Arch. Biol. Sci., Belgrade, 63 (2), 299-307, 2011 DOI:10.2298/ABS1102299B

A MINIREVIEW: RUBISCO SMALL SUBUNIT AS A STRONG, GREEN TISSUE-SPECIFIC PROMOTER

ALLAH BAKHSH, ABDUL QAYYUM RAO, ZEESHAN SHAMIM and TAYYAB HUSNAIN

National Centre of Excellence in Molecular Biology, University of the Punjab, Lahore, 53700 Pakistan.

Abstract - Plant genetic transformation is a powerful application used to study gene expression in plants. Transcriptomics has the potential to rapidly increase our knowledge of spatial and temporal gene expression and lead to new promoters for research and development. The availability of a broad spectrum of promoters with the ability to regulate the temporal and spatial expression patterns of transgenes can increase the successful application of transgenic technology. A variety of promoters is necessary at all levels of genetic engineering in plants, from basic research, to the development of economically viable crops and plant commodities, it can address legitimate concerns raised about the safety and containment of transgenic plants in the environment. Compared with temporal- or spatial-specific expression of a toxin, constitutive expression of foreign proteins in transgenic plants can cause adverse effects. The constitutive overexpression of transgenes that interferes 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 fulfill that need. Therefore, in certain circumstances it is desirable to use expression-specific promoters which only express the foreign gene in specific plant tissues or organs. This review highlights the uses and benefits reaped by the use of green tissue-specific promoter for the RuBisCo small subunit 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.

Key words: RuBisCo Small Subunit, green tissue-specific promoter

UDC 577.2

INTRODUCTION

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, whether used in basic research, crop improvement or in biopharming, depends on their efficacious selection and use (Potenza et al. 2004).

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.

Constitutive promoters are widely used in insect-resistant transgenic rice to express *Bt* genes, such as the 35S CaMV promoter (Cheng et al. 1998; Alam et al. 1999), the ubiquitin promoter (Chen et al. 2005; Tang et al. 2006) and the 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 can have adverse effects, such as the metabolic burden imposed on plants for constant synthesis of foreign gene products that can increase the potential risk of resistance of the target insects to Bt. There is also concern about 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 on plant growth and development and potentially on 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 and Custers 2001; Berrocal-Lobo et al. 2002). Concerns that 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, under certain circumstances it is desirable to use expression-specific promoters which only express 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 be more likely to accept 'less intrusive' expression of the transgene. For example, 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 and Goldman, 2002).

The second most common group of promoters after viral promoters for plant biotechnology have come from highly-expressed plant genes, such as those for seed storage proteins, photosynthetic protein s or housekeeping genes, all of whose mRNAs were easily cloned and characterized (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 improves, 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).

Ribulose Small Subunit as Promoter

RuBisCo 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 each of two subunits, a large subunit (LSU) encoded by the chloroplast genome and the small subunit (SSU) polypeptide encoded in 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 (Fig. 1). RuBisCo is the most abundant protein found in plant leaves, representing up to 50% of the soluble protein. Thus, the SSU promoters and their transit peptides are attractive candidates for the expression of genes at high levels in green tissue and for the targeting of different proteins into the chloroplast (Ellis, 1981).

In plants, the best characterized light-inducible genes are members of the rbcS 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 and Chua 1990).

Kuhlemeier et al. (1987) performed deletion analysis of a model rbcS promoter, the pea rbcS-3A; 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, labeled 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).

In another study, the effects of light and development were analyzed 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).

In most plants, rbcS is encoded by gene families. 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 and Cashmore 1990; Manzara et al. 1991). Analysis of transgenic tomato plants expressing a 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 confers expression comparable to 35S CaMV promoter (Song et al. 2000).

