Temporal and spatial control of gene expression in horticultural crops

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Biotechnology provides plant breeders an additional tool to improve various traits desired by growers and consumers of horticultural crops. It also provides genetic solutions to major problems affecting horticultural crops and can be a means for rapid improvement of a cultivar. With the availability of a number of horticultural genome sequences, it has become relatively easier to utilize these resources to identify DNA sequences for both basic and applied research. Promoters play a key role in plant gene expression and the regulation of gene expression. In recent years, rapid progress has been made on the isolation and evaluation of plant-derived promoters and their use in horticultural crops, as more and more species become amenable to genetic transformation. Our understanding of the tools and techniques of horticultural plant biotechnology has now evolved from a discovery phase to an implementation phase. The availability of a large number of promoters derived from horticultural plants opens up the field for utilization of native sequences and improving crops using precision breeding. In this review, we look at the temporal and spatial control of gene expression in horticultural crops and the usage of a variety of promoters either isolated from horticultural crops or used in horticultural crop improvement.

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INTRODUCTION

Gene expression in prokaryotes as well as in eukaryotes is regulated quantitatively and qualitatively by specific upstream DNA sequences.¹ These DNA sequences are commonly known as gene promoters. Initiation of transcription is in turn mediated by proteins that recognize specific DNA sequences in the promoter, thereby inducing RNA polymerase activity.²,³ Promoters regulate gene expression through DNA recognition sequences, which interact with basic transcription initiation complexes and numerous transcription factors.⁴ Such DNA recognition sequences usually include a core promoter with upstream enhancer sequences located close to the structural portion of the gene.⁵ Transcription can be activated by these enhancer sequences independent of their location, distance or orientation with respect to the genes promoters.⁹

Promoters in general are divided into two regions: a core promoter region and upstream regulatory regions.⁶ The core promoter consists of a 50–100 bp sequence adjacent to the transcription initiation site and flanking sequences.⁷ This region interacts with the general transcription machinery ⁸ and ensures the accurate initiation of transcription by RNA polymerase II.⁹ The core promoter consists of two key genetic elements: the TATA box (present in most promoters) and/or an initiator (Inr) element overlapping the transcription start site.¹⁰,¹¹ The initiator element binds trans-acting factors for the placement of the start site¹²–¹⁴ and can also mediate transcription initiation in some TATA-less promoters.¹⁵,¹⁶ The upstream promoter regions of 1–2 kb or more contains cis-regulatory elements that serve as the binding sites for genespecific regulators.⁷ The regulatory sequences that play a role in the qualitative specificity of gene expression have been intensely studied.¹⁷–¹⁹ Several regulatory sequences present upstream of the S region of plant genes include multiple cis-regulatory elements whose distribution and presence contribute to the expression pattern of that particular gene. This interaction between the cis-acting elements and transcription factors is key in the regulation of gene expression.²⁰ The presence of several cis-acting elements can contribute to the complex expression profile of a particular gene² and their differential combinatorial interactions with the transcription factors result in expression of the adjacent gene to be either constitutive or inducible by external factors, tissue-specific or some combination of these.²¹,²²

The first biotech crop commercialized in the United States was a horticultural commodity: the Flavr Savr tomato, which was submitted for approval in 1992 and released for consumption in 1994.²³–²⁵ Numerous horticultural crops in the last 20 years have since been transformed with a wide range of genes and promoter elements. In most studies the introduced genes are controlled by constitutive promoters—the most popular being the 35S promoter obtained from the Cauliflower mosaic virus (CaMV).²⁶,²⁷ In many cases, constitutive gene expression may not be required, especially when this does not serve a beneficial purpose.²⁸,²⁹ In such cases, targeted gene expression using tissue-specific or inducible promoters can often provide advantages not seen using constitutive promoters.³⁰ In recent years, there has been a boom in the availability of promoter information in many promoter databases.³¹–³³ This wealth of information enables the researcher to better understand the role of promoters and their control on plant growth and development. It also allows for the development of improved cultivars containing desirable traits.³⁴,³⁵ In this review, we look at the different promoter elements either isolated from horticultural crops or used to genetically modify a horticultural crop for improved traits (Table 1).

PROMOTERS USED FOR CONSTITUTIVE GENE EXPRESSION

Constitutive promoters direct gene expression uniformly in most tissues and cells at all stages of plant growth and development. Constitutive promoters confer high levels of transgene expression when transferred to plant cells. They generally consist of a core DNA

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| Promoter | Origin | Crop use | References |
|----------|--------|----------|------------|
| BSV | Banana streak badnavirus | Banana, Sunflower | 96 |
| CaMV 35S | Cauliflower mosaic virus | Apple, broccoli, citrus, chrysanthemum, cocoa, collard, grape, Indian Mustard, Lilium, *Nicotiana glutinosa*, papaya, peach, petunia, plum, poplar, rose, strawberry, tomato, torenia | 41–44, 47–63, 65–79, 84, 85, 87 |
| CMPS | Cestrum Yellow Leaf curling virus | Grape | 95 |
| Lhca3.St.1 | Potato | Chrysanthemum | 100 |
| Mannopin synthase | Gladiolus | Gladiolus | 99 |
| RoID | *A. rhizogenes* | Gladiolus | 99 |
| Uep1 | Oilpalm | Oilpalm, tobacco | 98 |
| Ubiquitin | Grape, gladiolus | Grape, gladiolus | 49, 99 |
| ACC-oxidase | Peach, apple, tomato, banana | Tomato, banana | 104–107 |
| ADP-glucose pyrophosphorylase | Watermelon | Tomato | 115 |
| Expansin | Cherry, cucumber | Tomato, cucumber | 109, 110 |
| Cucumisin | Melon | Melon | 114 |
| C11 | Citrus | Lemon | 117 |
| CsACS1G/CsACS1 | Cucumber | *Arabidopsis* | 111 |
| CsExp | Cucumber | Cucumber | 110 |
| DeH9 | Grape | Grape | 138 |
| DFR | Grape | Grape | 124 |
| E8 | Tomato | Tomato | 133 |
| Faxy1 | Strawberry | Strawberry | 112 |
| GIZEP | *Gentiana lutea* | Tomato | 126 |
| Metallothionin | Citrus, oilpalm | *Arabidopsis* | 118, 119 |
| Pac1 | Yeast | Avocado | 136 |
| RolB | *A. rhizogenes* | Tomato | 134 |
| SPS | Banana | Banana | 116 |
| SIACS4/SIEXP1 | Tomato | Tomato | 127 |
| 2S | Grape | Grape, tobacco | 145, 147 |
| CuMFT1 | Citrus | *Arabidopsis* | 171 |
| Dc3 | Carrot | *Arabidopsis* | 163 |
| HaG3-A | Sunflower | Tobacco | 170 |
| LeB4 | *Vicia faba* | Tobacco | 156 |
| LegA | Pea | *Helianthus* | 143 |
| NapA | *Brassica napus* | Tobacco | 150 |
| Phas | Bean | Tobacco | 140, 172 |
| Psl | Pea | Tobacco | 168 |
| Str | *Catharanthus roseus* | Tobacco | 173 |
| USP | *Vicia faba* | Tomato | 158 |
| BAN215-6 | *Brassica campestris* | Tobacco | 249 |
| CHS | Bean | Petunia, tobacco | 197, 198 |
| END1 | Pea | Tobacco | 250 |
| GTC_HS1 | *Gentiana triflora* | Petunia | 210 |
| LAT52 | Tomato | *Lilium longiflorum* | 246 |
| Pyr5T1 | Tobacco | Tobacco | 214 |
| SK2 | Potato | Potato | 224 |
| TomA108 | Tomato | Tobacco | 248 |
| B33 | Potato | Potato | 284 |
| FaRB7 | Strawberry | Tobacco | 267 |
| Glb3 | *Sesbania rostrata* | Lotus | 273 |
| MipB | *Mesembryanthemum crystallinum* | Tobacco | 268 |
| Npv30 | Bean | *Vicia hisruta* | 274 |
| PsENOD12A/PsENOD12B | Pea | Tobacco | 267 |
| SLREO | Tomato | Tomato | 263 |
| VfLb29 | *Vicia faba* | *Vicia faba* | 271 |
| Sporamin | Sweet potato | Potato, tobacco | 287, 288 |
The promoter consists of two domains A and B, which are regulatory elements that are dispersed among the entire promoter. Analyses of the 35S promoter reveal the presence of several regulatory elements that function to provide high gene expression levels. Sequences (core promoter) along with other regulatory elements such as enhancers, silencers and other DNA sequences, which interact with DNA binding proteins (transcription factors) to drive transgene expression in various plant cells. Constitutive promoters may provide ectopic gene expression in transgenic plants, not otherwise observed under normal conditions. Significantly variable results may be observed from the use of a constitutive promoter in a monocotyledonous and dicotyledonous species, which makes it essential to identify candidate promoters for specific groups to ensure high transgene expression levels. Most constitutive promoters used for production of transgenic plants derive their origin from viral sequences. Advances in plant genome sequencing initiatives and availability of public genomic databases have led to the identification of numerous plant-derived constitutive promoters, which are increasingly being used in plant transformation.

The Cauliflower mosaic virus 35S (CaMV 35S or simply 35S) promoter is by far the most widely used promoter in plant transformation. The promoter is capable of conferring high gene expression levels in most cells when transferred to plants. The 35S promoter has been extensively studied to identify key regulatory sequences that function to provide high gene expression levels. Sequences analyses of the 35S promoter reveal the presence of several regulatory elements that are dispersed among the entire promoter length. The promoter consists of two domains A and B, which are further subdivided into several subdomains. Deletion analyses studies identified specific cis-elements in these subdomains that confer expression in specific tissues of above and below-ground plant parts. Various combinations of cis-elements of the 35S promoter can produce gene expression patterns that are not observed with the sole use of such elements, which suggests an interaction between cis-elements for expression at various plant growth and developmental stages. Although the 35S promoter is considered to direct constitutive expression, varied expression effects result from its interaction with environmental factors and physiological state of plant development. Gene expression by the 35S promoter also appears to be species-dependent. For instance, high GUS expression levels were observed in pollen of transgenic strawberry plants when a 35S promoter was used, but no expression could be detected in transgenic tomato or petunia plants with similar construct configurations. In other cases, transgenic chrysanthemum expressing a GUS gene under the control of the 35S promoter exhibited weak transgene expression levels.

In-depth functional analyses of regulatory elements present in the 35S promoter has increased our understanding of the role of individual cis and enhancer elements in driving gene transcription. Such information has been exploited to produce chimeric versions of the 35S promoter that contain duplicated cis or enhancer elements. Inclusion of additional viral- and plant-derived sequences in various combinations can provide additional synergy to the 35S promoter. Duplication of 35S enhancer elements in unique orientation along with the core promoter can greatly assist in driving high levels of several genes in a single transformation cassette.

Genetic constructs containing a 35S-derived core promoter and either single or duplicated enhancer elements that controlled fusion gene expression, were arranged in a unidirectional (tandem) or bidirectional (divergent) orientation. Significantly high levels of GFP and GUS expression was observed in grapevine somatic embryos and plants transformed with constructs containing a bidirectional duplex promoter complex, where core promoters and duplicated enhancer elements were arranged in a divergent orientation. This phenomenon was attributed to synergistic activity of core promoters and enhancers arranged in a unique orientation. Similar results were obtained when a grapevine MybA1 transcription factor encoding anthocyanin expression was fused to viral promoters in various arrangements.

