Antioxidant enzymes change in relation to superficial scald development in pear fruit during cold storage

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Abstract

Superficial scald is a physiological disorder of apple and pear fruits associated to the pre-/postharvest treatment and genetic traits of each cultivar. In the Po Valley, two pear cultivars (Abbé Fétel and Doyenne du Comice) and two advanced selections (CREA 171, CREA 264), obtained by CREA Centro di ricerca Olivicoltura, Frutticoltura e Agrumicoltura (Forlì, Italy) pear breeding program, picked in August-September, were stored at -1°C and 95% RH until January. In this study, antioxidant enzymatic system of four cultivars/selections was evaluated during storage to investigate differences in scald susceptibility. In pear peel, superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (POD), polyphenol oxidase (PPO), and lipoxygenase (LOX) were evaluated. Significant differences in scald incidence were observed both in pear cultivars and selections. Abbé Fétel and CREA 171 showed lower superficial scald susceptibility compared to Doyenne du Comice and CREA 264, after being cold stored for five months. In fruit peel, SOD and CAT activities were higher in scald-resistant than in scald-susceptible pear cultivars and selections. However, APX activity in peel was very similar in all tested pear fruits. Superficial scald induced an increase in PPO and POD activities involved in fruit-browning during cold storage. Furthermore, an increase in LOX activity due to deoxygenation of polyunsaturated fatty acids producing toxic hydroperoxy fatty acids and consequent membrane damage in fruit peel was observed. This study shows that some pear cultivars and selections have different superficial scald susceptibility enabling them to induce activities of several antioxidant enzymes following cold storage. Regulation of antioxidant enzymes alleviates oxidative damage and in addition to other biochemical features could be involved in determining the susceptibility/resistance to superficial scald development of pear fruit.

Keywords: oxidative stress, physiological disorder, pear cultivars and advanced selections

INTRODUCTION

Superficial scald is a physiological disorder of apple and pear fruits that influence their postharvest management. Scald symptoms are associated with chilling injury and oxidative stress during storage and characterized by the onset of irregular brown or black patches on fruit peel only on the surface layers of hypodermal cortical tissue cells making the fruit unmarketable (Lurie and Watkins, 2012).

Nowadays, the mechanism of superficial scald development is widely ascribed to the oxidation of α-farnesene, but its physiology has not been clearly elucidated (Busatto et al. 2018). Several studies have demonstrated that the oxidation of α-farnesene produces

coniugated trienols (CTols) that are key factors in the occurrence of superficial scald (Whitaker 2007).

Furthermore, ethylene also plays a pivotal role, regulating the accumulation of both α-farnesene and CTols in a pear cultivar-dependent manner (Calvo et al., 2015; Chiriboga et al., 2013; Larrigaudière et al., 2016). Other factors such as reactive oxygen species and antioxidant systems are likely to be involved, at least in part, in scald development (Ahn et al., 2007; Lurie and Watkins, 2012). Antioxidant systems are constituted by metabolites and antioxidant enzymes that prevent the oxidation of α-farnesene from influencing superficial development (Lurie and Watkins, 2012; Rao et al. 1998).

Oxidative phenomenon occurs during fruit postharvest life involving production and removal of ROS, such as H_2O_2, O_2^- and hydroxyl radicals, from the tissues (Pasquariello et al. 2015). The failure or reduction in the capability of the antioxidant system, which includes SOD, APX, POD and CAT enzymes, could cause oxidative damage to the membrane, that is involved in fruit superficial scald development (Li et al., 2016; Kuo et al., 2007; Zhao et al., 2016).

Nowadays, few attempts to relate the antioxidant enzymes activity to scald development in pear were carried out. Zhao and co-workers (2016) found that SOD and APX activities were correlated with scald susceptibility in blushed peel of 'd'Anjou' pear, while CAT activity was not related to the surface area of scald. Analysis of antioxidant enzyme activity in Japanese pear suggested that PPO activities were associated with scald susceptibility (Li et al., 2016; Kuo et al., 2007) while POD activity has no correlation to the incidence of superficial scald (Li et al., 2016). A 1-methylcyclopropene (1-MCP) postharvest treatment in 'Wujiuxiang' pear prevented from scald development and increased the APX, SOD and CAT activity and a lower activity of LOX (Gao et al., 2015). In Yali pear, 1-MCP and modified atmosphere packaging treatment enhanced the catalase and superoxide dismutase activities in the fruit, while reduced activities of lipoxygenase and polyphenol oxidase in the peel preceding the onset of superficial scald (Feng et al., 2018).

Different scald development in pears could also be due to the ability of each cultivar to produce ethylene and/or to regulate ethylene metabolism during cold stress, as demonstrated for apple fruits (Ju and Curry, 2000; Lurie and Watkins, 2012; Gapper et al., 2006). Although for pear fruits several studies have demonstrated that ethylene is involved in α-farnesene metabolism, other factors can contribute to the scald susceptibility, such as low temperature and endogenous antioxidant systems (Larrigaudière et al., 2016; Zhao et al., 2016).

