Cloning of dicot gene promoter in expression vector

S. Raza, S. farooqi and H. Mubeen

Abstract— Genetic engineering involves direct genetic modification of organisms using recombinant DNA technology. The expression of transgenes is regulated by the promoter attached upstream to the gene. The transformation of plants is very important to impart desirable traits. A variety of promoters is necessary at all levels of genetic engineering in plants to regulate gene expression. The plant promoters fall into various categories including constitutive, tissue specific, inducible and differentially regulated. The study was focused on identification and cloning of sucrose synthase gene promoter in modified expression vector pGR1 derived from (pJIT166). The cloned promoters can further be studied for transgene analysis and *GUS* studies.

Index Terms— Promoters, Transformation, Vector, *GUS*

1 INTRODUCTION

Plant transformation is important genetic engineering tool for introducing foreign genes into plant genomes. It is a powerful application used to study gene expression in plants. The expression of transgenes is regulated by the promoter attached upstream to the gene. A variety of promoters is necessary at all levels of genetic engineering in plants from basic research to the development of economically viable crops. The plant promoters fall into various categories including constitutive, tissue specific, inducible and differentially regulated. Moreover, different promoters in the same category may have different strengths for the expression of a gene being regulated by them. Gene promoters that direct high levels of constitutive gene expression are important for crop biotechnology applications. Currently, the most widely used plant promoters for gene expression in plants include, 2X35S, Ubiquitin and RolC etc. The 35S promoter and its derivatives can drive high levels of transgene expression in dicotyledonous plants (Battraw and Hall, 1990; Benfey et al., 1990), their activities are substantially lower in monocotyledonous plants
(Christensen et al., 1992; Gupta et al., 2001; Weeks et al., 1993). These promoters are very useful but have property rights. Moreover, additional promoters are always required to clone multiple genes in a vector, specifically when each of the gene is targeted to different tissues for appropriate expression.

The correct regulatory sequences are added to the gene of interest and the cassette in a transformation vector is transferred to the plant tissues using an appropriate methodology.

[1] *Dean, Faculty of Biotechnology and Agro- Sciences, University of South Asia, Lahore, Pakistan.* .PH-92-3004238303. E-mail: mianrs@yahoo.com

[2,3] Lecturer, Faculty of Biological Sciences, University of South Asia, Lahore, Pakistan*PH-92-3007943269. E-mail:hira_sh@hotmail.com, sahrishfarooqi5@gmail.com*

The transformation vector usually contains a selectable marker gene, which allows selection for the transgenics. Promoters are a set of transcription control modules clustered around the initiation site of RNA polymerase II (Russell, 1996). They are important in the control of the overall expression profile of a gene, either driving or preventing transcription at appropriate times and places. Plant transformation can be carried out in a number of different ways depending on the plant species. Both physical and biological methods exist for transformation. Physical methods include particle bombardment, electroporation, microinjection and sonication. On the other hand, biological methods include *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes* mediated plant transformation. The transformation of plants through *Agrobacterium* is very important to stably integrate desirable genes in the host genome.

Plant transfomation methods

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Particle Bombardment and Electroporation

Biolistic approach for gene transformation is also known as “Particle Bombardment”. It involves shooting a piece of DNA into the recipient plant tissue. This is carried out using a gene gun. Tungsten or gold particles are coated with the plasmid vector having gene of interest. The coated metal particles are accelerated by Helium and bombarded on plant tissues. The particles pass through the plant cell wall and most of them may enter the nucleus under suitable conditions. The electric field generates holes in the plasma membrane thus allowing DNA to be taken up by the cell. The mortality rate of cells is high (25-50% survival) in this method. This technique can be used for variety of species and tissue types but the draw back is that it requires an established

protoplast regeneration system for the plant species being manipulated with foreign DNA.

Transformation of dicotyledonous plants

Dicotyledonous plants are those which develop from two cotyledons in the seed. They can be recognized by the branching veins in their leaves. Dicots of commercial value include many horticultural plants such as petunias, and crops such as tobacco, tomatoes, cotton, soybean and potatoes. Tobacco, due to its ease of transformation initially became the workhorse of plant genetic engineering, but more recently the *Arabidopsis thaliana*, has become very popular. A large number of constitutive, tissue-specific and inducible promoters have been characterized and reported in dicot plants. Hence, these plant-derived promoters can be characterized and used for developing transgenic plants (Rushton *et al*., 2002). An example of constitutive dicot gene promoter is polyubiquitin promoter (Gmubi). This promoter from soybean (*Glycine max*) showed high levels of constitutive expression and was used as an alternative to viral promoters (CaMV35S; Potenza *et al*. 2004) for driving gene expression in soybean (Hernandez-Garcia *et al.*, 2009). The characterization and understanding the functions of other dicot plant promoters is essential to enhance the understanding of gene expression and to develop transgenic plants with specialized traits. The present study is planned to analyze and clone the *SUS* dicot gene promoter in plant expression vector pGA482.

