

HRGP genes expressed during physiological deterioration of cassava storage roots

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Introduction

Cassava is one of the most important tropical crops. Rapid post-harvest deterioration, which can occur within 24 hours after harvest has been a major problem in its production. The deterioration consists of two stages, primary or physiological deterioration, and secondary or microbial deterioration (Booth, 1976; Onwume, 1978). Visual evidence for the postharvest physiological deterioration (PPD) is vascular streaking, a blue-brownish coloration of the xylem vessels in the storage parenchymatous tissue. PPD is a special wound response lacking wound-healing process; in other words, PPD is initiated from wound sites and spreads through the root.

Hydroxyproline-rich glycoproteins (HRGPs) are abundant proteins in the plant cell wall. They strengthen cell walls and control cell wall extension by insolubilization of the molecules through intermolecular cross-links. HRGPs are induced in wound responses and are involved in the wound healing process (Hirsinger *et al.*, 1997; Bradley *et al.*, 1992; Ussuf *et al.*, 1996). No wound healing processes have been observed at the histochemical level during PPD. It remains to be established whether there are any wound healing responses at a molecular level during PPD. Here we report the isolation of HRGPs genes expressed during PPD.

Material and Methods

A PPD related cDNA library in λ gt10 (Beeching *et al.*, 1998) was screened with parsley hydroxyproline-rich glycoprotein (HRGP) cDNA EL19 as a probe (Kawalleck *et al.*, 1995). After 3 h of pre-hybridization at 50°C, hybridization was carried out at 50°C overnight. The membrane was washed to 0.1 \times SSC/0.1% SDS at 50°C for 2 \times 10 min. Then secondary screening was performed to obtain single positive plaques. The cDNA inserts in the isolated positive clones were then amplified by PCR using λ gt10 primers.

Results and Discussion

Nine positive clones were isolated from the PPD related cDNA library. Six of these positive clones were amplified by PCR and sequences were determined using the PCR products. The deduced amino acid sequence of clone G showed typical repeat pattern of HRGP, serine-proline-proline-proline-proline or SPPPP (Han *et al.*, 2000). Clone B showed 93% identity over 271 bp to clone G and all the identified nucleotide differences between these two clones in the compared region occurred in the third nucleotide of the codons for amino acid residues, which did not change the encoded amino acid residues.

The deduced amino acid sequence of clone C had SPPPP and YYY (tyrosine) repeats, two A(Ala)YG(Gly)K repeats, two polyproline

units W(Trp)P₄ and H(His)P₃, seven palindromes of at least four amino acid residues and many palindromes of three residues (Figure 1). The partial polypeptide is rich in proline (17mole%), lysine (16%), tyrosine (9%), valine (8%), glycine (7%) and histidine (5%).

The determined sequence of clone D was highly similar to the 3' end of clone G, with just 18 extra nucleotides compared to clone G. Clone F was identical to the 3' coding region of clone G and clone H showed 74% identity to clone G over 170 bp compared.

The SPPPP motif, which can lead to molecular inflexibility and wall-self assembly, is the most common repeat motif in HRGPs of many plant systems and it is also present in all the analysed HRGP clones. Palindromes including YYY, PYYYP, HPSPH, HHF(Phe)HH, VVKVV, YD(Asp)WDY and GKKG are present in the deduced amino acid sequence of clone C. The palindromic peptides could create centrosymmetric domains, which may act as self-assembly nucleation sites. In other words, an intermolecular interaction may establish a structure to initiate a succession of intermolecular reaction or growth (Kieliszewski and Lamport, 1994). The effect of HRGP gene expression on wound healing was demonstrated by the impaired healing response in potatoes after gamma irradiation (Ussuf *et al.*, 1996). It has been shown that proline-rich cell wall protein was rapidly insolubilized at wound sites (Bradley *et al.*, 1992). Increases in the level of HRGP transcripts upon wounding stimulation have been observed in many plant systems such as tomato and potato (Showalter *et al.*, 1992; Bown *et al.*, 1993). The isolation of HRGP cDNA clones from PPD related cDNA library indicated that they were expressed during the special wounding response, suggesting that at least one element of the healing process was present during PPD. However, there may be insufficient expression of HRGPs or insolubilization of existing HRGPs, or/and other healing components, which results in the development of PPD.

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121	ATGGCCACCACCCCAAAAATCACCAGCACCAACACCTTATTATTACCCTTCTCCACCACC	180
	<u>W P P P P K S P A P T P Y Y Y P S P P P</u>	
181	ACCTAAGGCTCATCTCCGCGTACTACTATACTTCTCCACCACCACCTGTTCTTATCC	240
	<u>P K A H P P P Y Y Y T S P P P P V P Y P</u>	
241	TCACCCGTCTCCACATCCCCATCATTTCGTCGTAAAGGTGGTAGGAAAGGTCTATTGCTA	300
	<u>H P S P H P H . H . F . V . V K V V G K V Y C Y</u>	
301	CAGATGCTATGACTGGGATTATCCTGAAAAATCACATGACAAGAAGCATCTCAAAGGTGC	360
	<u>R C Y D W D Y P E K S H D K K H L K G A</u>	
361	CGTAGTGGAGGTAACATGCAAGACAGGTGAAAAGGAGATCAAGGCTTATGGTAAAACCAA	420
	<u>V V E V T C K T G E K E I K A Y G K T K</u>	
421	GATCAACGGTAAATACAGTATCACCGTTGAGGGCTTTGCGTATGGCAAATATGGAGCCGA	480
	<u>I N G K Y S I T V E G F A Y G K Y G A E</u>	
481	GGCTTGCAAGGCTAAGCTCCATAAGGCACCCAAAGGCTCACCATGCAACATACCAACTAA	540
	<u>A C K A K L H K A P K G S P C N I P T N</u>	
541	CCTCCACTGGGGCAAGAAGGGTGCCAAGCTCAAGGTGAAGTCCAAGAC	
	<u>L H W G K K G A K L K V K S K T</u>	

Figure 1. Deduced amino acid sequence from the partial nucleotide sequence of cDNA clone C. SPPPP and YYY are in bold, other XP_n (X refers to any residues) and repeats are underlined. Palindromic regions of the amino acid sequence are over-lined. Palindrome-like structures are indicated with double underlines. The palindrome overlapping with another one is dot-underlined.

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