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Involvement of polyamines in creasing of sweet orange [*Citrus sinensis* (L.) Osbeck] fruit

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ABSTRACT

Creasing is a physiological disorder in the rind of sweet orange [*Citrus sinensis* (L.) Osbeck] fruit and causes serious economic losses in the world. The involvement of polyamines in creasing and rind thickness of sweet orange fruit was investigated employing exogenous applications of putrescine (PUT) and a reversible inhibitor of polyamine biosynthesis (guanylhydrazone; MGBG) at different fruit developmental stages through regulating endogenous levels of free PUT, spermidine (SPD), spermine (SPM) and total polyamines in albedo and flavedo tissues of the fruit. A spray application of PUT depending upon its concentration and time of application reduced creasing index percent (CI) and increased rind thickness in Washington Navel and Lane Late fruit during 2011 and 2012. Single spray of PUT (500–1000 μM) applied at fruit set or golf ball stage was more effective in reducing CI as compared to all other treatments, in both the cultivars. PUT applied at fruit set, golf ball or mature fruit stage resulted in increased levels of endogenous free polyamines (PUT, SPD, SPM and total free polyamines) in the flavedo and albedo tissues of fruit in both cultivars. Single spray application of MGBG (1000 μM) at the golf ball stage significantly increased CI in Washington Navel and Lane Late orange fruit. In conclusion, these results suggest the involvement of polyamines in creasing of sweet orange fruit.

1. Introduction

Creasing (albedo breakdown) is a peel-related disorder in sweet orange fruit. The symptoms of this disorder consist of small random cracks in the albedo tissue corresponding to sunken grooves on the fruit surface (Erickson, 1968). The fracturing of the albedo tissue is predominantly due to separation of adjacent cells rather than cleavage of individual cells (Storey and Treeby, 1994). Although, creasing is usually detectable at fruit maturity, its initiation seems to be associated with earlier stages of fruit growth and development (Storey and Treeby, 1994). Creasing affects different cultivars of sweet orange including Washington Navel (Gambetta et al., 2000; Ali et al., 2000), Valencia (Jones and Embleton, 1967; Monselise et al., 1976) and Nova mandarins (Greenberg et al., 2006). Creasing was first reported from South Africa during 1938 (Le Roux and Crous, 1938) and is currently a major problem in the sweet orange industry in different parts of the world such as Australia (Storey and Treeby, 1994), USA (Ali et al., 2000), Israel (Monselise et al., 1976; Greenberg et al., 2006), Uruguay (Gambetta et al., 2000), Spain (Agustí et al., 2001) and China (Li et al., 2009). More than 50% losses in individual orchards have been reported from South Africa (Gilfillan et al., 1981). In the Australian citrus industry, creasing is a major cause of fruit diversion from fresh markets to processing and 50% of the Navel orange crop may be affected to varying degrees due to this disorder (Treeby et al., 2000).

Presently, the exact cause of this physiological disorder is not known. However, several factors have been associated with this disorder, such as genotype (Agustí et al., 2003); climate (Jones and Embleton, 1967; Gambetta et al., 2000); rootstock (Storey et al., 2002); crop load (Jones and Embleton, 1967); rind thickness (Holtzhausen, 1981); irrigation (Agustí et al., 2004) and mineral nutrition (Ali et al., 2000; Bower, 2004). Five repeated sprays of an aqueous solution containing calcium (0.33%) at 10 day intervals commencing at the golf ball stage reduced creasing (25–30%) in sweet orange fruit (Treeby et al., 2000; Pham et al., 2012). Similarly, a spray application of GA_3 (20 mg l^{-1}) applied at 3 to 4 weeks prior to colour break or at colour break significantly reduced the

Abbreviations: CI, creasing index percent; Pas, polyamines; PUT, putrescine; SPD, spermidine; SPM, spermine; CAD, cadaverine; MGBG, guanylhydrazone; μM, micro moles; *exo*-PG, *exo*-polygalacturonase; *endo*-PG, *endo*-polygalacturonase; PE, pectin esterase; EGase, endo-1,4-β-D-glucanase.

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incidence of creasing in Navel oranges (Gilfillan et al., 1981), but resulted in poor rind colour development.

Citrus fruit growth and development is a complex process and coordinated by the changes in endogenous levels of one or more plant hormones, including polyamines (PAs) (El-Otmani et al., 1995). Endogenous polyamines are involved in flower development in sweet orange (Sagee and Lovatt, 1991) and fruit growth of Murcott mandarin (Nathan et al., 1984). PAs are involved in many plant developmental processes, including cell division and morphogenesis (Cona et al., 2006; Kusano et al., 2007); fruit development and ripening (Kakkar and Rai, 1993); as well as fruitlet abscission and senescence (Bais and Ravishankar, 2002; Rastogi and Davies, 1991). Exogenous application of PAs have been reported to reduce fruitlet abscission, (a cell separation process), depending upon type, concentration and time of application of PAs (Malik and Singh, 2003). The spray application of PAs at flowering markedly reduced fruitlet abscission in mandarin (Nathan et al., 1984) and sweet orange (Saleem et al., 2007).

