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Changes in Promoter and Inhibitor Substances During Dormancy Release in Apple Buds Under Foliar-Applied Dormancy-Breaking Agents

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Abstract

The effect of hydrogen cyanamide (Dormex) at different concentrations for reaching early break dormancy in buds of "Astrachan" apple (Malus sylvestris, Mill) trees and their effects on metabolic changes in the content of buds from promoter and inhibitor substances during their release from dormancy was investigated. The efficiency of early bud break was noticed in varying degrees with Dormex at different concentrations. All applied concentrations led to early bud break, short flowering duration, high percentages of bud break and fruit-set, high contents of total indoles, indole-3-acetic acid, gibberellic acid, total and conjugated phenols and low content of abscisic acid and free phenols. These results were positively reflected in the final yield. Accordingly, we recommend using Dormex at 3% for reaching the early break dormancy in buds of "Astrachan" apple trees under Egyptian winter conditions and maximizing the yield by regulating the hormonal and phenolic content in buds.

Keywords: Apple, Dormancy, Hydrogen cyanamide (Dormex), Hormones, Phenols, Yield.

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1. Introduction

Dormancy is "the inability to initiate growth from meristems or other organs and cells with the capacity to resume growth under favorable conditions" (Rohade and Bhalerao 2007). The main physiological stages of dormancy have been recently termed paradormancy, endodormancy, and ecodormancy. During paradormancy the growth is regulated by plant factors originating outside the bud, at endodormancy stage growth is repressed by physiological factors inside the bud. While during the ecodormancy stage bud break is avoided because of environmental factors that are inadequate to support growth (Lang 1987). Bud break can be poor in locations that have warm winters with insufficient winter chilling (Walton et al. 2009).

Cyanamide is an allelochemical (CA), named also hydrogen cyanamide (CN_2H_2), it is an organic compound commonly applied in agriculture. It has been produced worldwide since early twentieth century, and in its form of calcium salt ($CaCN_2$) has been used as synthetic fertilizer. It was demonstrated that cyanamide calcium salt, when applied to soil, may be hydrolyzed into active CA after contact with water molecules and then is converted into urea inter alia by cyanamide hydratase (Cah) produced by the fungus Myrothecium verrucaria (Maier-Greiner et al. 1991). Therefore, it may be considered as a source of inorganic nitrogen. Except of its fertilizing role, its more recent applications are mainly breaking dormancy of fruits (e.g. grape, apricot, apple), buds (Ben Mohamed et al. 2012 a &b; El-Sabagh et al. 2012; Vergara et al. 2012; Bill 2012 and Eshghi et al. 2012; Seif El-Yazal and Rady 2012&2013; Seif El-Yazal et al. 2012&2014; Ahmed et al.2014; Leonel et al.2015; İmrak et al.2016 and Mohamed and Gouda 2017).

Initiation and release of dormancy have been related in other plant species to changes in endogenous hormone concentrations (Jiménez et al. 2008). Therefore, the effect of the aforementioned dormancy release treatments (hydrogen cyanamide) on endogenous concentration of indole-3-acetic acid (IAA), abscisic acid (ABA), gibberellins GA1, GA3 and GA20 (GAs), zeatin/zeatin riboside and N6(Δ2-isopentenyl) adenine/N6(Δ2-isopentenyl) adenosine was evaluated in oil palm seeds. A sharp reduction in ABA concentration in embryos and endosperm was the most notable outcome during the HC treatments. HC treatment additionally caused an increase in IAA levels in embryos and endosperm during the imbibitions phase. Changes in concentration of other hormones (e.g., GAs and cytokinins) could not be directly related to dormancy release in this species. In this respect, the endogenous hormonal change in the bud dormancy inducing and releasing processes was studied by many researchers (Jiménez et al. 2008; Guevara et al. 2008; Dong et al. 2009; Mornya and Cheng 2011; Okay et al. 2011; Seif El-Yazal et al. 2014). They found that the occurrence, termination, regulation, and control of dormancy were regulated by hormones. A few number of studies have been done on the change of endogenous hormones from dormancy releasing to bud opining, but none on the relation between bud break and dynamic change of endogenous hormones, as well as the equilibrium of late-opining apple varieties.

