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Changes in nitrogen and polyamines during breaking bud dormancy in "Anna" apple trees with foliar application of some compounds

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ABSTRACT

The capacity of hydrogen cyanamide (Dormex), potassium nitrate (KNO_3) and mineral oil for breaking of dormancy in buds of "Anna" apple (*Malus sylvestris*, Mill) trees, and their effects on metabolic changes of nitrogen (N) and polyamines (PAs) in buds during bud break were determined. Our results showed that the capacity to release of buds from dormancy was noticed in varying degrees with these compounds. Breaking bud dormancy was correlated with the early date of bud break, the short duration of flowering, the high percentages of bud break and fruit-set, and the high contents of soluble N and PAs. The best results were obtained with Dormex; therefore we recommend using this compound for reaching the bud break as early as possible, the short period of flowering and the high percentages of bud break and fruit-set by regulating the contents of N and PAs in buds.

1. Introduction

Dormancy in deciduous fruit trees is a physiological syndrome occurring annually to enable plants to survive cold winters. Entrance into dormant state occurs in late summer and fall. Deciduous trees require low winter temperatures to overcome dormancy and grow in the following spring. Induction and release of buds from dormancy are linked to structural and metabolic modification occurring on the bud level (Ramina et al., 1995). Many deciduous, perennial fruit crops require winter chilling for adequate bud break and flowering. Recent research has shown that changes in polyamine profiles are associated with the release of buds from dormancy (Wang and Faust, 1994).

Several chemicals can be used to induce bud break of deciduous fruit trees in areas lacking sufficient chilling units. Hydrogen cyanamide (Dormex), potassium nitrate (KNO_3) and mineral oil have a synergistic effect on bud break and some chemical constituents of many deciduous fruit trees (Sagredo et al., 2005; De-Oliveira et al., 2008; Morsi and El-Yazal, 2008; Sabry et al., 2011).

Polyamines (PAs) are small aliphatic nitrogenous compounds present in all living organisms (Tassoni et al., 2008). Putrescine (Put), cadaverine (Cad), spermidine (Spd) and spermine (Spm) are major PAs involved in the regulation of a large number of physiological activities such as embryogenesis, cell division, morphogenesis, development, ethylene production, fruit ripening, flower development, dormancy and normal or stress induced senescence (Kakkar et al., 2000; Bagni and Tassoni, 2001; Sood and Nagar, 2005; Groppa and Benavides, 2008).

Stress protective properties indicate that PAs are an integral component of plant stress management. Variations in PAs contents have been associated with several types of stresses, e.g. salinity (Shevyakova et al., 2006; Liu et al., 2007; Tassoni et al., 2008); heavy metals (Liu and Moriguchi, 2007; Zapata et al., 2008; Vladimir et al., 2009) and dormancy (Huang et al., 2004; Sood and Nagar, 2005; Santanen and Simola, 2007; Krawiarz and Szczotka, 2008; Sińska and Lewandowska, 2010).

Since the winter in Egypt is short and does not meet the needs of the buds from the cold units, the delay in opening buds of apple trees up till late winter exposes buds to damage under the influence of high temperature and/or delays them in entering in dormancy in the following year leads to some physiological defects that may result in weakness and death. This threatens the apple productivity in Egypt, so this work focuses mainly to explain the behavior of nitrogen and polyamines in buds and their reflections in the duration to full buds break, and the percentages of bud break and fruit-set as a result of spraying "Anna" apple trees with Dormex, KNO_3 and mineral oil elucidating their potential in hastening the dormancy break.

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Table 1
Mean of the physical and chemical characteristics of the experimental site.

Composition (% (w/w))			pH	EC (dS m^{-1})	OC (g kg^{-1})	$CaCO_3$ (%)	N (mg kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})
Clay	Loam	Sand											
28.4	20.8	50.8	7.7	7.8	9.1	8.2	96.8	18.6	81.7	84.9	5.7	4.8	1.2

OC, organic content.

2. Materials and methods

2.1. Soil analysis, trees selection and treatments

The main characteristics of the experimental site (Southeast Fayoum; 29°17′N; 30°53′E) were determined according to Jackson (1973) and are shown in Table 1.

The 15-year-old trees of "Anna" apple (*Malus sylvestris*, Mill) grafted on Malling-Merton 106 (MM 106) root stock were randomly, uniformly selected for preliminary study in 2007/2008 and for research study in 2008/2009 and 2009/2010 seasons, in the Horticultural Orchard of Experimental Farm (newly reclaimed saline calcareous soil), Faculty of Agriculture, Fayoum University, Egypt. The selected trees were labeled in November 2008, received the foliar treatments in December 2008 and then sampled beginning from 5 January up to 19 February 2009 for the first study season. The experiment was repeated for the second one; 2009/2010. Each tree was designed as one replicate and each treatment included six trees.