2 Materials and Methods

The motive of this research was to clone the promoter sequence previously isolated from HTGS sequence database. The regulatory sequence was isolated from highly expressed dicot genes.

2.1 Cloning of *SUS* expression cassette in plant transformation vector (pGR1)

Construct having the promoter for *SUS* was provided by the Gene Isolation Group, National institute for biotechnology and genetic engineering, Faisalabad. The construct was cloned in a modified vector derived from pJIT166 (pGR1) that contains *GUS* with intron under 2X35S promoter followed by CaMV terminator. Glycerol stock of *SUS* was streaked on LB agar plates having ampicilin. The single colonies were cultured to isolate the plasmid and verified by restriction digestion of isolated plasmids with *Sac*I and *Hind*III. The construct was further verified by PCR amplification.

3 RESULTS

3.1 Cloning of *SUS* promoter clone in pGR1

The construct pGRSUSP was cloned in a modified form of pJIT166 (pGR1) containing *GUS* with intron under 2X35S promoter followed by CaMV terminator. The clone was confirmed by restriction and PCR analysis before proceeding for cloning in plant expression vector.

3.2 Confirmation of *SUS* gene promoter clone in pGR1 by double digestion and PCR

Double digestion of *SUS* promoter clone in pGR1 vectors (pGRSUSP) with *Sac*I and *Hind*III gave two discrete bands of vector backbone (5.1Kb) and *SUS* promoter (2700bp) on 1% agarose gel. Further, confirmed by PCR using the promoter specific primers. The gel electrophoresis of the fragments indicated discrete DNA fragments of expected amplicon sizes.

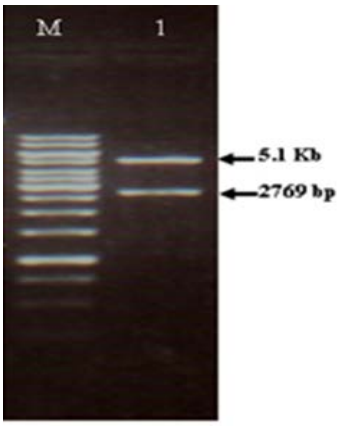

Fig: (1) Confirmation of *SUS* promoter clone in pGR1 vector, Lane M represents 1Kb DNA ladder, Lane 1: Digestion of pGRSUSP with Sac*I* and Hind*III* released a 2769bp fragment.

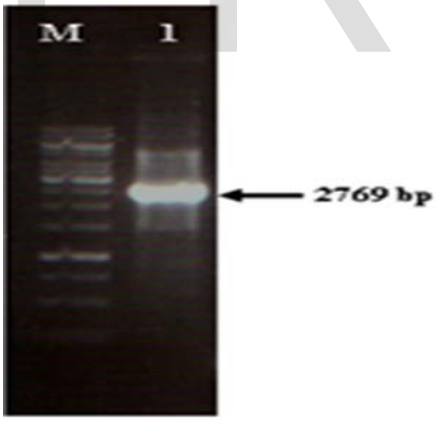

Fig: (2) Confirmation of promoter clones in pGR1 vecotor by PCR, M: 1Kb DNA ladder, Lane 1: PCR amplification of *SUS* promoter from pGRSUSP.

DISCUSSION

The present study was focused on cloning of dicot promoter in modified expression vector (pGR1). The promoter region from highly expressed constitutive gene sucrose synthase (*SUS*) was selected for cloning. The promoter is an important component in a plant transformation vector and is generally patented after its discovery and usefulness. To develop indigenous promoters free from IPR issues, it is a prerequisite to

clone characterize them by expressing in a model plant system. The novel constitutive gene promoters provide great asset for improvement and advancement in crop biotechnology applications.

CONCLUSION

These constitutive cloned promoters like *SuS* can be used for developing transgenic plants with controlled expression of desired genes. The use of alternative promoters with similar characteristics is essential for the stacking of several transgenes in order to avoid homology-dependent gene silencing, a phenomenon which often occurs in transgenic plants with multiple copies of the same promoter.

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