The relationship between plant phenols and bud dormancy as well as bud break were studied by several workers (Szecsko et al. 2002; Jindal and Mankotia 2004; Morsi and El-Yazal 2008; Zahra et al. 2009; Seif El-Yazal and Rady 2014)

Because the winter in Egypt is short and does not meet the chilling requirements of buds, the delay in opening the buds of " Astrachan " apple trees until late winter exposes them 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 " Astrachan " apple productivity in Egypt, so this work focuses mainly to explain the behavior of the inhibitor and promoter substances (hormonal and phenols contents) 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 " Astrachan " apple trees with Dormex at different concentrations elucidating their impacts in hastening the dormancy break.

2. Materials and methods

2.1. Trees selection and treatments

The 18-year-old trees of "Astrachan" apple (Malus sylvestris, Mill) buded on "Baladi" apple were randomly, uniformly selected for preliminary study in 2006 and for research study in 2007 and 2008 seasons, in the orchard of the Horticulture Station in Aboksha Abshawi, Fayoum Governorate, Egypt. The selected trees were labeled in November 2006, received the foliar treatments in January 2007 and then sampled beginning from 10 February up to 24 March 2007 for the first study season. The experiment was repeated for the second one; 2008. Each tree was designed as one replicate and each treatment included four trees.

The foliar applications were conducted as follows: the first treatment was the control trees that did not receive any of the three concentrations but received tap water. The second, the third and the fourth 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 1–1). All spray treatments were applied at 21 January with a volume of 4-liter tree–1. Triton B as a wetting agent at 0.1% was added to the spraying solutions. After our preliminary study, Dormex, at 1%, 2% and 3%, respectively, applied, were found to be the most significant in bud growth of "Astrachan" apple trees (data not shown). Therefore, these levels were used for our study.

2.2. Morphological characteristics and yield measurements

Buds 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. At harvest stage, apple fruits were harvested, counted and weighed for each examined tree.

2.3. Extraction and determination of endogenous hormone in apple buds

Bud samples were collected 7-day intervals beginning from 10 February up to 24 March for determining the metabolic changes in the hormonal content in buds. Buds were randomly sampled and immediately transported to the laboratory. Floral bud samples were taken from each tree of each treatment, frozen in liquid nitrogen and kept at –80°C until extraction and assay as described by (Gunes et al. 2010) Concisely, 1 g of homogenized bud tissue was kept in 20 ml of extraction solvent including methanol, chloroform, 2 N ammonium hydroxide, and BHT (12:5:3, V: `V: V, 0.001% BHT) within an amber dark bottle at –18°C for 3 weeks. Each week, the solvent was filtered and collected in another bottle (combined extract), and 40 ml of solvent was added to the samples. Both of them were kept at –18°C. At the end of 3 weeks, 25 ml of double distilled water was added to the combined extract in a separating funnel and the aqueous methanol on the top was put into a round-bottom flask. All organic solvents were evaporated under vacuum at 45°C. The pH value of the aqueous extract was adjusted to 2.5 for the extraction of indole-3-acetic acid (IAA), gibberellic acid (GA3), and abscisic acid (ABA). Growth regulators in this extract were transferred into ethyl acetate by washing with 15 ml of ethyl acetate 3 times. All ethyl acetate solvents were collected in a round-bottom flask. All hormones were separated onto thin layers and were collected separately in 1 ml of absolute methanol. All of the extraction was done under dark conditions. A 20 µl sample was analyzed using high pressure liquid chromatography (HPLC) (Bio Rad Model 2800) equipped with C18 column (Phenomenex Luna, 5 m) and UV detector (Bio Rad UV-1806) following straining the samples through 0.45 mm pore size filters (Millipore, SLGV013NL). All reagents used in this procedure were HPLC-grade obtained from detector wavelength settings. Solvents and conditions applied during HPLC analysis were as follows:

Hormone	Mobile phase	Wavelength (nm)	Flow rate (ml min^{-1})
IAA	CH_3CN: DDW (v/v, 1:1), %0.5 CH_3COOH	280	1.5
ABA	CH_3CN: DDW (v/v, 1:1), %0.5 CH_3COOH	265	1.5
GA_3	CH_3CN: DDW (v/v, 3:2)	208	3.0

Obtained peaks were defined according to retention times using external standards and quantified by the ratings of external standard peak areas. External standards such as IAA (I-2886), GA3, (G-7645) and ABA (A-1049) were obtained from Aldrich Co Ltd.