The 35S promoter has been extensively used in horticultural crops for improving abiotic and biotic stress tolerance and quality traits, and for modification of plant architecture. Transgenic papaya that expressed a viral coat protein gene driven by a 35S promoter exhibited enhanced resistance against papaya ring spot virus resistance. Following extensive field trials to confirm stability of resistance, the transgenic lines were used in breeding programs to produce virus resistant cultivars, which were deregulated and released for commercial production. Transgenic ‘Honey Sweet’ plums expressing a plum pox virus coat protein under the control of the 35S promoter exhibited enhanced resistance to plum pox virus, the causal agent of Sharka disease of plum. 'Honey Sweet'
was cleared for commercial production in the United States in 2010 following extensive studies by appropriate regulatory agencies.\textsuperscript{54} Similar strategies have been used to incorporate virus resistance in other fruit and vegetable crops.\textsuperscript{55,56}

The 35S promoter has also been fused to a number of genes coding for antimicrobial proteins to improve fungal and bacterial resistance. Improved scab resistance was demonstrated in transgenic apple that expressed a \textit{mbr4} gene driven by the 35S promoter.\textsuperscript{57} Genetically, engineered cacao plants constitutively expressing a chitinase gene showed decreased growth of \textit{Colletotrichum gloeosporiodes} and reduced symptoms of necrosis compared to the controls.\textsuperscript{58}

Transgenic \textit{Citrus} plants expressing an antimicrobial peptide under control of a double-enhanced 35S promoter exhibited reduced symptoms of \textit{Citrus} scab in greenhouse trials.\textsuperscript{59} Similar results were observed in transgenic strawberries expressing an antimicrobial protein.\textsuperscript{60} Transgenic grapevines expressing either anti-fungal or antibacterial genes under control of the 35S promoter exhibited enhanced disease resistance and are currently in advanced stages of field testing.\textsuperscript{61} A number of PR proteins under the control of the 35S promoter have also been employed to engineer disease resistance in ornamentals. Delayed symptoms of fungal diseases was observed in transgenic lines compared to the controls.\textsuperscript{62} Transgenic roses constitutively expressing an antimicrobial peptide exhibited resistance to powdery mildew in greenhouse trials.\textsuperscript{63} In other studies insect resistant transgenic fruits and vegetables have been produced by expressing a wide array of genes driven by the 35S promoter.\textsuperscript{64–67}

Transgenic horticultural crops with abiotic stress tolerance have been developed by constitutively expressing drought, cold and salinity-related genes. The \textit{Arabidopsis} CBF transcription factors and its homologues from several species have been transferred to a number of fruit and vegetable crops for improving cold/chilling and drought tolerance.\textsuperscript{68–72} A number of antipporter and vacuolar genes have been utilized for enhancing salinity tolerance in several plant species.\textsuperscript{73–75}

The 35S promoter has been frequently used to downregulate genes involved in ethylene biosynthesis or fruit ripening and subsequently enhance shelf life and fruit quality.\textsuperscript{76–78} Transgenic tomatoes expressing antisense versions of genes responsible for fruit softening under control of a 35S promoter exhibited enhanced shelf life due to their ability to inhibit or reduce fruit-specific enzymes responsible for softening of the fruit during the ripening process.\textsuperscript{79–81} Suppression of ripening-specific \textit{N}-glycoprotein modifying enzymes in tomato resulted in an increase in fruit shelf life without adversely affecting other qualitative characteristics.\textsuperscript{82}

The 35S promoter has also been used in a number of ornamental crops to modify plant structure, flower color and engineer floral scent in flowers that normally do not produce any fragrance. Enhanced anthocyanin production was observed in transgenic tobacco and petunia plants when a maize leaf color transcription factor was constitutively expressed by a 35S promoter.\textsuperscript{83,84} Transgenic flower crops with unique colorations not generally observed in wild populations have been created by isolating genes from the pigment biosynthesis pathway and placing them under control of the 35S promoter.\textsuperscript{85,86} Transgenic roses and carnations expressing unique colorations were also produced and released for commercial production. Transgenic petunia with reduced height and enhanced lateral branching were produced by constitutively expressing a zinc finger transcription factor.\textsuperscript{87} The enhanced branching patterns were attributed to alterations in cytokinin metabolism and increase in specific forms of cytokinins. Flowers with improved shelf life have also been produced by expressing various genes under the control of the 35S promoter.\textsuperscript{88} Other efforts to improve traits in ornamental plants include the production of dwarf and compact plants and enhanced leaf color.\textsuperscript{89} Several attempts to introduce floral scent have been made using genetic engineering; such efforts have achieved partial success, mainly in part due to the absence of key enzymes or precursors that are required for the biosynthesis of the final biochemical compound.\textsuperscript{90}

Chimeric promoters that drive constitutive gene expression are created by combining elements from viral-derived sequences other than the 35S promoter.\textsuperscript{91,92} The Cassava vein mosaic virus (CVMV), figwort mosaic virus and Cestrum Yellow Leaf Curling Virus (CMPS) have been used to identify regulatory elements that would drive high levels of constitutive gene expression in plants.\textsuperscript{93–95} Such chimeric promoters promoted through shuffling of regulatory elements and inclusion of plant-derived or other viral-derived sequences have shown high levels of transgene expression in several plant species. In some cases, the activity of viral-derived constitutive promoters has been less effective in monocotyledonous species compared to dicotyledonous plant species.\textsuperscript{96}

Advances in genome sequencing of major crops of commercial importance and availability of high throughput sequence analyses have led to the isolation of several constitutive promoters from plant species. Promoters of constitutively expressed genes such as ubiquitin are ideal candidates due to their ability to drive high gene expression levels in transformed cells. Several grapevine promoters have been isolated from the sequenced genome and analyzed for their ability to direct gene expression in various plant tissues.\textsuperscript{97} Among the various candidates tested, ubiquitin promoters exhibited the highest activity levels when tested in grape somatic embryos and tobacco callus cultures, leaves and floral tissues. Two promoters Ubi-6-1 and Ubi7-2 exhibited gene expression levels comparable to a doubly enhanced 35S promoter when fused to the \textit{gus} and anthocyanin reporter genes. Higher levels of gene expression could be correlated with an increased number of cis-elements in these promoters, which underlines the significance of identifying specific sequences in promoter regions for predicting expression levels. An ubiquitin extension promoter (\textit{uep1}) identified in oil palm exhibited constitutive expression in the native species as well as in tobacco, thereby indicating its utility in monocot and dicot groups of plants.\textsuperscript{98} A comparison of the activity of plant- and viral-derived promoter sequences in transgenic \textit{Gladiolus} found no differences in expression levels of \textit{GUS} during the culture stage.\textsuperscript{99} However greenhouse-grown transgenic lines exhibited higher gene expression levels when the \textit{gus} gene was driven by an \textit{Arabidopsis}-derived \textit{rolD} promoter. Transgenic chrysanthemums exhibited higher \textit{GUS} expression levels when fused to a potato \textit{Lhca3.St.1} promoter than the 35S promoter.\textsuperscript{100} Such effects were attributed to potential post-transcriptional modifications leading to greater stability of the mRNA and higher expression levels.

**PROMOTERS INVOLVED IN FRUIT-SPECIFIC GENE EXPRESSION**

The ability of constitutive promoters to direct high levels of transgene expression can be a limiting factor when temporal and spatial gene expression patterns are required to achieve manipulation of specific plant organs or developmental stages. Constitutive expression of transcription factors by the 35S promoter may interfere with normal plant development resulting in abnormal phenotypes.\textsuperscript{70,101} In other cases, the 35S promoter may not be active in specific plant tissues thereby rendering it ineffective for directing high levels of spatial transgene expression.\textsuperscript{43} Tissue-specific promoters may be useful for directing transgenic expression in specific plant tissues without interfering with normal plant growth and development processes. A number of promoters involved in various stages of fruit growth, maturity and ripening have been identified and can be used as genetic engineering tools to improve fruit yield, quality and post-harvest shelf life. Fruit-specific promoters with unique positive and negative regulatory elements may function efficiently in restricting
tissue-specific expression of genes and avoiding the possibility of abnormal plant growth often observed with constitutive promoters. Fruit-specific promoters from both plant species that exhibit climatic and non-climatic ripening patterns have been studied.

A number of fruit-specific promoters are regulated by ethylene, which is involved in a number of developmental processes including fruit maturity, ripening and senescence. Promoters of ethylene responsive genes such as the E4 and E8 genes have been well studied to identify activator and suppressor elements that ensure spatial and temporal gene expression.102,103 Promoters from genes such as the ACC oxidase and ACO synthase isofroms that catalyze the key steps in ethylene biosynthesis have also been analyzed in a number of plant species to identify specific cis-elements involved in the regulatory process.104–106 Deletion analysis of a peach ACC oxidase promoter fused to the GUS gene revealed the presence of regulatory regions that controlled gene expression at specific stages of fruit ripening.107 Longer sequences of the promoter enhanced GUS expression in transgenic tomato, which was attributed to the presence of an enhancer element. Genes involved in tomato fruit development from the immature-green to mature-green stages have been identified using large-scale microarray analysis to identify fruit-specific promoters that direct gene expression from ovary development to ripening.108 Analysis of a sour cherry expansin gene and its promoter region revealed the presence of a TATA box and several CAAT boxes that are conserved among promoter sequences.109 Additionally, sequences that were responsive to hormones (ethylene and gibberellins), an anarobic responsive element, GATA boxes, pyrimidine box and other cis-elements that conferred tissue specificity were identified in the 5′ upstream region. Such sequences were highly conserved with previously identified cis-elements in other plant species. Promoter deletion analysis studies confirmed specific cis-elements that acted as positive regulators of gene expression in fruits at various stages of development. Similar results were obtained with the analysis of a cucumber fruit-specific expansion gene, CsExp.110 In addition to the TATA and CAAT boxes, light and hormone-responsive cis-elements with a high degree of homology with other similar elements in other species were identified. Genes responsible for sex expression in cucumber and expressed during fruit development were studied along with their promoter regions.111 Sequence analysis for two female-specific genes revealed gene duplication except for differences in the promoter regions. No differences were observed in the proximal promoter region of the CsACS1G and CsACS1I genes, which has cis-elements that acted as repressors of gibberellins. In silico analysis of the distal regions indicated the presence of auxin-responsive elements in the CsACS1G promoter, which could potentially confer responsiveness of this gene to specific hormonal factors and control female sex expression.111

The strawberry gene Faxy1 coding for a fruit-specific β-xyllosidase and potentially involved in hemicellulose degradation during fruit ripening was isolated along with its 5′ flanking region.112 Analysis of the promoter region revealed the presence of several hormone, light and abiotic stress-related regulatory regions in addition to the TATA box and several CAAT boxes. While abscisic acid (ABA) treatment of peduncles enhanced gene expression and protein levels, a reduction was observed with NAA, GA3 and ethylene treatment thereby indicating the presence of cis-elements that were positively and negatively regulated by specific hormones. Light responsive cis-elements such as ACE, SP1 G-box and MRE sequences were identified. The promoter region also included a number of cold, drought and heat-responsive elements.

A number of promoter sequences that are involved in the expression of genes involved in biochemical changes of fruit composition during development and ripening have been studied.113–115 A banana sucrose phosphate synthase (SPS) promoter that is involved in sucrose accumulation during ripening was analyzed to identify regulatory elements and their interaction with transcription factors.116 The presence of cis-elements regulated by light and hormonal interactions in addition to the TATA box and CAAT box indicated an interaction of plant hormones and environmental factors during the process of fruit ripening. In watermelon, the ADP-glucose pyrophosphorylase gene, which is involved in carbohydrate metabolism during fruit ripening, was negatively regulated in the vegetative tissues.117 Removal of the cis-elements involved in negative regulation by fine promoter deletion analysis led to constitutive expression of the gene in leaf epidermal cells. Novel fruit-specific elements were identified in a cucumisin gene that is expressed in ripe melons.118 Deletion analysis identified a fruit-specific enhancer element, and an I-box-like sequence, which negatively regulated cucumisin biosynthesis in tissues other than the fruit. Similar elements with positive and negative regulatory functions were identified in a Citrus C11 promoter that was specifically expressed in juice sacs of ripening lemon fruit.119 Heterologous expression of the promoter: gus chimeric fusion in tomato revealed GUS expression specifically in the anthers and ovaries but not in vegetative tissues.

Promoters coding for metallothionin expression have been isolated from oil palm and Citrus.118,119 The oil palm promoter exhibited higher activity in the mesocarp tissue compared to leaf tissues. A core sequence that specified mesocarp expression while negatively regulating constitutive expression was identified in addition to specific enhancer elements that promoted expression in fruit tissues. Thus, tissue-specific expression appeared to be controlled by the combination of the positive and negative regulatory elements in the promoter region.120 Analysis of the Citrus metallothionin gene indicated the promoter to be in the TATA-less group of plant promoters such as those involved in photosynthesis. A number of fruit-specific cis-elements were identified in the promoter region and their function was confirmed by heterologous expression in Arabidopsis.

A number of genes for pigment production in fruits during the ripening phase have been well characterized.113,120–122 The WMYbA1 transcription factor is known to bind to specific regulatory elements of genes involved in the phenylalanine pathway, thereby promoting anthocyanin biosynthesis in grape berries post-veraison. A difference in the production of red and white colored berries in various grape cultivars is attributed to the insertion of a grape retrotransposon element GRET 1, which causes lack of pigment production resulting in white colored berries.123 Analysis of the grape dihydroflavonol reductase (dfr) gene promoter region revealed the presence of regulatory elements that conferred expression in fruits during ripening.124 A transcription factor LcMybA1 that accumulated anthocyanin initchi pericarp was found to be upregulated by light and ABA.125 Promoter analysis of the LcMybA1 gene revealed the presence of light, hormone and abiotic stress-responsive cis-elements that were involved in positive and negative regulation of gene expression. A Gentiana lutea carotenoid-related zeaxanthin epoxidase (GIZEP) gene and promoter region was analyzed for its function in carotenoid biosynthesis.126 Heterologous expression of a GIZEP:gus fusion in transgenic tomato specified carotenoid expression in flowers and ripe fruit but minimal levels in vegetative tissues, roots and immature fruit containing chloroplast. Cis-elements that are responsive to hormones and abiotic stress factors were identified in the promoter region and may be involved in carotenoid biosynthesis at specific developmental stages. In other studies, two fruit-specific promoters in tomato, SIACS4 and SIEXP1 contained regulatory elements that conferred gene expression specifically in seed, embryo and endosperm tissues.127 Candidate promoter sequences have also been identified from other fruits that exhibit seed-specific expression in heterologous species, indicating the presence of conserved cis-elements.128

Fruit-specific promoters have been used to either express or downregulate transgenic proteins at specific stages of development for enhancing fruit yield and quality.129–131 Transgenic tomatoes expressing miraculin, a taste modifying glycoprotein under control
of an E8 promoter accumulated uniformly high levels of the transgenic protein in ripening fruits compared to fruits expressing the protein under a 35S promoter, where protein accumulation occurred predominantly in the exocarp. Targeted expression of a yeast S-adenosylmethionine decarboxylase gene under the control of a fruit-specific E8 promoter significantly increased spermine and spermidine levels in transgenic tomato fruit, resulting in enhanced shelf life and higher lycopene content. Transgenic tomatoes expressing an Agrobacterium rolB gene under control of an ovary-specific promoter produced parthenocarpic fruit. No differences in fruit morphology were observed compared to the non-transformed fruit. In other studies, tomato fruits with enhanced rot resistance and shelf life were obtained by expressing a tomato anionic peroxidase under control of a fruit-specific E8 promoter. Transgenic avocado plants harboring a S-adenosine L-methionine hydrolyase gene under control of a fruit-specific cellulose promoter have been produced to study the potential for improving fruit shelf life. Targeted expression of a bacterial-derived auxin biosynthesis gene under control of a fruit-specific promoter have significantly enhanced fecundity of transgenic ‘Sicora’ and ‘Thompson Seedless’ grapevines by improving berry and cluster size without compromising qualitative characteristics. Similar results of improved yield along with the production of parthenocarpic fruit were obtained in transgenic strawberry and raspberry plants.

