Functional analysis of homologous and heterologous promoters in strawberry fruits using transient expression*

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Abstract

The isolation and characterization of fruit-specific promoters are critical for the manipulation of the nutritional value and quality of fruits by genetic engineering. The analysis of regulatory sequences of many ripening-related genes has remained elusive for many species due to their low transformation efficiency and/or lengthy regeneration of a small number of transgenic plants. Strawberry is an important crop and represents one of the most widely studied non-climacteric model systems. However, until recently, its difficult regeneration has limited the functional study of promoters by stable transformation. A protocol based on biolistic transient transformation has been developed in order to study the function of promoters in a fast and efficient manner in strawberry fruits. The protocol has been applied to the study of the GalUR promoter, a gene involved in the biosynthesis of vitamin C in this fruit. The activity of the GalUR promoter is restricted to the fruit, being strictly dependent on light. The analysis of deletion series revealed the presence of a minimum activation region 397 bp upstream of the gene with a putative G-box motif, and a negative regulatory region between −397 and −518 bp, where an I-box was identified. The transient assay has been used to study the activity of the tomato polygalacturonase and the pepper fibrillin promoters in strawberry fruits. Whereas slight activity was observed with the fibrillin promoter, no significant activity was found with the polygalacturonase promoter. The GalUR promoter in transiently transformed ripe tomato fruits showed no activity, indicating the presence of regulatory sequences specific for its function in strawberry fruit.

Key words: D-galacturonate reductase, fibrillin, fruit promoters, polygalacturonase, strawberry, transient expression.

Introduction

The study of fruit development and ripening has received considerable attention mainly due to their uniqueness as plant developmental processes as well as for the importance that fruits have in the human diet. Extensive genetic and molecular analyses have provided substantial information about genes participating in several aspects of fruit ripening, such as the disassembly of the cell wall structure, changes in soluble sugars, pigment biosynthesis, or the production of vitamins, antioxidants, flavour, and aromatic volatiles (Giovannoni, 2001). However, there are many questions that remain unanswered regarding the genetic regulatory elements that control this process. Analyses of regulatory sequences related to fruit ripening have been the focus of a number of studies, but in most cases they have been restricted to tomato (Giovannoni, 2001). This species has also been used as a heterologous system to study promoters from other plants such as apple, pepper, or kiwifruit (Atkinson et al., 1998; Kuntz et al., 1998; Wang et al., 2000).

Although the majority of fruits display similar changes during ripening, two major types of fruits can be distinguished: climacteric, such as tomato, and non-climacteric fruits, such as pepper and strawberry. Broadly, the difference between these types is that only the former requires ethylene for complete and co-ordinated ripening. In non-climacteric fruits other developmental and non-ethylene-mediated regulation occurs which may be common with an additional ethylene-independent control in climacteric...
fruits (Giovannoni, 2001). In strawberry, a non-climacteric fruit, the application of ethylene apparently does not affect ripening although a low level is produced throughout the development of the fruit (Given et al., 1988). Thus, it is likely that gene expression in non-climacteric fruits is differently regulated and promoters from strawberry genes may contain different regulatory elements not active in climacteric fruits such as tomato. It has been found that auxins produced in the achenes govern the growth of the receptacle in strawberry and that a decline in the concentration of auxin in expanded green fruits induces fruit ripening (Given et al., 1988; Lis et al., 1978). Many ripening-specific cDNA clones have been isolated in strawberry (Agius et al., 2003; Aharoni et al., 2002; Manning, 1998; Medina-Escobar et al., 1997). However, analysis of their promoter regions has been scarce and only recently have the promoters of two endo β1-4 glucanases and STAG1, a putative AGAMOUS homologue from strawberry, been analysed (Rosin et al., 2003; Spolaore et al., 2003). Spolaore et al. used a method based on the injection of an Agrobacterium suspension into the fruits that allows quantitative assays to be carried out in strawberry fruit (Spolaore et al., 2001), whereas Rosin et al. (2003) analysed the expression level in different strawberry tissues from transgenic plants.