In this study, fruits of two low susceptible cultivars/selections, 'Abbé Fétel' (Murayama et al., 2002; Rizzolo et al., 2010; Vanoli et al., 2010) and 'CREA 171' coming from CREA breeding program and two high susceptible cultivars/selections, 'Doyenne du Comice' and 'CREA 264' coming from CREA breeding program, were evaluated for scald susceptibility during cold storage conditions. The objective of this research was to evaluate the enzymatic antioxidant system in pear cultivars/selections during cold storage and to improve understanding of the inter-relationship of oxidative stress parameters and their association with scald susceptibility.

MATERIAL AND METHODS

Fruit material and storage

In 2016, in an orchard located in Emilia-Romagna region (Campogalliano-MO), fruits of 'CREA 264', 'Abbé Fétel', 'Doyenne du Comice' and 'CREA 171' were picked at flesh firmness of 5.5 kg cm^{-2}, as indicated in table 1 and stored at -1°C and 90% RH. From the first month of cold storage and then every month for five months, the stored fruits of each sample were evaluated for scald susceptibility. Scald index measurement at 1, 3 and 5 months was expressed as percentage of the fruit surface area affected, where no scald = 0, <25% = 1, 25–50% = 2, and >50% = 3 and was normalized to 100 by multiplying values by 33.3 (100:3, Wang and Dilly, 2000). In January, after five months of cold storage, ten fruits of each variety were analyzed for scald susceptibility evaluating the enzymatic antioxidant system.

Table1. Harvest date and fruit qualitative traits at harvest time

Variety/ selection	Fruit firmness, kg cm^{-2}	Harvest date	Fruit average weight, g	Fruit size, mm	Soluble solid content, °brix
Abbé Fétel	**5.5±0.5**	**22-Aug**	**315±21**	**72±2.5**	**16.6±0.6**
FRF 171	**5.5±0.3**	**14-Sep**	**218±15**	**70±2.0**	**16.1±0.4**
FRF 264	**5.5±0.4**	**13-Aug**	**233±16**	**72±1.5**	**13.9±0.7**
Doyenne du Comice	**5.5±0.4**	**26-Aug**	**250±18**	**75±3.0**	**18.2±0.3**

[a] Data are the mean of 10 replicates.
[b] ± Standard deviations

Enzyme extraction and activity assays

Frozen fruit tissue powder (1 g), obtained by pear peel, was extracted using 5 mL of 500 mM potassium phosphate buffer (pH 7.8) containing 10 mM sodium EDTA (pH 7), 40 mM PEG, 2 mM DTT, 5% (w/v) PVPP, and 5 mM ascorbic acid (the ascorbic acid was only used for APX enzyme extraction). After centrifugation at 12000 × g for 30 min at 4 °C, the obtained supernatant was used for catalase, superoxide dismutase, guaiacol peroxidase and ascorbate peroxidase activity determinations. Total protein content was estimated by the Bradford assay (Bradford, 1976) using bovine serum albumin as a standard.

Catalase (CAT, EC 1.11.1.6) activity was assayed according to the method described by Pasquariello et al. (2015) with slight modifications. The reaction medium consisted of 50 mM potassium phosphate buffer (pH 7), 20 mM H_2O_2 and 20 µL of crude enzyme extract in a final 1.5 mL volume. The reaction was started by adding H_2O_2, and the decrease in absorbance at 240 nm, caused by its breakdown, was monitored. The specific activity was expressed as the specific rate of molar change in H_2O_2 on a fresh weight basis as µmol g^{-1}.

Ascorbate peroxidase (APX; EC 1.11.1.11) activity was monitored according to Adiletta et al. (2018) with some changes. The reaction mixture consisted of 100 mM potassium phosphate buffer (pH 7), 0.25 mM ascorbic acid, 0.70 mM H2O2, 0.66 mM sodium EDTA (pH 7) and 20 µL of crude enzyme extract in a final 1.5 mL volume. The oxidation of ascorbic acid was evaluated by the decrease at 290 nm and the APX activity was expressed as the specific rate of molar change in ascorbate on a fresh weight basis as µmol g^{-1}.

Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured by monitoring the inhibition of photochemical reduction at 560 nm of nitroblue tetrazolium (NBT) in the presence of riboflavin, as described by Pasquariello et al. (2015) with minor modifications. The reaction assay consisted of 50 mM potassium phosphate buffer pH 7.8, 0.1 mM sodium EDTA, 13 mM methionine, 75 µM NBT, 2 µM riboflavin and 100 µL of crude enzyme extract in a total 1.5 mL volume. One SOD unit was defined as the amount of enzyme that inhibits

the rate of NBT reduction by 50% under the above assay conditions. The specific activity was expressed on a fresh weight basis as U g^{-1}.