PROMOTERS ACTIVE IN THE SEEDS

The expression of genes that produce seed storage proteins is highly regulated. Deletion analysis of seed-specific promoters has led to identification of proximal regions that confer seed specificity and distal regions that are responsible for modulating gene expression. Many seed storage protein genes have been cloned from diverse plant species, and their promoters have been analyzed in detail to identify several cis- and trans-acting elements involved in gene regulation. Although such proteins exhibit wide structural variations, their promoters have a number of common properties. They allow the synthesis of proteins at high levels in specific tissues of the seed and at certain stages of plant development. The tightly regulated promoters make them ideal candidates for improving seed-specific traits such as nutritional value without potentially altering existing desirable characteristics.

The 25 albumin gene promoter from a number of horticultural species has been used to direct seed-specific gene expression. DNA sequence analysis of a seed-specific 2S albumin promoter region derived from grape (Vitis vinifera L.) indicated that several conserved seed-specific regulatory motifs were clustered within a 0.6 kb region upstream of the transcription start site. A high level of GFP expression was observed in the cotyledonary but not hypocotyl and vegetative tissues of grape and tobacco indicating the ability of the promoter to direct seed-specific gene expression. This promoter region contained DNA motifs with core sequences identical to that of cotyledon box (CATGCA), F1 (ACGT) motif, F2 (CACCTC) motif, F3 (CACGTC) and AGGA box that have been previously characterized in 2S albumin and related seed-specific promoters of other species. Substitution mutation analysis of the napA promoter using promoter-reporter gene fusions in stable transgenic tobacco showed synergistic interactions between the B-box and RY/G cis-elements within these complexes. It was further determined that elements in the B-box constitute an ABA-responsive complex and the seed-specific activity of the napA gene promoter relies on the combinatorial interaction between the RY/G complex and the B-box ABA-responsive complex during ABA response in seed development. The B-box is highly conserved in all 2S promoters and displays similarity to abscisic acid response elements.

Legumin gene promoters have also been well studied in a number of plant species. In Pisum sativum, they are coded for by a multigene family. The promoter regions of legA, legB and legC were analyzed and were found to be identical including the TATA box and CAAT box. Deletion analysis of the pea legA major seed storage protein gene identified a minimal 549 bp upstream flanking sequence that was required for seed-specific expression. This fragment contained the leg box element, a 28 bp conserved sequence found in the legumin-type genes of Vicia, Pisum, Glycine and Helianthus. Larger promoter fragments significantly increased levels of seed-specific gene expression. DNA binding assays, however, indicated that the leg box element is not the sole promoter determinant in legumin gene expression since the −124 bp fragment which included the leg box did not bind to nuclear proteins. In addition, deletion of the leg box with its seed protein gene-specific CATGCATG motif has no obvious effects on expression levels. A 2.4 kb fragment containing the 5′-flanking region and the 5′-noncoding sequence of the Vicia faba legumin gene LeB4 was observed to mediate high level of seed-specific expression in transgenic tobacco plants. Deletion analysis revealed that a 1 kb of 5′-flanking sequence was sufficient for high-levels of expression. Similar to that observed with the pea legA promoter, positive regulatory elements are present within 566 bp of the upstream sequence. However, these elements are only fully functional in conjunction with the core motif CATGCATG of the legumin box present around position −95. Seed specificity within the 5′-upstream region of a Vicia faba non-storage seed protein gene, called usp was mainly determined by the −68/−51 region. Deletion analysis of the promoter revealed the 0.4 kb of usp upstream sequence contain at least six distinct interspersed cis-elements including an AT-rich sequence, a G-box element and a CATGCATG motif. The beta-phaseolin gene (phas), encoding the major seed storage protein of bean (Phaseolus vulgaris), is confined to the cotyledons of developing embryos. Deletion analysis revealed that a 1 kb of 5′-flanking sequence was sufficient for high-levels of expression. Similar to that observed with the pea legA promoter, positive regulatory elements are present within 566 bp of the upstream sequence. However, these elements are only fully functional in conjunction with the core motif CATGCATG of the legumin box present around position −95. Seed specificity within the 5′-upstream region of a Vicia faba non-storage seed protein gene, called usp was mainly determined by the −68/−51 region. 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inducible by ABA in vegetative tissues.162 Following a 3-day water stress cycle, leaf GUS expression increased about 200-fold while there was a 16-fold increase in free ABA. These effects were reversed by re-watering indicating the drought inducibility of this promoter. In addition, 10 μM ABA resulted in more than 10-fold induction within 8 h.166

Other cis-elements involved in seed-specific promoter expression such as a number of A/T-rich sequences and a CATG/CAT/G sequence are present in the 5’-upstream regions of genes encoding concanavalin A (ConA) and canavalin, two major seed storage proteins of *Canavalia gladiata*, the sword bean. Deletion analysis of the promoter regions of both genes revealed positive regulatory elements located in the −894/-602 and -602/-74 regions of the ConA gene, and in the −428/-376, −281/-155 and −155/-50 regions of the canavalin gene.167

Progressive 5’ deletions of the pea lectin (PsL) gene promoter identified a 22 bp element (W1), important for seed-specific expression when coupled as a trimer to a heterologous TATA box.168 Within the 469 bp upstream region of the seed-specific pea lectin gene, a trimer of the 22 bp fragment conferred high gene expression in seeds. This 22 bp fragment contains the binding site for the cloned basic domain/leucine zipper (bZIP) proteins TGA1α and Opaque-2 (O2), which in turn binds to the C-box cis-element (ATGAGTCAT).169 In a majority of the promoters, most of the cis-elements are located within 1 kb upstream of the ATG sequence. However, in the HaG3-A sunflower promoter that directs helianthinin gene expression, cis-regulatory elements located in a 2.4 kb upstream region were responsible for expression in a heterologous system.170 Similarly, the 2.4 kb in the 5’ upstream region of the *CumMT1* (citrus FT/TFL1 homolog from Satsuma mandarin (*Citrus unshiu* Marc.)) contained RY (CATG/CAT), E-box (CANTG) and distant B-box (GCCACTGTC) cis-elements, all of which have been reported to promote seed-specific gene expression in plants. Seed-specific expression was confirmed by expressing the *gus* gene in *Arabidopsis*.171 A 0.8 kb fragment from the 5’ flanking region of a French bean *beta-phaseolin* gene yielded strong, temporally regulated and embryo-specific GUS expression in transgenic tobacco plants.140 Expression levels were observed to be similar as that obtained using the phaseolin seed protein promoter.172

Several promoters expressed in the seeds can also be expressed in other plant organs. *The strictosidine synthase* (*Str*) gene promoter from *Catharanthus roseus* contains a G-box sequence which helps to direct seed-specific expression independently of other regulatory sequences. G-box-directed expression in leaves, however, required the presence of an enhancer region from the 35S promoter.173 The fruit and seed-specific expression of two tomato fruit-specific promoters *SIA54* and *SIEP1* was analyzed in transgenic tomato lines expressing the promoter: *gus* fusion constructs. The *SIA54* promoter (−1 to −373) showed GUS activity restricted specifically to flower buds and seeds in fruits. On the contrary, the *SIEP1* promoter (−1 to −769) showed high level of expression in seeds as compared to fruit tissues at different stages of fruit ripening.127 The seed-specific expression shown by these promoters might be due to the presence of *Prolamin* box and E-boxes, which are conserved sequences found in the promoters of many seed storage proteins.130

**PROMOTERS ACTIVE IN THE FLORAL TISSUES**

In contrast to other plant organs, flowers are composite structures composed of several organs that form an ordered pattern.174 The typical flower consists of four organs arranged in whorls. The sepals consist of the outermost whorl followed by the petals in the next whorl and stamens (male reproductive organs) in the third whorl and carpels (female reproductive organs) in the innermost whorl.175 Each of these whorls consist of unique genes targeted to the specific organ and several homeotic genes that affect the fate of organ primordia.176 Targeted genetic engineering, by utilizing promoters obtained from genes specifically expressed in a specific whorl is highly desirable for targeted gene expression and can be exploited by using specific promoters.177 Some of the traits that can be engineered in the floral tissues include increased vase life,178–182 flower color modification,181,183–185 fragrance185–187 and male and female sterility188–192 among others.

Chalcone synthase (CHS) is synthesized in the flower corolla, tube and anthers193 and is important for the biosynthesis of flavonoid antimicrobial phytoalexins and anthocyanin pigments in plants.194 Various CHS promoters has been studied extensively in many plants, especially in *Phaseolus vulgaris*, antirrhinum, petunia and parsley.195–197 A 1.4 kb promoter fragment of the bean *CHS* gene was highly active in the root apical meristem and in petals and weakly expressed in other floral organs, mature leaves, and stems.198 Gene expression strongly depended on the G-box and H-box,199 as a synthetic 39 bp DNA fragment containing the two elements and linked to the minimal cauliflower mosaic virus 35S promoter conferred a high level of tissue-specific expression. Mutations in either the G-box or H-box motifs abolished tissue-specific gene expression.195 A mutation in the G-box did not exhibit impaired promoter response to wounding, but demonstrated a 19% reduction in the response to HgCl2 and TMV. A mutation at the H-box resulted in a 30% increase in promoter response to wounding and reductions of 36% and 54% in the response to HgCl2 and TMV, respectively, demonstrating the differential utilization of regulatory cis-elements.200 A silencer element present between positions −140 and −326 contained three binding sites for a bean nuclear factor (SBF-1).201 The region between −326 and −130 contained both activator and silencer elements.202 The petunia genome contains eight chalcone synthase genes, of which four are differentially expressed in floral tissues and UV light-induced seedlings.197 The *chsA* promoter contains a 220 bp cis-acting element region conferring flower-specific and UV-inducible expression196,197 and its expression was enhanced when plant tissues were exposed to high carbohydrate levels.204 A promoter fragment from −67 to +1, was able to direct low level flower-specific gene expression, but could not drive UV-inducible expression in transgenic *Petunia* seedlings.205 Histochemical analyses of GUS expression demonstrated that CHS promoters are not only active in pigmented cell types (epidermal cells of the flower corolla and tube and subepidermal cells of the flower stem), but also in a number of non-pigmented cell types (mesophytic cells of the corolla, several cell types in the ovary and the seed coat).199 The highest level of expression directed by the 1.1 kb snapdragon chalcone synthase promoter was observed in immature seeds. Deletions analysis identified regions of the promoter required for expression in roots, stems, leaves, seeds and flower petals of transgenic plants. A promoter fragment truncated to −39 activates transcription in roots of 4-week-old seedlings, whereas a fragment extending to −197 bp directed expression in petals and seeds.206,207 The positive regulatory element in the promoter consists of a 47 bp direct repeat between positions −564 and −670.195 150 bp of the 5’ flanking region contained cis-acting signals for UV light-induced expression.209 The GTCHS1 promoter from *Gentiana triflora* contains a sequence of the MYB protein-binding site, five consensus sequences of the MYC protein-binding site, one core sequence of a G-box and three P-box-like sequences. Gene expression is strongly directed flower limbs and the inner epidermis210 and is dependent on the G-box.211

In efforts to produce high transgene expression in petal tissue of *Rosa* florrets of chrysanthemum, expression levels were compared with four petal-specific promoters: ubiquitin extension protein (*UEP1*) promoter from chrysanthemum chalcone synthase (*chs-A*) a zinc finger transcription factor (*EFP2-5*) from petunia, *eceriferum* (*CER6*) from *Arabidopsis* and multicystatin (*PMC*) from potato. The highest expression in petal tissue of *Rosa* and disc florrets was
conferring by the UE1 promoter, followed by CER6 and EPF2-5. The UE1 promoter in ray florets was reported to confer over 50-fold enhancement in expression as compared to CaMV 35S-based promoters.212