Traditional analysis of regulatory sequences requires well-established protocols of plant transformation. Therefore, in many plants in which permanent transformation is difficult, this is a limiting factor for a functional analysis of promoters. Alternatively, transient gene expression systems allow direct comparison of the activity of different promoters and can be used for a wide range of research applications in plant molecular biology and plant biotechnology (Baum et al., 1997; Manzara et al., 1994). Protoplasts have frequently been used for transient expression analyses of regulatory sequences in promoters activated by external stimuli (Abel and Theologis, 1994). However, the results obtained in intact tissues and organs can provide more valuable information since they reflect the in vivo situation in plants. Thus, particle bombardment of intact plant organs allows the analysis of the transcriptional regulation of organ-specific genes in their native environment (Langenkämper et al., 2001; Manzara et al., 1994; Xu et al., 1996). The rapidity of analysis is the major advantage of these techniques compared with stable transformation, particularly in species with limited and variety-specific regeneration capacity such as strawberry (El Mansouri et al., 1996). Only very recently, has efficient regeneration been shown in a number of strawberry cultivars (Passey et al., 2003).

A biolistic transient gene expression assay has been developed here that allows a rapid analysis of promoters in strawberry fruits. Using this assay, the expression of several promoters has been evaluated. First the tomato polygalacturonase promoter was analysed whose expression is fruit-specific and regulated by ethylene (DellaPenna et al., 1986; Grierson et al., 1986; Sitrit and Bennett, 1998). Second, the expression of the fibrillin promoter from pepper, a non-climacteric fruit, was analysed (Derrière et al., 1994a, b). Third, the expression of the GalUR promoter from strawberry was analysed. This gene encodes a β-galacturonide acid reductase, an enzyme involved in the biosynthesis of vitamin C in strawberry fruits (Agius et al., 2003). The expression of GalUR is fruit-specific and increases during the ripening process to reach very high level in mature red fruits. Therefore, the promoter region of GalUR has a clear biotechnological interest. Analyses of the GalUR promoter using the transient assay developed here in strawberry fruits indicated a level of expression similar to that of the constitutive CaMV 35S promoter. Deletion analyses of the promoter also identified a ~400 bp region required for expression in fruits.

Materials and methods

Plant material and treatments

Strawberry plants (Fragaria×ananassa cv. Chandler) were grown under field conditions in Huelva in the southwest of Spain. Ripe red fruits and leaves were harvested on the day of bombardment. Dark treatments were performed by covering individual fruits with aluminium foil (while attached to the plant) for 24 h and 48 h under the same field conditions. Five days under low-light (30–40 μmol m⁻² s⁻¹) or high-light (150–200 μmol m⁻² s⁻¹) exposures were performed with plants that had been grown in pots in a growth chamber under a 16/8 h light/dark cycle at 22 °C. Cherry tomato fruits (Lycopersicon esculentum cv. Josefina) were harvested on the day of bombardment from plants growing under standard greenhouse conditions. All the fruits were harvested, immediately frozen in liquid N₂, and stored at −80 °C until analysed.

Reporter gene constructs

A 5′-proximal 3.5 kb fragment of the PG promoter (Nicholass et al., 1995) was excised with EcoRI and BamHI restriction enzymes from plasmid pPGGUSI and subcloned in the polylinker of the pBluescript SK vector (Strategene) digested with the same enzymes. The resulting plasmid was digested with BamHII, filled in with Klenow and digested with KpnI. The promoter fragment was purified and inserted in the KpnI and Smal sites of the pGL3 vector (Promega), upstream of the luciferase (LUC) reporter gene (Ow et al., 1986).

A 2.3 kb XhoI/NcoI fragment containing the fibrillin promoter (Kuntz et al., 1998) was excised from plasmid pSIGN and inserted upstream of the LUC reporter gene in the pGL3 vector (Promega).

The GalUR promoter was isolated by screening a genomic library of Fragaria×ananassa using 750 bp of the 5′ cDNA as probe. Hybridizing plaques were purified and phage DNA prepared as described by Sambrook et al. (1989). A fragment of 1.2 kb corresponding to the sequence upstream of the ATG of GalUR was subcloned into the pBluescript SK vector (Strategene). The GalUR promoter fragment was ligated into the polylinker of the pGL3 vector, upstream of the LUC reporter gene. Deletion fragments of the promoter were generated by PCR using the following forward primers: Primer 1, AGTACCCATCATCACATCCAGTTA; Primer 2, AGGTAGCATAGGATGTCGA; Primer 3, AGTACCGCCGTTTAAAGACTATG; Primer 4, AGGTACCAAATCTCACGGTTATAAATGATGAT; Primer 5, AGTACCTTGGGAAATTGATGACCAA; Primer 6, AGTACCTGGCACTCTCAGGTTT, all with a KpnI site added.
random primer labelling using [α-32P] dCTP and the High-Prime Kit (Roche Diagnostics) and hybridized at 60 °C. The membranes were exposed to X-ray films (X-Omat AR, Kodak) at ~80 °C.

