Title Identification and subcellular localisation of *Sl;INT7*: A novel tomato (*Solanum lycopersicum* Mill.) fruit ripening-related and stress-inducible gene

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Abstract

The key step in ethylene (C_2H_4) signalling during tomato fruit ripening is initialized via the direct interaction between C_2H_4 and specialized membrane-bound receptors, including *N*ever-*R*ipe (*NR*), which is strongly induced during ripening. In order to identify novel ripening-related C_2H_4-dependent components, a yeast two-hybrid interaction screen has previously been employed, in which *NR* cDNA has been used as bait. This screen has identified a clone corresponding to interacting protein 7 (*SI;INT7*), through its specific and strong interaction with the NR receptor (L. Alexander, Z. Lin, R. Hackett, I. Wilson and D. Grierson, unpublished work). In this work, our objective was to identify the corresponding NR-interacting gene and subsequently characterize its expression response to various stress treatments, as well as unravelling its subcellular location in the cell. By sequencing and plant database interrogation, *SI;INT7* was found to be a small gene with an open reading frame (ORF) of ~243 bb encoding a protein composed of 77 aa that shares no sequence homology with any known gene. Notably, northern analyses demonstrated that *SI;INT7* gene expression is up-regulated in response to various stress signalling molecules such as salicylic acid (SA), abscissic acid, jasmonic acid, nitric oxide (NO) and salt, implicating *SI;INT7* in biotic and abiotic stress signalling transduction responses. To gain more insight into the possible function of *SI;INT7*, a construct in which SI;INT7 is C-terminally fused to the green fluorescent protein (GFP) was generated. Subsequently, *35S::SI;INT7::GFP*-containing constructs were transiently expressed in both tobacco leaves and onion peels via microprojectiles bombardment. Subsequently, confocal laser microscopic examination of bombarded tobacco and onion tissues revealed that the expression of GFP-SI;INT7 was observed predominantly in the plasma membrane, compared to the location throughout the cell observed with the control GFP construct alone. These results are discussed in the light of our present knowledge of C_2H_4-mediated control over fruit ripening and degree of cross-talk with other stress signalling pathways.