Promoters targeting other parts of the flower have also been evaluated. When a 2.4 kb fragment of the pistil-specific thaumatin/PR5-like protein (PsTL1) promoter from Japanese pear (Pyrus serrata) was evaluated,213 it was observed that PsTL1 accumulated in pistils but not in other floral and vegetative organs which constitute a novel pistil-specific class of thaumatin/PR5-like protein.214,215 Other parts of the flower targeted include the flower receptacle. Promoters targeting other parts of the flower have been evaluated. When a 2.4 kb fragment of the pistil-specific thaumatin/PR5-like protein (PsTL1) promoter from Japanese pear (Pyrus serrata) was evaluated,216 it was observed that PsTL1 accumulated in pistils, but not in other floral and vegetative organs which constitute a novel pistil-specific class of thaumatin/PR5-like protein.214,215 Several reports exist on the isolation, characterization and use of promoters targeted to the flower receptacles,216–218 stamen,219–220 anthers221–223 and ovaries.134 The potato SK2 gene promoter directed pistil-specific gene expression. It was observed that the regulatory elements responsible for pistil-specific expression were located within a 230 bp fragment.224

Numerous genes and their promoters that are expressed at the various stages during male gametogenesis have been cloned.225 Most of these have been isolated from agronomic crops such as maize,226,227 rice,228,229 tobaco2,230,231 and wheat2,232 as well as the model plant Arabidopsis.233–236 A few have also been isolated from horticultural crops.237 These promoters fused to a cytotoxic gene have been used to induce male sterility.226,230 The LAT52 and LAT59 anther-specific gene promoters from tomato have been evaluated in various crops for their anther-specific activity.237,239,240 These genes are very critical during tomato pollen development. In their absence, pollen germinates abnormally and is sterile.241 All major cis-regulatory elements required for pollen-specific transcription in the LAT52 promoter were located within −492 to −52.242 Both promoters became active with the onset of microspore mitosis and increased progressively until anthesis,243 although the LAT52 promoter demonstrated a minor temporal difference in activity when tested in different plant species.244–246 The LAT52 promoter was highly active in electroporated pollen protoplasts isolated from Lilium longiflorum.247 The antisense Bcp1 gene under the control of the LAT52 promoter induced sterility in cauliflower pollen.247 Similarly, a 0.44 kb chia PA2 promoter fragment from petunia drove pollen-specific gene expression and a 1.75 kb chib PB promoter fragment conferred anther-specific (pollen and tapetum cells) expression to the gus gene.222 The TomA108 gene promoter from tomato was also highly active from early-meiosis to free microspores production in the tapetum.248 Deletion analysis of the BANZ215-6 gene promoter isolated from the Chinese cabbage identified a 383 bp (−274–109) region that was observed to be sufficient for the anther-specific expression of the gus gene.223 GUS expression was first detected in uninucleate microspores, increased during anther development and reached its highest level in mature pollen.248 Similar observation were made with the 2.7 kb promoter fragment of a pea END1 gene. This promoter was evaluated in several species and observed to be fully functional in the anthers.250

PROMOTERS ACTIVE IN THE ROOT SYSTEM

Plant roots have been essential for the evolution of vascular plants enabling them to meet the requirements for anchorage and the acquisition of water and nutrients.251,252 Roots are multifunctional and involved in the acquisition of water and nutrients, anchorage of the plant and storage functions.252,253 In fact, plant productivity is directly affects the above ground part.254,255 Roots interact with its surrounding environment256 and can be susceptible to a multitude of problems stemming from the environment in which it lives.252,255–260 Targeted gene expression by using root-specific promoters can allow for the development of horticultural plants better suited for growth in a range of soil types, soil pH and under microbial stress.261,262 Several root-specific promoters have been evaluated in horticultural plants. The SLREO gene isolated from tomato is highly expressed in roots, but had a very low level of expression in aerial plant organs.263 The RB7 protein from tobacco,264 is a membrane channel aquaporin, allowing the diffusion of amino acids and/or peptides from the vacuolar compartment to the cytoplasm.264,265 This promoter is root-specific and has been used to drive the Arabidopsis thionin (Thi2.1) gene in tomato.266 A strawberry homolog (FaAR87) behaves in the same way as the tobacco RB7 promoter.267 Other promoters identified include a 2 kb promoter fragment of the MipB gene from Mesembryanthemum crystallinum that was observed to be expressed strongly in the tobacco root. However, gene expression was also observed in other rapidly expanding cells and cells with high water flux capacity.268

Several root nodule-specific promoters have been identified from leguminous plants.269 A 1.3 kb fragment of the French bean gln-gamma gene promoter is strongly induced during nodule development.270 The Vicia faba VfLb29 gene promoter was found to be specifically active not only in the infected cells of the nitrogen-fixing zone of root nodules but also in arbuscule-containing cells of transgenic V. faba roots colonized by the endomycorrhizal fungus Glomus intraradices.271 A promoter fragment (−692/41) encoding the Vicia faba early nodulin VfEnod12 and a putative binding site for the transcription factor ENBP1, mediated reporter gene expression in root cortical cells, nodule primordia and the prefixing zone II of transgenic Vicia hirsute root nodules.272 A 1.9 kb fragment of the Sesbania rostrata leghemoglobin gbl3 S′-upstream region was found to direct a high level of nodule-specific GUS activity in lotus. Replacement of the −161 to −48 region, containing the gbl3 CAA1 and TATA boxes, with the heterologous truncated promoters delta-p35S and delta-pnps, resulted in a loss of nodule specificity and reduction of GUS activity restricted to the Rhizobium-infected cells of the nodules.273 Promoter analyses of pea PsENOD12A and PsENOD12B, nodulin gene promoters showed that the 200 bp immediately upstream of the transcription start are sufficient to direct nodule-specific and Nod factor-induced gene expression.274 GUS activity was only detected in the infected cells of the nodules of Lotus transgenic plants when a Np3v promoter isolated from Phaseolus vulgaris fused to the gus reporter gene was used.275

Several genes are highly upregulated in tubers.276–278 Many of these storage gene promoters have been exploited for horticultural crop improvement. Patatin is a major tuber protein and is very tissue-specific.279 The 1.5 kb 5′-upstream region of the class I patatin gene B33 directed strong expression of the GUS reporter gene in potato tubers which was on average 100- to 1000-fold higher in tubers as compared to leaf, stem and roots.280 Gene expression was also induced by sucrose application.278 Deletion analysis identified a tuber-specific element located downstream from position −195. Sequences between −40 and −400 bp and between −40 and −957 bp of the transcriptional start site were able to confer tuber-specific expression on a heterologous truncated promoter.281 Sucrose inducibility was controlled by sequences downstream of position −228.282,283 High levels of mature human serum albumin was expressed in potato tubers using the potato patatin B33 tuber-specific promoter.284

Sporamin accounts for more than 80% of the total soluble proteins of tuberous roots of sweet potato285 and can be induced by wounding and sucrose.286 Two wound response-like elements, a G box-like element and a GCC core-like sequence, were found in the sporamin gene promoter.287 When overexpressed in potato, the sporamin promoter was highly active in leaves, stems and different size tubers.288 Deletion of the sporamin A promoter sequences extending from position −305 (relative to the transcription start
motif implicated in xylem expression. Similarly, the citrus PAL exclusively in the xylem parenchyma. PAL promoter when transformed into citrus expressed flowers or fruits. Targeting a transgene into the vasculature of expression in non-target tissues. Deletion analysis of the CsPPsively, conferred gene expression in xylem tissues of tobacco. tobacco and 'Valencia' sweet orange preferentially, but not exclu-
sively, expressed during differentiation of both primary and secondary vascular tissue and was also rapidly induced (within <30 min) after excision-wounding of young stems. The bean phenylalanine ammonia-lyase gene 2 (PAL2) is expressed in the early stages of vascular development at the inception of xylem differentiation. Deletion analysis revealed the presence of cis-elements located between nucleotides −289 and −74 relative to the transcription start site being essential for xylem expression. Expression of the PAL2 promoter in the vascular system involves positive and negative regulatory cis-elements. Among these elements is an AC-rich motif implicated in xylem expression. Similarly, the citrus PAL gene (CsPPP) promoter fused to the gus gene and transformed into tobacco and 'Valencia' sweet orange preferentially, but not exclusively, conferred gene expression in xylem tissues of tobacco. Weaker GUS staining was also detected throughout the petiole region in tobacco and citrus CsPPP transgenic plants. The Arabidopsis PAL promoter when transformed into citrus exclusively in the xylem parenchyma. The full-length promoter and a series of 5’ deletions of the pea cytosolic glutamine synthetase G3SA gene were fused to the gus gene and introduced into tobacco and alfalfa. The G3SA promoter directed GUS expression in the phloem cells of the vasculature in leaves, stems and roots. Interestingly, the promoter was found to be active even when deleted to −132 relative to the start of transcription. The Arabidopsis sucrose-H⁺ symporter AtSUC2 has been used to direct phloem-specific gene expression in a number of horticultural crops, such as Mexican lime, sweet orange, pears and strawberries. Two alleles of the Citrus sinensis sucrose synthase-1 promoter (CsSUS1p) were inserted upstream of the gus gene to test their ability to drive expression in the phloem of transgenic A. thaliana and N. tabacum. Although both promoter variants were capable of conferring localized GUS expression in the phloem, the CsSUS1p-2 allele also generated a significant level of expression in non-target tissues. Deletion analysis of the CsSUS1p suggested that a fragment comprising nucleotides −410 to −268 relative to the transcriptional start site contained elements required for phloem-specific expression, while nucleotides −268 to −103 contained elements necessary for wound-specific expression. In citrus, the CsSUS promoter appeared leaky with some laminar tissue staining. A citrus phloem protein 2 (CsPP2) promoter was also evaluated in sweet orange and gene was observed to be preferentially expressed in the phloem. Two heterologous promoters, rolC and CoYMVP, were fused with the gus reporter gene and evaluated in the vegetative tissues of apple. It was observed that the CoYMV promoter was slightly more active than the rolC promoter, although both expressed GUS at a lower level than the CaMV 35S promoter. This analysis demonstrated that with both the rolC and CoYMV promoters the reporter gene activity was primarily localized to vascular tissues, particularly the phloem.

### INDUCIBLE PROMOTERS

These promoters are induced by either physical factors such as biotic and abiotic factors or chemical agents and is a powerful tool to regulate the expression of genes at certain stages of plant or tissue development. Examples of physically regulated promoters include heat shock promoters, cold inducible promoters, light inducible promoters, repressor regulated promoters such as glucocorticoid receptor promoters, estrogen and ec dysone receptor promoters, metal-responsive promoters, and pathogenesis related promoters. Some of these promoters have been isolated from horticultural crops or used for horticultural plant improvement.

The potato proteinase inhibitor II gene (pinII) is a chymotrypsin and trypsin inhibitor and is wound and UV irradiation inducible. The sequence TATAAA is found 26 nucleotides upstream of the transcription initiation site and the sequence CAAAT at position −103 in the promoter. The wound inducibility of this promoter has been evaluated in several plant species to test gene function that involve cell-specific and systemic induction. The PinII promoter has been utilized in the wound-inducible expression of the bacterial isopentenyl transferase (ipt) gene into Nicotiana plumbaginifolia. In transgenic rice plants, the expression of the pinII-gus fusion gene displayed a systemic wound response. In alfalfa, GUS expression was observed in leaf and root vascular tissue, and in some plants, expression was observed in leaf mesophyll cells. Mechanical wounding of leaves increased GUS expression approximately twofold over 24 h. The PinII promoter is active in monocot species also. Localized induced gene expression was obtained in white spruce seedlings (Picea glauca) using a similar pinII-gus construct. In rice, the wound-inducible expression of the pinII gene driven by its own promoter, together with the first intron of the rice actin 1 gene (act1), resulted in high-level accumulation of the PINII protein in the transgenic plants. The wun1 gene is another wound inducible gene from potato. Histochemical analysis of transgenic tobacco plants that expressing the wun1-gus fusions demonstrated the wound-inducible and cell-specific wun1 promoter activity in plants containing the −1022 bp fragment. The tomato LehsP23.8 heat shock protein gene's expression is induced by treatment with high or low temperatures, heavy metal or ABA. Using the gus reporter gene system, the developmental and tissue-specific expression of the gus gene controlled by the LehsP23.8 promoter was characterized in transgenic tomato plants. The optimal heat-shock temperatures leading to the maximal GUS activity in the pericarp of green, breaker, pink and red fruits were 42, 36, 39 and 39 °C, respectively. Deletion analysis of the LehsP23.8 promoter revealed a proximal region (~565 to ~23 bp) to harbor cis-regulatory elements that conferred high levels of heat-induced expression in transgenic tobacco. Mutation of the five proximal
HSEs (HSE1 to 5) led to an absence of heat inducibility. The tomato chloroplast small heat shock protein (HSP), HSP21, is also induced by heat treatment in leaves. Several sunflower genes encode small HSPs. In vegetative tissues, these mRNAs accumulated in response to either heat shock (42 °C), ABA or mild water stress treatments. The Hahsp17.7 mRNA is also active during zygotic embryogenesis at 25 °C. Developmental induction of the G4 promoter was faithfully reproduced during zygotic embryogenesis in transgenic plants containing G4: GUS translational fusions. Distal sequences of this promoter (between −1132 and −395) were needed to confer a preferential spatial expression of GUS activity in the cotyledons while proximal regions confer responses to ABA and heat shock. This −83 to +163 fragment was observed to be sufficient to support a promoter activity in tobacco galls induced by the root–knot nematode Meloidogyne incognita. GUS activity was largely restricted to giant cells within the galls. However, the Hahsp17.6 (GI) promoter which is not induced by heat shock, was observed to be silent in these giant cells, indicating that the high metabolic rate of giant cells produced as a result of nematode infection may somehow mimic heat-shock and/or other stress responses. Other examples include the strong oxidative stress-inducible peroxidase SWPA2 promoter from sweet potato. This promoter contained several cis-element sequences implicated in oxidative stress such as GCN-4, AP-1, HSTF and SP-1 reported in animal cells and a plant-specific G-box. A 1314 bp promoter fragment fused to the gus gene and transformed into tobacco exhibited about 30 times higher GUS expression than the CaMV 35S promoter in response to environmental stresses including hydrogen peroxide, wounding and UV treatment. Similarly, when potatoes were transformed with a stress inducible Arabidopsis rd29A promoter driving the cold tolerance CBF genes, freezing tolerance was increased by 2 °C.

