Isolation and characterization of a cDNA encoding glutathione transferase from ripe Eksotika 2 papaya
(Pengasingan dan pencirian cDNA yang mengekod transferase glutation daripada betik Eksotika 2 yang masak)

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Key words: glutathione transferase, papaya fruit ripening

Abstract
A cDNA clone encoding glutathione transferase, PGST1, was isolated from a ripe Eksotika 2 papaya cDNA library. The cDNA is 968 bp. The 218 deduced amino acid sequence has a calculated molecular weight of 25.4 kD and an isoelectric point of 5.9. It has amino acid sequence similarities to other glutathione transferases. The gene is highly expressed in ripe fruit.

Introduction
Glutathione transferases (GSTs) (EC 2.5.1.18) are a family of multifunctional dimeric enzymes that conjugate glutathione to a wide range of xenobiotic compounds, forming a glutathione S-conjugate leading to their detoxification. In animals, GSTs catalyse the conjugation and detoxification of structurally diverse electrophilic environmental carcinogens and drugs (Gulick and Fahl 1995). In plants, GSTs are known to have roles in endogenous cellular metabolism (Marrs 1996), herbicides metabolism (Riechers et al. 1997, Wiegand et al. 1986) and in defense against pathogen attack (Mauch and Dudler 1993). GST gene expressions are inducible by a wide range of endogenous and xenobiotic chemical compounds although some are constitutively expressed. To date, several types of GSTs cDNA clones have been isolated from several different plants. Here, we report a cDNA isolated from a ripe Eksotika 2 papaya library that has homology to GSTs.

Materials and methods
Isolation of PGST1
A few cDNA clones were obtained from a ripe Eksotika 2 papaya cDNA library and sequenced. One of these clones showed high homology to glutathione transferase and was named, pPGST1. The cDNA library was

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constructed in this laboratory using the UNIZAP XR vector (Stratagene) (manuscript submitted for publication).

**Sequencing and computer aided analysis**
PGST1 was sequenced by a commercial vendor using automatic sequencing in a ABI Model 377 sequencer (ACGT Inc. USA) and its deduced amino acid sequence compared to the SwissProt database using the BLASTP programme at the National Centre for Bioinformatics, USA (Altschul et al. 1990).

**Northern analysis**
Total RNA was extracted from normal and wounded papaya tissues using the method of Covey and Grierson (1976). Wounded tissues were those cut into small pieces and left at room temperature (28 °C) for one hour. Tissues were then frozen in liquid nitrogen and stored at –70 °C until they were used for extraction of total RNA. Northern analysis was done according to Sambrook et al. (1989). The insert of PGST1 was used as a probe and labelled with α32P-dCTP according to the manufacturer’s protocol (Stratagene). The final washing condition was 2 x SSPE, 0.1% SDS at 65 °C for 20 min.

**Results**
The isolated PGST1 cDNA was fully sequenced. The cDNA size is 968 bp, the 5’ untranslated region is 76 bp while the 3’ untranslated region is 235 bp. The open reading frame is 654 bp and has a 218 deduced amino acid sequence (Figure 1). The deduced protein has a calculated molecular weight of 25.4 kD and an isoelectric point of 5.9. The PGST1 cDNA sequence was deposited as Accession AJ000923 in the EMBL nucleic acid database.

A search of the protein database using the deduced amino acid sequence of PGST1 showed that it has homology to glutathione transferases (Table 1). It is interesting to note that there are no fruit glutathione transferases in the list of 50 sequences picked from the protein databases with similarities to PGST1. Furthermore, most of the GSTs cDNA clones are auxin induced/regulated. The deduced amino acid sequence of PGST1 has identities of 73.2%, 72.3%, 70.9% and 69.7% with tobacco GST cDNA clones, namely, CNT107 (van der Zaal et al. 1991), PARC (Takahashi and Nagata 1992), PAR (Takahashi et al. 1989) and LS216 (Dominov et al. 1992) respectively. PGST1 also showed 70.1% amino acid sequence identity to a soybean GST cDNA clone.

