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# Stimulated auxin levels enhance plum fruit ripening, but limit shelf-life characteristics

Islam El-Sharkawy[a,b], Sherif Sherif[b,c], Tawfiq Qubbaj[a], Alan J Sullivan[c], Subramanian Jayasankar[a,*]

[a] University of Guelph, Department of Plant Agriculture, 4890 Victoria Av. N., P.O. Box 7000, Vineland Station, ON L0R 2E0, Canada
[b] Faculty of Agriculture, Damanhour University, Damanhour, Egypt
[c] University of Guelph, Department of Plant Agriculture, Guelph, ON N1G2W1, Canada

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ABSTRACT

Ripening is a highly coordinated, irreversible event involving a series of physiological and biochemical changes, leading to the development of a soft fruit. One of the limiting factors that influence the economic value of fruit is the relatively short ripening period and limited shelf-life. In climacteric fruit, ethylene is a key regulator of ripening; however, recent research has shown that auxin also plays an important role during the event. To understand the contributions of ethylene, auxin and their interaction in ripening, two plum cultivars with widely varying fruit ripening behaviors were compared. The early cultivar, EG, exhibited a brief ripening process in association with rapid decline in firmness. The late cultivar, V9, displayed slow ripening behavior accompanying by notable extension in fruit firmness, resulting in prolonged shelf-life along with preserved fruit quality traits. Auxin has been suggested to play an indirect role in promoting fruit ripening via stimulating the transcription of several ethylene components, resulting in ethylene-induced fruit ripening and softening. This study shows further that there is a direct involvement of auxin in advancing ripening events independent of ethylene action through stimulating the transcription of several genes that encode cell-wall metabolism-related proteins critical for determining the fruit softening rate and potential shelf-life. These results support the hypothesis that the autonomous role played by auxin is as important as that of ethylene in determining not only fruit ripening behavior, but also in mediating other fruit quality traits including shelf-life.


## 1. Introduction

Fruit ripening is a genetically programmed event that is characterized by a number of biochemical, physiological and structural alterations. These include production of aromatic compounds, nutrients, pigmentation, and softening of flesh to an edible texture (Singh and Khan, 2010). These processes have direct impacts not only in general fruit quality traits, but also in shelf-life, consumer acceptability, and postharvest disease incidence (Giovannoni, 2004). The fundamental importance of these processes has prompted considerable research into how they are governed. Research into fruit developmental processes has been greatly aided by analyzing the outcomes of both naturally occurring and induced genetic diversity (Kelly and Bradford, 1986; Lau et al., 2008). One outcome of the research has been the identification of phytohormones as master regulators of the many processes involved (McAtee et al., 2013; Kumar et al., 2014). While the role of ethylene in triggering and regulating the ripening of climacteric fruit has been clearly demonstrated, little is known about the contribution of other hormones (Giovannoni, 2004; Bouzayen et al., 2010). The characterization of mutant plants in some species like tomato, whose fruit are unable to ripen even when treated with ethylene has helped to identify the developmental factors that act upstream of ethylene and control the ripening process. Given its almost ubiquitous importance, it was not unexpected that auxin would be shown to play a prominent role (Friml, 2003). Several studies showed the involvement of auxin in mediating the fruit ripening process and other fruit quality traits in many crop species (Vendrell, 1985; Cohen, 1996; Sagar et al., 2013). Collectively, data from hormone application and quantification have shown that auxin is an important component in the regulation of the onset and coordination of ripening

*Abbreviations:* DAB, Days after bloom; EG, Early Golden; V9, V98041 plum genotype.

* Corresponding author.
*E-mail address:* jsubrama@uoguelph.ca (S. Jayasankar).

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processes (Miller et al., 1987; Gillaspy et al., 1993; El-Sharkawy et al., 2008, 2009, 2010, 2014). For instance, Trainotti et al. (2007) highlighted the contribution of ethylene, auxin, and more importantly their interaction in maintaining peach fruit ripening. Further, recent studies have shown the impact of auxin in accelerating the onset of ripening-associated ethylene production in plum and peach, acting at least partially by triggering the expression of several ethylene synthesis and response components (El-Sharkawy et al., 2008, 2009, 2010; Tatsuki et al., 2013).

