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PPO expression and accumulation during pollen germination and pollen tube growth

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Tomato (*Lycopersicon esculentum* Mill.) polyphenol oxidases (PPOs) are encoded by a seven-member gene family that exhibits complex patterns of differential expression during reproductive development. PPO activity is induced during tomato pollen germination and pollen tube growth. Cytochemical studies showed intense brown quinone products in in vitro germinated pollen following incubation with dihydroxy phenylalanine (DOPA), a PPO substrate. Microscopic observation showed that 95% of germinated pollen (4 h of pollen culture) stained for PPO activity, while only 3% of ungerminated pollen stained. After 6 h of culture, pollen possessed a 3-fold increase in immunodetectable PPO. Cycloheximide inhibition showed that a large fraction of the total PPO is translated within the first hour of pollen culture, the critical phase of protein synthesis required for pollen germination. However, PPO synthesis appeared to continue after 2 h of pollen culture. The increase in PPO and PPO activity appeared to be accounted for by the expression of PPO B. Addition of actinomycin D to the germination medium inhibited increased PPO B mRNA levels at 6 h of pollen culture, suggesting that PPO B is transcriptionally activated during pollen germination and tube growth. Analysis of PPO B promoter:GUS fusion construct in transgenic tomato confirms the result and demonstrates that cis-element(s) sufficient for PPO B inducibility reside in the 5' flanking region.
