REVIEW

Endeavours of RuBisCO Small Subunit Promoter as a Tool of Green Tissue Specific Expression

Allah BAKHSH[1,2] and Tayyab HUSNAIN[2]

[1]Department of Field Crops, Faculty of Agriculture, Ankara University, Ankara, Turkey; [2]National Centre of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan

Abstract: Transcriptomics has the potential to rapidly increase our knowledge of spatial and temporal gene expression and contributes to the characterization of new promoters for research and development. The successful application of transgenic technology has been further strengthened by the availability of a broad spectrum of promoters having the ability to regulate the temporal and spatial expression patterns of the transgene. A variety of promoters is necessary at all levels of genetic engineering in plants, from basic research discoveries, to development of economically viable crops and plant commodities, to addressing legitimate concerns raised about the safety and containment of transgenic plants in the environment. Compared with the temporal- or spatial-specific expression of the toxin, constitutive expression of foreign proteins in transgenic plants may cause adverse effects. Constitutive overexpression of transgenes that interfere with normal processes in a plant underscores the need for refinement of transgene expression. The development of tissue-specific promoters to drive transgene expression has helped us to fulfil that need. Therefore, in certain circumstances, it is desirable to use expression-specific promoters which express only the foreign gene in specific plant tissues or organs. This review highlights the uses and benefits reaped by researchers by using a green tissue specific promoter, RuBisCO small subunit promoter, in different crops and systems and thus establishing a broad range of tissue specific promoters. Such plant promoters that are activated precisely when and where they are needed would be ideal for genetic engineering strategies.

Keywords: constitutive expression; *rbcS* promoter; spatio-temporal expression

Promoters are regions of the DNA upstream of a gene's coding region that contain specific sequences recognized by proteins involved in the initiation of transcription (Buchanan *et al.* 2000). Numerous promoters have been isolated from a wide variety of organisms over the years and applied to plant genetic engineering systems. Promoters affect transcription both quantitatively and qualitatively. The success of gene transfer technologies varying from basic research to crop improvement for biopharming depends on the efficacious selection and use of promoters (Potenza *et al.* 2004).

Constitutive promoters are widely used in insect-resistant transgenic rice to express *Bt* genes, such as 35S CaMV promoter (Cheng *et al.* 1998; Alam *et al.* 1999), ubiquitin promoter (Chen *et al.* 2005; Tang *et al.* 2006) and actin promoter (Wu *et al.* 1997; Tu *et al.* 2004). However, compared with the temporal or

spatial specific expression of the toxin, constitutive expression of foreign proteins in transgenic plants may cause adverse effects, such as the metabolic burden imposed on plants by constant synthesis of foreign gene products, and these may increase the potential risk of resistance of the target insects to *Bt*. There is also a concern about the food safety of genetically modified plants (Kuiper *et al.* 2001; Shelton *et al.* 2002; Conner *et al.* 2003).

Constitutive expression can be problematic for several reasons. If a specific transgene is overexpressed at the wrong time in development, in tissues where it is not normally expressed, or at very high levels, it can have unexpected consequences for plant growth and development, and potentially for the environment. For instance, the constitutive expression of signal-transduction 'master-switches' for pathogen resistance can lead to decreased growth (Bowling *et al.* 1994, 1997) or enhanced susceptibility to other pathogens (Stuiver & Custers 2001; Berrocal-Lobo *et al.* 2002). Concerns that the constitutive overexpression of *Bacillus thuringiensis* insect toxins in commodity crop plants will increase targeted insect resistance (Huang *et al.* 1999) have led the Environmental Protection Agency (EPA) to announce rules for resistance management by planting refuges of conventional crops. Therefore, in certain circumstances, it is desirable to use expression-specific promoters which express only the foreign gene in specific plant tissues or organs (Cai *et al.* 2007).

Targeted expression has become particularly important for the future development of value-added crops because the public may more likely accept the 'less intrusive' expression of the transgene. For example, the confinement of an insecticidal transgene product to tissue besieged by insect pests instead of harvestable material could have potentially defused the Starlink corn fiasco (Bucchini & Goldman 2002).

The second most common group of promoters after viral promoters for plant biotechnology has come from highly-expressed plant genes, such as those for seed storage proteins, photosynthetic proteins or housekeeping genes, whose mRNAs (messenger RNA) were easily cloned and characterized in all cases (Potenza *et al.* 2004). Actin, ubiquitin and tubulin gene promoters have all been used in various plant species for expressing transgenes or selectable markers. As sophistication in biotechnology proceeds, the need for more developmentally or environmentally regulated promoters has become evident and considerable effort is going into the discovery of specific tissue or biotic, hormonal or abiotic stress responsive genes and promoters (Potenza *et al.* 2004).

RuBisCO small subunit promoter as a green tissue-specific promoter tool

RuBisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase) is the bifunctional enzyme found in the chloroplasts of plants that catalyzes the initial carbon dioxide fixation step in the Calvin cycle and functions as an oxygenase in photorespiration. In higher plants it consists of eight molecules each of two subunits, a large subunit (LSU) encoded by the chloroplast genome and a small subunit (SSU) polypeptide encoded by the nuclear genome (Ellis 1981). The SSU polypeptides are formed as precursors containing an amino-terminal extension termed a transit peptide that is involved in the transport of the SSU polypeptide into the chloroplast during which the transit peptide is removed (Figure 1). RuBisCO is the most abundant protein found in plant leaves, representing up to 50% of the soluble proteins. Thus, the SSU promoters and their transit peptides are attractive candidates for expression of genes at high levels in green tissue and for targeting of different proteins into the chloroplast (Ellis 1981).

