Comparison of the efficiency of some novel maize promoters in monocot and dicot plants

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ABSTRACT

Three novel maize promoters (Gos-2, Enolase and Actin-2) were evaluated for their efficiency in driving the GUS gene expression in plant tissues. Three plasmids were constructed to contain the GUS gene in combination with the three promoters and another three plasmids were constructed to contain the GUS gene in combination with the three promoters and the INTIUBIZM intron. The six plasmids were employed in the transformation of maize immature embryos (as a monocot system) and tomato leaflets (as a dicot system). The transformation was conducted using the biolistic particle delivery system. Our results revealed the efficiency of the newly isolated maize promoters in both monocots and dicots. However, the level of activity of the different promoters was variable in both systems. Among the six plasmids, the Gos-2 was determined as the most efficient promoter in driving the transient GUS gene expression in maize, while maize Actin-2 in the plasmid bearing an intron revealed the highest GUS expression in tomato leaflets.

Key words: *Transformation, GUS gene, promoters, immature embryos, tomato leaflets.*

INTRODUCTION

Improvement of the major cereal crops by molecular biological approaches is one of the major goals in plant biotechnology (Shimamoto, 1994). During the past few years progress in monocot (cereal) transformation has been rapid, and transformation of all the major cereals has now been achieved. These advances have made it possible to analyze monocot gene expression using transgenic monocots, instead of transgenic dicots in which monocot genes are not always expressed in a regulated manner. The first field trials using genetically engineered maize have been reported by Shimamoto (1994), and genetic engineering has proven to be an important tool for improvement of cereal crops. Consequently, it is becoming increasingly clear that the use of monocot promoters is desirable for expression of economically important genes in transgenic cereals.

Expression of heterologous DNA sequences in a plant host is dependent upon the presence of an operably linked promoter that is functional within the plant host (Christou, 1996). Choice of the promoter sequence will determine when and where

within the organism the heterologous DNA sequence is expressed. Where expression in specific tissues or organs is desired, tissue-specific promoters may be used. In contrast, where gene expression in response to a stimulus is desired, inducible promoters are the regulatory element of choice. Where continuous expression is desired, throughout the cells of a plant, constitutive promoters are utilized. Additional regulatory sequences upstream and/or downstream from the core promoter sequence may be included in expression constructs of transformation vectors to bring about varying levels of expression of heterologous nucleotide sequences in a transgenic plant (Christou, 1996). Frequently, it is desirable to have constitutive or inducible expression of a DNA sequence throughout the cells of an organism in a tissue-independent manner.

Currently, only few promoters that exhibit a constitutive pattern of expression in plants are available, examples of which include the CaMV 35S, nopaline synthase, rice actin and the ubiquitin promoters. Thus, isolation and characterization of constitutive promoters that can serve as regulatory regions for expression of heterologous nucleotide sequences of interest are needed for genetic manipulation of plants. Promoters derived from monocot species often fail to exhibit a regulated pattern of expression in transgenic dicots, whereas in transgenic monocots, they show a highly regulated expression pattern (Shimamoto, 1994).

Rapid progress has been made in maize transformation since the first fertile transgenic plants of maize were generated in 1990 (Fromm *et al*., 1990; Gordon-Kamm *et al*., 1990). Recently, the biolistic particle delivery system has become an established procedure for maize transformation. Immature embryos are the most widely used explants for plant regeneration in cereals and has proven to be particularly useful for biolistic DNA delivery and the production of transgenic plants (Songstad *et al*., 1996; Van der Geest and Petolino 1998; Bohorova *et al*., 1999).

The main objective of the present work was to evaluate the efficiency of three novel maize promoters in driving the GUS gene transient expression. As target plants, we used maize (as a monocot) and tomato (as a dicot). This is of practical importance for selecting the most useful promoter(s) for functional gene transfer to maize and also to determine the usefulness of these promoters in the transformation of tomato as a representative of dicotelydonous plants.

MATERIALS AND METHODS

Plant material and culture media

The explants used for transformation of maize were the immature embryos of the elite Egyptian maize inbred line Gz624. Seeds were provided by the maize research program of the Field Crops Research Institute, Agricultural Research Center, Giza, Egypt. The ears were harvested from field grown plants when immature embryos were 1.0 to 2.0 mm long, sterilized, then immature embryos were excised and cultured on N6 nutrient medium (Chu *et al*., 1975) supplemented with 100 mg/l myo-inositol, and 2 mg/l 2,4-D. Immature embryos were incubated in the dark on this medium for 48 hrs prior to bombardment.

