an unsuitable technique for all the *in vivo* transient or stable gene expression studies. Another difficulty encountered during the *gusA* transient visualization occurs when the material has been moved to observe. This can result in an error of staining or observation. In order to resolve this inconvenience, this group used a small sterile plastic ring around the transformed callus and did not disperse the X-Gluc substrate (5-Br-4-Cl-3-indolyl-b-D-glucuronic acid) (Figure 1).

One way to overcome this situation is provided by the *gfp* gene, which encodes a modified version of the green fluorescent protein from the jellyfish *Aqueorea victoria* (Haseloff et al., 1997). The *gfp* gene has been used as a vital marker in maize transformation (Chiu et al., 1996; Amien et al., 2010; Décima et al., 2010). Both genes admit being used together or in a single construction. Recent studies indicate that the co-bombardment of maize calluses using the *gusA* and *gfp* gene markers simultaneously does not show differences in the number of gene insertions as compared with the assays with only one gene marker. This suggests that there are no interferences in the gene insertion efficiency between both genes (Décima et al., 2010).

Table 4. Promoter and selectable marker genes used in maize transformation (Shrawat et al., 2006).

Promoter and selectable gene	Reference
Ubi1:pmi	Negrotto et al., 2000
CaMV 35S:bar	Zhao et al., 2001
CaMV 35S:bar	Frame et al., 2002
LIR:RepAll	
CaMV 35S:barI Ubi1:moGFP:PinII	Gordon-Kamm et al., 2002
CaMV 35S:bar+Ubi1:FLP:PinII	
Ubi1:PPO (Y426M + S305L) Ubi1:pmi//ubi:PPO	Li et al., 2003
e35S/HSP70:nptII Act1:cre//35S:npII	Zhang et al., 2003
e35S/HSP70:11// HSP17.5E:cre	
Act1:epsps-cp4	Huang et al., 2004
Ubi:bar	Décima et al., 2010

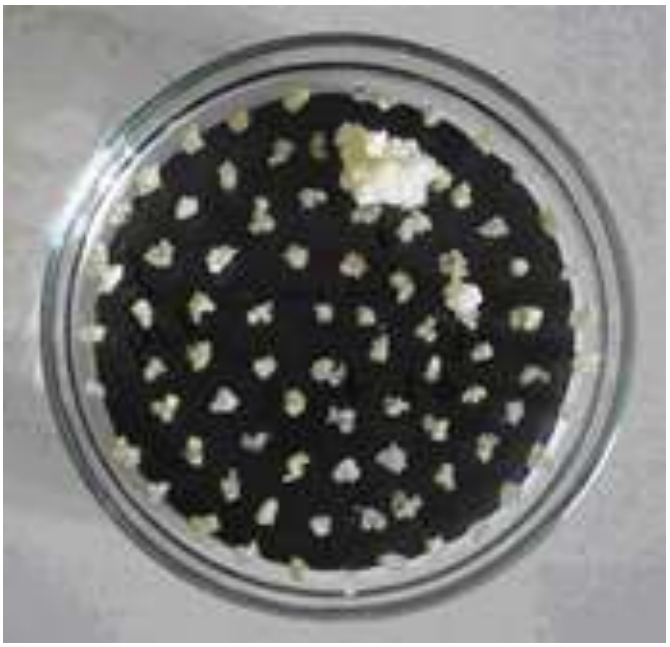

Figure 2. Differential growth in selective medium.

In contrast to reporter genes, selectable genes allow survival of the transformed cells in a selection medium enriched with a selective agent. The selection regime for transformed cells is based on the expression of a selectable gene that produces an enzyme which confers resistance to a cytotoxic substance (Wilmink et al., 1993; Angenon et al., 1994). This topic becomes a critical factor in discriminating between transformed and non-transformed cells. In direct DNA transfer methods, the selectable marker and non-selected transgenes may be linked in the same co-integrate vector or introduced in separate vectors (co-transformation) (Cooley et al., 1995). A list of selectable marker genes used in maize transformation along with their respective promoters is summarized in Table 4.