2.4. Extraction and determination of total indoles in apple buds

Procedure

Indoles was extracted from apple buds by grinding 2 g fresh buds with 50 mL toluene and 5 mL 5% TCA for 1 min. The purée was centrifuged for 30 min at 3500 rpm (2534 × g) to separate the toluene from the apple pulp.The toluene layer was decanted and filtered through a 0.45 µm syringe filter into a beaker containing anhydrous Na2SO4 (Aldrich).

Total indols in buds were determined as µg /g dry weight according to Snellings et al. (2003)with some modification .The derivatizing reagent used in the colorimetric method consisted of 1.25 g (4-dimethyl- amino-benzaldehyde (DMAB)) in 100 mL MeOH and 25.6 mL concentrated HCl (Snell and Snell 1967). In the procedure, 4mL apple extract was diluted to 10 mL with toluene, after which 2 mL was vortexed for 15 min with 2 mL derivatizing reagent. The resulting mixture was centrifuged for 6 min at 3500 rpm to separate the MeOH and toluene layers. The absorbance of the MeOH (bottom) layer was measured with spectrophotometer at 567 nm.

Estimation of free, conjugated and total phenolics content in apple buds using folin-ciocalteu reagent.

Total and free phenols in buds were determined as mg/g dry weight using folin-ciocalteu reagent and Sodium carbonate solution according to Galicia et al. (2009) with some modification.

Procedure

Take a random sample of 20-30 buds as a representative of your material. Dry the buds at 64-65 ºC for 16 hours. Grind each sample to a very fine powder.

Extraction of free phenolics

For each sample, weigh 100 mg of powder in an eppendorf tube. Add 6.5 ml of methanol (50%). Close the tubes, ensuring no evaporation will take place during extraction. Vortex thoroughly the samples and place them in a thermomixer at 65 ºC and 900 rpm for 30 minutes. Take the tubes out of the thermomixer and let them cool at room temperature. Centrifuge the tubes at 14,000 rpm for 5 minutes. Ensure that the supernatant does not have sample particles floating in it; if it does, centrifuge again. Make the colorimetric reaction.

Extraction of total phenolics

For each sample, weigh 100 mg of powder in an eppendorf. Add 6.5 ml of hydrochloric acid in methanol (10 ml of HCl 1.2 M with 90 ml methanol). Close the tubes, ensuring no evaporation will take place during extraction. Vortex thoroughly the samples and place them in a thermomixer at 42 ºC and 1100 rpm for 30 minutes. Take the tubes out of the thermomixer and let them cool at room temperature. Centrifuge the tubes at 14,000 rpm for 5 minutes. Ensure that the supernatant does not have sample particles floating in it; if it does, centrifuge again. Take 2.5 mL of supernatant, put it in new eppendorf. Reduce to dryness and resuspend the precipitate resulting in 6.5 ml of methanol. Vortex thoroughly and make the colorimetric reaction.

