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# ACCEPTED MANUSCRIPT

## Identification and Phylogenetic Analysis of the POLYGALACTURONASE

### Gene Family in Apple

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#### Abstract

In this study, a total of 85 apple polygalacturonase genes were characterized and clustered into seven groups based on the *Malus*  $\times$  *domestica* whole-genome sequence. These genes coded for proteins containing 176–1 125 amino acids with isoelectric points ranging from 4.68–9.58. The predicted *MdPG* genes were distributed on all chromosomes except the 14th. We then systematically analyzed conserved MdPG protein motifs and the structures of *MdPG* genes. We identified MdPG proteins containing four conserved motifs that are widely found in different PG proteins. Additionally, we found that *MdPG75* was the largest gene, encompassing 18 exons. Finally, we systematically analyzed the functional connection network of MdPG proteins and predicted the functions of related *MdPG* genes before undertaking a preliminary validation. Overall, we have described the genome-wide identification and analysis of the apple PG gene family.

Keywords: apple; polygalacturonase; gene family analysis; bioinformatic

#### 1. Introduction

Fruit ripening and softening is caused by a series of physical and chemical reactions regulated by various genes (Vrebalov et al., 2002; Manning et al., 2006; Prasanna et al., 2007) and the actions of enzymes on plant cell walls, particularly hydrolases, that cause the loosening and decomposition of plant cell walls through depolymerization and dissolution (Redgwell et al., 1992; Villarreal et al., 2009). As polygalacturonase (PG) catalyzes the fractures of  $\alpha$ - (1 $\rightarrow$ 4) glycosidic bonds in different sites, PG may help accelerate cell wall decomposition. According to their different actions, the PG family can be divided into endo-PG, exo-PG, and rhamno-PG (Markovič and Janeček, 2001).

The identification and functional analysis of PG genes has shown that in the late stages of maturity of many climacteric fruits, PG genes mediate fruit softening by degrading pectin (Hadfield et al., 1998; Rose et al., 1998; Callahan et al., 2004; Hiwasa et al., 2004; Asif and Nath, 2005). In plants, PG genes are also involved in a range of processes including responses to abiotic stresses, anther dehiscence, pollen development, and organ shattering (Hadfield et al., 1998; Hadfield and Bennett, 1998; Mohnen, 2008; Ogawa et al., 2009). Homologs of *Arabidopsis thaliana* PG have been identified in a number of plant species including *Malus* × *domestica, Zea mays, Oenothera biennis, Brassica campestris, Nicotiana tabacum, Medicago sativa,* and *Musa nana* (Brown and Crouch, 1990; Niogret et al., 1991; Allen and Lonsdale, 1992; Rober et al., 1993; Tebbutt et al., 1994; Qiu and Erickson, 1996; Asif and Nath, 2005).

A previous study has shown that PG protein levels in M. × domestica tended to peak and then gradually decline as fruit

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