Exopolygalacturonase Elicits Ethylene Production in Tomato

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Abstract. Exopolygalacturonase (exo-PG) (EC 3.2.1.67) was investigated for ability to induce ethylene production in green cherry tomatoes (*Lycopersicon esculentum* Mill.). The fruit were vacuum-infiltrated with various levels of exo-PG from green tomato fruit, squash flower, or oak pollen and compared to boiled enzyme or salt controls for ethylene production. In all cases, fruit treated with active enzymes produced significantly higher levels of ethylene than did control fruit. The ethylene response was evident 2 hours after treatment and was transient in nature, returning to basal levels by 22 hours. The amount of ethylene produced did not appear to be influenced by the source of exo-PG.

Preclimacteric tomatoes have no detectable endo-polygalacturonase (endo-PG) (EC 3.2.1. 15), an enzyme that is thought to play a major role in tomato softening (Tucker and Grierson, 1982; Grierson and Tucker, 1983; Grierson et al., 1985; Smith et al., 1988). Both tomato and fungal endo-PG, fungal endo-pectin lyase (EC 4.2.2.10), and uronic acid oligomers have been shown to induce ethylene when infiltrated into preclimacteric green tomato fruit (Baldwin and Pressey, 1988, 1989; Brecht and Huber, 1988). The appearance of endo-PG in tomatoes, however, occurs only after the onset of climacteric ethylene (Grierson and Tucker, 1983). This sequence precludes a role for endo-PG as an inducer of climacteric ethylene in tomato ripening. Exo-PG, however, has been detected in low levels in green preclimacteric tomato (Pressey, 1987) as well as in other fruits and plant tissues (Pressey, 1986). Exo-PGs from various systems, including tomato, have been characterized and were found to be exo-hydrolyses with optimum activities at pH 5.0 and require 0.4 mM Ca for activity (Pressey, 1987; Pressey and Avants, 1977). In previous studies, 400 µg galacturonic acid/g fresh weight, the product of exo-PG action on polygalacturonic acid (Pressey, 1987), was reported to induce ethylene in mature green 'Rutgers' fruit (Kim et al., 1987). The pur-

pose of our study was to determine whether exo-PG was capable of inducing ethylene production in preclimacteric tomatoes.

Green cherry tomatoes, cultivars Short Red Cherry and Cherry Sweet 100, were harvested at the Russell Research Center in Athens, Ga., during Summer 1987. The fruit were allowed to equilibrate to room temperature for several hours before surface sterilization with 0.02% NaOCl and analysis for ethylene production. The fruit were sealed in containers for 1 hr, after which a 1-ml sample of the headspace was analyzed on a Perkin Elmer Sigma 300 gas chromatography equipped with an activated alumina column and flame ionization detector. Only those fruit producing basal levels of ethylene (<0.2 nl·g⁻¹·hr⁻¹) were used in experiments. Four fruits were vacuum-infiltrated with treatment solutions equivalent to 1% of the fruit fresh weight as described by Baldwin and Pressey (1988). The production of ethylene was monitored at various times after infiltration. Data for ethylene measurements were tested by analysis of variance using the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS Institute Inc., Cary, N.C.). Specific differences between treatments were determined by least significant difference and Tukey's studentized range. All comparisons were done at $P = 0.05$.

Exo-PG was separated using ion-exchange chromatography as reported by Pressey (1987). One unit of enzyme activity is defined as the amount that catalyzes the hydrolysis of polygalacturonic acid, releasing 1 µmol of galacturonic acid in 1 hr at 37°C in reaction mixtures containing polygalacturonic acid (Sigma, St. Louis) (Pressey and Avants, 1977).

Enzyme treatments consisted of exo-PG from 'Better Boy' tomato fruit, yellow squash

flowers (*Cucurbita pepo* L.), or white oak pollen (*Quercus alba* L.) at 6.5 to 42 units of activity and 0.8 to 5 mg protein/ml in 0.15 M NaCl. Either 0.15 M NaCl or identical boiled (5 rein) enzyme solutions (no precipitation was observed) served as controls. Some treatment solutions contained 0.5 mM Ca.