Several promoters are chemically induced. Ethylene treatment or leaves wounding rapidly induced the melon ACC oxidase gene, CMACO1-gus gene in transgenic tobacco plants. Jasmonates and alpha-linolenic acid strongly induced the expression of the wound-induced 4CL promoter in parsley cell cultures and transgenic tobacco plants expressing 4CL-GUS gene fusions. This supported a role for jasmonates in mediating wound-induced gene expression. Two wound response-like elements, a G box-like element and a GCC core-like sequence were found within a 1.25 kb sporamin promoter. Transgenic tobacco containing this promoter driving the gus gene was wounded and a high level of GUS activity was observed in stems and leaves of, but not in roots. Exogenous application of methyl jasmonate also activated the sporamin promoter in leaves and stems of sweet potato.

The chemically inducible PR-1a tobacco promoter was fused to the Bacillus thuringiensis cry1Ab gene and transformed into broccoli. Two progeny lines expressed the cry1Ab gene and provided insect resistance when treated with the chemical inducers 2,6-dichloroisonicotinic acid or 1,2,3-benzothiadiazole-7-carboxylic acid S-methyl ester. Other examples include the alfalfa pathogen-inducible PR10 promoter. This promoter fused to the Vitis stilbene synthase 1 (WvSS1) gene was introduced into the grape rootstock genome. Transgenic plants accumulated 5- to 100-fold resveratrol in leaves infected with Botrytis using an in vitro test.

Some promoters can be regulated both physically and chemically. A 2.2 kb promoter region of the tomato prosystemin gene fused to the gus gene and transformed back into tomato contains elements conferring its correct temporal and spatial expression in the vascular bundles of transgenic tomato plants by wounding and by treatment of the plants with methyl jasmonate.

**CONCLUSIONS**

The global human population is increasing at an unprecedented rate and is projected to cross 11 billion before the end of this century. This doubling of the population and a rapid increase in global food demand creates huge challenges for the sustainability both of food production and the ability to grow more from a shrinking cultivable land mass. Thus far, the combined effects of improved varieties, increased fertilizer use and irrigation coupled with increased pesticide use have been instrumental in allowing world food production to double in the last 35 years. A multifaceted and linked global strategy to increase food production from shrinking land and water resources will ensure sustainable and equitable food security. Fruits and vegetables claimed an increasing share of the world agricultural trade, from 10.6% in 1961 to 17% in 2001. It is expected that demand for horticultural commodities, especially fruits, vegetables and flowers will continue to increase with the increase in the purchasing ability of the expanding middle class and an growing awareness of the many health benefits associated with an increased consumption of fruits and vegetables.

Acreage under genetically modified crop plants has increased substantially in recent years as more and more acreage is consumed to feed, clothe and sustain a growing world population. However, there has been limited progress in the commercialization of genetically modified horticultural commodities, with the exception of the Hawaiian papaya cultivars resistant to papaya ringspot virus and color-altered varieties of carnation flowers. Development of genetically modified horticultural cultivars that can alleviate consumer concerns and the related reluctance of food processors and marketers to accept new biotech horticultural commodities can speed up the introduction of horticultural products already developed.

In recent years, molecular advancement in the field of bioinformatics has been rapid. With the genome of a number of horticultural species being sequenced and the availability of numerous online databases for analyzing, identifying and characterizing promoters from different horticultural species, it has become relatively easier to identify and characterize plant derived promoters and other genetic elements. Identification and incorporation of plant promoter and other genetic sequences by exploiting the expanding public databases and bioinformatics services can potentially alleviate some of the public concerns about safety issues with the use of a genetically modified horticultural crop. Development of precision breeding techniques (previously termed as cisgenic or intragenic genetic improvement) will enable more precise genetic modification of plants. The resulting horticultural plant, devoid of DNA from other gene pool and restricted to a module of existing traits from the sexually compatible gene pool, could also result in less comprehensive regulation towards the release of a precision bred plant, thereby decreasing the regulatory approval costs.

**COMPETING INTERESTS**

The authors declare no conflict of interest.

**REFERENCES**

1. Mitsuhara, I, Ugaki M, Hirochika H et al. Efficient promoter cassettes for enhanced expression of foreign genes in dicotyledonous and monocotyledonous plants. *Plant Cell Physiol* 1996; 37: 49–59.
2. Komamutsy S, Borjisvik N. Functional analysis of promoter elements in plants. In: Setlow J (ed). *Genetic Engineering*. Vol. 25. Berlin: Springer, 2003: 113–141.
3. Lee TI, Young RA. Transcription of eukaryotic protein-coding genes. *Annu Rev Genet* 2000; 34: 77–137.
4. Katagiri F, Chuah NH. Plant transcription factors: present knowledge and future challenges. *Trends Genet TIG* 1992; 8: 22–27.
5. Banerji J, Ruscioni S, Schaffner W. Expression of a beta-globin gene is enhanced by remote SV40 DNA sequences. *Cell* 1981; 27: 299–308.
6. Singh KB. Transcriptional regulation in plants: the importance of combinatorial control. *Plant Physiol* 1998; 118: 1111–1120.
7. Wu K, Malik K, Tian L et al. Enhancers and core promoter elements are essential for the activity of a cryptic gene activation sequence from tobacco, tCUP. *Mol Gen Genomics* 2001; 265: 763–770.
Novina CD, Roy AL. Core promoters and transcriptional control. Trends Genet 1996; 12: 351–355.

Juven-Gershon T, Kadaona JT. Regulation of gene expression via the core promoter and the basal transcriptional machinery. Dev Biol 2010; 339: 225–229.

Smale ST, Baltimore D. The “initiator” as a transcription control element. Cell 1989; 57: 103–113.

Breathnach R, Champoun P. Organization and expression of eucaryotic split genes. Annu Rev Biochem 1981; 50: 349–383.

Means AL, Farnham PJ. Transcription initiation from the dihydrofolate reductase promoter is positioned by HP1 binding at the initiation site. Mol Cell Biol 1990; 10: 653–661.

Mukherjee B, Burma S, Hasnain SE. The 30-kDa protein binding to the “initiator” of the baculovirus polyhedrin promoter also binds specifically to the coding strand. J Biol Chem 1995; 270: 4405–4411.

Sato E, Shi Y, Shenk T. YY1 is an initiator sequence-binding protein that directs and activates transcription in vitro. Nature 1991; 354: 241–245.

Smale ST, Baltimore D. The “initiator” as a transcription control element. Cell 1989; 57: 103–113.

Zenzie-Gregory B, O’Shea-Greenfield A, Smale ST. Similar mechanisms for transcription initiation mediated through a TATA box or an initiator element. J Biol Chem 1992; 267: 2823–2830.

Gudynaste-Savitch L, Johnson DA, Miki BL. Strategies to mitigate transgene-promoter interactions. Plant Biotechnol J 2009; 7: 472–485.

Wen Z, Yang YZ, Zhang JI et al. Highly interactive nature of flower-specific enhancers and promoters, and its potential impact on tissue-specific expression and engineering of multiple genes or agronomic traits. Plant Biotechnol J 2014; in press.

Koltunow AM, Trautvetter J, Cox KH, Wallroth M, Goldberg RB. Different temporal and spatial gene expression patterns occur during anther development. Plant Cell 1990; 2: 1201–1224.

Yang Y, Li R, Qi M. In vivo analysis of plant promoters and transcription factors by agroinfiltration of tobacco leaves. Plant J 2000; 22: 543–551.

Hartmann U, Sagasser M, Mehrfens F, Stracke R, Weisshaar B. Differential promoter interactions. Plant J 2002; 31: 189–194.

Dhekney SA, Li ZT, Gray DJ. Grapevines engineered to express cisgenic Vitis vinifera antimicrobial gene Attacin E. Plant Biotechnol J 2013; 11: 391–404.

Scorza R, Ravelonandro M, Callahan AM et al. Transgenic plums (Prunus domestica L) express the plum pox virus coat protein gene. Plant Cell Rep 1994; 14: 18–22.

Scorza R, Callahan A, Levy L, Damsteegt V, Webb K, Ravelonandro M. Post-transcriptional gene silencing in plum pox virus resistant transgenic European plum containing the plum pox potyvirus coat protein gene. Transgenic Res 2001; 10: 201–209.

Nakamura Y, Theoharides CT. Analyses of plant promoters and transcription factors by in silico and bioinformatic tools. Mol Biotechnol 2009; 42: 103–113.

Fitch MM, Manshardt RM, Gonzalves D, Slichtom J, Sanford JC. Stable transformation of papaya via microprojectile bombardment. Plant Cell Rep 1990; 9: 189–194.

Gonzalves D, Gonzalves C, Ferreira S et al. Transgenic virus resistant papaya: From hope to reality for controlling papaya ringspot virus in Hawaii. APSNet Feature, July 2004.

Scorza R, Callahan A, Darlick C et al. Genetic engineering of Plum pox virus resistance: ‘HoneySweet’ plum–from concept to product. Plant Cell Tissue Organ Culture (PCTOC) 2013; 115: 1–12.

Zanek MC, Reyes CA, Cervera M et al. Genetic transformation of sweet orange with the coat protein gene of Citrus psorosis virus and evaluation of resistance against the virus. Plant Cell Rep 2008; 27: 57–66.

Fuentes A, Ramos PL, Fiallo E et al. Intron–harpin RNA derived from replication associated protein C1 gene confers immunity to tomato yellow leaf curl virus infection in transgenic tomato plants. Transgenic Res 2006; 15: 291–304.

Roudis C, Tsamandas I, Kartz T et al. Transgenic plants overexpressing the LC gene of maize show an altered growth habit and increased resistance to apple scab and fire blight. Plantas 2010; 231: 623–635.

Maximova S, Miller C, Antúnez de Mayolo G, Piskash S, Young A, Guitian MJ. Stable transformation of Theobroma cacao L. and influence of matrix attachment regions on GFP expression. Plant Cell Rep 2003; 21: 872–883.

Mondal S, Dutt M, Grosser J, Dewdney M. Transgenic citrus expressing the antimalarial gene Attacin E (attl) reduces the susceptibility of ‘Duncan’ grapefruit to the citrus scab caused by Elsinoe fawcettii. Eur J Plant Pathol 2012; 133: 391–404.

Qi Y, Teixeira da Silva JA, Zhang L, Zhang S. Transgenic strawberry: state of the art. Biotechnol Adv 2011; 29: 325–327.

Fitch MM, Manshardt RM, Gonzalves D, Slichtom J, Sanford JC. Stable transformation of papaya via microprojectile bombardment. Plant Cell Rep 1990; 9: 189–194.

Gonzalves D, Gonzalves C, Ferreira S et al. Transgenic virus resistant papaya: From hope to reality for controlling papaya ringspot virus in Hawaii. APSNet Feature, July 2004.

Scorza R, Callahan A, Darlick C et al. Genetic engineering of Plum pox virus resistance: ‘HoneySweet’ plum–from concept to product. Plant Cell Tissue Organ Culture (PCTOC) 2013; 115: 1–12.

Zanek MC, Reyes CA, Cervera M et al. Genetic transformation of sweet orange with the coat protein gene of Citrus psorosis virus and evaluation of resistance against the virus. Plant Cell Rep 2008; 27: 57–66.

Fuentes A, Ramos PL, Fiallo E et al. Intron–harpin RNA derived from replication associated protein C1 gene confers immunity to tomato yellow leaf curl virus infection in transgenic tomato plants. Transgenic Res 2006; 15: 291–304.

Roudis C, Tsamandas I, Kartz T et al. Transgenic plants overexpressing the LC gene of maize show an altered growth habit and increased resistance to apple scab and fire blight. Plantas 2010; 231: 623–635.

Maximova S, Miller C, Antúnez de Mayolo G, Piskash S, Young A, Guitian MJ. Stable transformation of Theobroma cacao L. and influence of matrix attachment regions on GFP expression. Plant Cell Rep 2003; 21: 872–883.