Colorimetric reaction

Take 1 mL of supernatant and carefully transfer it to test tube. Add 0.8 mL of 25 % Folin-Ciocalteu reagent (dissolve 10 g sodium tungstate and 2.5 g sodium molybdate in 70 ml water. Add 5 ml 85% phosphoric acid and 10 ml concentrated hydrochloric acid. Reflux for 10 hr. Add 15 g lithium sulfate, 5 ml water and 1 drop bromine. Reflux for 15 min. Cool to room temperature and bring to 100 ml with water. Then take 2.5 ml of F-C 2N with 7.5 ml of deionized water and vortex thoroughly). The F-C reagent should be added before the alkali to avoid the air-oxidation of phenolics. Add 2.2 mL of 400 mM Na2CO3(4.25 g of Na2CO3 (99.9%) in 100 ml of deionized water). Cover the tubes with adhesive aluminum tape to avoid dropping of samples. Vortex the tubes at 800 rpm for 10 seconds. Incubate tubes at 42 ºC for 9 minutes for color development. Take the tubes out of the oven and let them cool at room temperature, protect them from direct light. Read absorbance at 765 nm in a spectrophotometer.

These estimates represented total phenlos (conjugated and free phenols) and thus conjugated compounds were obtained by subtracting free phenols from total phenols.

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 hormones and their relations are represented in combined analysis system for the two examined seasons.

3.1. Date of floral bud break

The foliar application with hydrogen cyanamide (Dormex) at three concentrations for "Astrachan" apple trees was hastened the floral bud break as compared to the control in which trees were sprayed with tap water (Table 1). The period to the first floral bud break was shortened by 8–7 days, 10–8 days and 12–12 days with Dormex, at 1, 2 and 3%, respectively when compared to the control. In addition, the period to full flowering were shortened by 8–9 days, 9–9 and 13-16 days with the same three concentrations, respectively as compared to the control. Dormex at 3% was found to be most effective in shortening the period of full flowering, since shortened flowering period to 19–19 days, while Dormex at 2% was shortened it to 20–21 days and Dormex at 1% was shortened it to 20-21 days compared to 21-22 days in the control.

Table 1. Effect of dormex at (1 ,2 and 3%) treatments on the date of flower bud opening and flowering period in "Astrachan" apple trees.

Treatments	Date of flower bud opening											
	Beginning		25% flowering		50% flowering		75% flowering		End		Flowering period (day)	
	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
Control	4 April	6 April	12 April	14 April	17 April	18 April	22 April	22 April	25 April	28 April	21	22
Dormex1%	28 March	29 March	9 April	10 April	12 April	11 April	13 April	12 April	17 April	19 April	20	21
Dormex2%	27 March	27 March	7 April	7 April	13 April	13 April	15 April	16 April	16 April	17 April	20	21

Dormex3%	24 March	25 March	5 April	6 April	10 April	10 April	11 April	12 April	12 April	12 April	19	19

3.2. Floral bud break and fruit set

Table 2 shows that, all tested compounds significantly increased the percentages of floral bud break and fruit-set when compared to the control. However, Dormex at 3% was the most effective than all others. It surpassed the tap water by 19–18% and 55–48% for bud break and fruit-set, respectively. Dormex at 1% was less effective among the three compounds. It exceeded the control by 13–13% and 28–28% for the same two parameters, respectively.

Table 2. Effect of dormex at (1 ,2 and 3%) treatments on the percentage of bud break and fruit set in "Astrachan" apple trees

Treatments	Bud break (%)		Fruit-set (%)	
	2007	2008	2007	2008
Control	71.25c	72.04b	13.96d	14.77c
Dormex1%	80.29b	81.35a	17.87c	18.93b
Dormex2%	81.65ab	82.16a	18.85b	19.07b
Dormex3%	84.55a	84.86a	21.61a	21.92a

Mean pairs followed by different letters are significantly different (p = 0.05) by Duncan's test; n=6

3.3. Fruit yield

Table 3 shows that, all tested concentrations increased number of apple fruits tree–1 and total fruit yield tree–1 when compared to the control. Dormex at 3% was most effective in this concern. It exceeded the control by 14–14% and 52–33% for number of fruits tree–1 and fruit yield tree–1, respectively. Dormex at 1% was found to be less effective when compared to Dormex at 2 and 3%, although it exceeded the control by only 5–5% and 20–12% for the same two parameters, respectively.

