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Exogenous onion extract hastens bud break, positively alters enzyme activity, hormone, amino acids and phenol contents, and improves fruit quality in 'Anna' apple trees

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ABSTRACT

Metabolic changes in the activity of catalase, and the contents of hydrogen peroxide, proline, total free amino acids, total indoles, anthocyanins and free phenols in floral buds of apple (*Malus domestica* Borkh. cv. 'Anna') trees were investigated during dormancy and bud break under the effect of foliar application of two onion extract rates (100 and 200 ml l^{-1}). Flowering percentage, fruit yield characteristics (i.e., fruit-set, number of fruits $tree^{-1}$ and fruit yield $tree^{-1}$) and fruit quality measurements were increased, while number of days recorded to reach full bloom were reduced with the application of both onion extract rates. In addition, contents of hydrogen peroxide, proline, total free amino acids, total indoles and anthocyanin were significantly increased in floral buds, particularly during bud break. In contrast, the activity of catalase and the content of free phenols were reduced. The best results were obtained from the extract rate of 200 ml l^{-1} that could be recommended for early and high percentage of flowering and increased yield with high quality by regulating the metabolism of amino acids, including proline and indoles, and the activities of catalase and hydrogen peroxide in apple floral buds.

1. Introduction

Winter dormancy is a phenomenon observed during winter in deciduous trees and evergreen tree species such as tea (Kumar and Paul, 2011). In late summer, declining the photoperiods and temperatures cause shoot extension growth to cease and the initiation of apical buds to protect the apical meristem (Heide and Prestrud, 2005). A specific signal (environmental or endogenous) perceived within the bud, induces and maintains these buds in dormancy state (Espinosa-Ruiz et al., 2004; Bohlenius et al., 2006). These dormant buds, in temperate perennial species, require a period of low temperatures (winter chilling) to release from dormancy. However, there is no a sufficient period of low temperatures in the short-winter countries, including Egypt.

Nowadays, there is an urgent request to find a tool for organic apple production in the short-winter countries. Therefore, it is necessary to find eco-friendly and safer early bud break promoters suitable for this purpose. Although many researchers used synthetic chemicals for breaking bud dormancy in different fruit species (Germchi et al., 2011; Theron et al., 2011; Seif El-Yazal et al., 2012; Seif El-Yazal and Rady, 2012, 2013), a number of investigations have been successfully carried out for artificially interrupting the bud dormancy in areas lacking the sufficient chilling units by using natural extracts such as olive oil (Abd El-Rzek et al., 2011), green algae extract (Abd El-Moniem and Abd-Allah, 2008), garlic extract (Botelho et al., 2010; Biasia et al., 2010; Abd El-Rzek et al., 2011). The beneficial effect of onion extract on bud break, flowering, fruit yield and some chemical components of different fruit species were recently studied (Kim et al., 2000a, 2000b; Kubota et al., 2002; Rady and Seif El-Yazal, 2013).

Therefore, the present work was prepared in an attempt to produce, to some extent, organic fruits by the exogenous application of eco-friendly natural materials such as onion extract in Egypt as one of the short-winter countries. The effect of onion extract on date of full bloom and flowering percentage as a result in floral bud break, and yield and quality characteristics was studied on 'Anna' apple trees in short-winters. In addition, changes in amino acids, indoles, phenols, hydrogen peroxide, anthocyanin metabolism and catalase enzyme activity in floral buds during different stages of dormancy and its release were also investigated.

2. Materials and methods

2.1. Selection of the tested trees

This work was conducted during the two growing seasons of 2011/2012 and 2012/2013, in the Orchard of the Horticulture

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Station in Aboksah, Abshwai, Fayoum Governorate, Egypt, in an attempt to break bud dormancy of apple (*Malus domestica* Borkh. cv. 'Anna') grafted on Malling-Merton 106 (MM 106) rootstock as early as possible and to obtain high quality fruits. The 15-year-old trees grown in a loamy sand soil were selected in November 2010 as uniform as possible for a preliminary study to select the effective rates of onion extract. The rates of 100 and 200 ml l^{-1} were generated higher flowering percentage and fruit yield per tree, and recorded the minimum number of days to reach full bloom in the season of 2010/2011 (Rady and Seif El-Yazal, 2013). Onion extracts were applied in the two selected rates (i.e., 100 and 200 ml l^{-1}) in addition to tap water used as a control. In November 2011, 18 trees were selected for the season of 2011/2012. Also, in November 2012, another 18 trees were selected for the season of 2012/2013. In a random arrangement, each treatment included six trees. Onion extracts were sprayed at two equal doses; the first was applied at 5 December and the second one was applied 2 weeks later with an extract volume of 4 l $tree^{-1}$ in both seasons. All the agricultural and horticultural practices such as pruning, irrigation, hoeing, suitable fertilization, and pest, diseases and weed controls were carried out according to the bulletin of the Egyptian Ministry of Agriculture (2002).