Truncated cry1Ab 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 CryIA(b) expression. The level of the Bt protein was generally high in the leaves for the PEPC promoter which was comparable with the levels of a few high Bt protein plants with the 35SP or Actin-1 promoter (Datta et 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 analyzed in transgenic tobacco plants. Examination of leaf tissue revealed that the *atslA* promoter with its transit peptide sequence fused to the truncated CryIA(c) protein provided a 10- 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 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 against the rice leaf folder (*Cnaphalocrocis medinalis* L.) and yellow stem borer (*Chilo suppressalis 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 to GUS gene separately, were transferred into cotton (*Gossypium hirsutum* L.cv. Coker 312) by *Agrobacterium*-mediated transformation. Transgenic plants from the T_0 generation were analyzed for expression of the GUS reporter gene in different tissues and de-

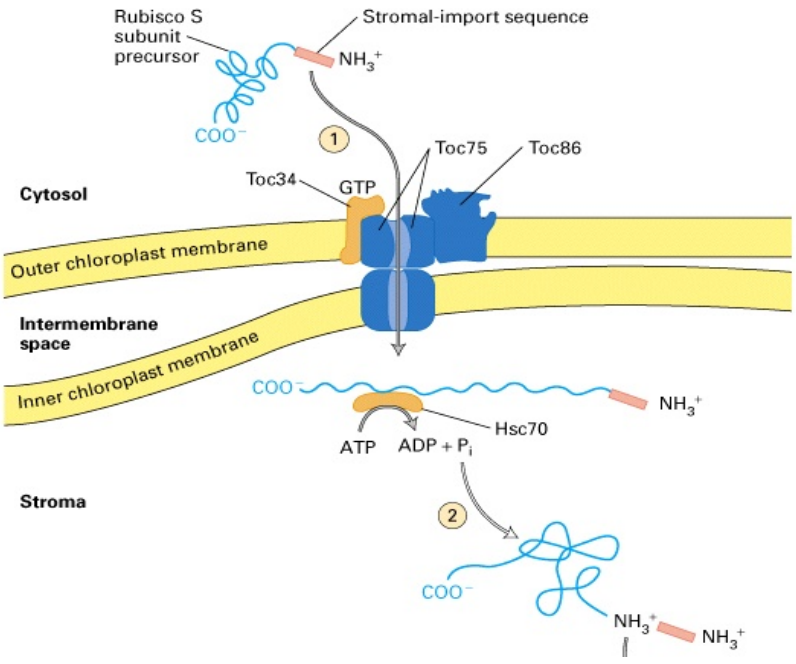

Figure-1: RuBisCo small subunit encoded by the nuclear genome. These nucleus encoded subunits are synthesized as precursors on cytosolic 80S ribosomes and targeted to the chloroplast. Adapted from webpage of Dr. Jean David.

velopmental stages of cotton. Qualitative and quantitative analyses indicated that the GUS gene driven by the *Gh-sp* promoter was expressed only during seed maturation, beginning approximately 25 days post-anthesis. Expression of the GUS reporter gene driven by the *Gh*-rbcS promoter was detected in the leaf tissue of transgenic plants. Levels of GUS expression in the leaves of *Gh*-rbcS/GUS transgenic plants were comparable to that of transgenic cotton plants containing a GUS gene construct controlled by the CaMV 35S promoter.

Gittins et al. (2000) studied the ability of the 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 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 was compared with that 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.

Baranski et al. (2004) compared five heterologous promoters fused to the 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 form soybean, 1.5 kbp), and introduced into cauliflower seedling explants using *Agrobacterium rhizogenes*-mediated transformation. Quantitative and histochemical GUS assays confirmed tissue-specific GUS expression. It was found that the extAP promoter was the most active in petioles but also caused a significant GUS expression in curds. In the case of the PsMTAP promoter, GUS activity was hardly observed in curd and limited only to its epidermis. RbcS3CP and SRS1P promoters controlled similar expression of the GUS gene in plant parts, except for the curd where RbcS3CP was almost inactive.

A 5'-upstream regulation region of rice RuBisCo small subunit gene (rbcS) was cloned from a 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 region 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 the GUS fusion gene was significantly stronger in the leaf blade and sheath than in other organs of transgenic rice plants. 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 β-glucuronidase controlled by rbcS promoter. The results clearly indicate this promoter was as strong as the pea rbcS3A promoter that was characterized earlier. Sequence homology studies with the 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 the 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). A cry1Ca or a H04 hybrid gene from *Bacillus thuringiensis*, driven by the *Chrysanthemum* rbcS promoter, along with the hygromycin phosphotransferase (hpt) gene 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 the GUS reporter gene linked with 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 the T2 lines revealed that cotyledons and leaves had very high expression of the GUS gene. The roots had almost no detectable GUS expression. Young stems 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, stigma and pollen, but not in the ovules.

