CHANGES IN ANTIOXIDANT COMPOUNDS IN FLOWER BUDS OF TWO APRICOT CULTIVARS DURING WINTER SEASON.

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Abstract

The antioxidant metabolism of higher plants undergoes alterations according to a seasonal cycle. Studies have demonstrated that free radicals are formed in dormant buds, where their removal seems to be associated with bud break. Protection against free oxygen radicals is achieved through the action of antioxidant compounds and enzymes. In flower buds of two apricot cultivars with different chilling requirement, changes in antioxidant enzymes (catalase, peroxidase, ascorbate peroxidase), sulfhydryl compounds and glutathione content during the winter season were determined. In both cultivars, the activities of oxygen-scavenging enzymes and glutathione underwent change during the winter season. During deep dormancy such activities were low, subsequently increasing but reaching different levels between cultivars. Increases were particularly high in the cultivar with low chilling requirement, and occurred in concomitance with the end of endo-dormancy. Lowest antioxidant activity was detected in the cultivar with high chilling requirement. These results suggest that minimum thresholds in antioxidant activities could be crucial for scavenging free radicals generated during the winter season.

1. Introduction

Free radicals can be generated enzymatically but also from non-enzymatic reactions of oxygen with organic compounds. These radicals initiate self-propagating reactions leading to peroxidation of membrane lipids and destruction of proteins (Lin and Kao, 1998). The plant antioxidant defense system provides protection against high levels of free oxygen radicals induced by unfavorable environmental conditions such as low temperature, drought and high light intensities (Taulavuori *et al*., 1998). Removal of oxyradicals and their intermediates is controlled both by enzymatic and non-enzymatic mechanisms.

The antioxidant system undergoes seasonal fluctuation in perennial species and the cold hardening process may alter antioxidant metabolism. Studies have shown that free radicals are formed in dormant buds of some fruit species, where their removal seems to be associated with bud break as a result of changes in antioxidant systems (Wang *et al*., 1991; Siller-Cepeda *et al*., 1992). Catalase and peroxidase are antioxidant enzymes that participate in this process. Moreover, it has been suggested that glutathione is an important metabolite in stabilizing the cell redox state during the cold hardening process (De Kok and Stulen, 1993). In particular, in stressed plants reduced glutathione (GSH) protects protein thiol groups from auto-oxidation (Kranner and Grill, 1996). GSH is the main reserve and long-distance transport form of reduced sulfhydryls, which are indispensable for protein synthesis (Rennenberg, 1982), and participate in the detoxification system (Taulavuori *et al*., 1998). The major portion of the glutathione in the cell is maintained in the reduced state, and a high reduced/oxidized ratio (GSH/GSSG) is necessary for numerous physiological functions (Wang *et al*., 1991). In apple flower buds, the end of endo-dormancy was found to be associated with increased ascorbic acid, GSH and correlated enzymes (Wang and Faust, 1994). High GSH level during resumption of growth in peach

buds was associated with increased capacity for free-radical scavenging (Siller-Cepeda *et al.*, 1992). Furthermore, in apricot flower buds enhanced GSH levels and catalase activity were observed just before the resumption of growth (Viti and Bartolini, 1998).

The aim of this research was to study the changes in antioxidant enzymes and sulfhydryl compounds and their relationship with dormancy in flower buds of two apricot cultivars.

2. Material and Methods

Trials were carried out during winter 1999-2000 on mature apricot trees growing in the climatic conditions of the Tuscan coastal area. We used two cultivars characterized by different chilling requirements: 'Canino' with low–medium chilling requirement and 'Polonais' with high chilling requirement.

Flower buds were collected from uniform sized twigs (50-60 cm) for the following observations:

(a) *flower bud growth* – determined from December to February, at 15-7 day intervals. Samples of 25 flower buds were fresh and dry weighted before and after forcing. Forcing was conducted on twigs maintained in water in a climatized chamber for one week in the following environmental conditions: 23°C, 60% relative humidity, photoperiod 12 hours at 300-400 $\mu E\ m^{-2}\ s^{-1}$;

(b) *biochemical assays* - determined in three different periods from deep dormancy to resumption of growth. The following analyses were carried out:

