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CHARACTERIZATION OF A *VITIS VINIFERA* GH3 GENE FAMILY INVOLVED IN THE CONTROL OF HORMONE LEVELS

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Grapevines (*Vitis vinifera* L.) produce non-climacteric fruit that exhibit a double sigmoidal pattern of growth. Ripening occurs during the second growth phase when grapes change colour, start to soften, accumulate reducing sugars, metabolise organic acids and synthesise flavour compounds. All these biochemical and physiological changes affect the quality of the fruit and therefore of the wine. Although the physiological processes underlying the ripening have been described the mechanisms that control the ripening of grape berries are not well known. Abscisic acid, ethylene and brassinosteroids are considered as promoters of ripening, as treatments of immature berries with these hormones can advance ripening. In grape, auxin levels are high early in development, then decline towards the onset of ripening (veraison). Indole-3-acetic acid (IAA) is the most abundant auxin in grape berries. Auxins can delay ripening when applied at an appropriate time prior to veraison. One important mechanism for controlling the levels of free, biologically active IAA is its enzymatic conjugation to amino acids. GH3 enzymes, encoding IAA-amido synthetases, are responsible for this conjugation. Previous phylogenetic analyses of *Arabidopsis thaliana* GH3 proteins classified them into three groups based on sequence similarity. Group II enzymes have been shown to be active on IAA and a member of group I conjugates jasmonic acid to amino acids.

In order to elucidate the involvement of GH3 genes in grape berry ripening, we studied seven GH3 genes, six of which are IAA-amido synthetases, the other is a jasmonic acid-amido synthetase. The primary objective was to determine the subcellular localization of these enzymes. GFP-protein fusion constructs for all seven enzymes were transiently expressed in capsicum by biolistic bombardment and the transformed cells were scanned by fluorescence microscopy. All of these proteins displayed a cytosolic localization, confirming the *in silico* prediction. In order to further understand the likely function of these genes their expression patterns were analysed in different tissues comparing the varieties Shiraz and Cabernet Sauvignon. All of the IAA-amido synthetase genes showed different patterns of expression suggesting that although they all conjugate IAA to amino acids there is a degree of specialisation at the organ level.