For tomato transformation, leaflets of the tomato cultivar (Castlerock) were taken from greenhouse grown plants and placed on petri dishes on a wet cotton pad prior to bombardment.

Promoters and plasmids

Three novel maize promoters vz., enolase, maize actin and Gos-2 were evaluated

for their efficiency in driving the transient GUS expression. These promoters were fused with the *uidA* reporter gene coding for the -glucuronidase (GUS) enzyme in three different plasmid constructs, vz., pAGS-Gos-2, pAGS-Enolase, pAGS-Actin-2. Moreover, plasmids pAGS-GOS-2in, pAGS-Enolase-in and pAGS-Actin-2in, contained the same promoters with the INTIUBI1ZM intron, Fig. (1). Plasmid Ubi-GUS containing the GUS gene driven by Ubi promoter was used as a positive control for the maize transformation experiments, while plasmids pBI 221 (5.611 Kb) and pILTAB 380 (5.271 Kb) containing the GUS gene driven by CaMV-35S and CsVMV promoters, respectively were used as positive control plasmids for tomato transformation experiments.

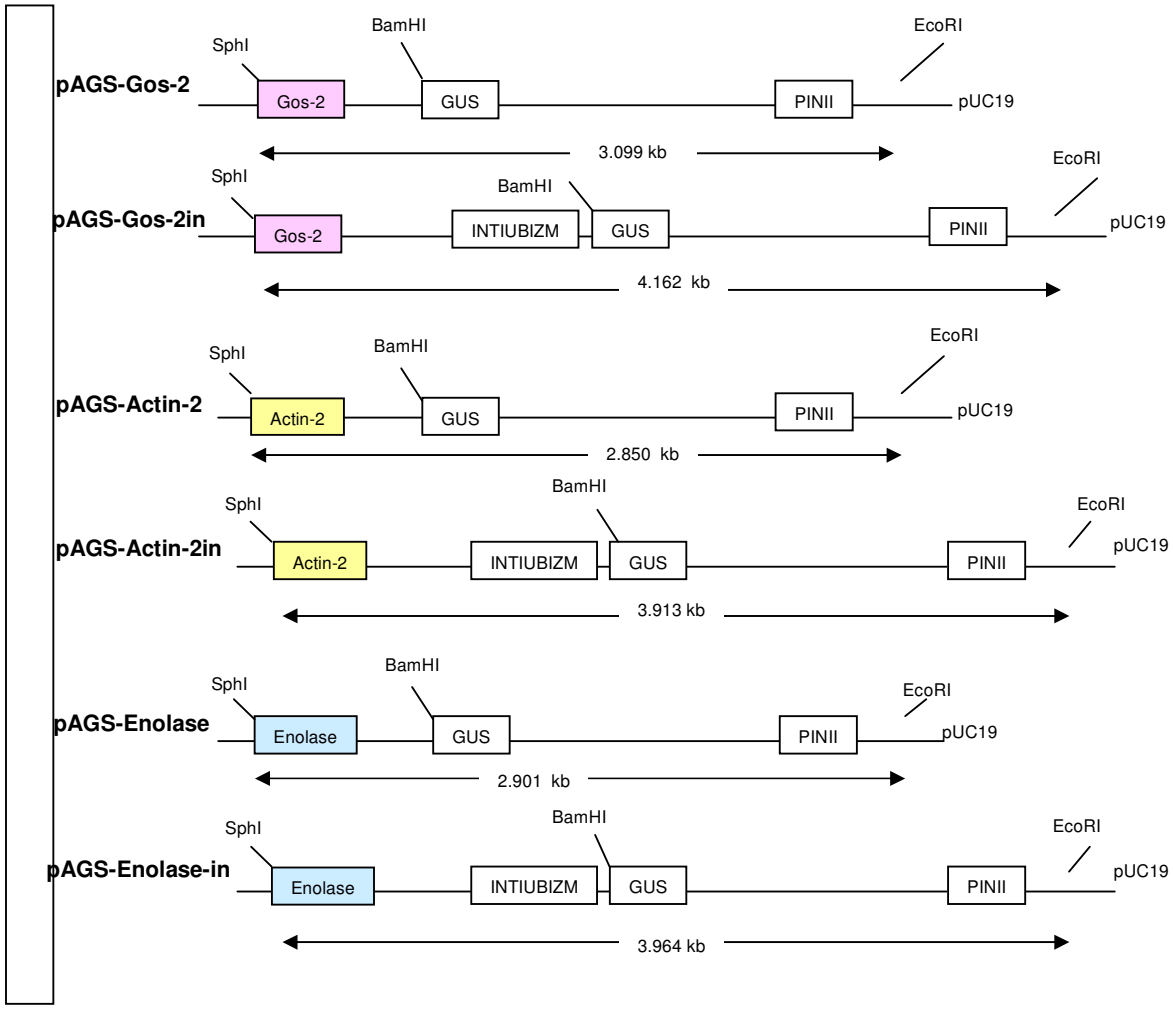

Fig. (1): Plasmids used in transformation experiments.