**Table 1. Amino acid identities of papaya glutathione transferase cDNA (PGST1) with other glutathione transferases**

<table>
<thead>
<tr>
<th>Accession no.</th>
<th>Gene name</th>
<th>Organism</th>
<th>Type</th>
<th>Length (aa)*</th>
<th>% Identities (aa overlap)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF048978</td>
<td>GSTA</td>
<td>Soybean</td>
<td>auxin induced</td>
<td>219</td>
<td>76.1 (218)**</td>
<td>McGonigle and O’Keefe (1998)</td>
</tr>
<tr>
<td>X56266</td>
<td>CNT107</td>
<td>Tobacco</td>
<td>auxin induced</td>
<td>221</td>
<td>73.2 (217)</td>
<td>van der Zaal et al. (1991)</td>
</tr>
<tr>
<td>X64398</td>
<td>PARC</td>
<td>Tobacco</td>
<td>auxin induced</td>
<td>221</td>
<td>72.3 (217)</td>
<td>Takahashi and Nagata (1992)</td>
</tr>
<tr>
<td>Y10820</td>
<td>GSTS2</td>
<td>Soybean</td>
<td>not determined</td>
<td>216</td>
<td>70.1 (218)</td>
<td>Andrews et al. (1997)</td>
</tr>
<tr>
<td>X64399</td>
<td>C-7</td>
<td>Tobacco</td>
<td>not auxin induced</td>
<td>219</td>
<td>70.1 (218)</td>
<td>Takahashi and Nagata (1992)</td>
</tr>
<tr>
<td>M29274</td>
<td>PARA/Par</td>
<td>Tobacco</td>
<td>auxin induced</td>
<td>220</td>
<td>70.9 (217)</td>
<td>Takahashi et al. (1989)</td>
</tr>
<tr>
<td>U80615</td>
<td>EGPAR</td>
<td>Blue Gum</td>
<td>auxin induced</td>
<td>220</td>
<td>67.4 (209)</td>
<td>Nehls et al. (1998)</td>
</tr>
<tr>
<td>AF079511</td>
<td>R6-R37</td>
<td>Ice Plant</td>
<td>NaCl induced</td>
<td>224</td>
<td>66.6 (222)</td>
<td>Michalowski and Bohnert (1998) unpublished</td>
</tr>
<tr>
<td>S44036</td>
<td>LS216</td>
<td>Tobacco</td>
<td>auxin induced</td>
<td>219</td>
<td>67.7 (217)</td>
<td>Dominov et al. (1992)</td>
</tr>
<tr>
<td>Y12862</td>
<td>GST5</td>
<td>Maize</td>
<td>auxin induced</td>
<td>224</td>
<td>59.1 (213)</td>
<td>Dixon et al. (1998)</td>
</tr>
</tbody>
</table>

*aa = amino acid residues  
**numbers in brackets indicate number of overlapping amino acid residues
GSTS2 (Andrews et al. 1997). GSTS2 was obtained from a cDNA library using a degenerate oligonucleotide probe of a 2-4-D-inducible GST, but GSTS2 has not been shown to be auxin induced. The alignment with these GST amino acid sequences is shown in Figure 2.

The Northern analysis showed that the transcript size of PGST1 is about 0.9 kb. This suggests that PGST1 is a full length clone since the cDNA length is 968 bp. Northern analysis also showed that PGST1 is expressed in the mature fruit (green to ripe) but not in the immature fruit (Figure 3a and Figure 3b). Papaya leaf and flower showed low levels of expression and none in the stem (Figure 3b). The expression is increased in wounded leaf, but wounding did not induce the expression in immature green fruit (Figure 3c).