Treatment of tomato fruit with exo-PG from three sources resulted in a transient increase in ethylene production that peaked between 3 and 8 hr after treatment and returned to near control levels by 22 hr (Table 1). A variable wound ethylene response due to the vacuum-infusion process was observed in the boiled or salt controls, but the level of ethylene produced by control fruit was significantly lower during peak production than that produced in response to the active enzymes from all three sources. The rates of ethylene production in response to tomato fruit, squash flower, or oak pollen exo-PG at 26, 27.5, or 42 units of activity per milliliter and 5, 0.8, and 5 mg protein/ml, respectively, ranged from 8 to 11 nl·g⁻¹ fresh weight/hr. This quantity did not appear to be related to the amount of protein infiltrated, since squash flower exo-PG treatments elicited more ethylene production than tomato fruit or oak pollen PG with less protein per unit. This result confirms observations in an earlier study with endo-pectinases (Baldwin and Pressey, 1989) where elicitation of ethylene production in tomato and citrus was not found to be dependent on amount of protein infiltrated, protein size (within 25,000-36,500 Da) or ionic properties. Baldwin and Pressey (1988) also showed that ethylene produced by green tomatoes, infiltrated with tomato endo-PG solutions of identical units of activity and quantity of protein per unit, depended on the pH of the treatment solution. Significantly higher ethylene production was obtained at the pH that was optimal for tomato endo-PG activity.

Tomato exo-PG was applied to green 'Cherry Sweet 100' tomatoes at three levels to study the effect of concentration. The highest activity of tomato exo-PG (26 units of activity) induced significantly higher ethylene production than did diluted treatments (13 and 6.5 units of activity) or controls at 4 and 8 hr after treatment (Table 1A).

Squash exo-PG was applied to 'Cherry Sweet 100' with and without 0.5 mM Ca. Although endogenous levels of Ca in tomato may be sufficient for exo-PG activity, it is possible that the enzyme is directly or indirectly regulated by the concentration of free Ca in vivo. Squash exo-PG without Ca induced slightly higher ethylene levels than squash exo-PG with Ca, although the difference was not significant. (Table 1B). Both exo-PG treatments resulted in significantly higher ethylene levels than either Cain NaCl or NaCl alone between 4 and 8 hr after treatment.

Oak pollen exo-PG also induced ethylene levels that were significantly higher than the boiled control in 'Short Red Cherry' for up to 6 hr after treatment (Table 1C). Although this treatment solution contained double the enzyme activity of tomato or squash exo-PG,

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Table 1. Effect of tomato fruit, squash flower (± 0.5 mM Ca), or oak pollen exo-PG in 0.15 M NaCl on green 'Cherry Sweet 100' (for tomato and squash exo-PG) and 'Short Red Cherry' (for oak pollen exo-PG) ethylene production.^{a, y}

Treatment solution	Enzyme activity (units/ml)	C ₂ H ₄ (nl·g ⁻¹ ·hr ⁻¹)							
		Hours after treatment							
		0	2	3	4	6	8	22	45
A) Tomato fruit exo-PG (Expt. 1)									
NaCl	0.0	0.100 a	0.60 b		0.00 c		0.22 b	2.05 a	
PG	6.5	0.015 a	2.67 a		3.33 b		1.03 b	1.03 a	
PG	13.0	0.015 a	1.23 ab		3.05 b		0.98 b	0.82 a	
PG	26.0	0.020 a	2.83 a		8.10 a		6.12 a	1.42 a	
df		3	3		3		3	3	
F value		0.24	7.31		41.51		19.41	2.09	
P value		0.8700	0.0057		0.0001		0.0001	0.1603	
HSD		0.035	1.739		2.300		2.712	1.629	
B) Squash flower exo-PG (Expt. 2)									
NaCl	0.0	0.06 a	0.67 b		1.55 b		1.08 b	0.40 a	
NaCl + Ca	0.0	0.02 a	0.60 b		1.12 b		0.03 b	0.42 a	
PG + Ca	27.5	0.02 a	5.40 a		11.17 a		10.30 a	0.57 a	
PG	27.5	0.02 a	3.00 ab		8.06 a		8.43 a	1.97 a	
df		3	3		3		3	3	
F value		0.43	6.16		38.28		120.44	3.16	
P value		0.7384	0.1078		0.0001		0.0001	0.0786	
HSD		0.105	4.162		3.446		2.068	1.843	
C) Oak pollen exo-PG (Expt. 3)									
Boiled PG	0.0	1.13 b		2.70 b		2.67 b		0.10 a	1.87 a
Active PG	42.0	1.47 a		6.27 a		9.40 a		0.67 a	0.17 a
df		1		1		1		1	1
F value		20.0		11.12		26.86		7.22	1.98
P value		0.011		0.0290		0.0066		0.0548	0.2326
HSD		0.207		2.972		3.609		0.586	0.593

^aBoiled exo-PG, 0.5 mM Ca, and 0.15 M NaCl (± 0.5 mM Ca) served as controls. All points are means of four replications.

^yMean separation for each experiment and within columns at $P = 0.05$.

it induced similar amounts of ethylene production (9 compared to 8 and 11 nl·g⁻¹·hr⁻¹ for tomato and squash exo-PG, respectively).