In plants, the best-characterized light-inducible genes are members of the *rbcS* (RuBisCO small subunit) multigene family encoding the small subunit of ribulose-1,5-bisphosphate carboxylase. It has been found that promoters from *rbcS* genes contain an intricate assortment of positive and negative regulatory elements that are able to confer light-inducible and tissue-specific expression in transgenic plants (Gilmartin & Chua 1990).

The expression of a member of the multigene family encoding the small subunit (*rbcS*) of ribulose-1,5-bisphosphate carboxylase was examined in various tissues of pea. The *rbcS* gene, *pPS-2.4*, was characterized by DNA sequence analysis and 5' and 3' end mapping of its mRNA transcript. *rbcS* polypeptides were shown to be differentially present in various tissues of light- and dark-grown plants. It was concluded that the *pPS-2.4* gene is expressed in a tissue-specific, light-regulated fashion and that transcriptional controls of individual *rbcS* genes vary (Coruzzi *et al.* 1984).

Kuhlemeier *et al.* (1987) performed deletion analysis of a model *rbcS* promoter (pea *rbcS-3A*).

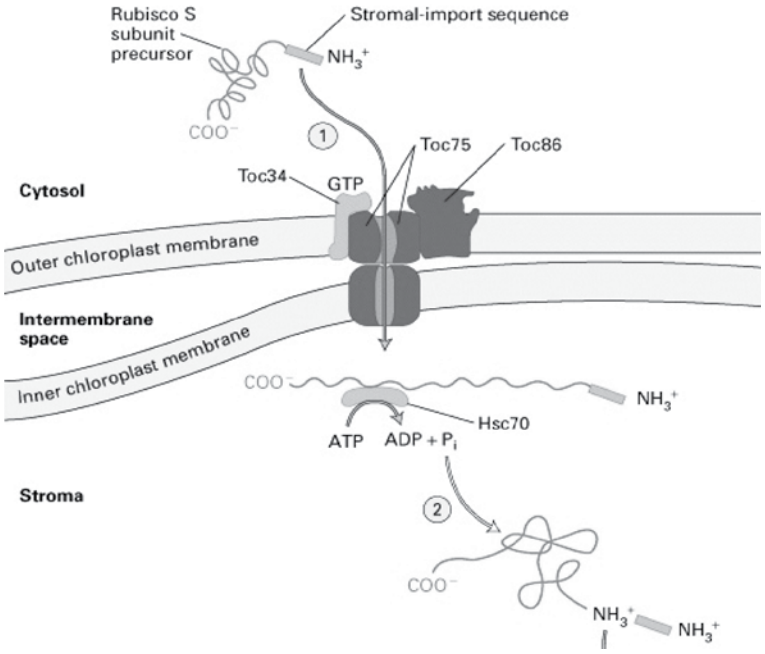

Figure 1. RuBisCo small subunit encoded by the nuclear genome; these nucleus encoded subunits are synthesized as precursors on cytosolic 80S ribosomes and targeted to the chloroplast; adapted from Molecular Cell Biology, a book by Lodish *et al.* (2000)

It revealed redundant light-specific response elements (LREs), which, when removed, greatly reduced light-induced expression. The LRE located between 169 and 2112 bp contained two binding sites for the transcription factor GT-1 (Green *et al.* 1987). These binding sites, labelled box II (2151 to 2136 bp) and box III (2125 to 2114 bp), are both required for transcriptional activation by light (Kuhlemeier *et al.* 1988) (Figure 2).

In another study, the effects of light and development were analysed on *rbcS-3A* expression in transgenic tobacco. Two highly conserved sequences (boxes II and III) around nucleotide position -150 (relative to the transcription initiation site, +1) are required for *rbcS-3A* expression. The sequences upstream and downstream of nucleotide -170 are capable of directing organ-specific and light dependent transcription (Kuhlemeier *et al.* 1988).

rbcS is encoded by gene families in most plants. Promoters from one group of these genes contain two *cis*-acting elements, the I-box and the G-box, that are important for tissue-specific expression (Donald & Cashmore 1990; Manzara *et al.* 1991). Analysis of transgenic tomato plants