- *enzymatic activities:* bud samples of 0.5g fresh weight were collected and stored at -80° C until enzyme analysis. Buds were ground by pestle in a mortar; the frozen powder was then added to Tris buffer pH 8.0 (1:1 w/v) and the supernatant was used to determine: catalase (CAT), guaiacol dependent peroxidase (GPX) and ascorbate peroxidase (APX) according to the Aebi (1984), Maethy and Chance (1954), Nakano and Asada (1981) methods, respectively. Total protein was determined according to Bradford (1976) using bovine serum albumin as a standard;
- *total water extractable sulfhydryl compounds (protein SH + non-protein SH)*: bud samples of 1.0 g fresh weight were ice-cold homogenized with 0.85 mM ascorbic acid. The supernatant was used for total sulfhydryl compounds (SH), adding 5,5'-dithio-bis- (2-nitrobenzoic acid) (DTNB) according to Grill *et al* (1979), and for glutathione determination;
- *reduced (GSH) and oxidized (GSSG) glutathione content*: detected in deproteinized supernatant according to De Kok *et al.* (1981). Absorbance at 412 nm was measured by spectrophotometer to calculate SH, GSH and GSSG content. Each assay was the result of three replications.

In field conditions, on tagged twigs the blooming and the flower bud drop percentages were recorded. From November (at 50% of leaf drop), minimum and maximum daily temperatures were used to calculate Chilling Units (C.U), on the basis of an Asymkur program (Pitacco *et al.*, 1992).

3. Results

3.1. Flower bud growth

The fresh weight trend showed that in cv. Canino a significant increase occurred after forcing in January, when 1040 C.U. had been accumulated (Fig. 1a). By this time, it can be assumed that endo-dormancy had been broken since, in accordance with Guerriero *et al.* (2000), a roughly 30% weight increase was observed after forcing and this increase was confirmed in subsequent determinations. 'Polonais', on the other hand, never responded to the forcing test, showing a later weight increase only after 1200 C.U. (Fig. 1b). This cultivar, at blooming time, presented only 5% of flowering with an elevated percentage (57%) of unopened buds , while in 'Canino' blooming reached 33% with roughly 11% of unopened buds (Fig. 1 a, b).

3.2. Enzymatic activity

In general, total soluble protein content underwent little variation from December to February, ranging from 0.10 to 0.12 in 'Canino' and from 0.12 to 0.15 in 'Polonais' (Fig. 2a). A slight decrease was detected at the end of endo-dormancy, which was estimated on January 18, for 'Canino' only. Dynamic enzymatic activities (Fig. 2b, c, d) revealed that 'Canino' presented a sharp increase in CAT and GPX activities (3- to 4-fold higher) in the middle of January. After this period, CAT and GPX tended to decrease while APX activity increased further and subsequently peaked in February (4-fold higher). A different trend was observed in cv. Polonais, which initially presented slightly higher CAT and GPX activity, followed by a gradual increase in enzymatic activities that reached 2-fold higher values. Maximum CAT activity was detected in February, later than the other two enzymes.

3.3 Sulfhydryl compounds and glutathione content

Concentrations in total water extractable sulfhydryl compounds (SH) showed no substantial change during the observation period. In both cultivars, a similar content and trend was observed with a slight decrease in January, returning to initial levels in February (Fig. 3).

Total glutathione concentration ranged from 144 to 179 nmol g^{-1} FW in 'Canino' flower buds, and from 140 to 160 nmol g^{-1} FW in 'Polonais'. A considerable enhancement of reduced glutathione percentage (GSH) was detected in 'Canino' flower buds in January (Fig 4). A concomitant decrease in GSSG percentage occurred, altering the GSH/GSSG ratio (Fig. 5). Thus while the ratio was similar in both cultivars in December, it underwent a sudden increase in January, remaining elevated in February as well. In cv. Polonais, the GSH and GSSG proportion remained unchanged, although a small increase in GSH/GSSG ratio was detected in January (Fig.4 and 5).

4. Discussion

The forcing method allowed identification of the end of endo-dormancy only in 'Canino', a cultivar with low-medium chilling requirement. In the case of cv. Polonais, which has a high chilling requirement, no definition of the end of dormancy could be obtained by the forcing method since no weight increase was observed in comparison to out conditions.

There was no significant change in the protein content in both cultivars, during dormancy period. This behaviour would appear to confirm that the protein level of woody perennials increases dramatically during the initial 300 Chill Units, declining slightly when the chilling requirement has been met and at the resumption of growth (Rowland and Arora, 1997). In our work, this significant increase probably occurred prior to the first observation.

Results of the biochemical analyses showed that the activity of oxygen-scavenging enzymes underwent changes in both cultivars during the winter season. During deep dormancy (December) activity was low, subsequently increasing once around 1000 C.U. had been accumulated, but with different levels between cultivars. Increases were more pronounced in 'Canino' flower buds compared to 'Polonais', though the latter cultivar initially presented a little bit higher enzymatic activity.