Table 3. Effect of dormex at (1, 2 and 3%) treatments on No. of fruit tree–1 and total yield tree–1 of "Astrachan" apple trees

Treatments	No. of Fruit $tree^{-1}$		Total yield $tree^{-1}$ (kg)	
	2007	2008	2007	2008
Control	124.69c	130.00c	10.10d	11.36c
Dormex1%	130.42bc	135.9 4bc	12.15c	12.76bc

Dormex2%	138.50a	145.77a	13.33b	13.22b
Dormex3%	142.00a	148.78a	15.33a	15.11a

Mean pairs followed by different letters are significantly different (p = 0.05) by Duncan's test; n=6

3.4. Hormonal content in buds

Foliar-applied Dormex at 1, 2 and 3% increased the contents of Total indole, indole-3-acetic acid (IAA) and gibberellic acid (GA3) and decreased the contents of abscisic acid (ABA) in the floral buds of " Astrachan " apple trees when compared to the control in which trees were sprayed with tap water (Table 4).

Table 4. Effect of dormex at (1, 2 and 3%) treatments on hormonal content in buds of "Astrachan" apple trees

Treatments	Indole acetic acid (IAA) (µg g^{-1} DW)						
	10 Feb	17 Feb	24 Feb	3 Mar	10 Mar	17 Mar	24 Mar
Control	1.54c	1.48c	4.33b	4.68b	5.01a	4.65b	4.31b
Dormex1%	1.79c	1.68c	4.54b	4.80a	5.16a	4.88a	4.58b
Dormex2%	1.75c	1.71c	4.58b	4.86a	5.18a	4.90a	4.61b
Dormex3%	1.97c	1.82c	4.62b	4.88a	5.20a	4.92a	4.64b
	Gibberellic acid (GA_3) (µg g^{-1} DW)						
Control	1.21d	1.16d	1.64c	1.83c	3.60a	3.52a	2.77b
Dormex1%	1.41d	1.19d	1.86c	1.95c	3.86a	3.64a	2.81b
Dormex2%	1.43d	1.20d	1.87c	1.97c	3.89a	3.66a	2.83b
Dormex3%	1.46d	1.22d	1.89c	2.01c	3.93a	3.68a	2.88b
	Abscisic acid (ABA) (µg g^{-1} DW)						
Control	0.50b	0.84a	0.108a	0.56b	0.40c	0.39c	0.26d
Dormex1%	0.65b	0.72b	0.98a	0.54b	0.35c	0.33c	0.24d
Dormex2%	0.62b	0.70b	0.93a	0.50b	0.33c	0.31c	0.22d
Dormex3%	0.59b	0.69b	0.89a	0.48c	0.31c	0.30d	0.20d
	IAA/ABA ratio						
Control	3.08d	1.76d	4.01c	8.28b	12.35a	11.87b	16.07b
Dormex1%	2.75d	2.32d	4.62c	8.86b	14.67a	14.40a	18.86a
Dormex2%	2.81d	2.42d	4.91c	9.69a	15.39a	15.70a	20.55a

Dormex3%	3.32d	2.04d	5.17c	10.04a	16.55a	16.22a	22.44a
	GA_3/ABA ratio						
Control	2.42b	1.38d	1.51d	3.24b	8.88a	8.99a	10.33a
Dormex1%	2.16b	1.65d	1.89c	3.59b	10.99a	10.74a	11.59a
Dormex2%	2.29b	1.70d	2.01c	3.93b	11.55a	11.72a	12.61a
Dormex3%	2.46b	1.76d	2.11c	4.13b	12.50a	12.13a	13.93a

Mean pairs followed by different letters are significantly different (p = 0.05) by Duncan's test; n=6

The results of all hormones obtained with Dormex at 3% treatment were surpassed the results found with Dormex at 2% and Dormex at 1% in most of the sampled dates. The maximum contents of total indols, IAA and GA3 were obtained from buds applied with Dormex at 3% and collected in 10 March (buds were released from dormancy), while their minimum contents were obtained from buds applied with Dormex at 2% and Dormex at 1% and sampled in 10 & 17 Februry (buds were still dormant). For ABA, the opposite result was found. In addition, the maximum ratios of IAA/ABA and GA3/ABA were noted in the 24 March sample (buds were released from dormancy), while the minimum ones were found in the 17 & 24 Februry samples (buds were still dormant).