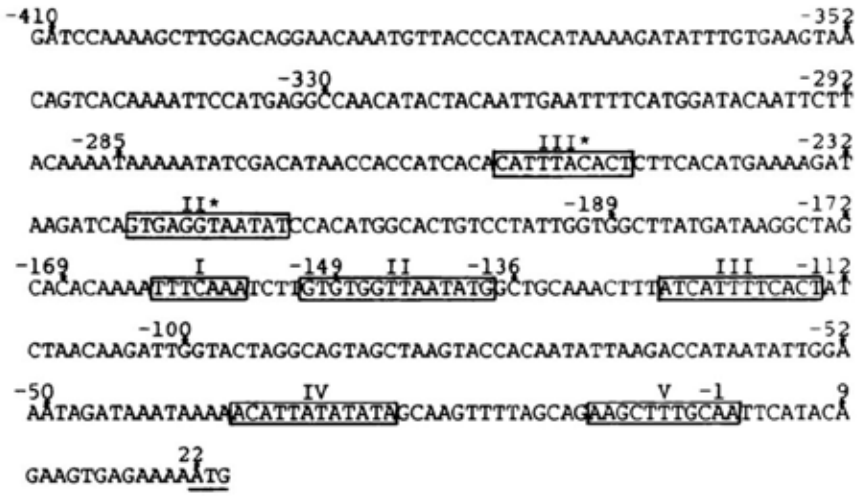

Figure 2. Nucleotide sequence of pea *rbcS3A* promoter as described by Kuhlemeier *et al.* (1987); two highly conserved sequences (Box I and Box II) around -150 bp related to the transcriptional start site are required for *rbcS3A* expression and sequences at both 5' and 3' of -170 direct light regulated and organ specific expression

expressing an rbcS-promoter/GUS fusion gene confirmed that promoter fragments ranging from 0.6 to 3.0 kb of *rbcS1*, *rbcS2*, and *rbcS3A* genes were sufficient to confer the organ-specific expression pattern (Manzara *et al.* 1993; Meier *et al.* 1995). In these genes, the I-box and G-box are located within -600 to -100 bp upstream of the transcription initiation site. The 560-bp promoter fragment from the cotton *rbcS* gene used to assemble the GUS reporter gene construct includes putative I-box (-287 to -274 base pair) and G-box (-260 to -252 base pair) sequences conferring expression comparable to 35S CaMV promoter (Song *et al.* 2000).

Truncated c*ryIA(b)* gene has been introduced into several cultivars of rice (indica and japonica) by microprojectile bombardment and protoplast system. The expression was driven by two constitutive promoters (35S from CaMV and *actin-1* from rice) and two tissue specific promoters (pith tissue and PEP carboxylase /PEPC/ for green tissue from maize). The results demonstrated that PEPC in general and 35S in some lines act as strong promoters in c*ryIA(b)* expression. The level of *Bt* protein was generally high in the leaves under the PEPC promoter which was comparable with the levels of a few high *Bt* protein plants with the 35SP or actin-1 promoter (Datta e*t al.* 1998).

The expression of the modified gene for a truncated form of the *cryIA(c)* gene, encoding the insecticidal portion of the lepidopteran-active *cryIA(c)* protein from *Bacillus thuringiensis* var. *kurstaki* (*B.t.k.*) HD73, under control of the *Arabidopsis thaliana* ribulose-l,5-bisphosphate carboxylase (RuBisCO) small subunit *atsiA* promoter with and without its associated transit peptide was analysed in transgenic tobacco plants. The examination of leaf tissue revealed that the *atslA* promoter with its transit peptide sequence fused to the truncated *cryIA(c)* protein provided a 10-fold to 20-fold increase in *cryIA(c)* mRNA and protein levels compared to gene constructs in which the cauliflower mosaic virus 35S promoter with a duplication of the enhancer region (CaMV-En35S) was used to express the same *cryIA(c)* gene (Wong *et al.* 1992).

Fujimoto *et al.* (1993) developed insect resistant rice by introducing a truncated δ-endotoxin gene, *cryIA(b)* of *Bacillus thuringiensis* (*B.t.*) driven by a green tissue specific promoter (*rbcS*). Transgenic plants efficiently expressed the modified *cryIA(b)* gene at both mRNA and protein levels. The transformed plants had nearly 0.05% toxin of the total soluble leaf protein and showed good resistance to the rice leaf folder (*Cnaphalocrosis medinalis* L.) and yellow stem borer (*Chilosuppressalis* sp).

Song *et al.* (2000) isolated two promoters. These promoters were *Gh-sp*, derived from a seed protein gene, and *Gh-rbcS*, obtained from a nuclear encoded chloroplast gene. These two promoters fused separately to GUS gene were transferred into cotton (*Gossypium hirsutum* L. cv. Coker 312) by *Agrobacterium*-mediated transformation. Transgenic plants from T_0 generation were analysed for expression of the GUS reporter gene in different tissues and developmental stages of cotton. Qualitative and quantitative analyses indicated the *GUS* gene driven by the *Gh-sp* promoter was expressed only during seed maturation, beginning approximately 25 days post-anthesis. The expression of the *GUS* reporter gene driven by the *Gh-rbcS* promoter was detected in leaf tissue of transgenic plants. Levels of GUS expression in leaves of *Gh-rbcS/GUS* transgenic plants were comparable to those of transgenic cotton plants containing a *GUS* gene construct controlled by the CaMV 35S promoter.

Gittins *et al.* (2000) studied the ability of heterologous RuBisCO small-subunit gene promoters, *RbsS3CP* (0.8 kbp) from tomato (*Lycopersicon esculentum* Mill.) and *SRS1P* (1.5 kbp) from soybean (*Glycine max*) to drive the expression of the β-glucuronidase (*GUS*) marker gene in apple (*Malus pumila* Mill.). Transgenic lines of cultivar were produced by *Agrobacterium*-mediated transformation and the levels of GUS expression in the vegetative tissues of young plants were compared with those produced using the cauliflower mosaic virus (CaMV) 35S promoter. The heterologous SSU promoters were active primarily in the green vegetative tissues of apple. The mean GUS activity in the leaf tissue of the SSU promoter transgenics was approximately half that of plants containing the CaMV 35S promoter. Histochemical analysis demonstrated that GUS activity was localized to the mesophyll and palisade cells of the leaf.