'Cherry Sweet 100' fruit infiltrated with 50 or 125 µg galacturonic acid/ml showed a similar pattern of ethylene production when compared to the enzyme-treated fruit, although the levels of ethylene were slightly lower (data not shown). This result confirms earlier reports by Kim et al. (1987) that galacturonic acid, the product of exo-PG action on cell walls, elicits ethylene production in tomato even at these low levels. In general, green tomatoes treated with either enzymes or galacturonic acid solutions showed accelerated red pigment development compared to control fruit (visual observation), although this appeared to depend on the physiological maturity of the green fruit at harvest.

Variation in ethylene evolution in response to infiltration treatments was apparent in this study. This variation may have been influenced by differences in the ethylene production capacity of the tomatoes over the growing season due to the physiological maturity of the fruit at time of harvest (Baldwin and Pressey; 1988) and the physiological state as influenced by environmental factors before harvest.

It is not known whether the terminal residues of cell wall polysaccharides are important to cell wall structure, but if they involve Ca crosslinks between adjacent pectin chains or are attached to critical neutral sugar sidechains, their removal by exo-PG could lead to cell wall alteration that could

signal ethylene production. Apparently, the ethylene response to exo-PG in tomatoes is not specific to tomato exo-PG, since similar responses were obtained when exo-PGs from other plant sources were used. This is not surprising, considering the similarity of the exo-PG enzymes thus far characterized. It is conceivable that endogenous exo-PG in green tomatoes could promote ethylene production, via the release of galacturonic acid monomers or other effects on cell wall structure, and at some critical point during fruit maturation influence the initiation of autocatalytic ethylene production.

Whether the ethylene response to infiltration of exo-PG and galacturonic acid is a ripening response to a ripening signal or a wound response to a wound signal is unclear, as either response can initiate ripening. Infiltration of other cell wall-degrading enzymes or their products (cell wall sugars) has been shown to elicit ethylene production in tomato (Baldwin and Pressey, 1988, 1989; Brecht and Huber, 1988; Gross, 1985; Kim et al., 1987). It cannot be assumed, however, that these treatments truly mimic the in vivo ripening situation involving endogenous cell wall-degrading enzymes and their cell wall sugar products.

Literature Cited

- Baldwin, E.A. and R. Pressey. 1988. Tomato polygalacturonase elicits ethylene production in tomato fruit. *J. Amer. Soc. Hort. Sci.* 113:92-95.
- Baldwin, E.A. and R. Pressey. 1989. Pectic enzymes in pectolyase: Separation, characterization and induction of ethylene in fruits. *Plant Physiol.* 90:191-196.
- Brecht, J.K. and D.J. Huber. 1988. Products released from enzymically active cell wall stimulate ethylene production and ripening in preclimacteric tomato (*Lycopersicon esculentum* Mill.) fruit. *Plant Physiol.* 88:1037-1041.
- Grierson, D. and G.A. Tucker. 1983. Timing of ethylene and polygalacturonase synthesis in relation to control of tomato fruit ripening. *Planta* 157:174-179.
- Grierson, D., A. Slater, J. Spiers, and G.A. Tucker. 1985. The appearance of polygalacturonase mRNA in tomatoes: one of a series of changes in gene expression during development and ripening. *Planta* 163:263-271.
- Gross, K.C. 1985. Promotion of ethylene evolution and ripening of tomato fruit by galactose. *Plant Physiol.* 79:306-307.
- Kim, J., K.C. Gross, and T. Solomos. 1987. Characterization of the stimulation of ethylene production by galactose in tomato (*Lycopersicon esculentum* Mill.) fruit. *Plant Physiol.* 85:804-807.
- Pressey, R. 1986. Polygalacturonases in higher plants, p. 157-174. In: M.L. Fishman and J.J. Jen (eds.). *Chemistry and function of pectins*. American Chemical Society Press, Washington, D.C.
- Pressey, R. 1987. Exopolygalacturonase in tomato fruit. *Photochemistry* 26:1867-1870.
- Pressey, R. and J.K. Avants. 1977. Occurrence and properties of polygalacturonase in *Avena* and other plants. *Plant Physiol.* 60:548.
- Smith, C. J., C.F. Watson, J. Ray, C.R. Bird, P.C. Morris, W. Schuch, and D. Grierson. 1988. Antisense RNA inhibition of polygalacturonase in transgenic tomatoes. *Nature (London)* 334:724-726.
- Tucker, G.A. and D. Grierson. 1982. Synthesis of polygalacturonase during tomato fruit ripening. *Planta* 155:64-67.