Guaiacol peroxidase (POD; EC 1.11.1.7) activity was assayed, spectrophotometrically by recording the formation of tetraguaiacol and its consequent increase in absorbance at 470 nm, according to Petriccione et al. (2015) with some modifications. The reaction mixture contained 100 mM potassium phosphate buffer pH 7, 0.15 mM sodium-EDTA pH 7.0, 6.6 mM H2O2, 8 mM guaiacol and 250 µL of crude enzyme extract in a final 1 mL volume. The specific enzyme activity was expressed on a fresh weight basis as µmol tetraguaiacol g^{-1}.

Polyphenol oxidase (PPO) was extracted and its activity determined following the methods described by Petriccione et al. (2015) with some modifications. Crude enzyme extract (10 µL) was incubated with a buffered substrate (500 mM catechol in 100 mM sodium phosphate buffer pH 6.4) in a final 1.5 mL volume and monitored by measuring the increase in absorbance at 398 nm. The specific activity on a fresh weight basis for molar change in catechol was expressed in µmol g^{-1}.

Lipoxygenase (LOX) was extracted re-suspending 1 g of frozen fruit tissue powder with 3 mL of extraction buffer (50 mM potassium phosphate buffer pH 7.8, 1 mM sodium-EDTA pH 7, 2% PVPP). LOX activity was quantified following the method described by Pasquariello et al. (2015). The lipoxygenase activity was detected spectrophotometrically by recording the formation of hydroperoxides and the resulting 234 nm increase in absorbance. LOX activity was expressed as the specific rate on a fresh weight basis of molar change of hydroperoxides in µmol g^{-1}.

Statistical analysis

All data represent the mean of three biological and three technical replicates ± standard deviation (SD). In order to determine the difference between different pear cultivars/selections, one-way ANOVA and the Tukey test for mean comparisons were used. Differences at $P<0.05$ were considered significant and are indicated with different letters. All analyses were performed using the SPSS software package, version 20.0 (SPSS Inc., Chicago, IL, USA).

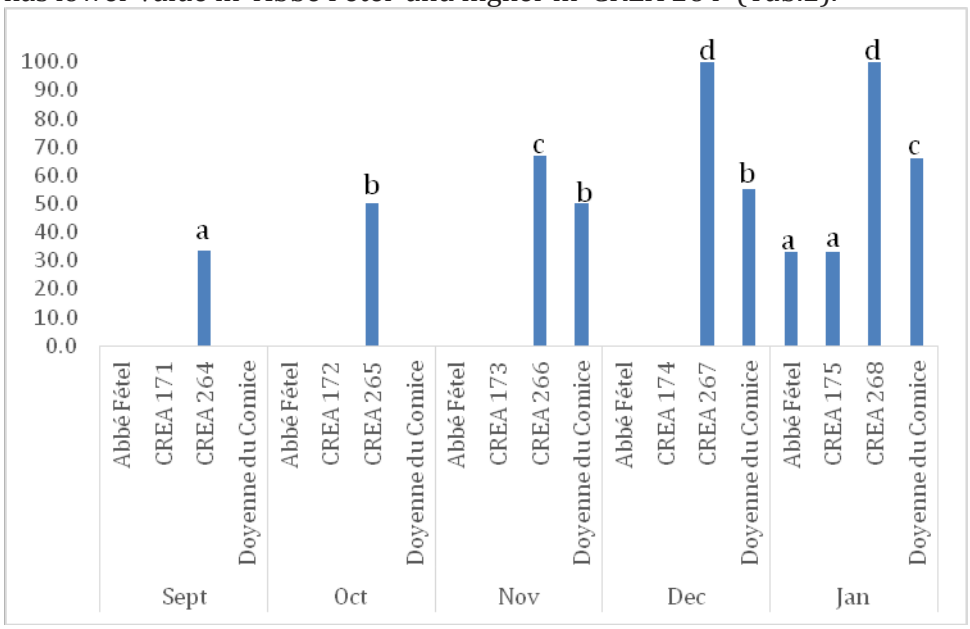
RESULTS AND DISCUSSION

In this study, the incidence of superficial scald was significantly different among different pear cultivars and selections during cold storage (CS). 'Abbé Fétel' and 'CREA 171' showed lower superficial scald susceptibility compared to 'Doyenne du Comice' and 'CREA 264'. 'Abbé Fétel' and 'CREA 171' did not show sintoms from September to December. First symtoms appeared in January, although significantly lower than 'Doyenne du Comice' and 'CREA 264'. In 'CREA 264' fruit, the first symptoms of superficial scald appeared after one month of CS, reaching the maximum value after four months and in 'Doyenne du Comice' after three months reaching higher values after five months (fig. 1). These results confirm that scald susceptibility in pears is cultivar-dependent. Larrigaudière and co-workers (2016) demonstrated that 'Packham Triumph' fruits were less susceptible than 'Beurré d'Anjou' ones and that the standard parameters used to evaluate fruit ripeness at harvest cannot be used to predict the differences in scald susceptibility between two pear cultivars, as previously described (Calvo et al. 2015).