The changes in enzyme level could be related to membrane changes, since membranes became highly permeable prior to budbreak, for solute transport (Faust *et al.*, 1997). Moreover, it has been suggested that the desaturation process requires a high level of reducing power and concomitant activity to detoxify H_2O_2 (Norman *et al.*, 1991). The process that provides protection against active oxygen damage involves several antioxidant enzymes such as CAT, GPX and APX. These enzymes catalyze H_2O_2 breakdown into H_2O and O_2 but they are

under different regulatory mechanisms and/or they are compartmentalized in different organelles (Abassi *et al*., 1998).

Investigation of non-enzymatic scavenging mechanisms showed that in both cultivars GSSG was depleted and GSH increased, altering the GSH/GSSG ratio. This ratio was particularly high in the low-medium chilling requirement cultivar and occurred in concomitance with the end of endo-dormancy, in agreement with results obtained in other species (Siller-Sepeda *et al*., 1992; Wang and Faust, 1994). A high GSH/GSSG ratio could promote protein synthesis by controlling monosome formation, activating and deactivating redox-dependent enzyme systems, and allowing the removal of free radicals that are associated with budbreak (Rennemberg, 1982; Wang *et al*., 1991). The lower activity in the antioxidant system detected in the high chilling requirement cultivar may play an important role determining the incomplete overcoming of flower bud dormancy that led to a very low blooming percentage. These results suggest that minimum thresholds in antioxidant activities could be crucial for scavenging free radicals generated during the winter season.

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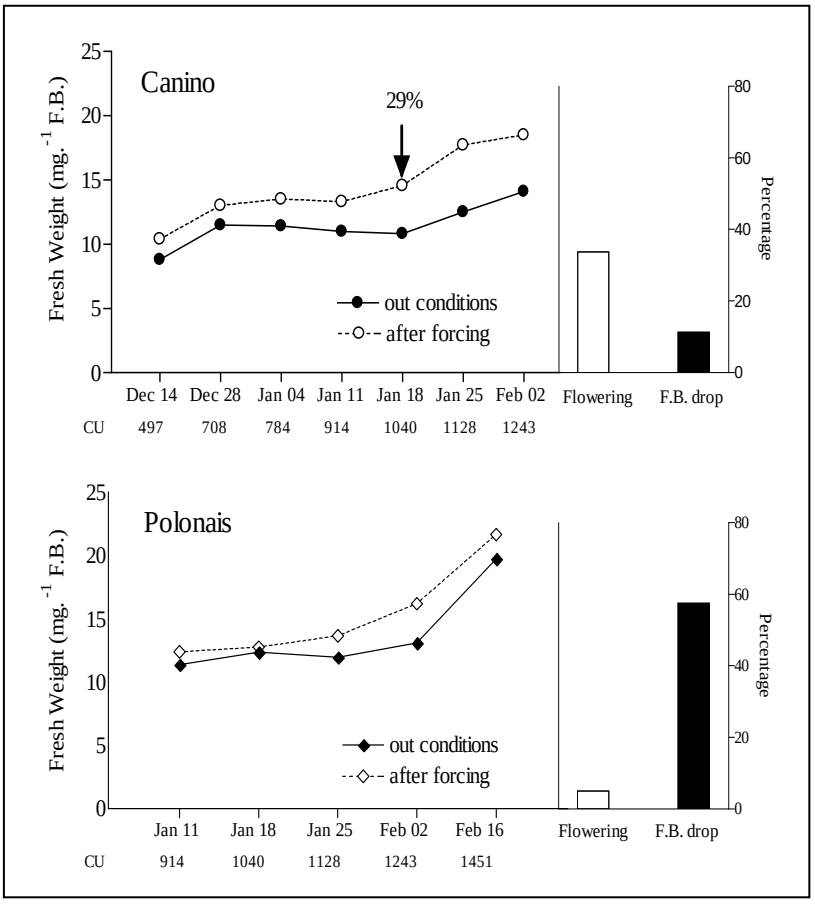

Fig. 1 - Cultivar 'Canino' (a) and 'Polonais' (b): on the left, flower bud fresh weight (mg/flower bud) detected from December to February, in out conditions and after forcing. On the right, flowering and flower bud drop percentage. The corresponding C.U. accumulated are indicated.