The spray applications were conducted as follows: the first treatment that the control trees did not receive any of the three compounds, but received tap water. The second treatment was the foliar spray with hydrogen cyanamide commercially known as "Dormex" (molecular weight 42.04 g mol^{-1} and formulation 49% hydrogen cyanamide, density 1.065 g l^{-1}). The third treatment was the foliar application with potassium nitrate (KNO_3; containing 13% N and 44% K). The fourth treatment was the foliar application with mineral oil (regular winter oil having a UR of 75%). All spray treatments were supplied twice. The first one was applied at 11 December (before the end of dormancy duration) and the second was applied two weeks later with a volume of 4 l $tree^{-1}$. Triton B as a wetting agent at 0.1% was added to the spraying solutions. After our preliminary study, Dormex, KNO_3 and mineral oil at 4%, 8% and 6%, respectively, applied twice, were found to be the most significant in bud growth of "Anna" apple trees (data not shown). Therefore, these levels were used for our study.

2.2. Morphological characteristics measurements

Bud count was made for each tree of all treatments. The dates on which floral and vegetative buds started to open were recorded. Number of vegetative and floral buds was counted when all buds were opened and the percentages were estimated. The dormant buds were also counted and were expressed as a percentage of the total number of buds. The dates at which flowering reached 25%, 50%, 75% and 100% of the total flowers were estimated in each treatment. Flowers whose calyx began to extend were tagged in order to measure the percentage of fruit-set.

2.3. Determination of nitrogen (N) fractions and polyamines (PAs) contents

Bud samples were collected 15-day intervals beginning from 5 January up to 19 February for determining the seasonal changes in bud components. Buds were randomly sampled and immediately transported to the laboratory for determining the N fractions and PAs.

Total N and soluble N (%) in dried material of buds were determined by using micro-Kjeldahl method described by the A.O.A.C. (1995).

PAs were extracted and quantified according to the method of Fontaniella et al. (2001). For extraction of PAs, 2 g of buds was homogenized with 5% perchloric acid (PCA) (v/v) in a pre-chilled pestle and mortar at 4 °C. Homogenized extracts were kept at 4 °C for 1 h, and then centrifuged at 15,000 rpm for 25 min at 4 °C. Supernatant was dried in vacuo at 45 °C and residues were redissolved in 5% PCA and were stirred with polyvinylpolypyrrolidone (PVPP) (50 mg ml^{-1}) for excluding the impurities like polyphenolics.

PAs were analyzed on Waters 515 (USA) Chromatograph HPLC equipped with Waters (717) Plus Autosampler and Photodiode Array Detector (2996). They were eluted in a RP-C-18 column (15 cm × 4 mm i.d.), (5 M particle sizes) reversed phased column at 30 °C using methanol:water gradient. Gradient elution was carried out using Methanol-Milli-Q water in a linear gradient from 50:50 (v/v) to 80:20 (v/v) for 30 min. The last proportion was maintained for 15 min until the end of the analysis. Mobile phase was maintained at a flow rate of 1 ml min^{-1}. Fluorescence intensities of the extracted PAs were compared; peaks and retention times of samples were compared to those of standard PAs (Spm, Spd, Put and Cad, purchased from Sigma Aldrich Ltd., India).

2.4. Statistical analysis

The values for the determined characters were subjected to statistical analysis, following the standard procedure described by Gomez and Gomez (1984). The '*F*' test was applied to assess the significance of the treatment, at 5% level of probability.

3. Results

Because of the matched trends of the results, the data of nitrogen fractions and polyamines represented in combined analysis system for the two examined seasons.

3.1. The date of floral bud break

The foliar application with hydrogen cyanamide (Dormex), KNO_3 or mineral oil for "Anna" apple trees was hastened the flower bud break compared to the control in which trees were sprayed with tap water (Table 2). These compounds shortened the duration to the first flower bud break by 31–32 days, 26–27 days and 24–25 days for Dormex, KNO_3 and mineral oil, respectively when compared to the control. The duration to full flowering was shortened by 36–38 days, 30–31 days and 29–30 days for the same compounds, respectively as compared to the control. In addition, flowering duration was shortened by the three compounds when compared to the tap water. Dormex was found to be more effective in this concern. It was shortened flowering period to 20–21 days, while mineral oil was shortened it to 20–22 days and KNO_3 was shortened it to 22 days compared to 26 days in the control.