Several fruit parameters such as firmness, size, weight, TSS, and acidity are used to specify the progression of ripening. However, the significant postharvest losses of fresh fruits due to excessive softening have justified considerable research into the mechanism of fruit softening (Brummell and Harpster, 2001; Li et al., 2010). In plums, a typical climacteric fruit, cultivars considerably vary in their ripening behavior. Some cultivars show a brief ripening pattern associated with rapid decline in firmness, limiting their storage and shelf-life; however, others exhibit notable extension in both processes resulting in relatively firm fruit with prolonged shelf-life. Fruit softening during the ripening process results in part from numerous modifications of the cell-wall architecture, leading to a reduction in intercellular adhesion, depolymerization and solubilization of pectins, depolymerization of hemicelluloses, and loss of pectic galactose side chains (Brummell and Harpster, 2001; Rose et al., 2003; Fry, 2004). These modifications in cell-walls involve the coordinated and interdependent action of many of cell-wall modifying enzymes and proteins (Rose et al., 2003). Thus, investigating the developmental process and signal mechanisms involved in cell-wall protein regulation and fruit softening remain an important area of research (Li et al., 2010). Generally, the decline in fruit firmness due to softening in many fleshy fruits, including plum, is accompanied by elevated expression of numerous cell metabolism enzymes, including polygalacturonase, endo-1,4-β-mannanase, pectin methylesterase, pectate lyase, endo-1,4-β-glucanase, and β-galactosidase (Pressey and Avants, 1973; Carpita and Gibeaut, 1993; Smith and Gross, 2000; Brummell et al., 2004; Hayama et al., 2006; Khan and Singh, 2007) in which all these enzymes have the capacity to reduce the apparent molecular size of pectic polymers by cleaving the backbone or side chain residues (Ranwala et al., 1992; De Veau et al., 1993; Hadfield et al., 1998). Based on enzyme activity and genetic studies, other classes of proteins have been suggested to participate in ripening-related cell-wall disassembly. For instance, it has shown that down-regulation of genes encoding the *N*-glycan processing enzymes α-mannosidase and β-D-*N*-acetylhexosaminidase significantly increased fruit shelf-life (Meli et al., 2010). These enzymes act through breaking the glycosidic bonds between carbohydrates, or between carbohydrates and non-carbohydrate structural molecules (Brummell and Harpster, 2001). Further, phospholipase D-α is a key enzyme involved in membrane deterioration that occurs during fruit ripening and senescence. This enzyme catalyzes the hydrolysis of membrane phospholipids, which maintain cell viability and homeostasis into phosphatidic acid (Dawidowicz, 1987; Exton, 1997). Finally, expansins are cell wall-localized proteins facilitating cell-wall loosening. They are involved in many aspects of cell-wall modification during development through disruption of non-covalent bonds between matrix glycans and cellulose microfibrils (Rose et al., 1997; Rose and Bennett, 1999; Brummell and Harpster, 2001).

In climacteric fruits, although ethylene is the key regulator of the ripening process, not all of the ripening-related events are dependent on ethylene action as some other processes are controlled by other hormonal or developmental factors. For instance, the suppression of ethylene in many fruit species had impact on delay and reduce fruit softening, but was never be able to prevent the occurrence, indicating that the fruit firmness events are not exclusively regulated by ethylene (Murray et al., 1993; Flores et al., 2001; Nishiyama et al., 2007). Apparently, both ethylene-dependent and -independent pathways coexist to coordinate climacteric fruit ripening process (Lelièvre et al., 1997; Alexander and Grierson, 2002).

Several researchers have investigated the role of ethylene in mediating cell-wall metabolism-related gene expression and enzyme activity. However, little is known about the contribution of other ethylene-independent factors that can play an important role as that of ethylene in synchronizing fruit softening process. In this study, the role of auxin and its joined effect with ethylene in coordinating fruit ripening of two plum cultivars that vary in their ripening and shelf-life characteristics was studied. El-Sharkawy et al. (2014) previously showed that the diversity in fruit ripening behaviors between the early- (EG) and late-ripening (V9) cultivars (i.e., the same cultivars studied in the present work) was associated with the levels of auxin and ethylene that occurred during fruit maturation and ripening. In this study, the importance of auxin in accelerating ripening through stimulating autocatalytic ethylene production by activation of the transcription of several ethylene signaling components is assessed along with the potential autonomous role of auxin in advancing ripening events by triggering the transcription of several cell-wall disassembly-related genes that contribute to fruit softening independently of ethylene action.

## 2. Materials and methods

### 2.1. Plant materials and postharvest treatments

Japanese plum (*Prunus salicina* L.) cultivars Early Golden (EG) and V98041 (V9) were harvested at commercial maturity in 2011 and 2012 from the experimental farm at the Vineland Research and Innovation Center (Vineland Station, ON, Canada). These two cultivars were chosen due to the diversity of their fruit ripening behavior (early and late, respectively) and shelf-life characteristics (short and extended, respectively). Uniform sized fruit from both cultivars were collected from early maturation until a post-climacteric stage of fruit ripening; 50–82 days after bloom (DAB) for EG and 50-128 DAB for V9. Tissue from nine fruit exhibiting similar ethylene production and firmness at every stage were collected and frozen for further analysis.

To evaluate the effect of hormones in fruit ripening, mature fruit of EG (76 DAB) and V9 (108 DAB) were harvested before autocatalytic ethylene production had risen, surface sterilized, and subjected to various treatments, including: 1-naphthalene acetic acid (NAA), propylene ($C_3H_6$), the ethylene-inhibitor 1-methylcyclopropene (1-MCP), and 1-MCP followed by dipping in NAA as described previously (El-Sharkawy et al., 2014). Water-dipped fruit were used as controls. After assessing ethylene production and flesh firmness at different intervals post-treatment, climacteric V9 fruit from each treatment were collected (9 fruits/replicated treatment, three independent biological replicates). Fruit were sampled ~6 d and ~15 d after treatment for propylene and NAA treatment, respectively. In case of treatments with no alteration in ethylene emission as MCP and MCP/NAA treated fruit, samples were collected ~24 d after treatment. All samples were frozen and stored for further analysis.

### 2.2. Fruit shelf-life assessment and other quality traits determination

To determine shelf-life characteristics, fruit of similar size from both cultivars were harvested at commercial maturity (76 and 108 DAB for EG and V9, respectively), surface sterilized, stored at 4 °C for 30 d, and then were transferred to 20 °C. Physical and chemical properties of fruit, such as flesh firmness, weight loss, total soluble