**Western blot**

Protein extracts used for SDS-PAGE analyses were prepared from strawberry tissues according to Agnis et al. (2003). Polyclonal antibodies against the GST-GalUR recombinant protein were used for immunoblotting. Immunodetection was conducted with a chemiluminescence ECL kit (Amersham Pharmacia Biotech) in accordance with the manufacturer’s instructions.

**Results**

**Biolistic transient transformation of red strawberry fruit**

To optimize the transient assay, the CaMV 35S promoter was fused to the LUC gene. This promoter has been shown to be functional in transgenic strawberry fruits (El Mansouri et al., 1996; Jiménez-Bermúdez et al., 2002). The main parameters that can be optimized in the biolistic transformation of plant tissue are the size of the gold particle, the distance between the sample and the macro-carrier, the amount of DNA used in each bombardment, as well as the number of shots per sample (Sanford et al., 1993). All of them were optimized for red strawberry fruits (data not shown). The addition of an osmoticum to the bombardment medium has been shown to increase the efficiency of transformation in a number of tissues, since it provides osmotic support for the tissue and minimizes cell damage (Baum et al., 1997; Sanford et al., 1993). In the case of red strawberry fruits, which contain large cells, the incubation of fruit slices in 12% mannitol prior to and during bombardment was found to be essential (data not shown). The particular fruit texture and cell composition of ripe strawberry meant that the bombardment pressure and the ionic strength of the lysis buffer were critical in the transformation process. As shown in Fig. 1A, 1800 psi bombardment pressure was needed to detect activity of the reporter gene over the background level. Since strawberry fruits are highly acidic, it was found that the buffer capacity of the commercial lysis reagent CCLR was not enough to maintain the pH optimum for assaying LUC activity. As shown in Fig. 1B, only when the concentration of TRIS-phosphate buffer in the lysis reagent was raised from 25 mM to 300 mM was the luciferase activity detected above background (Fig. 1B).

**Analysis of ripening-regulated promoters in strawberry and tomato fruits**

In a search for promoters that could be used in strawberry fruits in addition to the CaMV 35S, two heterologous fruit ripening-regulated promoters were analysed. One was the tomato polygalacturonase (PG) promoter whose expression is confined to the tomato fruit and which is transcriptionally activated during ripening (Nicholass et al., 1995). This PG activity during ripening appears to be regulated by...
ethylene (Sitrit and Bennett, 1998). The second promoter was from the Fibrillin (Fib) gene, which encodes a protein that accumulates during ripening in the chromoplasts of red bell pepper fruits (Deruère et al., 1994a, b). More recently, it has been shown that Fib mRNA can be induced in leaves as a consequence of stresses such as wounding or drought (Chen et al., 1998; Langenkämper et al., 2001). Using the transient assay on strawberry fruit, no significant PG promoter activity was found, whereas the Fib promoter was active in strawberry but at a significantly lower level than the 35S promoter (Fig. 2A). The activity of these promoters was analysed next in tomato fruit. The protocol used for transient analysis in tomato was optimized to these experimental conditions and is described in the Materials and methods. As shown in Fig. 2B, transient assays using the CaMV 35S promoter revealed much larger activity in ripe tomato fruits relative to ripe strawberry fruit. Thus the luciferase activity increased around 25 times (from ~600 ALU mg⁻¹ protein to ~15 000 ALU mg⁻¹ protein). The 35S and PG promoters showed similarly high levels of activity, indicating that the PG promoter used in this study had all the regulatory elements necessary for a high expression in tomato. It was also found that the Fib promoter had significant activity in ripened tomato fruits, as described previously (Kuntz et al., 1998). These results suggest that, although these promoters could be used in tomato fruits, they could not drive high expression of a gene of interest in strawberry fruits.