Mondal S, Dutt M, Grosser J, Dewdney M. Transgenic citrus expressing the antimalarial gene Attacin E (attl) reduces the susceptibility of ‘Duncan’ grapefruit to the citrus scab caused by Elsinoe fawcettii. Eur J Plant Pathol 2012; 133: 391–404.

Qi Y, Teixeira da Silva JA, Zhang L, Zhang S. Transgenic strawberry: state of the art for improved traits. Biotechnol Adv 2008; 26: 219–232.

Dhekney SA, Li ZT, Gray DJ. Grapevines engineered to express cisgenic Vitis vinifera thaumatin-like protein exhibit fungal disease resistance. In Vitro Cell Dev Biol Plant 2011; 47: 458–466.

Marchant, R, Davey MR, Lucas JA et al. Expression of a chitinase transgene in rose (Rosa hybrida L.) reduces development of blackspot disease (Diplocarpon rosae Wolf). Mol Breed 1998; 4: 187–194.

Li X, Gasic K, Cammue B, Broekaert W, Korban SS. Transgenic rose lines harboring an antimicrobial protein gene, Ace-AMP1, demonstrate enhanced resistance to powdery mildew (Sphaerotheca pannosa). Planta 2003; 218: 226–232.
Applications and functions of promoters for the improvement of horticultural crops

M Dutt et al

64 Graham IA, Besser K, Blumer S et al. The genetic map of Artemisia annua L. identifies loci affecting yield of the antimarial drug artemisinin. Science 2010; 327: 328–331.

65 McCafferty HR, Moore PH, Zhu YJ. Improved Carica papaya tolerance to carnine spider mite by the expression of Manduca sexta chitinase transgene. Transgenic Res 2006; 15: 337–347.

66 Cao J, Tang JD, Strizhov N, Shelton AM, Earle ED. Transgenic broccoli with high levels of Bacillus thuringiensis Cry1C protein control diamondback moth larvae resistant to Cry1A or Cry1C. Mol Breed 1999; 5: 131–141.

67 Shelton A, Hatch SL, Zhao JZ, Chen M, Earle EB, Cao J. Suppression of diamondback moth using Bt-transgenic plants as a trap crop. Crop Prot 2008; 27: 403–409.

68 Dehney SA, Litz RE, Amador DAM, Yadav AK. Potential for introducing cold tolerance into potato by transformation with C-repeat binding factor (CBF) genes. In Vitro Cell Dev Biol Plant 2007; 43: 195–202.

69 Jin Y, Ni DA, Ruan YL. Posttranslational elevation of cell wall invertase activity by silencing its inhibitor in tomato delays leaf senescence and increases seed weight and fruit hexose level. Plant Cell Online 2009; 21: 2072–2089.

70 Hsieh TH, Lee JT, Yang PT et al. Heterology expression of the Arabidopsis C-repeat/ dehydration response element binding factor 1 gene causes elevated tolerance to chilling and oxidative stresses in transgenic tomato. Plant Physiol 2002; 129: 1086–1104.

71 Tillet RL, Wheatley MD, Tattersall EA, Schlauch KA, Cramer GR, Cushman JC. The NIV/FRD1 C-repeat binding protein 4 (VvCBF4) transcriptional factor enhances freezing tolerance in wine grape. Plant Biotechnol J 2012; 10: 105–124.

72 Wisniewski M, Norelli J, Basset C, Artlip T, Macarion D. Ecopic expression of a novel peach (Prunus persica) CBF transcription factor in apple (Malus × domestica) results in short-day induced dormancy and increased cold hardiness. Plant Sci 2011; 233: 971–983.

73 Li F, Guo S, Zhao Y, Chen D, Chong K, Xu Y. Overexpression of a homopeptide methylesterase gene alters pectin chemistry and soluble solids in tomato fruit. J Genet Genomics 2012; 39: 667–679.

74 Tian L, Tan L, Liu F, Cai H, Sun C. Identification of quantitative trait loci associated with salt tolerance at seedling stage of Oryza rupiglumae. J Genet Genomics 2011; 38: 593–601.

75 Gaxiola RA, Li J, Undurraga S et al. Drought-and salt-tolerant plants result from overexpression of the A1PVH 1–pump. Proc Natl Acad Sci USA 2001; 98: 11444–11449.

76 Dandekar AM, Teo G, Defilippig BG et al. Effect of down-regulation of ethylene biosynthesis on fruit flavor complex in apple fruit. Transgenic Res 2004; 13: 373–384.

77 Tiemann DM, Harrisman RW, Ramahoman G, Handa KA. An antiseptic pentin methylsterase gene alters pectin chemistry and soluble solids in tomato fruit. Plant Cell Online 1999; 4: 667–679.

78 Klee HJ, Hayford MB, Kretzmer KA, Barry GF, Blommers DF. Control of ethylene synthesis by expression of a bacterial enzyme in transgenic tomato plants. Plant Cell Online 1991; 3: 1187–1193.

79 SheehyRE, Kramer M, Maiti IB, Gomm JR. Reduction of polygalacturonase activity in tomato fruit by antisenesne. Proc Natl Acad Sci USA 1998; 85: 8805–8809.

80 Bruening G, Lyons J. The case of the FLAVR SAVR tomato. Calif Agric 1998; 78: 107–112.

81 Xiong AS, Yao GH, Peng RH, Li X, Han PL, Fan HQ. Different effects on ACC oxidase gene silencing triggered by RNA interference in transgenic tomato. J Biotechnol 2003; 107: 493–504.

82 Xiong AS, Yao GH, Peng RH, Li X, Han PL, Fan HQ. Different effects on ACC oxidase gene silencing triggered by RNA interference in transgenic tomato. Plant Cell Rep 2005; 23: 639–646.

83 Meli VS, Ghosh S, Prabha TN, Chakraborty N, Chakraborty S, Datta A. Enhancement of fruit shelf life by suppressing N-glycan processing enzymes. Proc Natl Acad Sci USA 2010; 107: 2413–2418.

84 Lloyd AM, Walbot V, Davis RW. Arabidopsis and Nicotiana anthocyanin production activated by maize regulators R and C1. Science 1992; 258: 1773–1775.

85 Bradley JM, Davies KM, Deroles SC, Bloor SJ, Lewis DH. The maize Lc regulatory gene up-regulates the flavonoid biosynthetic pathway of Petunia. Plant J 1998; 13: 381–392.

86 Ueyama Y, Suzuki K, Fukushi-Mizutani M et al. Molecular and biochemical characterization of torenia flavonoid 3'-hydroxylase and flavone synthase II and characterization of 1-aminocyclopropane-1-carboxylate synthase 1 and study of its interaction with pyridoxal-5'-phosphate and aminooxyacetic acid. J Biotechnol 2010; 6: 291–294.

87 Moon H, Callahan AM. Developmental regulation of peach ACC oxidase promoter–GUS fusions in transgenic tomato fruits. J Exp Botany 2004; 55: 1519–1528.

88 Hiwas-Tanase K, Kuroda H, Hirai T, Aoki K, Takane K, Ezura H. Novel promoters that induce specific transgene expression during the green to ripening stages of transgenic tomatoes. Plant Cell Rep 2012; 31: 1415–1424.

89 Karaslan M, Huzdiana G. Characterization of an expansin gene and its ripening-specific promoter fragments from soy cherry (Prunus cerasus L.) cultivars. Acta Physiol Plant 2010; 32: 1073–1084.

90 Unni SC, Vivek PJ, Maju TT, Varghese RT, Soniya EV. Molecular cloning and characterization of fruit specific promoter from Cucumis sativus L. Am J Mol Biol 2012; 2: 132.

91 Wu T, Qin ZW, Feng Z, Zhou YJ, Xin M, Du YL. Functional analysis of the promoter of a female-specific cucumber CsaC51G gene. Plant Mol Biol Rep 2012; 30: 235–241.

92 Bustamante CA, Civello PM, Martinez GA. Cloning of the promoter region of β-xylodeside (FaXyl) gene and effect of plant growth regulators on the expression of FaXyl in strawberry fruit. Plant Sci 2009; 177: 49–56.

93 Rahim MA, Busatto N, Trainotti L. Regulation of anthocyanin biosynthesis in peach fruits. Planta 2014; in press.
114 Yamagata H, Yonesu K, Hirata A, Azmony Y. TGTCCACA motif is a novel cis-regulatory enhancer element involved in fruit-specific expression of the cucumisin gene. J Biol Chem 2002; 277: 11582–11590.

115 Yin T, Wu H, Zhang S et al. Two negative cis-regulatory regions involved in fruit-specific promoter activity from watermelon (Citrus vulgaris S.). J Exp Botany 2009; 60: 169–185.

116 Choudhury SR, Roy S, Das R, Sengupta DN. Differential transcriptional regulation of banana sucrose phosphate synthase gene in response to ethylene, auxin, wounding, low temperature and different photoperiods during fruit ripening and functional analysis of banana SPS gene promoter. Planta 2008; 229: 207–223.

117 Sorkina A, Bardosh G, Liu YZ et al. Isolation of a citrus promoter specific for reproductive organs and its functional analysis in isolated juice sacks and tomato. Plant Cell Rep 2011; 30: 1627–1640.

118 Endo T, Shimada T, Fuji H, Moriguchi T, Omura M. Promoter analysis of a type 3 Mehta RA, Cassol T, Li N, Ali N, Handa AK, Mattoo AK. Engineered polyamine Omidvar V, Abdullah SN, Izadfard A, Ho CL, Mahmood M. The oil palm Costantini E, Landi L, Silvestroni O, Pandolfini T, Spena A, Mezzetti B, Auxin retrotransposon-induced Plant Physiol 2009; 149: 1347–1361.

120 Palapol Y, Ketsa S, Lin-Wang K, Ferguson IB, Allan AC. A MYB transcription factor regulates anthocyanin biosynthesis in mangosteen (Garcinia mangostana L.) fruit during ripening. Planta 2009; 229: 1323–1334.

121 Takos AM, Robinson SP, Walker AR. Transcriptional regulation of the flavonoid pathway in the skin of dark-grown 'Cripps' Red apples in response to sunlight. J Hort Sci Biotechnol 2006; 81: 735–744.

122 Kobayashi S, Goto-Yamamoto N, Hirochika H. Retrotransposon-induced mutations in grape skin color. Science 2004; 304: 982–982.

123 Gollrop R, Even S, Colova-Tsolova V, Perl A. Expression of the grape dihydroflavonol reductase gene and analysis of its promoter region. J Exp Botany 2002; 53: 1397–1409.

124 Lai B, Li XJ, Hu B et al. LCMYB1 is a key determinant of differential anthocyanin accumulation among genotypes, tissues, developmental phases and ABA and light stimuli in Lotus Chinensis. PLoS ONE 2014; 9: e86293.

125 Yang Q, Yuan D, Shuai Y et al. Functional characterization of the Gentiana lutea zeaxanthin epoxidase (GzZEP) promoter in transgenic tomato plants. Transgenic Res 2012; 21: 1043–1056.

126 Bhat D, Anjanasree K, Lenka S, Bansal K. Isolation and characterization of fruit-specific promoters ACS54 and EXP1 from tomato (Solanum lycopersicum L.). J Plant Biochem Biotechnol 2010; 19: 51–57.

127 Lin E, Burns DJ, Gardner RC. Fruit developmental regulation of the kiwifruit actin 1 promoter is conserved in transgenic petunia plants. Plant Mol Biol 1993; 23: 489–499.

128 Litz RE, Padilla G. Genetic transformation of tree fruits. In: Genomics of Tree Crops. Berlin: Springer, 2012: 117–153.

129 Lücker J et al. Monoterpenene biosynthesis in lemon (Citrus limon). Eur J Biochem 2002; 269: 3160–3171.

130 Rai MK, Shekhawat N. Recent advances in genetic engineering for improvement of fruit crops. Plant Cell Tissue Organ Culture (PCTOC) 2014; 116: 1–15.

131 Hirai T, Duhita N, Hwasa-Tanaka K, Ezuru H. Cultivation under salt stress increases the concentration of recombinant miraculin in transgenic tomato fruit, resulting in an increase in purity. Plant Biotechnology 2011; 28: 387–392.

132 Mehta RA, Cassol T, Lin N, Ali N, Handa AK, Matteo AK. Engineered polypolyamine accumulation in tomato enhances phytonutrient content, juice quality, and vine life. Nat Biotechnology 2002; 20: 613–618.

133 Carmi N, Salts Y, Dedica B, Shabtai S, Barg R. Induction of parthenocarpy in tomato via specific expression of the rolB gene in the ovary. Planta 2003; 217: 726–735.

134 Kesanaikuri D, Kolattukudy PE, Kirti PB. Fruit-specific overexpression of wound-induced tap1 under E8 promoter in tomato confers resistance to fungal pathogens at ripening stage. Physiol Plant 2012; 146: 136–148.

135 Raharjo S, Witjaksono ED, Gomez-Lim M, Suarez I, Litz R. Genetic transformation of avocado. ISHS Acta Hort 2003; 692: 115–118.

136 Costantini E, Landi L, Silvestroni O, Pandolfini T, Spena A, Mezzetti B. Auxin synthesis-encoding transgene enhances grape fecundity. Plant Physiology 2007; 143: 1689–1694.