The regulatory 5' and 3' non-translated regions of *rbcS1* were engineered to drive the heterologous expression of various genes. The homologous *rbcS1* cassette resulted in a β-glucuronidase accumulation of 0.88% of total soluble protein in *Chrysanthemum morifolium*. In tobacco (*Nicotiana tabacum* L.), the *gusA* expression reached 10% of total soluble protein. The population mean of 2.7% was found to be 7 to 8 fold higher than for the commonly used cauliflower

mosaic virus (CaMV) 35S promoter (population mean 0.34%). The *RbcS1*-driven expression of sea anemone equistatin in potato (*Solanum tuberosum* L.) and potato cystatin in tomato (*Lycopersicon esculentum* Mill.) yielded maximum levels of 3–7% of total soluble protein. The results demonstrated that the compact 2-kb *rbcS1* expression cassette provides a novel nuclear transformation vector that generates plants with expression levels of up to 10% of total protein (Outchkourov *et al.* 2003).

Baranski and Puddephat (2004) compared five heterologous promoters fused to β-glucuronidase gene to evaluate their influence on the localization of GUS activity in cauliflower (*Brassica oleracea* var. *botrytis*) tissues: roots, leaves, petioles and curds. A constitutive promoter CaMV 35S and four tissue specific promoters were used: *extAP* (extensin A, targeting root tissue in rape 1.0 kbp), *PsMTAP* (metallothionein-like, targeting root tissue in pea 0.8 kbp), *RbcS3CP* (ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit from tomato 0.8 kbp) and *SRS1P* (ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit from soybean, 1.5 kbp), and introduced into cauliflower seedling explants using *Agrobacterium rhizogenes* mediated transformation. Quantitative and histochemical GUS assays confirmed the tissue specific GUS expression. It was found that the *extAP* promoter was the most active in petioles but it also caused a significant GUS expression in curds. In the case of *PsMTAP* promoter, GUS activity was hardly observed in curd and limited only to its epidermis. *RbcS3CP* and *SRS1P* promoters controlled a similar expression of the *GUS* gene in plant parts except for curd where *RbcS3CP* was almost inactive.

A 5'-upstream regulation region of the rice RuBisCO small subunit gene was cloned from the Chinese cultivar Wuyunjing 8, and its sequences were confirmed by comparison with the known genome sequences of both japonica and indica rice. The cloned *rbcS* promoter was fused to the 5'-upstream of the GUS (β-glucuronidase) coding region in a binary vector, and was introduced into rice by *Agrobacterium*-mediated transformation. The results of both histochemical staining and quantitative analysis of GUS activity showed that the expression level of GUS fusion gene was significantly stronger in leaf blade and sheath than in other organs of transgenic rice plants, and the GUS activity was restricted to the mesophyll cells of leaf tissue, which showed that the rice *rbcS* promoter could control not only the tissue- but also the cell-specific expression of foreign genes in transgenic rice. Transgene expression regulated by the rice *rbcS* promoter in transgenic rice is significantly enhanced by light induction. The rice *rbcS* promoter might be very useful for the expression of target genes in transgenic rice, with particularly high efficiency in leaf tissues (Liu *et al.* 2005).

Panguluri *et al.* (2005) isolated green tissue-specific promoters of the *rbcS* gene family from pigeon pea (*Cajanus cajan* L.) and transformed tobacco plants with *uidA* gene encoding beta-glucuronidase controlled by *rbcS* promoter. The results clearly showed that this promoter was as strong as the pea rbcS3A promoter that was characterized earlier. Sequence homology studies with pea *rbcS3A* promoter, especially the region (boxes I and III) that is required for *rbcS3A* expression, showed more than 50% divergence. In contrast, this pigeon pea promoter sequence was more similar to that of spinach and rice *rbcS* promoters.

Zheng *et al.* (2005) applied *Agrobacterium*-mediated genetic transformation to produce beet armyworm (*Spodoptera exigua* Hubner) resistant tropical shallots (*Allium cepa* L. group Aggregatum). *Cry1ca* or *H04* hybrid gene from *Bacillus thuringiensis*, driven by *Chrysanthemum rbcS* promoter, along with the hygromycin phosphotransferase gene (*hpt*) driven by the CaMV 35S promoter, was used for genetic transformation. Molecular analysis confirmed the integration and expression of foreign genes. The amount of *cry1ca* expressed in transgenic plants was higher than the expression levels of *H04* (0.39 vs. 0.16% of the total soluble leaf proteins, respectively). There was a good correlation between protein expression and beet armyworm resistance.