3.5. Phenolic content in buds

Foliar-applied Dormex at 1, 2 and 3% increased the contents of total soluble phenols and conjugated phenols and decreased free phenols in the floral buds of "Astrachan" apple trees when compared to the control in which trees were sprayed with tap water (Table 5).

Table 5. Effect of dormex at (1 ,2 and 3%) treatments on total indole, total, free and conjugated phenols content in buds of "Astrachan" apple trees

Treatments	Total Indole (μg g^{-1} DW)						
	10 Feb	17 Feb	24 Feb	3 Mar	10 Mar	17 Mar	24 Mar
Control	4.80c	4.35c	4.96c	5.51b	6.43a	6.04a	5.92a
Dormex1%	4.86c	4.54c	5.12b	5.66b	6.68a	6.45a	6.41a
Dormex2%	4.92c	4.65c	5.24b	5.71ab	6.89a	6.56a	6.49a
Dormex3%	4.99c	4.67c	5.26b	5.98ab	7.59a	7.21a	7.09a
	Total phenols (mg g^{-1} DW)						
Control	31.15a	25.54b	27.30b	29.55b	32.10a	32.61a	26.24b
Dormex1%	32.15a	25.90b	28.11a	30.02a	33.98a	34.14a	28.15b
Dormex2%	34.22a	26.54b	28.50a	31.06a	34.51a	35.00a	28.60b
Dormex3%	36.55a	27.11b	29.77a	32.21a	35.20a	36.11a	29.21b

	Free phenols (mg g^{-1} DW)						
Control	11.25a	10.10a	9.55a	8.91a	8.64a	8.31b	8.06b
Dormex1%	10.85a	8.60b	8.24b	8.16b	8.10b	7.90c	7.65c
Dormex2%	10.71a	8.41b	8.21b	8.12b	8.09b	7.62c	7.52c
Dormex3%	10.15a	8.23b	8.14b	8.09b	7.81c	7.25c	7.01c
	Conjugated phenols (mg g^{-1} DW)						
Control	19.90c	15.44c	17.75c	20.64b	23.46b	24.30b	18.18c
Dormex1%	21.30b	17.30c	19.87c	21.86b	25.88a	26.24a	20.50c
Dormex2%	23.51b	18.13c	20.29c	22.94b	26.42a	27.38a	21.08c
Dormex3%	26.40a	18.88c	21.63b	24.12b	27.39a	28.86a	22.20b
	Conjugated /Free phenols ratio						
Control	1.76c	1.52c	1.85c	2.31b	2.71b	2.92b	2.25b
Dormex1%	1.96b	2.01b	2.41b	2.67a	3.19a	3.32a	2.67b
Dormex2%	2.19b	2.15b	2.47b	2.82a	3.26a	3.59a	2.80b
Dormex3%	2.60b	2.29b	2.65b	2.98a	3.50a	3.98a	3.16a

Mean pairs followed by different letters are significantly different (p = 0.05) by Duncan's test; n=6

The data of all phenols obtained with Dormex at 3% treatment were surpassed the data found with 1% and 2% in most of the sampled dates. The maximum contents of total soluble phenols and conjugated phenols were obtained from buds applied with Dormex at 3% and collected in 17 March (buds were released from dormancy), while their minimum contents were obtained from buds applied with Dormex at 2% and Dormex at 1% and sampled in 17 February (buds were still dormant). For free phenols, the opposite result was found. In addition, the maximum ratios of conjugated phenols /free phenols were noted in the 17 March sample (buds were released from dormancy), while the minimum ones were found in the 10 & 17 Februry samples (buds were still dormant).