2.2. Preparation of onion extracts

Onion samples were washed and ground using mortar and pestle. The active ingredients were then extracted by ethyl alcohol (95%) as described by Rady and Seif El-Yazal (2013). The ethanol mixture was filtered and the alcohol was evaporated under vacuum (30 °C) using a Buchi Rotary evaporator R-200 with "V" assembly (vertical water condenser). The produced paste was termed as onion extract which was kept cool in refrigerator (4 °C) until use. The onion extract was diluted by water to give the final concentration required (100 and 200 ml onion extract l^{-1} tap water) directly before use. A surfactant "Triton B" at 0.1% was added to the spraying solution. The trees were sprayed using a backpack sprayer gun to the spur surface until well wetted.

2.3. Phenological and yield characteristics

In both seasons, number of days beginning from the second spray application (20 December) to full bloom (50% flowering) was recorded for each treatment, and at the end of flowering period the percentage of flowering of each treatment was estimated. Flowers in which calyx began to extend were tagged in order to determine the percentage of fruit-set. In addition, numbers of mature/full colored fruits and fruit yields (in kg per tree) were recorded. Samples of mature fruits were taken from each treatment for fruit quality determinations (vitamin C, total soluble solids, acidity, total free amino acids, total phenols and water content).

2.4. Floral bud sample preparations for chemical analyses

At 20-day intervals beginning from 2 January to 3 March, floral bud samples were randomly collected from each replicate of each treatment and were immediately transported to the laboratory. Collected samples were frozen using liquid nitrogen until they used for determining the seasonal changes in free proline, total free amino acids and hydrogen peroxide. On the other hand, total indoles, free phenols and catalase activity were determined in fresh buds.

2.5. Free proline determination

According to Ennajeh et al. (2006), free proline was determined as follows: Sample (200 mg) of frozen buds was extracted with 5 ml of 40% methanol heated to 80 °C for 30 min. The supernatant (1 ml) was mixed with 2 ml glacial acetic acid, 1 ml ninhydrin solution (25 mg ml^{-1}) and 2 ml of a mixture consisting of 24% distilled water, 60% glacial acetic acid and 16% orthophosphoric acid. The mixtures were heated for 30 min in a water bath set to 100 °C, then cooled on ice and 3 ml of toluene was added and the mixture was shaken vigorously. The colored toluene phase (upper phase) was read at 528 nm with a Shimadzu UV-160 spectrophotometer.

2.6. Hydrogen peroxide (H_2O_2) determination

The content of H_2O_2 was determined based on Loreto and Velikova (2001). Frozen bud sample (0.35 g) was ground in liquid nitrogen, and then homogenized in an ice bath with 5 ml of 0.1% TCA. The homogenate was centrifuged at 12,000 × *g* for 15 min, then 0.5 ml of the supernatant was added to 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0) and 1 ml of 1 M potassium iodide. The absorbance was measured at 390 nm with a Shimadzu UV-160 spectrophotometer and the content of H_2O_2 was calculated and expressed in μmol g^{-1} fresh weight.

2.7. Determination of total free amino acids in floral buds and fruits

Total free amino acids were determined according to Jayarman (1981) with some modifications (Chen et al., 2009). A sample (500 mg) of frozen buds or fruit pulp was extracted with 50 ml of 80% ethanol and filtered to remove insoluble materials, then 1.0 ml of ethanol extract was added. Then, 0.5 ml of 0.07 mol l^{-1} phosphate buffer solution (pH 8.04) and 0.5 ml of 2% ninhydrin solution containing 0.8 mg ml^{-1} of $SnCl_2$–$2H_2O$ was added. The mixtures were then placed on a boiling water bath for 15 min, and then quickly cooled with cold water, and adjusted to 25 ml with water. After leaving to stand still for 10 min, the absorbance values of these blue-purple products were measured against a reagent blank at 550 nm.

2.8. Extraction and determination of total indoles

Total indoles were extracted from fresh floral buds by grinding 2 g with 50 ml toluene and 5 ml 5% TCA for 1 min. The mush was centrifuged at 2500 × *g* for 30 min to separate the toluene layer that was filtered through a 0.45 μm syringe filter into a beaker containing anhydrous Na_2SO_4 (Aldrich). Total indoles were determined (as μg g^{-1} fresh weight) according to Snell and Snell (1967) with some modifications (Snellings et al., 2003). The extract (4 ml) was diluted to 10 ml with toluene, after which 2 ml was vortexed for 15 min with 2 ml reagent (1.25 g [4-dimethyl-aminobenzaldehyde (DMAB)] in 100 ml MeOH and 25.6 ml concentrated HCl). The mixture was centrifuged at 3500 rpm for 6 min to separate the MeOH (bottom) layer that was measured with spectrophotometer at 567 nm.

2.9. Anthocyanin determination

Total anthocyanin content was determined by the method of Mancinelli (1984). Dried floral buds were extracted with 1% HCl–MeOH for 24 h at room temperature in darkness with occasional shaking. The extract was carefully decanted and their absorbance was measured at 530 and 657 nm. The formula A_{530}–0.25 A_{657} was used to compensate the absorption of chlorophyll degradation products. Anthocyanin content was expressed as mg of cyaniding 3-glucoside in 100 g dry buds, using 29,600 as molecular extinction coefficient.