**Light is required for the activity of the GalUR promoter**

Previous studies have shown that GalUR is expressed specifically in fruits during ripening (Agius et al., 2003). Additional studies have shown that its expression is very high in red strawberry fruits (~0.01% abundance in the mRNA population) and specific to the receptacle (data not shown). As a result it was decided to isolate and analyse the promoter region that drives the expression of GalUR. A region of 1.2 kb upstream of the ATG initiation codon of the GalUR cDNA was cloned from a strawberry genomic library (see Materials and methods). The promoter activity of this fragment in ripe strawberry fruit was comparable to the 35S promoter (Fig. 2A). In tomato fruit there was no induced expression of the reporter gene under the 1.2 kb fragment of the GalUR promoter (Fig. 2B).
Computer analysis of the promoter sequence identified cis elements with significant homology to the light-responsive elements G-Box, I-box, and GT-1 (Terzaghi and Cashmore, 1995), and to the wound-responsive element Wun (Matton et al., 1993) (Table 1). A putative TATA-box was located −174 bp upstream of the ATG initiation codon. The significance of these putative light-responsive elements in the promoter activity of GalUR was tested by the transient expression assay. It was found that the GalUR promoter was active in red strawberry fruits only when the bombarded tissue was maintained in continuous light prior to the LUC assay (Fig. 3A). Luciferase activity was ~5-fold higher in fruits incubated in these conditions than in fruits incubated during the same period in the dark, whose activity was not significantly different from the control. A general effect of light on gene expression was excluded since the activity of the 35S promoter was similar in dark and light conditions (Fig. 3A). The GalUR promoter also contains regulatory regions that are potentially induced by mechanical damage, like the Wun-motif elements (Table 1). However, the absence of luciferase activity in the dark (Fig. 3A), when there is some wounding caused by the bombardment, seems to exclude any functionality to this wun box in the absence of light. Northern analyses showed expression of GalUR in ripening fruits but not in leaves (Agius et al., 2003). The biolistic assay developed here confirmed this result, since no luciferase activity was observed after bombardment of strawberry leaf (Fig. 3B), by contrast with the activity under the CaMV 35S promoter that was used as positive control.

A requirement for the activity of the GalUR promoter has been shown to be light (Fig. 3A). To determine whether this reflects the in vivo expression of GalUR, it was next examined if light regulates mRNA and protein accumulation of GalUR. Red fruits were maintained in the dark in planta by covering them with aluminium foil for 24 h and 48 h. Red fruits that remained uncovered were used as the control. There was a continuous decrease of GalUR mRNA after 24 h and 48 h in fruits maintained in the dark, in accordance with the data obtained from the promoter analysis (Fig. 4A). While a slight reduction of GalUR protein was also observed in the covered fruits after 24 h and 48 h (Fig. 4B), no significant changes were observed in either GalUR mRNA or protein levels in uncovered fruits (Fig. 4C, D). It was further determined whether light intensity regulates GalUR expression in vivo. It was found that the level of GalUR protein is dependent on light intensity as observed in plants that were maintained for 5 d under low-light (LL, 30–40 μmol m⁻¹ s⁻¹) or high-light (HL, 150–200 μmol m⁻¹ s⁻¹) exposures (Fig. 4E).

**Table 1. Cis-acting regulatory elements in the strawberry GalUR promoter**

The GalUR promoter sequence was screened against a database of plant transcription cis elements (http://intra.psb.ugent.be:8080/PlantCARE/). Only cis element sites with a point score >94.0 are shown. The positions are given relative to the ATG initiation codon in the strawberry GalUR gene.

<table>
<thead>
<tr>
<th>Cis-elements</th>
<th>Motif and position</th>
<th>Putative function</th>
</tr>
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<tbody>
<tr>
<td>G-box-like consensus:</td>
<td>−183 TACG TG −178</td>
<td>Cis-acting regulatory element</td>
</tr>
<tr>
<td>CACG TG</td>
<td></td>
<td>involved in light responsiveness</td>
</tr>
<tr>
<td>I-box consensus:</td>
<td>−455 GATAAGTA −446</td>
<td>Light-responsive element</td>
</tr>
<tr>
<td>GATAAGAT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GT-1 consensus:</td>
<td>−655 GGT TAA −649</td>
<td>Light-responsive element</td>
</tr>
<tr>
<td>GGTTAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wun-motif consensus:</td>
<td>−759 AATT TCC −752</td>
<td>Cis-acting regulatory element</td>
</tr>
<tr>
<td>AATT TCC</td>
<td>−769 AATT TCC −762</td>
<td>involved in wound responsiveness</td>
</tr>
<tr>
<td></td>
<td>−24 CATT TCC −18</td>
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**Fig. 3. Analysis of the GalUR promoter in fruit and leaves of strawberry.**