Plant transformation

Transformation experiments were carried out using the Biolistic particle acceleration device (PDS 1000/He, Bio-Rad). Microcarriers were prepared by precipitation of plasmid DNA onto gold particles 1.0 µm in diameter

(Bio-Rad) following a modified protocol for the Biolistic PDS-1000/He Particle Delivery System (Bio-Rad) (Zhong *et al*., 1996 ;Assem, 1999). Thirty immature embryos of maize were placed in the center of each petri dish and bombarded once at 1350 psi with the particles coated with plasmid DNA (8 replicates for each plasmid). Leaflets of tomato were placed in the center of petri dishes (one leaflet per plate), on cotton pads and were bombarded (one shot per plate) with acceleration pressure of 900 psi as recommended by Gaafar (1999). Eight replicates were performed for each plasmid. Bombardment without any DNA was also performed as negative control for maize and tomato transformation. Tomato leaflets and maize embryos were then incubated at 25°C. GUS activity assay was performed according to Jefferson (1987), 24 hrs after bombardment. The embryos and leaflets were incubated with the substrate-GUS buffer in the dark at 37°C for 20 hrs and the number of transient signals (blue spots) that represent GUS gene expression was determined under the binocular stereomicroscope.

Statistical Analysis

The data were analyzed using randomized complete block design (RCBD) with eight replicates, and then the results were compared using L.S.D. test at 0.05 levels of probability.

RESULTS AND DISCUSSION

Effective transformation systems require appropriate genetic constructs to facilitate integration and expression of the transgene(s). Regulatory sequences or promoters constitute an essential part of genetic constructs. Therefore, an essential requirement in transformation experiments is the availability of efficient promoters.

A powerful tool for the rapid analysis of promoters is the use of marker genes from which expression can be easily monitored by autoradiography (*NPT II, CAT*), light emission (*LUC,* GUS), or color production (GUS). Among these reporter genes GUS and *LUC* are of special interest since their assays do not involve radiolabeling (Reichel *et al*., 1996).

In the present investigation, an attempt has been made to evaluate the efficiency of three novel promoters, isolated from maize (Barbour *et al.*, 2000), in driving the transient expression of GUS gene in two different plant systems, i.e. maize as a monocot and tomato as a dicotelydonous plant species.

Effect of promoters on transient GUS activity in bombarded maize embryos

As shown in Fig. (2), the histological GUS assay revealed considerable variation in GUS activity among immature embryos for most promoters. The data revealed that the highest average number of blue spots was given by plasmids bearing the GUS gene driven by the Gos-2 promoter with intron followed by enolase promoter with intron (1014.0 and 980.3, respectively). However, the transient GUS expression shown by these promoters was not significantly different than that given by the Ubi promoter (985.0), which was used as a positive control. The maize actin promoter revealed the lowest GUS expression amongst plasmids bearing introns upstream the GUS gene. In this respect, Cornejo *et al*. (1993) reported that the maize ubiquitin promoter has been shown to give high level of reporter gene expression in transgenic rice.

Among the plasmids that contain the promoters without an intron, GOS-2 promoter gave the highest significant average value of GUS expression (551.1), (Fig.4-A), when compared to the average values of GUS expression driven by maize actin and enolase

promoters (48.38 and 2.125, respectively). However, the average value of GUS expression given by the intronless GOS-2 promoter was not significantly different from that of the maize actin promoter with intron (551.1 and 456.6, respectively).

Therefore, the present results demonstrated that the two newly isolated promoters GOS-2 and enolase were efficient promoters for maize transformation at the level of transient GUS gene expression, particularly when fused to an intron. Similarly, in the literature, a number of investigators have shown enhanced gene expression in monocots by introducing a monocot intron between the promoter and reporter gene (Callis *et al*., 1987; Vasil *et al*., 1989; Mascarenhas *et al*., 1990; Last *et al*., 1991; Rathus *et al*., 1993; Wilmink *et al*., 1995). Wilmink *et al*. (1995) added that although the relative activities vary among the experiments, but it was clear that the presence of an intron downstream the promoter increased GUS expression in most monocots.