Amarasinghe *et al.* (2006) isolated the *rbcS* promoter from *Gossypium hirsutum* L. cv. Coker 315 and transformed *Arabidopsis* and cotton with GUS reporter gene linked with the isolated *rbcS* promoter to determine its expression level. They reported that *rbcS* had the highest expression throughout the vegetative and reproductive stages. Histochemical results in T_2 lines revealed that cotyledons and leaves had a very high expression of the *GUS* gene. The roots had hardly any detectable GUS expression. Young stem and young floral buds also had low levels of GUS expression. In mature open flowers, staining was observed in the green bract surrounding the flower, in the

green ovary wall and in the stigma, and in pollen, but not in ovules.

Lee *et al.* (2006) reported the integration and expression of the c*ry1A(b)* insecticidal gene under *rbcS* promoter in transformed oil palm. A biolistic method was used to transform immature embryos (IEs) of oil palm. More than 700 putative transformed IEs from independent transformation events were generated. Transient transformation efficiency of 81–100% was reached. The presence of c*ry1A(b)* mRNA transcripts showed that it is fully functional in oil palm, at the transcriptional level.

Anisimov *et al.* (2007) studied the activity of the highest expressing RuBisCO small subunit promoters (*prbcS*) from the cotyledons of germinating seedlings of *Brassica rapa* var. oleifera and *Nicotiana tabacum* plants that were transformed using an *Agrobacterium*-mediated transformation strategy to drive high-level and preferably stage-specific transgenic protein expression in plants. The mRNA levels of rbcS and of GUS were quantified in transformed plants. The results demonstrated that the most active promoter in seedlings under native conditions was also the most active in transgenic constructs at the same stage of plant development.

Two truncated versions of *prbcS-2* (360 bp and 624 bp), a full version of *prbcS-2* (1.6kb) and 35S CaMV promoter, were fused to the *gusA* gene to determine a minimal length of the most active promoter in transgenic tobacco plants after *Agrobacterium* transformation. GUS protein expression was determined quantitatively in recovered shoots by an enzymatic assay *in vitro*. GUS expression levels under both truncated *prbcS* versions did not differ significantly from each other; however, it appeared to be more than fourfold lower than that of GUS under the 1.6-kb *prbcS-2* promoter, while being higher than the 35S-GUS expression (Anisimov *et al.* 2007).

RuBisCO contents were substantially increased in rice (*Oryza sativa* L.) plants after *Agrobacterium*-mediated transformation with the rice *rbcS* sense gene under the control of the rice *rbcS* promoter. The primary transformants were screened for the ratio of RuBisCO to leaf-N content, and the transformants with > 120% of wild-type levels of RuBisCO were selected. In the progeny of the selected lines of transformants, the mRNA levels of one member of the *rbcS* gene family were increased from 3.9- to 6.2-fold, whereas those of other members of the *rbcS* gene family were unchanged. The total levels of *rbcS* mRNA were increased from 2.1- to 2.8-fold. The levels of *rbcL* mRNA were increased from 1.2- to 1.9-fold. RuBisCO protein content was significantly increased by 30% on a leaf area basis (Suzuki *et al.* 2007)

Cai *et al.* (2007) isolated a tissue specific promoter (P_{D54O}) from rice and used it to drive the expression of *cry1Ac* encoding *Bacillus thuringiensis* endotoxin against rice leaf-folders. No *cry1Ac* protein was found in endosperm or embryo. A reporter gene regulated by a series of truncated P_{D54O} showed various tissue-specific expression patterns.

Suzuki *et al.* (2009) reported that four out of five members of the *rbcS* multigene family (*OsrbcS2-Os rbcS5*) were highly expressed in leaf blades of rice (*Oryza sativa* L.) irrespective of plant growth stage, whereas the accumulation of all *rbcS* mRNAs in leaf sheaths, roots and developing spikelets was quite low. A highly positive correlation was observed between total *rbcS* and *rbcL* mRNA levels and RuBisCO content at their maxima, irrespective of tissues and growth stage. The results indicated that the total *rbcS* mRNA levels may be a primary determinant for maximal RuBisCO protein content and that the RuBisCO gene expression is well coordinated through the whole life of rice.

The insect resistant gene *cry1c* under rice *rbcS* promoter was transformed in the Zhonghua 11 cultivar of rice (*Oryza sativa* L. sp. *japonica*) via *Agrobacterium*-mediated transformation to confer resistance to yellow stem borer (*Tryporyza incertulas* Walker), striped stem borer (*Chilo suppressalis* Walker) and leaf folder (*Cnaphalocrocis medinalis* Guenec). An elite transgenic line RJ5 was selected which possessed high resistance to leaf folders and stem borers and had a very good agronomic performance. The levels of *cry1c* remained undetectable in the endosperm. It was noted as only 2.6 ng/g in the endosperm (Ye *et al.* 2009).

The synthetic truncated *cry1Ac* gene was linked to the rice *rbcS* promoter and its transit peptide sequence (*tp*) and was transformed in rice using an *Agrobacterium*-mediated transformation method. The use of the *rbcS-tp* sequence increased the *cry1Ac* transcript and protein levels by 25- and 100-fold, respectively, with the accumulated protein in chloroplasts comprising up to 2% of the total soluble proteins. The high level of *cry1Ac* expression resulted in high levels of plant resistance to three common rice pests, rice leaf folder, rice green caterpillar, and rice skipper, as evidenced by insect feeding assays. Transgenic plants were also

evaluated for resistance to natural infestations by rice leaf folder under field conditions. Throughout the entire period of plant growth, the transgenic plants showed no symptoms of damage, whereas non-transgenic control plants were severely damaged by rice leaf folders (Kim *et al.* 2009).