SOD is the first Reactive Oxygen Species (ROS) scavenging enzyme involved in the dismutation of toxic oxygen (radical superoxide) to H_2O_2 and molecular oxygen (O_2) (Hodges et al., 2004). SOD activity in 'Doyenne du Comice' peel registered lower value compared to 'CREA 264', 'Abbé Fétel' and 'CREA 171', indicating a ROS reduced detoxification ability (Tab. 2). Hydrogen peroxide is detoxified by two different classes of H_2O_2-scavenging enzymes such as CAT and APX that showed different affinities to the substrate (Hodges et al., 2004). APX is involved in the detoxification of hydrogen peroxide, using ascorbate as an electron donor to reduce H_2O_2 to H_2O. CAT and APX activity showed significant lower values in 'Abbé Fétel' fruits compared to 'CREA 264' ones (Tab.2).

Reactive oxygen species are involved in lipid peroxidation that is closely related to the scald development (Rao et al. 1998). LOX catalyzes the dioxygenation of polyunsaturated fatty acids producing toxic hydroperoxy fatty acids and consequent membrane damage. In superficial scald, the onset of irregular brown or black patches on fruit peel is also due to oxidation of phenolic substrates mediated by PPO (Fernández-Trujillo et al. 2003). Furthermore, guaiacol peroxidase (POD), which catalyses single-electron oxidation of diverse antioxidant compounds in the presence of hydrogen peroxide, is also a candidate enzyme to scald susceptibility (Fernández-Trujillo et al. 2003).

POD activity, in fruit peel, showed higher values in scald-susceptible Decana and CREA 264 pear. Our results are in line with previous studies carried out in apple that proved Peroxidase activity to be related to scald susceptibility (Fernandez-Trujillo et al. 2003).
PPO and LOX are involved in scald development as suggested by several studies in which the diphenylamine (DPA) is considered as a treatment controlling the scald in Empire apple fruit peels and in Dangshansuli pear fruits and having an effect both on metabolization of α-farnesene and conjugated trienes, and on the inhibition of the LOX and PPO activities (Hui et al. 2010; Whitaker 2000). The activity of PPO was significantly different in pear samples with lower value in'Abbé Fétel' peel and higher in 'Doyenne du Comice' while LOX activity has lower value in 'Abbé Fétel' and higher in 'CREA 264' (Tab.2).



Mean separation using Tukey's HSD post-hoc test at P≤0.05.

Figure 1. Superficial scald of Abbè Fétel, CREA 171, CREA 264 and Doyenne du Comice stored at -1°C and 95% R.H. for one, three and five months

Table2. Changes in superoxide dismutase (SOD; U g^{-1}.), catalase (CAT; µmol g^{-1}), ascorbate peroxidase (APX; µmol g^{-1}), guaiacol peroxidase (POD; µmol tetraguaiacol g^{-1}), polyphenol oxidase (PPO; µmol g^{-1}), lipoxygenase (LOX µmol g^{-1}) activity of peel pear cultivars/selections after five months of cold storage.

Pear cultivars/ selections	Peel					
	SOD	CAT	APX	POD	PPO	LOX
Abbé Fétel'	4.07 b	8.09 a	1.30 a	0.28 a	1.40 a	0.22 a
CREA 171	4.64 c	9.39 ab	2.77 b	0.43 b	2.44 ab	0.25 ab
Doyenne du Comice	3.31 a	9.16 ab	2.16 ab	0.64 c	8.84 c	0.31 ab
CREA 264	3.96 b	10.97 b	2.24 b	0.89 d	3.55 b	0.39 b

Mean separation using using Tukey's HSD post-hoc test at P≤0.05.

CONCLUSIONS

This study suggests that enzymatic antioxidant system would be a key factor in occurrence of superficial scald. These results demonstrate that some pear cultivars and selections have different superficial scald susceptibility enabling them to induce activities of several antioxidant enzymes following cold storage. Regulation of antioxidant enzymes alleviates oxidative damage and, in addition to other biochemical features, could be involved in determining the susceptibility/resistance to superficial scald development of pear fruit. Further studies, on the antioxidant enzymatic mechanisms in peel and flesh of scald-resistant/susceptible pear cultivar/selection, are necessary to predict the occurrence of this physiological disorder and to develop new strategies to control scald in pear fruit postharvest.

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