

Update section

Sequence

Sequence analysis of three members of the maize polygalacturonase gene family expressed during pollen development

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Received 23 March 1992; accepted in revised form 28 April 1992

Key words: maize, microsporogenesis, pollen, polygalacturonase

We have previously reported [1] the isolation of a maize pollen-specific cDNA, 3C12, which exhibits homology in its predicted amino acid sequence to polygalacturonases of various species [2, 3, 4, 5]. We have used a 300 bp fragment from the 5' end of this clone to isolate the corresponding genomic clones from maize libraries made by partial *Sau* 3A digests of genomic DNA cloned in the λ vector EMBL3. Four such clones were isolated: two from a library of maize variety W22 (purchased from 'Clontech') and two from a library of B73. The λ clones isolated from the W22 library, W2247 and W2265, had insert sizes of 10 and 16 kb respectively and lacked the 3' end of the coding sequence. The λ clones isolated from the B73 library, B7317 and B7339, had insert sizes of 17 and 13 kb respectively and contained the whole coding region. The inserts were subcloned and a comparison of the restriction maps of subclones of the four genomic clones and the cDNA, 3C12, made (Fig. 1). Sub-clones p47.42 and p65.3 have identical restriction maps. While there are no obvious differences between the four clones over the coding regions, there are differences both in the 5' and 3' untranslated regions

between the clones. The nucleotide sequences of the coding regions for three of these (W2247, W2265 and B7317) have been determined. We have predicted the ATG start in the three genomic clones based on it being the first in frame ATG after the transcription start mapped on W2247 (unpublished data). The coding sequences are colinear with the cDNA, there being no introns. Genomic clones B7317 and W2247 are identical in their predicted amino acid sequence to the cDNA, 3C12. Clone W2265 has a one amino acid difference; a conservative substitution of serine for asparagine due to a nucleotide substitution of adenine for guanine at position 167 bp relative to the ATG start. B7317 and W2247 are also identical in their predicted amino acid sequence to the homologous maize polygalacturonase cDNA clone, PG1, isolated by Niogret *et al.* [6] from maize line MO17. A further PG cDNA clone isolated by Niogret *et al.* [6], PG2, has a one amino acid difference compared with PG1, 3C12, B7317 and W2247 due to a substitution at 1076 bp.

We have compared the nucleotide sequence of the coding regions of the three genomic clones

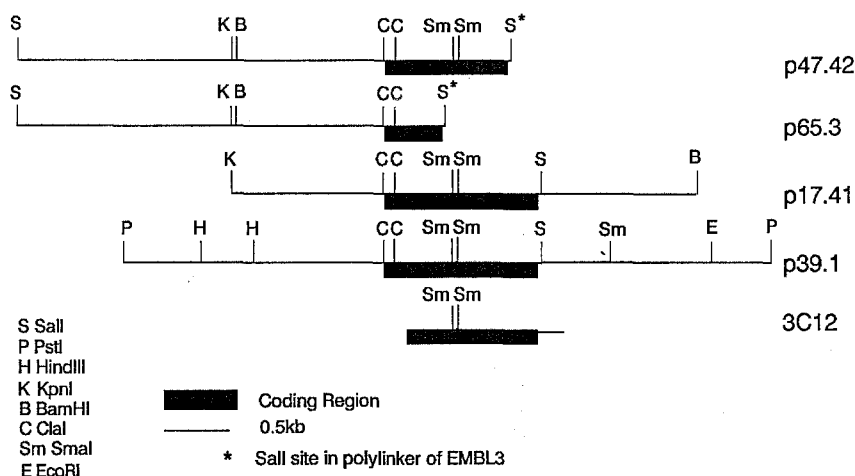


Fig. 1. Restriction maps of cDNA and genomic PG subclones.