A gradual decline in insecticidal gene (*cry1Ac*) expression was observed in advance cotton lines along with the age of plant (Bakhsh *et al.* 2010, 2011a). In order to combat the variation in insecticidal gene expression, *rbcS* promoter was isolated from *Gossypium arboreum* var. 786. The promoter was fused with the insecticidal gene *cry1Ac* to confer resistance in cotton (*Gossypium hirsutum*) against lepidopteran pests, especially American boll worm. The local cotton variety NIAB-846 was transformed using this construct via *Agrobacterium tumefaciens* strain LB4404. The same cotton variety was transformed with another construct pk2Ac harbouring *cry1Ac* under 35S promoter. The comparative study for insecticidal gene expression in Rb-Ac plants (transformed with *cry1Ac* driven by *rbcS* promoter) and pk2Ac plants (transformed with *cry1Ac* driven by 35S promoter) showed that *rbcS* is an efficient promoter to drive the expression of *cry1Ac* gene in green parts of cotton plants as compared to 35S promoter (Bakhsh 2011b).

Schaart *et al.* (2011) cloned promoter and terminator sequences of an apple (*Malus* × *domestica*) ribulose biphosphate carboxylase small subunit gene (*MdRbcS*) to test the regulatory activity of the isolated promoter and terminator sequences in transgenic tobacco. The *MdRbcS* promoter itself seemed to be less strong than the CaMV35S promoter when both were used in combination with the *nos* terminator. However, the combination of the promoter and terminator of *MdRbcS* was able to drive *GUS* gene to similar expression levels as the reference construct with CaMV35S promoter and nos terminator. It is concluded that the combination of the *MdRbcS* promoter and terminator is a suitable regulatory sequence set for the expression of transgenes to a high level in plants and for intragenesis in apple specifically.

CONCLUSION

New technologies from transcriptomics via proteomics to genome sequencing projects will open up new approaches to the isolation of promoters tailored to answer specific questions in research or create new transgenic crops and products to feed and help the people in the world. Indeed, transcriptomics has the potential to rapidly increase our knowledge of spatial and temporal gene expression and lead to new promoters for research and development. Additionally, the expanding field of bioinformatics will lead to computational analysis of primary structure and function of single promoters, helping define enhancer function and relationships with other conserved motifs in the genome *in silico*. Because only a small portion of an organism's genome is actually composed of transcribed sequences, there is a large, untapped resource of promoter sequences to be studied that can help us to focus on global views of the evolutionary relationship within and across species.

References

Alam M.F., Datta K., Abrigo E., Oliva N., Tu, J., Virmani S.S., Datta S.K. (1999): Transgenic insect-resistant maintainer line (IR68899B) for improvement of hybrid rice. Plant Cell Reports, **18**: 572–575.

Amarasinghe B.H.R., Nitschke E.F., Wu Y., Udall J.A., Dennis E.S., Constable G., Llewellyn D.J. (2006): Genomic approaches to the discovery of promoters for sustained expression in cotton (*Gossypium hirsutum* L.) under field conditions: expression analysis in transgenic cotton and *Arabidopsis* of a RuBisCO small subunit promoter identified using EST sequence analysis and cDNA microarrays. Plant Biotechnology, **23**: 437–450.

Anisimov A., Koivu K., Kanerva A., Kaisalainen S., Juntunen K., Kuvshinov V. (2007): Cloning of new RuBisCO promoters from *Brassica rapa* band determination of their activity in stably transformed *Brassica napus* and *Nicotiana tabacum* plants. Molecular Breeding, **19**: 241–253.

Bakhsh A., Rao A.Q., Shahid A.A., Husnain T., Riazuddin S. (2010): CaMV 35S is a developmental promoter being temporal and spatial in expression pattern of insecticidal genes (*cry1ac* & *cry2a)* in cotton. Australian Journal of Basic and Applied Science, **4**: 37–44.

Bakhsh A., Shahzad K., Husnain T. (2011a): The variation in the spatio-temporal expression of insecticidal genes in cotton. Czech Journal of Genetics and Plant Breeding, **47**: 1–9.

Bakhsh A., Siddique S., Husnain T. (2011b): A molecular approach to combat spatio-temporal variation in insecticidal Gene (*cry1Ac*) expression in cotton. Euphytica, **183**: 65–74.

Baranski R., Puddephat I.J. (2004): Tissue specific expression of ß-glucuronidase gene driven by heterologous promoters in transgenic cauliflower plants. Acta Physiologiae Plantarum, **26**: 307–315.

Berrocal-Lobo M., Molina A., Solano R. (2002): Constitutive expression of *ETHYLENE-RESPONSE-FACTOR1* in *Arabidopsis* confers resistance to several necrotrophic fungi. Plant Journal, **29**: 23–32.

Bowling S.A., Guo A., Cao H., Gordon A.S., Klessig D.F., Dong X. (1994): A mutation in *Arabidopsis* that leads to constitutive expression of systemic acquired resistance. Plant Cell, **6**: 1845–1857.

