Title Role of polyamines in regulating growth and development in tomato (*Solanum lycopersicum*)

Author Savithri Nambeesan, Avtar K. Handa, Mario G. Ferruzzi, Nicholas C. Carpita and David Rhodes

Citation Thesis, Doctor of Philosophy, Purdue University. 152 pages. 2009.

Keywords Abiotic stress; *Botrytis cinerea*; Flowering; Fruit set; Ripening; Shelf life; Polyamines; *Solanum lycopersicum*

Abstract

Polyamines (PAs) such as putrescine (Put), spermidine (Spd) and spermine (Spm) are polycationic and biogenic amines. To study the role of PAs in fruit set and ripening, and stress response, transgenic tomato plants expressing a yeast spermidine synthase (ySpdSyn), an enzyme that converts Put to Spd were generated under the constitutive CaMV 35S promoter and a fruit ripening specific E8 promoter. CaMV 35S-ySpdSyn transgenic plants exhibited increased accumulation of Spd in leaves and fruits, whereas E8-ySpdSyn plants had higher Spd levels at initiation of fruit ripening only. Fruits from CaMV 35S-ySpdSyn and E8-ySpdSyn homozygous lines exhibited delayed ripening and an increase in shelf-life by up to 7 days. Transcript levels of cell wall modifying enzymes such Expansin1 and xyloglucan endotransglucosylase/hydrolase was higher and that of β-galactosidase was lower at later stages of fruit development in the transgenic fruits. Expression of *SlLOXB* and *SlPLDα* , enzymes involved in membrane deterioration were also reduced in the transgenic fruits. These studies suggest a role for PAs in enhancing fruit shelf-life through alterations in fruit cell-wall and membrane associated properties. Additionally several enzymes involved in cell wall and membrane deterioration decreased at a lower rate during later stages of ripening in transgenic fruits suggesting a role for PAs in mRNA stabilization or enhancing transcription.

CaMV 35S-ySpdSyn leaves exhibited enhanced susceptibility to infection by the necrotrophic fungus, *Botrytis cinerea* . Treatment with PA biosynthesis inhibitors and an ethylene precursor reversed the enhanced susceptibility-response. During *Botrytis* infection, transcript levels of *SlACS, SlEIL1, SlEIL2, SlERF1* and *SlERF2* , genes involved in ethylene biosynthesis and signaling were reduced in transgenic lines. Additionally, transgenic lines displayed tolerance to salt and mannitol stress during seed germination but not during vegetative growth. These results indicate that PAs act as specific upstream regulators of ethylene regulated pathogenesis responses to Botrytis cinerea infection.

Certain CaMV 35S-ySpdSyn plants had increased flower size, impaired staminal cone fusion, reduced length and curvature of the style, enlarged ovaries and parthenocarpic fruit development. Expression of floral homeotic genes, *TM4, TM5, TM6, TM29* and *TAG1*, was up-regulated in developing flower buds in the transgenic lines. Additionally, transcript accumulation of *DELLA*, a negative regulator of gibberellin (GAs) signaling, and *GA-20oxidase-1* and *GA-3oxidase-2* , genes involved in GA biosynthesis, was reduced in developing flower buds of these transgenic plants. These results suggest that PAs regulate floral morphogenesis and fruit set in tomato by enhancing the expression floral homeotic genes and genes associated with GA biosynthesis and signaling. The above studies provide direct genetic evidence supporting a role for PAs in regulating flower development, delaying fruit ripening and senescence, and enhancing susceptibility to *Botrytis* infection in tomato.