(A) Luciferase activity measured in fruit incubated in the dark (dark) and in continuous light (light) transformed with the 35S–LUC and GalUR–LUC constructs. Transformation with the empty vector (pGL3) was used as a negative control. (B) Luciferase activity in leaves (incubated in 16/8 h light/dark photoperiod) transformed with GalUR–LUC construct. Values corresponding to leaf transformed with the empty vector (pGL3) and the 35S–LUC construct (incubated in 16/8 h light/dark photoperiod) are also shown. Luciferase activity is expressed as Arbitrary Light Unit (ALU) mg⁻¹ protein.
However, computer analysis of the GalUR promoter did not identify putative regulatory cis-elements in this region with significant homology to others in the databank (Table 1). The decreased activity in the −518 bp, −678 bp, and −950 bp constructs compared with the −397 construct indicates the presence of a negative regulatory element in the region from −397 to −518 bp of the GalUR promoter (Fig. 5). In this region between −518 and −397, the promoter contains an I-box-like sequence that could be a negative cis-acting element. The construct containing 397 bp upstream of ATG showed 62% of the activity of the 1.2 kb GalUR promoter, indicating that the −397 bp fragment upstream of the ATG is sufficient to confer high expression in red fruits.

Therefore, this region could be defined as the minimum sequence that showed promoter activity for the GalUR gene, and included a G-box-like element.

The transient expression using the biolistic technique developed here was compared with the method previously reported in strawberry fruits that used Agrobacterium infiltration (Spolaore et al., 2001). The expression cassettes were identical for both methods with the difference that the constructs for the infiltration method were inserted in a binary vector. As shown in Fig. 5 (column b) similar results were obtained using this transient assay method when compared with the biolistic method developed here (column a). Again, a reduced activity of the promoter was observed when the −1200 to −950 fragment was removed confirming the presence of positive cis-acting elements. Also, putative negative regulatory elements in the region from −397 and −518 bp of the GalUR promoter were detected.

**Discussion**

Transient transformation of intact plant organs by particle bombardment has proved to be a useful approach to study the promoter activity of organ-specific genes, as the
transformed cell can be monitored in its native organ environment (Goff et al., 1990; Potrykus, 1991). The use of biolistics has the advantage of allowing a fast analysis, but it has to be adapted to the specific characteristics of the plant tissue. Ripe fleshy fruits usually have large vacuolated cells whose walls undergo marked changes in their structure as a result of the presence of many cell wall hydrolytic enzymes. These physiological and anatomical peculiarities must be considered when fruit is transformed by particle bombardment. Results obtained in tomato fruits (Baum et al., 1997) showed that osmotic treatment of the fruit slice before, during, and after particle bombardment, together with optimization of bombardment conditions resulted in a 100-fold increase in the transient expression of luciferase driven by the ribulose 1,5-bisphosphate carboxylase/oxygenase small subunit promoter (Manzara et al., 1993; Montgomery et al., 1993). It was observed that osmotic treatments of red strawberry fruit slices before, during, and after bombardment were critical for the detection of luciferase activity. These results might be explained by a loss in the turgor of the cell at the cut surface and the subsequent improved viability of the cells hit by the gold particles, as was observed in tomato fruit (Baum et al., 1997). Thus, using the osmotic treatment together with optimized bombardment conditions and two shots per slice with the CaMV 35S promoter, a reporter gene activity that was about 7–10-fold higher than the background was consistently obtained. Co-bombardment with a 35S–GUS construct to be used as an internal control among different experiments could not be used due to the internal fluorescence of the red fruit tissue that made it difficult to assay the GUS activity. Therefore, the intrinsic variability of the protocol was minimized by increasing the number of replicates. Another important parameter to be optimized was the assay of the luciferase activity in the transformed strawberry tissue. The optimum pH for luciferase is 7.8; however, it was found that the pH of the extract prepared from strawberry red fruit ranged from 5.0 to 5.4. This suggested the need to increase the buffering capacity of the TRIS-phosphate extraction buffer by raising its concentration to 300 mM. The same requirements were reported in the transient expression of tomato red fruits transformed with 35S–LUC, RBSC3b–LUC and HMG2–LUC (Manzara et al., 1994).

The strawberry GalUR gene that encodes a D-galacturonic acid reductase involved in the biosynthesis of L-ascorbic acid in strawberry fruit has been isolated and characterized (Agius et al., 2003). The results suggested that the activity of this protein regulates the levels of vitamin C. Northern studies revealed that the expression of GalUR was restricted to the ripening fruit. It has been shown here that 1.2 kb of the promoter region contains cis elements that determine a high and specific activity of the promoter in strawberry fruit. Other plant promoter regions, with a size in the range of several hundreds bp to 1 kb, have also been found to reproduce faithful expression patterns of reporter genes in vivo (Yamagata et al., 2002). An important characteristic of the GalUR promoter is that its activity seems to be strictly dependent on light. This light-dependence was confirmed by following the levels of the gene expression products during the dark/light treatments. Slight discrepancies between the mRNA and protein levels may reflect differences in the turnover rate of mRNA versus the protein. Nevertheless, the result is consistent with the old finding that the content of vitamin C in strawberry fruit is dependent on light intensity (Hansen and Waldo, 1944).