Bowling S.A., Clarke J.D., Liu Y., Klessig D.F., Dong X. (1997): The cpr5 mutant of *Arabidopsis* expresses both NPR1-dependent and NPR1-independent resistance. Plant Cell, **9**: 1573–1584.

Bucchini L., Goldman L.R. (2002): Starlink corn: a risk analysis. Environmental Health Perspectives, **110**: 5–13.

Buchanan B.B., Gruissem W., Jones R.L. (2000): Biochemistry and Molecular Biology of Plants. American Society of Plant Biologists, Rockville, 340–342.

Cai M., Wei J., Li X.H., Xu C.G., Wang S.P. (2007): A rice promoter containing both novel positive and negative cis-elements for regulation of green tissue-specific gene expression in transgenic plants. Plant Biotechnology Journal, **5**: 664–674.

Coruzzi G., Broglie R., Edwards C., Chua N.H. (1984): Tissue-specific and light-regulated expression of a pea nuclear gene encoding the small subunit of ribulose-1,5-bisphosphate carboxylase. EMBO Journal, **3**: 1671–1679.

Chen H., Tang W., Xu C.G., Li X.H., Lin Y.J., Zhang Q.F. (2005): Transgenic indica rice plants harboring a synthetic *Cry2A* gene of *Bacillus thuringiensis* exhibit enhaced resistance against rice lepidopteran pests. Theoretical and Applied Genetics, **111**: 1330–1337.

Cheng X., Sardana R., Kaplan H., Altosaar I. (1998): *Agrobacterium tumefaciens*-transformed rice plants expressing synthetic *cryIAb* and *cryIAc* genes are highly toxic to striped stem borer and yellow stem-borer. Proceedings of National Academy of Sciences, **95**: 2767–2772.

Conner A.J., Glare T.R., Nap J.P. (2003): The release of genetically modified crops into the environment. Part II. Overview of ecological risk assessment. Plant Journal, **33**: 19–46.

Datta K., Vasquez Z., Tu J., Torrizo L., Alam M.F., Oliva N., Abrigo E., Khush G.S., Datta S.K. (1998): Constitutive and tissue-specific differential expression of the *cryI(A)b* gene in transgenic rice plants conferring resistance to rice insect pests. Theoretical and Applied Genetics, **97**: 20–30.

Donald R.G., Cashmore A.R. (1990): Mutation of either G box or I box sequences profoundly affects expression from the *Arabidopsis* rbcS-1A promoter. EMBO Journal, **9**: 1717–1726.

Ellis R.J. (1981): Chloroplast proteins: Synthesis, transport, and assembly. Annual Review of Plant Physiology, **32**: 111–137.

Fujimoto H., Itoh K., Yamamoto M., Kyozuka J., Shimamoto K. (1993): Insect resistant rice generated by introduction of a modified δ-endotoxin gene of *Bacillus thuringiensis.* Biotechology, **11**: 1151–1155.

Gilmartin P.M., Chua N.H. (1990): Spacing between GT-1 binding sites within a light-responsive element is critical for transcriptional activity. Plant Cell, **2**: 447–455.

Gittins J.R., Pellny T.K., Hiles E.R., Rosa C., Biricolti S., James D.J. (2000): Transgene expression driven by heterologous ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit gene promoters in the vegetative tissues of apple (*Malus pumila* Mill.). Planta, **210**: 232–240.

Green P.J., Kay S.A., Chua N.H. (1987): Sequence-specific interactions of a pea nuclear factor with light-responsive elements upstream of the *rbcS-3A* gene. EMBO Journal, **6**: 2543–2549.

Huang F., Buschman L.L., Higgins R.A., Mcgaughey W.H. (1999): Inheritance of resistance to *Bacillus thuringiensis* toxin (Dispel ES) in the European corn borer. Science, **284**: 965–967.

Kim E.H., Suh S.C., Park B.S., Shin K.S., Kweon S.J., Han E.J., Park S.H., Kim Y.S., Kim J.K. (2009): Chloroplast- targeted expression of synthetic *Cry1Ac* in transgenic rice as an alternative strategy for increased pest protection. Planta, **230**: 397–405.

Kuhlemeier C., Fluhr R., Green P.J., Chua N.H. (1987): Sequences in the pea *rbcS-3A* gene have homology to constitutive mammalian enhancers but function as negative regulatory elements. Genes and Developments, **1**: 247–255.

Kuhlemeier C., Cuozzo M., Green P., Goyvaerts E., Ward K., Chua N.H. (1988): Localization and conditional redundancy of regulatory elements in *rbcS-3A*, a pea gene encoding the small subunit of ribulose-bisphosphate carboxylase. Proceedings of National Academy of Sciences, **85**: 4662–4666.

Kuiper H.A., Kleter G.A., Noteborn H.P., Kok E.J. (2001): Assessment of the food safety issues related to genetically modified foods. Plant Journal, **27**: 503–528.

Lee M., Yeun L., Abdullah R. (2006): Expression of *Bacillus thuringiensis* insecticidal protein gene in transgenic oil palm. Electronic Journal of Biotechnology, **9**: 117–126.