Lee et al. (2006) reported the integration and expression of the Cry1Ab insecticidal gene under the control of the 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 achieved. The presence of Cry1Ab 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 (rbcS) 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 promoter most active in seedlings under native conditions was also most active in transgenic constructs at the same stage of plant development.

Two truncated versions of the prbcS-2 (360 bp and 624 bp), a full version of prbcS-2 (1.6kb) and 35S CaMV promoters were fused to the gusA gene to determine the 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% wild-type levels of RuBisCo were selected. In the progeny of the selected lines of the 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)

Cia et al. (2007) isolated a tissue-specific promoter (PD540) 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 PD540 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 the leaf blades of rice (*Oryza sativa* L.) irrespective of the 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 RuBisCo gene expression is well coordinated through the whole life of rice.

The insect resistant gene Cry1C under rice rbcS promoter was transformed in Zhonghua 11 (*Oryza sativa* L. sp. japonica) via *Agrobacterium*-mediated transformation to confer resistance against 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 very good agronomic performance. The levels of Cry1C remained undetectable in endosperm. It was measured as only 2.6 ngg^{-1} in the endosperm (Ye et al. 2009).

A synthetic truncated Cry1Ac gene was linked to the rice rbcS promoter and its transit peptide sequence (*tp*), and was transformed in rice using the *Agrobacterium*-mediated transformation method.

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).

rbcS promoter was isolated from *Gossypium arboreum* Var. 786. The promoter was fused with an insecticidal gene Cry1Ac to confer resistance in cotton (*Gossypium hirsutum*) against lepidopteran pests, especially the American boll worm. A local cotton variety, NIAB-846, was transformed using this construct via the *Agrobacterium tumefaciens* strain LB4404. The same cotton variety was transformed with another construct pk2Ac harboring Cry1Ac under the 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 consistent in the green parts of cotton plants as compared to 35S promoter (Bakhsh, 2010).

New technologies, from transcriptomics to proteomics to genome sequencing projects, will open up new vistas in the isolation of promoters tailored to answer specific questions in research or create new transgenic crops and products to feed and help the people of 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 the 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 focus on global views of the evolutionary relationship within and across species.

REFERENCES

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

Amarasinghe, B.H.R., Nitschke, E.F. Wu, Y., Udall, J.A., Dennis, E.S., Constable, G., and *D.J. Llewellyn* (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., Kimmo, K., Anne, K., Seppo, K., Kari, J., and *K. Viktor* (2007). Cloning of new RuBisCo promoters from Brassica rapa band determination of their activity in stably transformed Brassica napus and Nicotiana tabacum plants. *Mol Breeding*, **19**, 241–253.

Bakhsh, A., (2010). Expression of two insecticidal genes in Cotton. PhD Thesis. University of the Punjab, Lahore, Pakistan: 112-113.

Baranski, R., and *I.J. Puddephat* (2004). Tissue-specific expression of β-glucuronidase gene driven by heterologous promoters in transgenic cauliflower plants. *Acta Physiologiae Plantarum* **26**(3), 307-315.

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

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

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

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

Buchanan, B.B., Gruissem, W., and *R.L. Jones* (2000). Biochemistry and molecular biology of plants. Rockville, MD: American Society of Plant Physiologists, 340–342.

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

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

Cheng, X., Sardana, R., Kaplan, H., and *I. Altosaar* (1998). *Agrobacterium tumefaciens*-transformed rice plants expressing synthetic cryIAb and cryIAc genes are highly toxic to striped stem borer and yellow stem borer. *Proc Natl Acad Sci*, **95**, 2767–2772.

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

Data, K., Vasquez, Z., Tu, J., Torrizo, L., Alam, M.F., Oliva, N., Abrigo, E., Khush, G.S., and *S.K. Datta* (1998). Constitutive and tissue-specific differential expression of the cryI(A)b gene in transgenic rice plants conferring resistance to rice insect pests. *Theor Appl Gene* **97**(2), 20-30.

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

Ellis, R.J., (1981). Chloroplast proteins: Synthesis, transport, and assembly. *Annu Rev Plant Physiol*, **32,** 111-137.