Ideal selection systems should kill non-transformed cells rapidly and the selective regime should be simple and inexpensive to implement. The most commonly used selection gene in maize transformation is the *bar* or the *pat* gene (bialaphos or ammonium glufosinate resistance gene) (Gordon-Kamm et al., 1990; Valdez et al., 2004; Yan et al., 2010). Both the *bar* and *pat* genes, isolated from different *Streptomyces* species, encode for phosphinothricin acetyl transferase.

The selection scheme carried out by this group was based on the *bar* gene regulated by the *Ubi* promoter and the *nos* terminator, with the addition to the medium culture a commercial herbicide with 150 g/l of ammonium glufosinate , as follows (Table 1):

(a) 10 days after bombardment, treated calluses growing in N6-1 were subcultured in selective-1 medium.
(b) 15 days after culture in selective-1 medium, transformed cells were cultured in selective-2 medium
(c) 15 days after subculture in selective-2 medium, transformed cells were cultured to selective-3 medium. At this stage, transformed cells showed a differential growth. The calluses with differential growth were identified as a potential event (Figure 2). This was confirmed by polymerase chain reaction (PCR) reaction on DNA extracted from the calluses.
(d) Each event detected in selective-3 medium was subcultured again in selective-2 medium for 20 days more.
(e) Once the calluses had at least two repetitions in