4. Discussion

It is clear nowadays that a wide variety of factors can break dormancy, in particularly environmental and hormonal influences have to be analyzed to understand the complex mechanism which start when the bud resume growth. During the process of the release of buds from dormancy, many changes in some chemical components in floral buds, particularly the contents of endogenous hormones; Total indoles (included, Indole-3-acetaldehyde, Indole-3-acetic acid, Indole-3-acetonitrile, Indole-3-ethanol etc ….), IAA, GA3 and ABA) and endogenous phenols (Total, free and conjugated phenols) play a vital role in regulating dormancy and bud break. Several studies focused on the relationship between the endogenous hormones and dormancy either in buds (Guevara et al. 2008; Dong et al. 2009; Seif El-Yazal et al. 2014; Seif El-Yazal and Rady 2014), in tubers (Liping et al. 2010) or in seeds (Jiménez et al. 2008; Seo et al. 2011). During the whole testing period, Total

indoles, IAA and GA3 concentrations in buds of "Astrachan" apple at their release from dormancy was higher than those of before bud break. In contrast, ABA concentration in floral buds was higher before bud break than that of at dormancy releasing (Table 4). This suggested that higher total indoles, IAA and GA3 contents and lower ABA content were needed for release of "Astrachan" apple buds from dormancy (Table 4). In this concern, Dong et al. (2009) showed that, growth-promoting hormones such as GA3 and IAA found to be gradually increased, while growth-inhibiting hormones such as ABA decreased during bud break. GAs and ABA had the same precursor (Mevalonic acid) for their synthesis and the increase in the lighting time promoted the synthesis of GA3 in buds. Moreover, the results showed that when the dormancy released, IAA/ABA ratio had the same changing tendency to IAA content (Table 4). Moreover, GA3/ABA ratio in buds had the same changing tendency to GA3 content during release from dormancy (Table 4) So, it could be presumed that IAA/ABA and GA3/ABA ratio determined the metabolism direction. In addition, the ratios of IAA/ABA and GA3/ABA in apple buds were decreased in dormant buds, while increased in opening ones. In this concern, Dong et al. (2009) suggested that the balance of several hormones played a more important role than the level of certain single hormone in the procedure of dormancy releasing and opining of buds. Endogenous IAA may have an effect on transcription of nuclear DNA that can contribute to cell enlargement, promote fruit development, and are involved in apical dominance and dormancy. Endogenous gibberellins (GAs) play a role in many developmental processes and have been proved to participate in the regulation of dormancy (Wang et al. 2006). They are necessary to floral bud break in several species (Rentzsch et al. 2011). Moreover, GAs could promote the synthesis of certain mRNA and enzymes and increased the activity of hydrolytic enzymes such as α- & β-amylases, proteinase, peptidase, Lipase, ribonuclease and isocitrate lyase … etc. These enzymes and an unknown factor cooperate with GA3 to relieve the inhibition of bud break and assure the transcription process and consequently promote the synthesis of mRNA and protein (Alexopoulos et al. 2008). In contrast, ABA could inhibit the production of certain RNA indispensable to the synthesis of α-amylase, and the main role of ABA in the process of germination was restraining GA3 and inducing the transforming of reserve substance (Gubler et al. 2005).The control of apple bud dormancy induction, maintenance and release therefore is mediated, at least in part, by changes in hormone signaling as it is also known for tree bud dormancy (Horvath et al. 2003; Rohde et al. 2007) and seed dormancy (Holdsworth et al. 2008). Gibberellic acid (GA3) and abscisic acid (ABA) signaling as well as the GA3/ABA ratios are important as known for tree bud sprouting (Horvath et al. 2003; Rohde et al. 2007), seed germination of dicots (Linkies et al. 2009; Voegele et al. 2011) and monocots (Barrero et al. 2009; Gerjets et al. 2010), and for α-amylases induction in the endosperm of germinated cereal grains (Leubner-Metzger 2007).