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

Gilmartin, P.M., and *N.H. Chua* (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., Biricolti, S., Rosa, C., and *D.J. James* (2000). Use of heterologous ribulose-1, 5- bisphosphate carboxylase/oxygenase small subunit gene promoters to drive transgene expression in the vegetative tissues of apple (*Malus pumila* Mill.). *Planta*, **210**, 232–240.

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

Huang, F., Buschman, L.L., Higgins, R.A., and *W.H. McGaughey* (1999). Inheritance to Bacillus thuringiensis toxin (Dispel ES) in 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., and *J.K. Kim* (2009). Chloroplast-targeted expression of synthetic Cry1Ac in transgenic rice as an alternative strategy for increased pest protection. *Planta*, **230**, 397–405.

Kuhlemeier, C., Cuozzo, M., Gren, P., Goyvaerts, E., Ward, K., and *N.H. Chua* (1988). Localization and conditional redundancy of regulatory elements in rbcS-3A, a pea gene encoding the small subunit of ribulose bisphosphate carboxylase. *Proc. Natl Acad. Sci*, **85**, 4662-4666.

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

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

Lee, M., Yeun, L., and *R. Abdullah* (2006). Expression of *Bacillus thuringiensis* insecticidal protein gene in transgenic oil palm. *Electronic Journal of Biotechnology*, **9** (2), 117-126.

Liu, Q.Q., Yu, H.X., Zhang, W.J., Wang, H., and *M.H. Gu* (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** (3), 247-253.

Manzara, T., P. Carrasco and *W. Gruissem* (1993). Developmental and organ-specific changes in DNAprotein interactions in the tomato rbcS*1*, rbcS*2,* and rbcS*3* promoter regions. *Plant Mol. Biol.* **21**, 69-88.

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

Meier, I., Kristie, L., Flemin, J.A., and *W. Gruissem* (1995). Organ-specific differential regulation of a promoter subfamily for the ribulose-1, 5-bisphosphate carboxylase/oxygenase small subunit genes in tomato. *Plant Physiol*, **107**, 1105-1118.

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

Potenza, C., Aleman, L., and *C. Sengupta-Gopalan* (2004). Targeting transgene expression in research, agricultural, and en-

vironmental applications: promoters used in plant transformation. *In Vitro Cell Devel Biol. Plant*, **40**, 1–22.

Russell, P.J., (1996). Genetics, 4th edn. New York: Harper Collins College.

Shelton, A.M., Zhao, J.Z., and *R.T. Zhao* (2002). Economic, ecological, food safety and social consequences of the development of Bt transgenic plants. *Annu Rev Entomol*, **47**, 845–881.

Song, P., Heinen, J.L., Burns, T.H., and *R.D. Allen* (2000). Expression of Two Tissue-Specific Promoters in Transgenic Cotton Plants. *The Journal of Cotton Science*, **4,** 217-223.

Stuiver, M.H., and *J.H.H.V. Custers* (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., and A. makino (2007). Increased RuBisCo content in transgenic rice transformed with the 'sense' rbcS gene. *Plant Cell Physiol*, **48**, 626 -637.

Suzuki, Y., Kaori, N., Ryuichi, Y., Tadahiko, M., and *M. Amane* (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**(10), 1851-1855.

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

Tu, J., Zhang, G., Data, K., Xu, C., He, Y., and *Q. Zhang* (2004). Field performance of transgenic elite commercial hybrid rice expressing *Bacillus thuringiensis* δ-endotoxin. *Nat Biotechnol*, **18**, 1101–1104.

Wong, E.Y., Hironaka, C.M., and *D.A. Fischhoff* (1992). *Arabidopsis thaliana* small subunit leader and transit peptide enhance the expression of *Bacillus thuringiensis* proteins in transgenic plants *Plant Molecular Biology*, **20**(1), 81-93.

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

Ye, R., Huang, H., Yang, Z., Chen, T., Liu, L., Li, X., Chen, H., and *Y. Lin* (2009). Development of insect-resistant transgenic rice with Cry1C-free endosperm. *Pest Mang Sci*, **65**(9), 1015-1520.

Zheng, S.J., Henken, B., Maagd, R.A., Purwito, A., Krens, F.A., and *C. Kik* (2005). Two different *Bacillus thuringiensis* toxin genes confer resistance to beet armyworm (*Spodoptera exigua* Hubner) in transgenic Bt-shallots (*Allium cepa* L.). *Transgen Res*, **14**, 261–272.