and the cDNA, 3C12, with each other and with PG1 and PG2. Table 1 illustrates the differences. Two of the cDNA clones, PG1 and 3C12, are identical, but the three genomic clones are different from each other and also from the cDNAs. All of the base substitutions (except for those giving rise to the amino acid substitutions mentioned above) are silent third-codon differences and are both transitions and transversions. The base substitutions are limited, only one of two nucleotides are present at any of the substituted positions. It is known that the maize pollen-specific polygalacturonase is a member of a small

multigene family [6] and it is probable that these clones are all different members of that gene family. It is possible that some of the differences between the clones may be due to varietal differences, however the examples in the literature of cDNA and genomic clones of low copy number genes being isolated from libraries made from different maize lines show that the differences between these clones appear only in the 3' untranslated region [7, 8]. It is interesting that two cDNA clones of the *Oenothera* pollen-expressed polygalacturonase gene family exhibit only 89% homology in the coding region [3], whereas the mem-

Table 1. Comparison of coding regions of cDNA and genomic polygalacturonase clones.

nt position relative to ATG start	B7317	W2247	W2265	3C12	M017PG1	M017PG2
6	A	G	A	?	G	A
167	G	G	A	G	G	G
243	C	T	C	T	T	C
378	C	T	C	T	T	C
432	T	C	C	C	C	T
573	G	G	T	G	G	G
669	C	G	?	G	G	C
696	C	C	?	T	T	C
783	A	C	?	C	C	A
1032	C	?	?	T	T	T
1076	C	?	?	C	C	T
1203	A	?	?	G	G	G

? Region not present on clone.

bers of the maize multigene polygalacturonase family are more highly conserved (>99%) in the coding region. Such a high degree of conservation is in contrast to reports of other maize multigene families [9, 10]. It is possible that this pollen-specific PG gene family has arisen due to recent gene duplications.

Acknowledgement

This work was supported by Pioneer Hi-Bred International Inc.

References

1. Rogers HJ, Allen RL, Hamilton WDO, Lonsdale DM: Pollen-specific cDNA clones from *Zea mays*. *Biochim Biophys Acta* 1089: 411–413 (1991).
2. Grierson D, Tucker GA, Keen J, Ray J, Bird CR, Schuch W: Sequencing and identification of a cDNA clone for tomato polygalacturonase. *Nucl Acids Res* 14: 8595–8603 (1986).
3. Brown SM, Crouch ML: Characterisation of a gene family abundantly expressed in *Oenothera organensis* pollen that shows sequence similarity to polygalacturonase. *Plant Cell* 2: 263–274 (1990).
4. Saarihtti HT, Heino P, Pakkanen R, Kalkkinen N, Palva I, Palva ET: Structural analysis of the *pehA* gene and characterisation of its protein product, endopolygalacturonase, of *Erwinia carotovora* subspecies *carotovora*. *Mol Microbiol* 4: 1037–1044 (1990).
5. Huang J, Schell MA: DNA sequence analysis of *pglA* and mechanisms of export of its polygalacturonase product from *Pseudomonas solanacearum*. *J Bact* 172: 3879–3887 (1990).
6. Niogret M-F, Dubald M, Mandaron P, Mache R: Characterisation of pollen polygalacturonase encoded by several cDNA clones in maize. *Plant Mol Biol* 17: 1155–1164 (1991).
7. Hanson DD, Hamilton DA, Travis JL, Bashe DM, Mascarenhas JP: Characterisation of a pollen-specific cDNA clone from *Zea mays* and its expression. *Plant Cell* 1: 173–179 (1989).
8. Stiefel V, Ruiz-Avila L, Raz R, Valles MP, Gomez J, Pages M, Martinez-Iquierdo JA, Ludevid MD, Langdale JA, Nelson T, Puigdomenech P: Expression of a maize cell wall hydroxyproline-rich glycoprotein gene in early leaf and root vascular differentiation. *Plant Cell* 2: 785–793 (1990).
9. Marks MD, Lindell JS, Larkins BA: Nucleotide sequence analysis of zein mRNAs from maize endosperm. *J Biol Chem* 260: 16451–16459 (1985).
10. Russell DA, Sachs MM: Differential expression and sequence analysis of the maize glyceraldehyde-3-phosphate dehydrogenase gene family. *Plant Cell* 1: 793–803 (1989).