137 Mezzetti B, Landi L, Pandolfini T, Spena A. The defh9-iaaM auxin-synthesizing gene increases plant fecundity and fruit production in strawberry and raspberry. BMC Biotechnology 2004; 4: 4.

138 Thomas TL. Gene expression during plant embryogenesis and germination: an overview. Plant Cell 1993; 5: 1401–1410.
Applications and functions of promoters for the improvement of horticultural crops

M Dutt et al

165 Chak RK, Thomas TL, Quaratro RS, Rock CD. The genes ABI1 and ABI2 are involved in abscisic acid-and drought-inducible expression of the Dcasa cautor l Dc3 promoter in guard cells of transgenic Arabidopsis thaliana (L.) Heynh. Planta 2000; 210: 875–883.

166 Vivekananda J, Drew MC, Thomas TL. Hormonal and environmental regulation of the carrot lea-class gene Dc3. Plant Physiol 1992; 100: 576–581.

167 Yamamoto S, Nishihara M, Morikawa H, Yamauchi D, Minamikawa T. Promoter analysis of seed storage protein genes from Canavalia gladiata D.C. Plant Mol Biol 1995; 27: 729–741.

168 de Pater S, Pham K, Kiltie I, Kijnke J. The 22 bp W1 element in the pea lectin promoter is necessary and, as a multimer, sufficient for high gene expression in tobacco seeds. Plant Mol Biol 1996; 32: 513–523.

169 de Pater S, Katagiri F, Kijnke J, Chua NH. bZIP proteins bind to a palindromic sequence without an ACGT core located in a seed-specific element of the pea lectin promoter. Plant J 1994; 6: 133–140.

170 Bogue MA, Vonder Haar RA, Nickol MC, Griffling LR, Thomas TL. Developmentally regulated expression of a sunflower 115 seed protein gene in transgenic tobacco. Mol Gen Genet 1990; 222: 49–57.

171 Nishikawa F, Endo T, Shimada T et al. Isolation and characterization of a citrus FT/ TFL1 homologue (CaMFT1), which shows quantitatively preferential expression in Citrus seeds. Jpn Soc Hort Sci 2008; 77: 38–46.

172 Sengupta-Gopalan C, Reichert NA, Barker RF, Hall TC, Kemp JD. Developmentally regulated expression of the bean beta-phaseolin gene in tobacco seed. Proc Natl Acad Sci USA 1985; 82: 3320–3324.

173 Ouwerkerk PB, Memelink J. A G-box element from the Caltharhanthus roseus strictosidine synthase (Str) gene promoter confers seed-specific expression in transgenic tobacco plants. Mol Gen Genet 1999; 261: 635–643.

174 Endress PK. Origins of flower morphology. J Exp Zool 2001; 291: 105–115.

175 Theiszen G, Sauder H. Plant biology: floral quadrates. Nature 2001; 409: 469–471.

176 Coen ES, Romero JM, Doyle S, Elliott R, Murphy G, Carpenter R. Floricula: a homeotic gene required for flower development in antirrhinum majus. Cell 1990; 63: 1311–1322.

177 Yang Y, Singer S, Lu Z. Petunia AGAMOUS enhancer-derived chimeric promoters specify a carpel, stamen, and petal-specific expression pattern sufficient for engineering male and female sterility in tobacco. Plant Mol Biol Rep 2003; 21: 2174–2183.

178 Seker M, Woltering E, Sider E, Frello S, Srikantharajah S. Controlling ethylene responses in flowers at the receptor level. Biotech Adv 2006; 24: 368–381.

179 Tanaka Y, Katsumoto Y, Bruglera F, Mason J. Genetic engineering in floriculture. Plant Cell Tissue Organ Culture 2005; 80: 1–24.

180 Savin KW, Baudinette SC, Graham MW et al. Antisense ACC oxidase RNA delays etr1-1 allele inhibits the senescence of carnation flowers. Mol Breed 1996; 15: 371–377.

181 Fritze K, Staiger D, Czaja I, Walden R, Schell J, Wing D. Developmental and UV light regulation of the Snapdragon chalcone synthase (chs-A) gene promoter. Plant Cell Online 1999; 3: 893–905.

182 Wang J, Letham DS, Cornish E, Stevenson KR. Studies of cytokinin action and metabolism using tobacco plants expressing either the ipt or the gus gene controlled by a chalcone synthase promoter. I. Developmental features of the transgenic plants. Funct Plant Biol 1997; 24: 661–672.

183 Faktor O, Kooter JM, Loake GJ, Dixona RA, Lamb CJ. Differential utilization of regulatory cis-elements for stress-induced and tissue-specific activity of a French bean chalcone synthase promoter. Plant Sci 1997; 124: 175–182.

184 Schmid M, Doerner PW, Clouse SD, Dixona RA, Lamb CJ. Developmental and environmental regulation of a bean chalcone synthase promoter in transgenic tobacco. Plant Cell Online 1990; 2: 619–631.

185 Loake GJ, Faktor O, Lamb CJ, Dixon RA. Combination of H-box (CCTAACC/TCTG) and G-box (CAGCTG) cis-elements is necessary for feed-forward stimulation of a chalcone synthase promoter by the phenylpropanoid-pathway intermediate p-coumaric acid. Proc Natl Acad Sci USA 1992; 89: 9230–9234.

186 Zon L, Zlotnik A, Czernicka G. Efficient production of male and female pollen from tobacco plants. Mol Gen Genet 1990; 224: 409–414.

187 Flores V, Fritze K, Czaja I, Wing D. Expression of a bean chalcone synthase promoter in transgenic tobacco plants. Mol Gen Genet 1997; 255: 488–496.

188 Yang Y, Singer S, Lu Z. Petunia AGAMOUS enhancer-derived chimeric promoters specify a carpel, stamen, and petal-specific expression pattern sufficient for engineering male and female sterility in tobacco. Plant Mol Biol Rep 2003; 21: 2174–2183.

189 Moulton AM, Griffling LR, Peret A, Thomas TL. The genes AB11 and AB12 are involved in abscisic acid- and drought-inducible expression of the Dcasa cautor l Dc3 promoter in guard cells of transgenic Arabidopsis thaliana (L.) Heynh. Planta 2000; 210: 875–883.
Castillo C, de la Fuente JJ, Iannetta P, Botella MÁ, Valpuesta V. Pectin esterase gene family in strawberry: fruit study of FaPE1, a ripening-specific isoform. J Exp Botany 2004; 55: 909–918.

Hill TA, Day CD, Zondlo SC, Thackeray AG, Irish VF. Discrete spatial and temporal cis-acting elements regulate transcription of the Arabidopsis floral homeotic gene APETALAS. Development 1998; 125: 171–1721.

Yang Y, Singer SD, Liu Z. Petunia AGAMOUS enhancer-derived chimeric promoters specify a carpel, stamen, and petal-specific expression pattern sufficient for engineering male and female sterility in tobacco. Plant Mol Biol 2011; 69: 162–170.

Nacken WK, Huisjper P, Beltran JP, Sandler H, Sommer H. Molecular characterization of two stamen-specific genes, tap1 and Nt, that are expressed in the wild type, but not in the deficient mutants of Antirrhinum majus. Mol Gen Genet 1991; 229: 129–136.

Choi YO, Kim SS, Lee S. Yang Y, Singer SD, Liu Z. Petunia AGAMOUS enhancer-derived chimeric promoters specify a carpel, stamen, and petal-specific expression pattern sufficient for engineering male and female sterility in tobacco. Plant Mol Biol 2011; 69: 162–170.

van Tunen AJ, Mur LA, Brouns CS, Rienstra JD, Koes RE, Moi JN. Pollen-and anther-specific genes encoding putative arabinogalactan proteins in Malus × domestica. Plant Cell Rep 2010; 29: 15–24.

van Tunen AJ, Mur LA, Brouns CS, Rienstra JD, Koes RE, Moi JN. Pollen- and anther-specific chi promoters from petunia: tandem promoter regulation of the chiA gene. Plant Cell Online 1990; 2: 393–401.

Towell D, Yamaguchi J, McCormick S. Pollen-specific gene expression in transgenic plants: coordinate regulation of two different tomato gene promoters during microsporogenesis. Development 1990; 109: 705–713.

Ficker M, Wemmer T, R. A promoter directing high level expression in pistils of transgenic plants. Plant Mol Biol 1997; 35: 425–431.

McCormick S. Molecular analysis of male gametogenesis in plants. Trends Genet 1991; 7: 298–303.

Kriete G, Niehaus K, Perlick A, Pühler A, Broer I. Male sterility in transgenic tobacco plants induced by tapetum-specific deacetylation of the externally applied non-toxic compound N-acetyl-D- phosphinothrin. Plant J 1996; 9: 809–818.

Guerrero FD, Crossland S, Smutzer GS, Hamilton DA, Mascarenhas JP. Promoter sequences from a maize pollen-specific gene direct tissue-specific transcription in tobacco. Mol Gen Genet 1990; 224: 161–168.

Wang Z, Zou YJ, Li XY, et al. Arabidopsis leaf-specific gene that can be regulated by 50VAP1, a novel gene isolated from tobacco. Plant J 1997; 109: 321–326.

Nacken WK, Huisjper P, Beltran JP, Sandler H, Sommer H. Molecular characterization of two stamen-specific genes, tap1 and Nt, that are expressed in the wild type, but not in the deficient mutants of Antirrhinum majus. Mol Gen Genet 1991; 229: 129–136.

Kim H, Park BS, Jin YM, Chung T. Promoter sequences of two homologous pectin esterase genes from Chinese cabbage (Brassica campestris L. ssp. pekinensis) and pollen-specific expression of the GUS gene driven by a promoter in tobacco plants. Mol Cells 1997; 7: 21–27.

Gómez MD, Beltrán JP, Cañas LA. The pea ENDO1 promoter drives anther-specific gene expression in different plant species. Planta 2002; 219: 967–971.

Boyce CK. The evolutionary history of roots and leaves. In: Vascular Transport in Plants. Amsterdam: Academic Press, 2002: 479–499.

Schiefelbein JW, Benfey PN. The development of plant roots: new approaches to understanding underground problems. Plant Cell 1991; 3: 1147.

Olsen S, Kemper W. Movement of nutrients to plant roots. Adv Agron 1968; 20: 91–151.

Lynch J. Root architecture and plant productivity. Plant Physiol 1995; 109: 7.

Aken R, Smucker A. Root system regulation of whole plant growth. Annu Rev Phytopathol 1996; 34: 325–346.

rost TL, Bryant JA. Root organization and gene expression patterns. J Exp Botany 1996; 47: 1613–1628.

Rovira A. Interactions between plant roots and soil microorganisms. Annu Rev Microbiol 1965; 19: 241–266.

Cook RJ. Advances in plant health management in the twentieth century. Annu Rev Phytopathol 2000; 38: 95–116.

Foy CD. Limitations to Plant Root Growth. Berlin: Springer, 1992: 97–149.

Neher DA. Role of nematodes in soil health and their use as indicators. J Nematol 2001; 33: 161.

Potenza C, Aleman L, Sengupta-Gopalan C. Targeting transgene expression in research, agricultural, and environmental applications: promoters used in plant transformation. In: In Vitro Cell Dev Biol Plant 2004; 40: 1–22.

Yamamoto VT, Taylor GZ, Grzybek RP, Gallardo WJ, Xing C, Conkling MA. Characterization of cis-acting sequences regulating root-specific gene expression in tobacco. Plant Cell Online 1991; 3: 371–382.

Jones MO, Kamarainen-Karpinnen T, Koskimaki JJ et al. The promoter from SIREO, a highly-expressed, root-specific Solanum lycopersicum gene, directs expression to cortex of mature roots. Funct Plant Biol 2009; 35: 1224–1233.

Maurel C, Verdoucq L, Luu DT, Santoni V. Plant aquaporins: membrane channels with multiple integrated functions. Annu Rev Plant Biol 2008; 59: 595–624.

Javot H, Maurel C. The role of aquaporins in root water uptake. Annu Botany 2002; 90: 301–313.

Chan YL, Prasad V, Sanjaya et al. Transgenic tomato plants expressing an Arabidopsis thionin (Thi2.1) driven by fruit-inactive promoter battle against phytopathogenic attack. Planta 2005; 221: 386–393.

Vaughan SP, James DJ, Lindsey K, Massiah AJ. Characterization of FabR7, a near root-specific gene from fababean (Vigna unguiculata) and barley (Hordia vulgare), and promoter activity analysis in homologous and heterologous hosts. J Exp Botany 2006; 57: 3901–3910.

Yamada S, Nelson DE, Ley E, Marquez S, Bohnert HJ. The expression of an aphid-specific gene from Vicia faba L. in tobacco. Mol Gen Genet 1989; 217: 260–245.

Raque E, Gómez MD, Ellul P et al. The pEND1 promoter: a novel tool to produce genetically engineered male-sterile plants by early anther ablation. Plant Cell Rep 2007; 26: 313–325.

Towell D, Klein TM, Fromm ME, McCormick S. Transient expression of chimeric genes delivered into pollen by microprojectile bombardment. Plant Physiol 1989; 91: 1270–1274.