Discussion
The protein database search showed that PGST1 has the highest amino acid sequence homology with deduced amino acid sequences of four tobacco GSTs and two of soybean (Figure 2). Most of these clones are auxin induced/regulated GSTs genes. The soybean cDNA GSTS2 and tobacco cDNA C-7 were isolated using GSTs which were auxin induced. The CNT107 clone was isolated after addition of auxin (2,4-D).
Figure 2. Comparison of the deduced amino acid sequences of PGST1 with other known plant glutathione transferase genes. The consensus sequence was determined using MEGALIGN in the DNAstar programme.
**Figure 3. Expression of PGST1 in papaya**

(a) in different developmental stages

(b) in different organs

(c) in wounded and normal leaf and fruit

IMGr = immature green stage
MGr = mature green stage
Turn = turning stage (first sign of colour change)
Ripe = ripe stage
Wleaf = wounded leaf
Nleaf = unwounded leaf
WIMGr = wounded immature green fruit
NIMGr = unwounded immature green fruit

Tobacco cells (van der Zaal et al. 1991). The mRNAs of CNT107 were not detected in any plant organ but found to accumulate transiently prior to cell division in response to the auxin treatment. PAR clone was isolated from an auxin-treated mesophyll protoplast (Takahashi et al. 1989). The mRNA was detected in cultured mesophyll protoplasts as early as 30 min after addition of 2,4-D, but not in leaves or freshly prepared protoplasts or protoplasts in the absence of 2,4-D. The accumulation of par mRNA before the initiation of DNA synthesis in tobacco mesophyll protoplasts suggests that the par gene product could play a role in the initiation of meristematic activity in differentiated mesophyll cells. PARC was also isolated from auxin induced tobacco mesophyll protoplasts (Takahashi and Nagata 1992). PARC expression was highest during G₀ to S phase of cell cycle. The expression was also inducible by other auxins such as NAA and IAA but not by GA, ABA and ethylene. PARC mRNA was detected throughout fully-grown tobacco plants (in shoot tips, flowers, leaves, stems, roots) and whole plant of one week old seedlings and was most abundant in seedlings and roots. LS216 gene expression was induced by auxin (2,4-D) and cytokinin (BA) in tobacco suspension culture cells. Cytokinin was found to further enhance the
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auxin response and prevent the stationary effect due to auxin feedback inhibition to the gene expression (Dominov et al. 1992). GSTS2 in soybean has been implicated as one of the bifunctional GST/GSH peroxidases having tolerance to oxidative stress as well as to herbicides (Andrews et al. 1997).

The PGST1 was isolated from a ripe papaya fruit cDNA library. The role of the GST gene in ripening papaya fruit is still unknown. Furthermore, no fruit GSTs clones have been reported to enable a deduction to be made on the possible functions of PGST1. The expression of PGST1 indicated that it is ripening related (not expressed in immature green fruit but expressed in the mature to ripe fruit) (Figure 3a). The gene expression is not fruit-specific as it is also expressed at low levels in the leaf and flower (Figure 3b). This suggested that the PGST1 may be involved in the ripening process of papaya either directly or indirectly. The PGST1 gene expression seemed to be slightly induced during wounding. However, the wounding process has no effect on the gene expression in the immature green fruit (Figure 3c).

The high amino acid sequence homology (70–76%) of PGST1 to the four tobacco and two soybean sequences and the similarity in the predicted protein size of 25 kD suggest that PGST1 may share similar functions to the GSTs described above. Plant GSTs has been known for their ability to detoxify chemically diverse herbicides or other toxic organic compound. It is possible that the PGST1 gene product is required to solubilize for easy removal of any by product of the ripening process or for proper localization of any products formed during ripening. Recently, Alfenito et al. (1998) reported the role of GST in the sequestration of anthocyanin in the vacuole.

While gene expression of all the five GSTs are auxin inducible, it is not known whether PGST1 is also auxin inducible as the studies have not been done. It is also interesting to know the effect of exogenous ethylene application on the expression of PGST1 as ethylene is known to control the ripening process in fruits. Further research to determine the functions of PGST1 need to be done to better understand its roles in the ripening papaya.

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References


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