Effect of promoters on transient GUS expression in bombarded tomato leaflets

In tomato, as a dicot., a different response was observed, Fig. (4-B). The highest GUS activity was expressed by the CsVMV promoter which is used in the present study as a positive control and achieved a far better expression than the most commonly used CaMV 35S promoter. This result is in good agreement with Saad (2001), who bombarded tomato leaflets with four constructs containing different promoters (Act-1, CsVMV, 35S CaMV and Ubi-1) and demonstrated that the highest GUS expression was generated by the construct pILTAB380 harbouring the CsVMV promoter. The activity of the maize actin promoter when fused to an intron was less than that of the CsVMV promoter, but the difference was statistically insignificant.

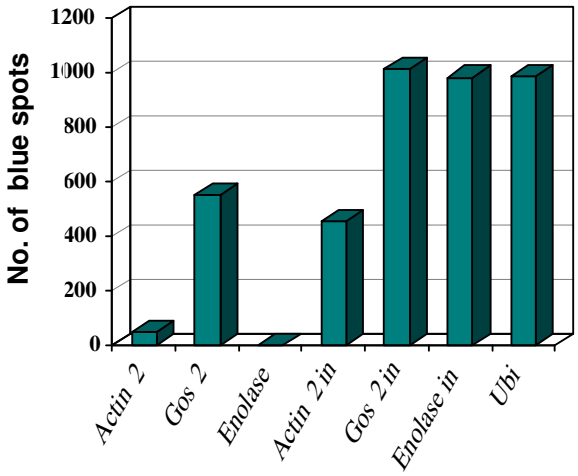

Fig. (2): Average number of GUS blue spots in transformed maize embryos.

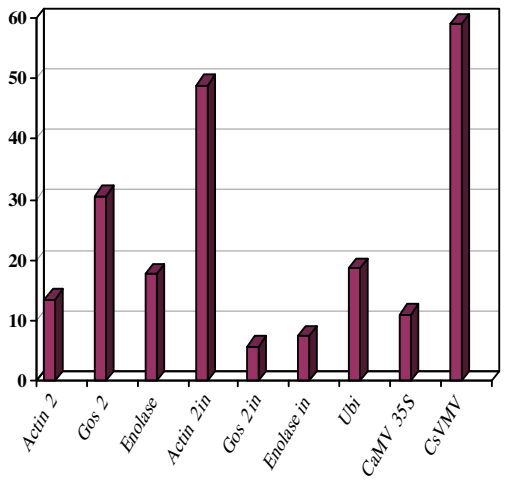

Fig. (3): Average number of GUS blue spots in transformed tomato leaflets.

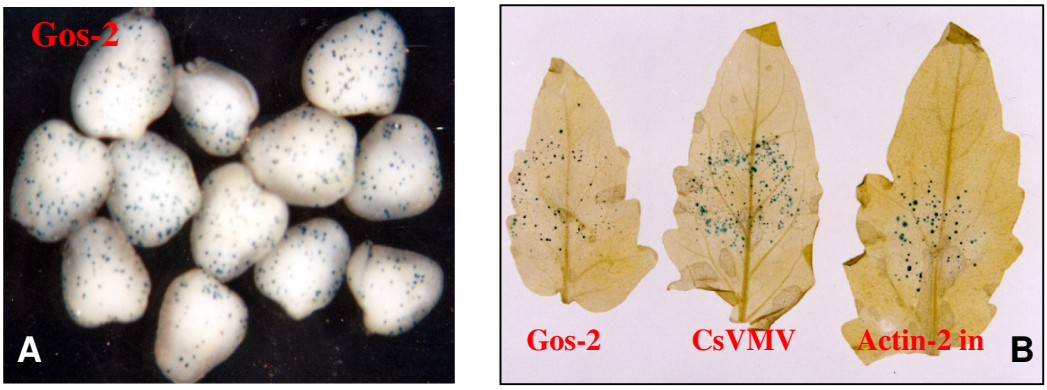

Fig. (4): (A) Transient GUS gene expression in immature embryos transformed with pAGS-Gos-2. (B) transient GUS gene expression in tomato leaflets transformed with pAGS-Gos-2, pILTAB 380 and pAGS-Actin-2in.