Mascarenhas JP. The male gametophyte of flowering plants. Plant Cell 1989; 1: 657.

Muscutti J, Dircks L, Vancanney G, McCormick S. LAT52 protein is essential for tomato pollen development: pollen expressing antisense LAT52 RNA hydrates and germinates abnormally and cannot achieve fertilization. Plant J 1994; 6: 321–338.
nODULES AND THE ARBUSCULE-CONTAINING CELLS OF MYCORRHIZAL ROOTS FROM DIFFERENT SPECIES. Mol Plant-Microbe Interact 2006; 19: 125–134.

277. Vierling E. The roles of heat shock proteins in plants. Annu Rev Plant Biol 1995; 46: 539–572.

278. Schena M, Lloyd AM, Davis RW. A steroid-inducible gene expression system for transgenic plants. Curr Opin Biotechnol 1996; 7: 235–244.

279. Miyata LY, Harakava R, Stipp LC et al. cis-regulatory elements in osmotic- and cold-stress-responsive promoters. Mol Genet Genom 2005; 26: 131–135. Chinese.

280. GUS gene expression driven by a DNA-binding protein that interacts with the phloem-specific cis elements of the phenylalanine ammonia-lyase promoter. Plant Mol Biol 1997; 35: 281–291.

281. de Azevedo FA, Mourão Filho FA, Schinor EH et al. GUS gene expression driven by a citrus promoter in transgenic tobacco and ‘Valencia’ sweet orange. Pesq Agropec Bras 2006; 41: 1623–1628.

282. Harakava, R. Tissue-specific and sucrose-inducible expression of a chimeric class I patatin promoter/tuber yield of transgenic potato. EMBO J 1990; 9: 1051–1061.

283. Dettmer and molecular studies of pathogenesis. PhD thesis, University of Florida, Gainesville, FL, USA, 2000.

284. Brase T, Walker EL, Coruzzi GM. A promoter sequence involved in cell-specific expression of the pea glutamine synthetase GS3A gene in organs of transgenic tobacco and alfalfa. Plant Mol Biol 1991; 14: 235–244.

285. de Azevedo FA, Mourão Filho FA, Schinor EH et al. GUS gene expression driven by a citrus promoter in transgenic tobacco and ‘Valencia’ sweet orange. Pesq Agropec Bras 2006; 41: 1623–1628.

286. Ohta S, Hattori T, Morikami A, Nakamura K. High-level expression of a sweet potato sporamin gene promoter region. Plant Cell Online 2003; 579–620.

287. Vijn I, Christiansen H, Lauridsen P et al. A novel repetitive element from sweet potato nodules and in the arbuscule-containing cells of mycorrhizal roots from different species. Mol Plant-Microbe Interact 2006; 19: 125–134.

288. Hong YF, Liu CY, Cheng KJ. The induction of vascular tissue by auxin and cytokinin. In: Aloni R, The induction of vascular tissue by auxin and cytokinin. In: Plant Hormones. Amsterdam: Springer, 1995: 531–546.

289. Tyree MT, Zimmermann MH. Xylem Structure and the Ascent of Sap. Berlin: Springer, 2002.

290. Crafts AS, Crisp CE. Phloem Transport in Plants. San Francisco, CA: WH Freeman & Co., 1971.

291. van Bel AJ. The phloem, a miracle of ingenuity. Plant Cell Environ 2003; 26: 125–149.

292. Hilder V, Powell KS, Gatehouse AM et al. Expression of snowdrop lectin in transgenic tobacco plants results in added protection against aphids. Transgenic Res 1995; 4: 18–25.

293. Stoger E, Williams S, Christou P, Down RE, Gatehouse JA. Expression of the insecticidal lectin from snowdrop (Galanthus nivalis agglutinin; GNA) in transgenic wheat plants: effects on predation by the grain aphid Sitobion avenae. Mol Breed 1999; 5: 65–73.

294. Saha P, Majumder P, Dutta I, Ray T, Roy SC, Das S. Transgenic rice expressing Allium sativum leaf lectin with enhanced resistance against sap-sucking insect pests. Plant Cell 2006; 22: 1339–1343.

295. Keller B, Baumgartner C. Vascular-specific expression of the bean GRP 1.8 gene is negatively regulated. Plant Cell 1991; 3: 1051–1061.

296. Liu YH, Jia SJ. [Vascular-specific promoters and cis-regulatory elements]. Sheng Wu Gong Cheng Xue Bao 2003; 19: 131–135. Chinese.

297. Keller B, Schmid J, Lamb CJ. Vascular expression of a bean cell wall glycin-rich protein beta-glucuronidase gene fusion in transgenic tobacco. EMBO J 1989; 8: 1309–1314.

298. Leyva A, Liang X, Pintor-Toro JA, Dixon RA, Lamb CJ. cis-element combinations determine the phenylalanine ammonia-lyase gene tissue-specific expression patterns. Plant Cell 1992; 4: 263–271.

299. Seguin A, Laible G, Leyva A, Dixon RA, Lamb CJ. Characterization of a gene encoding a DNA-binding protein that interacts in vitro with vascular specific cis elements of the phenylalanine ammonia-lyase promoter. Plant Mol Biol 1997; 35: 281–291.

300. Keller B, Schmid J, Lamb CJ. Vascular expression of a bean cell wall glycin-rich protein beta-glucuronidase gene fusion in transgenic tobacco. EMBO J 1989; 8: 1309–1314.
Feuillet C, Lauvergeat V, Deswarte C, Pilate G, Boudet A, Grima-Pettenati J et al. Tissue- and cell-specific expression of a cinnamyl alcohol dehydrogenase promoter in transgenic poplar plants. *Plant Mol Biol* 1995; 27: 651–667.

Weinmann P, Gossen M, Hillen W, Bujard H, Gatz C. A chimeric transactivator allows tetracycline-responsive gene expression in whole plants. *Plant J* 1994; 5: 559–569.

Martinez A, Sparks C, Hart CA, Thompson J, Jesop L. Edyscone agonist inducible transcription in transgenic tobacco plants. *Plant J* 1999; 19: 97–106.

Rutherford JC, Bird AJ. Metal-responsive transcription factors that regulate iron, zinc, and copper homeostasis in eukaryotic cells. *Eukaryot Cell* 2004; 3: 1–13.

Ulkes S et al. Regulation of pathogenesis-related protein-1a gene expression in tobacco. *Plant Cell Online* 1993; 5: 159–169.

Bryant J, Green TR, Gurussadaita T, Ryan CA. Proteinase inhibitor II from potatoes: isolation and characterization of its protemor components. *Biochemistry* 1976; 15: 3418–3424.

Berger DJ, Howe GA, Ryan CA. Polypeptide signaling for plant defensive genes exhibits analogies to defense signaling in animals. *Proc Natl Acad Sci USA* 1996; 93: 12035–12058.

Conconi A, Smedori MJ, Howe GA, Ryan CA. The octadecanoid signaling pathway in plants mediates a response to ultraviolet radiation. *Nature* 1996; 383: 826–829.

Keil M, Sanchez-Serrano J, Schell J, Wittenbriner L. Primary structure of a proteinase inhibitor II gene from potato (*Solanum tuberosum*). *Nucleic Acids Res* 1986; 14: 5641–5650.

Godard KA, Byun-McKay A, Levasseur C, Plant A, Seguin A, Bohlmann J. Testing of Smigocki A, Neal JW Jr, McCanna I, Doughton L. Cytokinin-mediated insect resistance in *Nicotiana* plants transformed with the *ipt* gene. *Plant Mol Biol* 1993; 23: 325–335.

Xu D, McElroy D, Thornburg RW, Wu, R. Systemic induction of a potato pin2 promoter by wounding, methyl jasmonate, and abscisic acid in transgenic rice plants. *Plant Mol Biol* 1993; 22: 573–588.

Samac DA, Smigocki AC. Expression of oryzacystatin I and II in alfalfa increases resistance to *N. tabacum* virus CM-ACD1. *Plant Mol Biol* 1993; 21: 799–804.

Godard KA, Byun-McKay A, Levasseur C, Plant A, Seguin A, Bohlmann J. Testing of a heterologous, wound and insect-inducible promoter for functional genomics studies in conifer defense. *Plant Cell Rep* 2007; 26: 2083–2090.

Smigocki A, Neal JW Jr, McCanna D, Douglass L. Cytokinoin-mediated insect resistance in *Nicotiana* plants transformed with the *ipt* gene. *Plant Mol Biol* 1993; 23: 325–335.

Xu D, McElroy D, Thornburg RW, Wu, R. Systemic induction of a potato pin2 promoter by wounding, methyl jasmonate, and abscisic acid in transgenic rice plants. *Plant Mol Biol* 1993; 22: 573–588.

Samac DA, Smigocki AC. Expression of oryzacystatin I and II in *A. aﬃnis* increases resistance to the root-lesion nematode. *Phytopathology* 2003; 93: 799–804.

Godard KA, Byun-McKay A, Levasseur C, Plant A, Seguin A, Bohlmann J. Testing of a heterologous, wound- and insect-inducible promoter for functional genomics studies in conifer defense. *Plant Cell Rep* 2007; 26: 2083–2090.

Duan X, Li X, Xue Q et al. Transgenic rice plants harboring an introduced potato proteinase inhibitor II gene are insect resistant. *Nat Biotechnol* 1996; 14: 494–498.

Logemann J, Schell J. Nucleotide sequence and regulated expression of a wound-inducible potato gene (*wea1*). *Mol Gen Genet* 1989; 219: 81–88.

Siebertz B, Logemann J, Willmitzer L, Schell J. cis-analysis of the wound-inducible promoter wun1 in transgenic tobacco plants and histochemical localization of its expression. *Plant Cell Online* 1989; 1: 961–968.

Yi SY, Sun AQ, Sun Y et al. Differential regulation of *Lehsp23* 8 in tomato plants: analysis of a multiple stress-inducible promoter. *Plant Physiol* 2006; 171: 398–407.

Yi SY, Liu J. Combinatorial interactions of two cis-acting elements, AT-rich regions and HSEs, in the expression of tomato *Lehsp23* 8 upon heat and non-heat stresses. *Plant Cell Online* 2007; 51: 965–976.

Neta-Sharir I, Isaacson T, Lurie S, Weiss D. Dual role for tomato heat shock protein *Hahsp17.7G4* promoter by root–knot nematodes: involvement of heat-shock proteins, correlate with promoter activation in root-knot nematode feeding. *Nat Biotechnol* 2001; 19: 560–568.

Huang S. Global trade of fruits and vegetables and the role of consumer demand. In: Trade, Food, Diet and Health: Perspectives and Policy Options. Oxford: Wiley-Blackwell; 2010: 60–76.

Liu RH. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *Am J Clin Nutr* 2003; 78: 5175–5205.

Liu RH. Potential synergy of phytochemicals in cancer prevention: mechanism of action. *J Nutr* 2004; 134: 3479S–3485S.

Barrows G, Sexton S, Zilberman D. Agricultural biotechnology: the promise and prospects of genetically modified crops. *J Econ Perspect* 2014; 28: 99–119.

Gonsalves C, Lee DR, Gonsalves D. The adoption of genetically modified papaya in Hawaii and its implications for developing countries. 1. *Dev Stud* 2007; 43: 177–191.

Feireira SA, Pitz KY, Manshardt R et al. Virus coat protein transgenic poppy provides practical control of papaya ringspot virus in Hawaii. *Plant Dis* 2002; 86: 101–105.

Tanaka Y, Brugliera, F, Chandler S. Recent progress of flower colour modification by biotechnology. *Int J Mol Sci* 2009; 10: 5350–5369.

Bradford K, Alston J, Lemaux P, Sunner D. Challenges and opportunities for horticultural biotechnology. *Calc Agric* 2004; 58: 68–71.

Mooney S. Bioinformatics approaches and resources for single nucleotide polymorphism functional analysis. *Brief Bioinf* 2005; 6: 44–56.

Lowe TM, Eddy SR. *InterProScan*-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res* 1997; 25: 9555–9564.

Nagaraj SH, Gasser RB, Ranganathan S. A hitchhiker’s guide to expressed sequence tag (EST) analysis. *Brief Bioinf* 2007; 8: 6–21.

Ashurst JL, Collins JE. Gene annotation: prediction and testing. *Annu Rev Genomics Hum Genet* 2003; 4: 69–88.

Portales-Casamar E, Thonguea S, Kwon AT et al. JASPAR 2010: the greatly expanded open-access database of transcription factor binding profiles. *Nucleic Acids Res* 2009; 38(Database issue): D105–D110.

Espinoza C et al. Cisregulation and intragenes: new tools for improving crops. *Biol Rec* 2013; 46: 323–331.

Holme IB, Wendt T, Holm PB. Intragenes and cisregulation as alternatives to transgenic crop development. *Plant Biotechnol J* 2013; 11: 395–407.

Nielsen KM. Transgenic organisms—time for conceptual diversification? *Nat Biotechnol* 2003; 21: 227–228.

Gray DJ, Li ZT, Dhewayka SA. Precision breeding of grapevine (*Vitis vinifera* L.) for improved traits. *Plant Sci* 2014; in press.

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