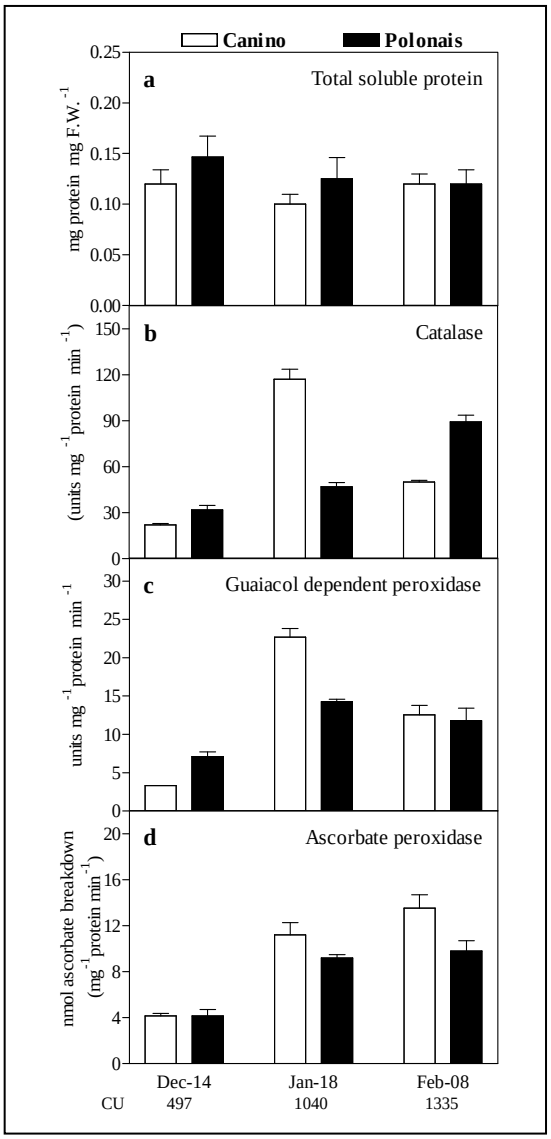

Fig. 2 - Total soluble protein (a), catalase (b), guaiacol peroxidase (c) and ascorbate peroxidase (d), in 'Canino' and 'Polonais' flower buds from December to February. The corresponding C.U. accumulated are indicated. Bars indicate standard errors of means

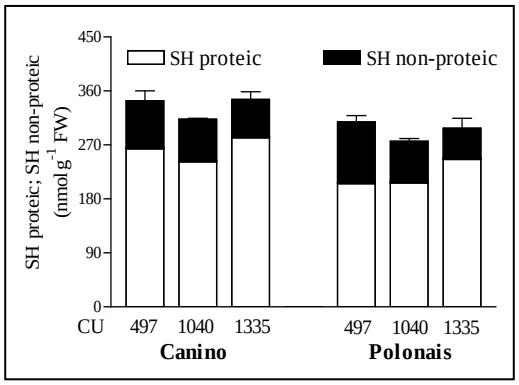

Fig. 3 - Total sulphydryl compound, proteic and non-proteic (nmol g^{-1} FW) detected in 'Canino' and 'Polonais' flower buds, from December to February. The corresponding C.U. accumulated are indicated. Bars indicate standard errors of means.

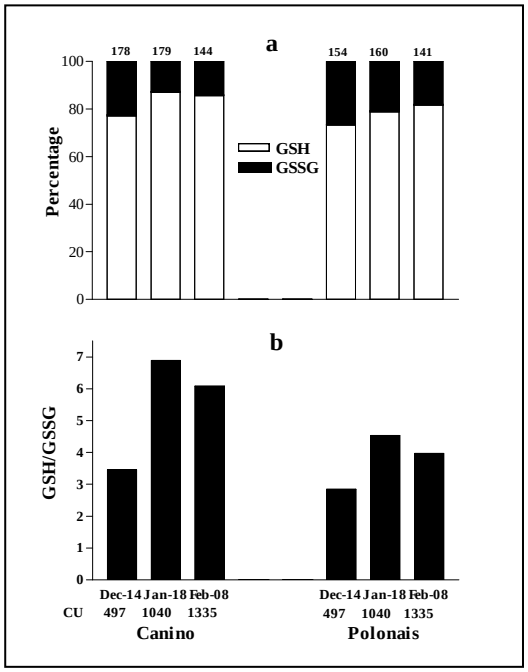

Fig. 4 - Cultivars Canino and Polonais: (a) changes in GSH and GSSG percentage. Total glutathione concentrations are shown at top of bars (nmol g^{-1} FW); (b) GSH/GSSG ratio. The corresponding C.U. accumulated are indicated.