In comparing the number of blue spots given by other promoters, significant differences in the means of transient GUS activity were found between maize actin without intron and GOS-2 without intron (13.63 and 30.63, respectively) and between Gos-2 without intron and Gos-2 with intron (30.63 and 5.875, respectively) and also between Gos-2 without intron and enolase with intron (30.63 and 7.625, respectively). There were no significant differences between the average value of GUS expression when driven by maize actin, or enolase without intron, or Gos-2, enolase with intron, or Ubi or 35S promoters (13.63, 17.75, 5.875, 7.625, 18.75 and 11.13, respectively). The lowest expression level of the GUS gene was obtained when driven by the GOS-2 with intron.

From the transient GUS expression results obtained from tomato transformation experiments, a conclusion can be drawn that the CsVMV promoter is more active than monocot promoters in tomato, while the activity of the monocot promoters was comparable to/or higher than that of the 35S promoter. This conclusion contradicts the results of Wilmink *et al*. (1995), which showed that the 35S promoter is more active than monocot promoters in *Nicotiana* species. This contradiction could be attributed to the use of different promoters. In this context, Wilmink *et al*. (1995) interpreted their results on the basis that the monocot promoter activity is inhibited in dicots by the presence of the intron between promoter and gene present in the constructs tested. On the other hand, Saad (2001) found no significant difference between the mean numbers of blue foci expressed by the two constructs pBI221 and pUBI:GUS harbouring the 35S and the Ubi-1 promoters, respectively. As shown in Fig. (3), the maize ubiquitin promoter was one of the promoters which gave relatively low expression of GUS gene in tomato leaflets. These results are in accordance with those reported by Schledzewski and Mendel (1994), who stated that the highest level of expression in tobacco cells was obtained in a low frequency by maize Ubi-1 promoter.

The present results revealed that the newly isolated Gos-2 promoter is highly efficient in increasing the transcription level in maize particularly in the presence of an intron. While in tomato, although the transcription level expressed by the GOS-2 promoter was higher than that obtained by the 35S promoter,

however the transcription inducing effect of the monocot promoter decreased significantly in the presence of an intron between the promoter and the reporter gene. An inverse relationship was observed with the Actin promoter in the presence and absence of an intron. The presence of an intron between this promoter and the reporter gene increased the transcription level significantly. In this respect, Last *et al*., (1991) and Wilmink *et al*. (1995) suggested that the molecular mechanisms underlying gene expression are not the same for monocots and dicots, there may be differences with respect to transcription factors. Keith and Chua (1986) concluded, from their studies on the processing of monocot and dicot pre-mRNAs in tobacco, that differences may exist in sequences required for RNA processing between monocot and dicot plants. Monocot introns are spliced at lower rates in dicots than in monocots. Similarly, Goodall and Filipowicz (1991) indicated that monocots differ from dicots in their mechanism of intron recognition. They showed that monocot splicing seems to be more "permissive" than dicot splicing since the monocot maize was shown to be able to recognize and splice many introns that were spliced poorly or not at all in tobacco.

In summary, our results reveal the efficiency of the newly isolated maize promoters in both monocots and dicots. However, it appears that the level of activity of a promoter cannot be generalized and it could be suggested that each plant species should be tested with a set of promoters in the presence and in the absence of intron.

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الملخص العربي

مقارنة كفاءة بعض المحفزات الجديدة المعزولة من الذرة الشامية في نباتات أحادية وثنائية الفلقة

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تم تقييم ثلاثة محفزات جينية جديدة معزولة من الذرة الشامية (Gos-2, Enolase and Actin-2) و قياس مدى كفائتهم في تحفيز التعبير الجينى لجين الـ *GUS* . وضعت هذه المحفزات في ثلاثة بلازميدات تحتوى على جين الـ *GUS* وثلاثة بلازميدات أخرى تحتوى على جين الـ GUS في وجود انترون (intron) . استخدمت هذه البلازميدات الستة فى إجراء التحول الوراثي باستخدام قاذفة الجينات (Biolistic gun) لأجنة الذرة الشامية غير مكتملة النمو كمثال لنباتات ذوات الفلقة الواحدة و وريقات الطماطم كمثال لنباتات ذوات الفلقتين . أظهرت النتائج كفاءة المحفزات الجينية الجديدة فى كلا النظامين . من بين الستة بلازميدات اظهر البلازميد المحتوى على المحفز الجينى Gos-2كفاءة عاليه فى تحفيز التعبير المؤقت لجين الـ *GUS* في أجنة الذرة الشامية . بينما كان البلازميد المحتوى على المحفز الجينى Actin-2فى وجود الـ intron هو الأكثر كفاءة في تحفيز التعبير الجينى المؤقت لجين الـ *GUS* في وريقات الطماطم.