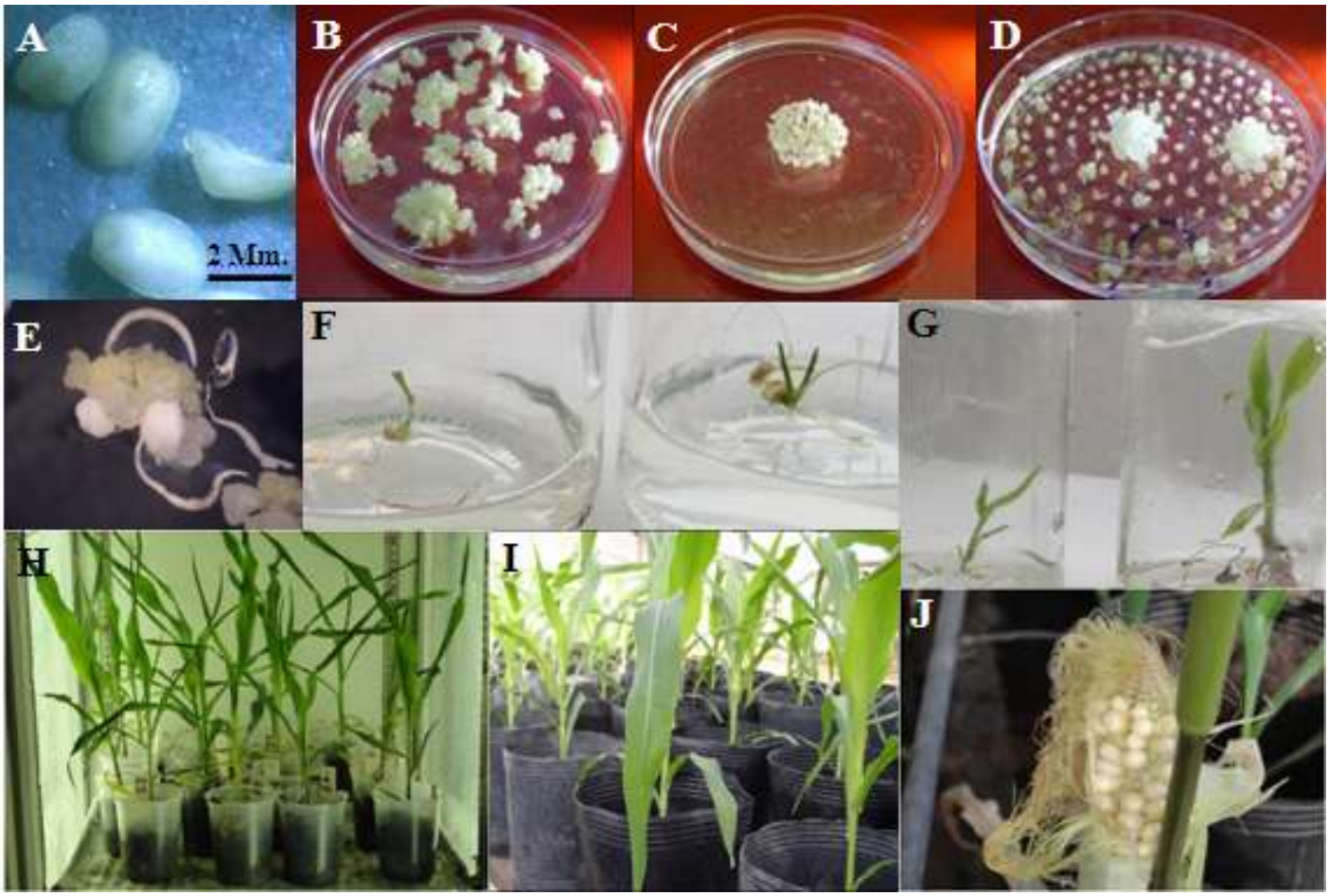

Figure 3. Scheme of maize tissue culture and plant regeneration; (A) HiII immature embryos; (B) embryogenic type II calluses (C); biolistic transformation; (D) differential growth in selective medium; (E) somatic embryogenesis; (F) and (G) germination; (H) acclimatization of plantlets in growth chamber; (I) plants growing in biosafety greenhouse and (J) mature seeds.

selective-2 medium, the regeneration stage began.

ACHIEVEMENT OF TRANSFORMED MAIZE PLANTS

Most of the transformation procedures have one major difficulty: the transitions from tissue culture to the whole plant regeneration (Figure 3).

The tissue culture requirement increases the time and cost of the procedure to obtain transgenic plants and is perhaps the most serious limitation to the technology because it restricts the range of species amenable to genetic manipulation. After the selection protocol, the regeneration stage takes place. The regeneration procedure for the regenerated plant requirements was adjusted in order to increase the frequency of transgenic maize plants (Décima et al., 2010). The critical steps were:

(a) After point 4, the calluses were subcultured in N6-proline medium for 15 days. This step promotes and improves the future shape of somatic embryos (Armstrong et al., 1985).

(b) Calluses growing in N6-proline medium were subcultured in regeneration-1 medium in order to obtain the somatic embryos.

(c) The somatic embryos obtained were incubated in regeneration-2 medium (in 15 cm-tall flasks) to finally obtain a maize seedling developing *in vitro*.

(d) At the V3 stage, the plantlets developed several kinds of roots (adventitious and hairy). Only the adventitious roots were functional when the plantlets had to grow at *ex vitro* conditions. For this reason, hairy roots were removed at least one week before the acclimatization step (this procedure is conducted under laminar flow).

(e) In order to acclimatize the transgenic maize plants it is necessary to use a growth chamber with 90% humidity and a 16/8 photoperiod (day/night). It is convenient to use soil enriched with a mix of mob and magnesium silicate in a 5:1:1 proportion for a 300 ml pot.

(f) At the V5/V6 stage, plants were moved to the greenhouse with a 16/8 photoperiod (day/night) and