PAs are biologically active compounds of low molecular weight with aliphatic nitrogen groups and are present in all living organisms (Cohen, 1978). The most common PAs are PUT, SPD, SPM and cadaverine (CAD). PAs exist in both free and conjugated forms in plants (Evans and Malmberg, 1989), however free PAs participate more actively in biological processes (Antognoni et al., 1998). Due to their cationic nature, PAs coordinate with anionic components of cell membranes, such as the phospholipids (Roberts et al., 1984). PAs have a physiological role in cell walls by interacting directly with pectins and cell wall components (D'Orazi and Bangi, 1987). PAs are essential for maintaining cell wall characteristics by strengthening the links between cell wall components (Berta et al., 1997). Creasing in sweet orange fruit is known to be associated with enhanced loss of pectins in the cell walls of the albedo tissue, leading to cell wall loosening, formation of cracks and consequently reduced hardness, stiffness and tensile force of the rind (Saleem et al., 2014; Monselise et al., 1976). However, no research work has been reported on the involvement of PAs in creasing of sweet orange fruit. Moreover, cell separation is associated with creasing, hence we hypothesised that PAs play a role in modulating creasing in sweet orange fruit. We investigated the involvement of PAs in creasing of sweet orange fruit by employing the exogenous application of different concentrations of PUT applied at fruit set, the golf ball or at the colour break stage and their effects on the endogenous levels of free PAs such as PUT, SPD, SPM in the albedo and flavedo tissues of rind at various fruit developmental stages, CI and rind thickness of the fruit. The effects of the reversible inhibitor of PAs biosynthesis (MGBG) in regulating the incidence of creasing in sweet oranges at the golf ball stage were also determined.

2. Materials and methods

2.1. Plant materials

Four experiments were conducted during 2010–2011 to 2011–2012 in a commercial orchard located at Gingin (latitude 31°21′South, longitude 155°55′East), Western Australia. Twenty-five year old uniform sweet orange scions grafted on trifoliate orange (*Poncirus trifoliate* Raf.) rootstock were used in the experiment. The trees were spaced 7.5 m between rows and 2.7 m within rows in the North-South orientation. These experiments were conducted on cv. 'Washington Navel,' and 'Lane Late' sweet oranges. All the experimental trees received similar cultural practices including fertilisers, irrigation, weed control and plant protection, except for the experimental treatments. The experimental site has a sandy loam soil dominated by cool wet winters and hot dry summers.

2.2. Effects of exogenous application of putrescine on endogenous levels of free polyamines in the albedo and flavedo tissues of sweet orange and incidence of creasing

An aqueous solution containing (100, 250, 500 or 1000 μM) of PUT and 0.05% (v/v) 'Tween 20' as a surfactant were sprayed on to whole 'Washington Navel' orange trees until run off at fruit set (fruit diameter: 15 ± 5 mm), golf ball (fruit diameter 40 ± 5 mm) or colour break (fruit diameter: 80 ± 5 mm) stage. Control trees were kept unsprayed. The experimental lay out was randomised block design with two-factor factorial (treatments and stage of application) with a single tree as an experimental unit and four replicates. The experiment was conducted in two consecutive seasons during 2010–2011 and 2011–2012. The data of two years were not pooled because error mean squares over two years were heterogeneous. In both years, 35 fruit per tree at commercial maturity were randomly harvested around the tree canopy. The incidence of creasing was examined on individual fruit, based on the appearance of the fruit surface and per cent creasing index (CI) was calculated. Rind thickness at commercial harvest was also measured by using a Vernier's calliper. In 2011–2012, the effects of different concentrations of exogenously applied PUT on the changes in the endogenous levels of free polyamines (PUT, SPD, SPM and total free PAs) in the albedo and flavedo tissues were also monitored from ten fruit harvested per tree were randomly 50 days after each spray.

The same experiment was repeated on the cv. 'Lane Late' during 2010–2011 and 2011–2012. In this experiment, the creasing index and rind thickness were recorded during both the years but the effects of PUT treatments on endogenous levels of free polyamines in the albedo and flavedo tissues were examined during 2011–2012 only.

2.3. Effects of exogenous application of a reversible inhibitor of biosynthesis of polyamines on the incidence of creasing in sweet orange

Uniform, 25-year old 'Washington Navel' sweet orange trees were sprayed with an aqueous solution containing (100, 250, 500 or 1000 μM) of methylglyoxal-bis (guanylhydrazone) MGBG and 0.05% 'Tween 20' as a surfactant at the golf ball (fruit diameter 40 ± 5 mm) stage on whole trees until run off during 2010–2011. Untreated trees served as the controls. The experiment was laid out by following a randomised block design using single trees as the experimental unit and replicated 4 times. Creasing index was recorded from 35 randomly harvested fruit per tree.

The experiment was repeated on 25-year old trees of cv. 'Lane Late' during 2010–2011. At the ripe stage, 35 fruit per tree were randomly harvested from around the tree canopy. Creasing index was recorded from these harvested fruit.

2.4. Creasing index (%)

Creasing symptoms on the whole fruit at commercial maturity stage were assessed using a subjective scale of 0 = no creasing; 1 = slightly creased (1 to 25% fruit surface with symptoms); 2 = moderately creased (26 to 50% fruit surface with symptoms); 3 = severely creased (>51% fruit surface with symptoms). Creasing index (CI) per cent was calculated by using the following formula

$$\text{Creasing index}\,\% = \frac{\left[\sum (\text{Rating number} \times \text{number of fruit in rating category})\right] \times 100}{\text{Highest rating value} \times \text{Total number of fruit assessed}}$$

2.5. Measurement of rind thickness

Rind thickness was measured from 10 randomly selected fruit (four side of each fruit) using a digital Vernier's calliper. The average fruit rind thickness was calculated and expressed as mm.