Sequence analysis revealed the presence of G-box-, I-box-, and GT-1-like elements in the GalUR promoter; these have previously been identified in promoters of light-regulated genes (Terzaghi and Cashmore, 1995). The G-box motif is currently one of the best characterized cis regulatory elements in plants and has been identified in the promoters of a diverse set of unrelated genes, including those controlled by visible and UV light (Hartmann et al., 1998),ABA (Chandrasekharian et al., 2003), wounding (Rosahl et al., 1986), and anaerobiosis (de Lisle and Ferl, 1990). A family of plant basic leucine zipper (bZIP) proteins has been identified that interacts with G-box elements to confer high promoter activity (Foster et al., 1994; Martínez-Hernández et al., 2002). The transient expression assay with −397 bp of the GalUR promoter fused to the LUC reporter gene suggests that the G-box is important for the fruit expression of GalUR, but also that some upstream sequences are necessary for full activity of the GalUR promoter. Most in vivo expression studies indicate that G-box elements cannot act on their own but require the presence of additional cis acting elements for their function (Foster et al., 1994; Martínez-Hernández et al., 2002). This analysis also indicates that a negative regulatory region is located between nucleotides −397 and −518 relative to ATG. This region contains an I-box-like element. This element has been shown to be involved in light-regulated and/or circadian clock-regulated expression of photosynthetic genes (Borello et al., 1993; Terzaghi and Cashmore, 1995). Another negative regulatory region in the GalUR promoter could be the GT-1-like box, since GT-1 sequences and sequences related to GT-1 can also act as negative regulators in different genes (Faktor et al., 1996; Zhou, 1999). However, an increase in activity was not observed when the fragment containing the GT-1-like sequence was deleted. Related GATA motifs are found in many other promoters, some of which are regulated by light (Anderson and Kay, 1995; Lam and Chua, 1989). In tomato, the I-box has been shown to be an activating cis element of the RBCS in leaves (Baum et al., 1997, 1999). Recently, it has been demonstrated that I-box-like elements down-regulate gene expression in melon fruit, suggesting a new function of the I-box-like element as a negative regulator (Yamagata et al., 2002). This could be the case for the I-box element of the strawberry GalUR promoter.
Sugar and osmotic conditions can modulate gene expression (Jang et al., 1997; Mikolajczyk et al., 2000). To determine whether the high osmotic solution used in the biolistic assays might affect the final result, the agroinfiltration-mediated transient gene expression method was also used (Spolaore et al., 2001, 2003). Similar data were obtained with the two methods, and this seems to exclude any effect of the experimental conditions on the final result. In addition, this supports the validity of the method developed in this work for the rapid analysis of fruit-specific promoters in strawberry. However, a drawback of this transient assay is that it cannot distinguish among specific tissues such as epidermis, vascular bundles, or achenes. The central part of the slice is hit more efficiently than the outer regions such as the epidermis of the receptacle and the achenes. Furthermore, induction by wounding cannot be completely eliminated due to the tissue damage by the bombardment. Therefore, this transient expression system cannot fully replace the permanent transformation system, which is more tissue-specific and sensitive.

Promoter regions of genes comprise highly divergent sequences visualized in modular terms whose activities are controlled by the combinatorial association of multiple proteins. Knowledge about this complex interaction that controls gene expression at the transcriptional level is still very limited. This makes it very difficult to predict the activity of a promoter in a different physiological context, either a different organ or a different species. In the case of strawberry this is more complex since the edible fruit is not a true fruit but an enlarged receptacle and the real fruits are achenes. Furthermore, induction by wounding cannot be completely eliminated due to the tissue damage by the bombardment. Therefore, this transient expression system cannot fully replace the permanent transformation system, which is more tissue-specific and sensitive.

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It was also found that the Fib promoter of pepper displays a significant activity in tomato fruits, as previously reported (Kuntz et al., 1998), but only a moderate activity in strawberry fruits. This low activity in strawberry would make this promoter of limited used for biotechnological purposes. Nevertheless, it has been established that the GalUR promoter is both highly specific and active in the receptacle of strawberry fruits and may well be used in the manipulation of fruit quality factors by genetic engineering.

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