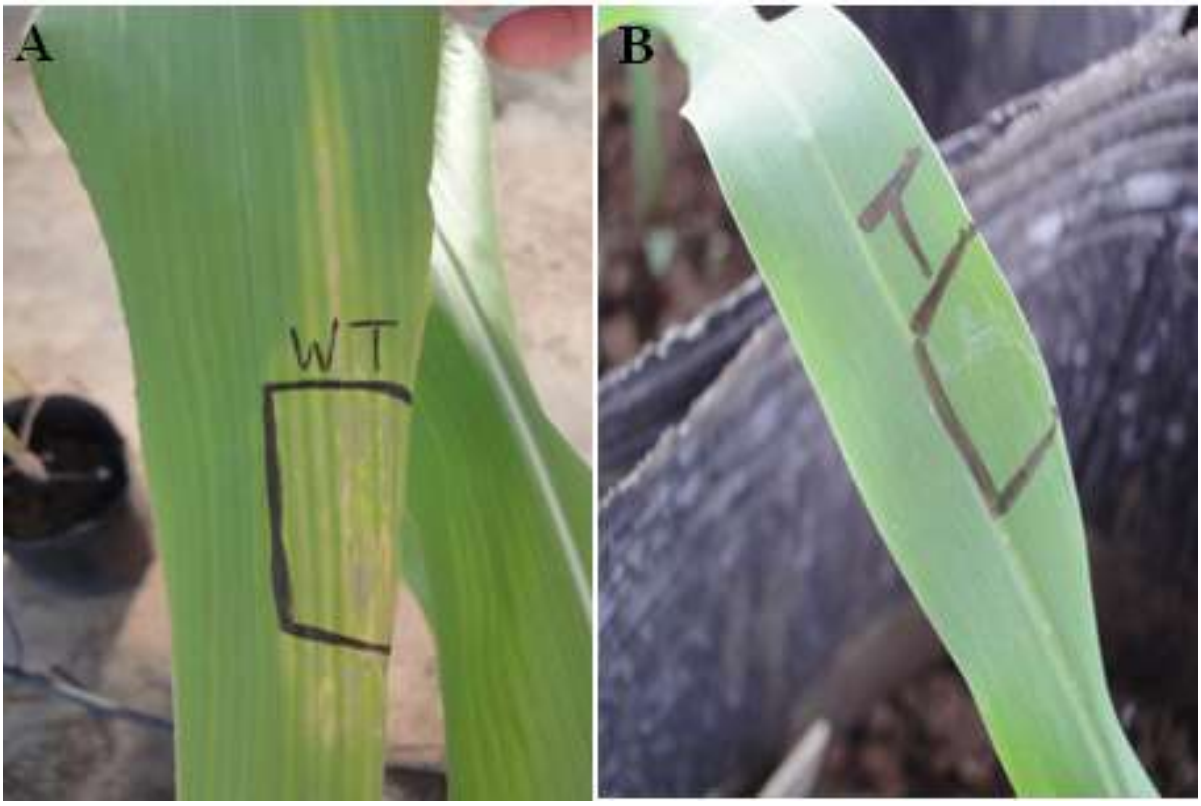

Figure 4. Plant painting with a 600 mg/l solution of commercial herbicide (A) wild type and (B) bar transgenic plant.

controlled watering. Three or four days later, plants were placed into 12 L pots. The maize plants were fertilized with a solid mix of N-P-K (15-15-6).

(g) Screening of herbicide resistance in plants was performed by painting an area of approximately 2 cm^2 with a 600 mg/l phosphinothricin/water solution of commercial herbicide (Figure 4).

(h) Once the plants were flowering (approximately 55 to 60 days after the germination of somatic embryos), the maize plants were pollinated to obtain seeds. To produce segregating T1 hybrids, crossing with a wild type line was preferred. This kind of crossing allows decrease in the transgene copy number in T1 seeds.

(i) Finally, to perform the molecular analysis of the T0 plants (PCR, Southern blot, etc), samples were obtained from young leaves without the main vascular tissues.

FUTURE PROSPECTS

This review attempted to provide an overview of the advances in maize biotechnology involving microprojectile bombardment. In the last 20 years, maize tissue culture and biotechnology have advanced and become an important option to improve the introduction of novel traits in this crop. Through these years of progress in maize tissue culture, it has been possible to establish the best explants for each transformation method. These advances, together with the improvement of the transformation systems, mean that in theory, any gene should be transferable, thus making possible to produce a large number of commercially available transgenic maize events. In addition, the transformation technology and its products have spread around the world and lots of research groups have been created around these developments. The group has acquired experience in maize transformation and has made some adjustments to the method described elsewhere. This work provides experience to all the groups working on maize transformation.

While the most important aim of plant transformation technology over the last years has been to introduce and stably express transgenes in plants, the challenge for the next decade is to improve these techniques and introduce single transgene copies at defined sites without extraneous DNA sequences such as parts of the vector backbone or marker genes. The ultimate aim is to produce transgenic plants with the transgene integrated at a known site, allowing the predictable control over transgene expression.

Finally, it is important to highlight that transgenic maize technology has become an important tool to improve food and agronomic quality and there are still lots of traits to improve owning to the climate change and the millennium

development goals.

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