Liu Q.Q., Yu H.X., Zhang W.J., Wang H., Gu M.H. (2005): Specific expression of the foreign gene regulated by the rice rbcS promoter in transgenic rice. Journal of Plant Physiology and Molecular Biology (Zhi Wu Sheng Li Yu Fen Zi Sheng Wu Xue Xue Bao), **31**: 247–253.

Lodish H., Berk A., Zipursky S.L., Matsudaira P., Baltimore D., Darnell J. (2002): Molecular Cell Biology. 4th Ed. W.H. Freeman, New York.

Manzara T., Carrasco P., Gruissem W. (1991): Developmental and organ-specific changes in promoter DNA- protein interactions in tomato *rbcS* gene family. Plant Cell, **3**: 1305–1316.

Manzara T., Carrasco P., Gruissem W. (1993): Developmental and organ-specific changes in DNA-protein interactions in the tomato *rbcS1*, *rbcS2,* and *rbcS3* promoter regions. Plant Molecular Biology, **21**: 69–88.

Meier I., Callan K.L., Flemin J.A., Gruissem W. (1995): Organ-specific differential regulation of a promoter subfamily for the ribulose-1,5-bisphosphate carboxylase/oxygenase small su bunit genes in tomato. Plant Physiology, **107**: 1105–1118.

Outchkourov N.S., Peters J., De Jong J., Rademakers W., Jongsma M.A. (2003): The promoter-terminator of chrysanthemum *rbcS1* directs very high expression levels in plants. Planta, **216**: 1003–1012.

Panguluri S.K., Sridhar J., Jagadish B., Sharma P.C., Kumar P.A. (2005): Isolation and characterization of a green tissue-specific promoter from pigeonpea (*Cajanus cajan* L. Millsp.). Indian Journal of Experimental Biology, **43**: 369–372.

Potenza C., Aleman L., Sengupta-Gopalan C. (2004): Targeting transgene expression in research, agricultural, and environmental applications: promoters used in plant transformation. In Vitro Cellular and Developmental Biology – Plant, **40**: 1–22.

Schaart J.G., Tinnenbroek-Capel I.E.M., Krens F.A. (2011): Isolation and characterization of strong gene regulatory sequences from apple, *Malus* × *domestica*. Tree Genetics & Genomes, **7**: 135–142.

Shelton A.M., Zhao J.Z., Roush R.T. (2002): Economic, ecological, food safety and social consequences of the deployment of *Bt* transgenic plants. Annual Reviews of Entomology, **47**: 845–881.

Song P., Heinen J.L., Burns T.H., Allen R.D. (2000): Expression of two tissue-specific promoters in transgenic cotton plants. Journal of Cotton Science, **4**: 217–223.

Stuiver M.H., Custers J.H.H.V. (2001): Engineering disease resistance in plants. Nature, **411**: 865–868.

Suzuki Y., Ohkubo M., Hatakeyama H., Ohashi K., Yoshizawa R., Kojima S., Hayakawa T., Yamaya T., Mae T., Makino A. (2007): Increased RuBisCO content in transgenic rice transformed with the 'sense' *rbcS* gene. Plant and Cell Physiology, **48**: 626–637.

Suzuki Y., Nakabayashi K., Yoshizawa R., Mae T., Makino A. (2009): Differences in expression of the *RBCS* multigene family and RuBisCO protein content in various rice plant tissues at different growth stages. Plant and Cell Physiology, **50**: 1851–1855.

Tang W., Chen H., Xu C.G., Li X.H., Lin Y.J., Zhang Q.F. (2006): Development of insect-resistant transgenic indica rice wıth a synthetic *Cry1C** gene. Molecular Breeding, **18**: 1–10.

Tu J., Zhang G., Datta K., Xu C., He Y., Zhang Q., Khush G.S., Datta S.K. (2004): Field performance of transgenic elite commercial hybrid rice expressing *Bacillus thuringiensis* δ-endotoxin. Nature Biotechnology, **18**: 1101–1104.

Wong E.Y., Hironaka C.M., Fischhoff D.A. (1992): *Arabidopsis thaliana* small subunit leader and transit peptide enhance the expression of *Bacillus thuringiensis* proteins in transgenic plants. Plant Molecular Biology, **20**: 81–93.

Wu C., Fan Y., Zhang C., Oliva N., Datta S.K. (1997): Transgenic fertile japonica rice plants expressing a modified *cry1A(b)* gene resistant to yellow stem borer. Plant Cell Reports, **17**: 129–132.

Ye R., Huang H., Yang Z., Chen T., Liu L., Li X., Chen H., Lin Y. (2009): Development of insect-resistant transgenic rice with Cry1C-free endosperm. Pest Managment Science, **65**: 1015–1520.

Zheng S.J., Henken B., Maagd R.A., Purwito A., Krens F.A., Kik C. (2005): Two different *Bacillus thuringiensis* toxin genes confer resistance to beet armyworm (*Spodoptera exigua* Hűbner) in transgenic Bt-shallots (*Allium cepa* L.). Transgenic Research, **4**: 261–272.

Received for publication April 13, 2011
Accepted after corrections November 28, 2011

Corresponding author:

Dr. Allah Bakhsh, Ankara University, Faculty of Agriculture, Department of Field Crops, Ankara, Turkey
e-mail: abthebest@gmail.com