Moreover, the favorable effect of Dormex at different concentrations on the date of flower bud opening may be due to their stimulation effect on natural GAs. In this connection, Subha-Drabandhu (1995) concluded that the induction of flowering could be correlated with a natural rise in GAs in plants to facilitate the formation of flowering hormone in the leaves or expressing it in the growing buds. GAs also may be a primarily responsible for bolting which may be essential for the formation of the floral stimulus in leaves. Dormancy is associated with down-regulated cell cycle genes and increased ABA content in the bud meristems when compared with the non-dormant state (Campbell et al. 2010). The induction and maintenance of tuber bud dormancy seems to involve ABA, while bud dormancy release seems to involve GAs and cytokinins (Hartmann et al. 2011). In our study, Dormex at different concentrations positively affected the date of flower bud opening. This may be due to the increase in GA3 and IAA and the decrease in ABA contents. The beneficial effect of Dormex on germination of dormant embryos is associated with a marked reduction in the generation of hydrogen peroxide and superoxide anions in the embryonic axes (Oracz et al. 2009). Most genes identified following Dormex application appear to be associated with responses to stress, but a number of genes appear to be associated with the reactivation of growth. Three patterns of gene expression were identified: Profile 1, a Dormex-induced transient activation of gene expression; Profile 2, a Dormex-induced transient activation followed by a bud opening-related activation; and Profile 3, Dormex-induced growth of buds. One group of genes that was rapidly up-regulated in response to Dormex was the glutathione S-transferase class of genes, which have been associated with stress and signaling (Walton et al. 2009).

High levels of endogenous IAA and GAs contents which noticed in "Astrachan" apple trees applied with Dormex at different concentrations agreed with the explanation of Kuroi (1985) and Yang et al. (1990). They concluded that, cyanide ion may play a role in inducing the enzyme activity, promoting the retranslocation of stored reserves and the uptake of nitrogen with water for bud break. The degradation of cyanamide was demonstrated to occur through urea to other compounds and both are utilized in production of amino acids such as tryptophan (a precursors of IAA) (Miller and Hall 1963). Also, Foott (1987) found that Dormex is easily absorbed in the buds and initiated the processes leading to bud break. Dormex is rapidly metabolized in the plant and helps in the synthesis of amino acids. Dormex may be metabolized into urea, arginine and probably further into guanidinium compounds (Wunsch and Amberger 1968). It caused a rapid increase of nitrogen level and growth promoting substances (Kuroi 1974) and may be involved in oxidative processes which are a prerequisite for bud break. Fuchigami and Nee (1987) suggested that, hydrogen cyanamide break dormancy by its involvement in the conjugation of the thiol group which is assumed to be involved in breaking dormancy. In addition, Dormex breaks dormancy by decreasing the endogenous ABA content in buds (Subha-

drabandhu 1995). On the other hand, the increase in ABA after HC treatments in the first sample may be due to a consequence of stress. In this respect Netting (2000) reported that HC caused a significant increase in endogenous ABA. Those levels were approximately three times higher in this treatment than in the control. The rise in ABA as a result of the higher HC dose may be more a consequence of stress than a direct stimulation of ABA synthesis by HC.

Moreover, Vergara et al. (2012) reported that dormancy-breaking compound hydrogen cyanamide (HC) stimulates the fermentative pathway and inhibits respiration in grapevine-buds, suggesting in this way, that a respiratory stress must be involved in the release of buds from dormancy. Moreover, the mode of action of allelochemicals (cyanamide) usually involves alteration in one of crucial physiological processes, e.g. photosynthesis, respiration, water or nutrient transport (Weir et al. 2004; Gniazdowska and Bogatek 2005). Inhibition of any of them results in disturbances in plant growth and development. Moreover, (Pérez and Lira 2005) observed a reduced catalase activity in dormant buds of grapevine treated with HC, followed by an increase in the H2O2 levels in the buds. It is known that accumulation of reactive oxygen species (ROS), such as H2O2, may cause lipid oxidation (Apel and Hirt 2004). Indole-3-acetic acid was the only hormone whose endogenous concentration responded to lower HC concentration (Guevara et al. 2008). IAA levels measured in control plants decreased significantly, while those in HC treatments increased. This may indicate that IAA, possibly together with H2O2, was among the initial reactions of the plant to the HC treatment. This increase in IAA could be a response to stress, such as the one mentioned above, as pointed out by (Havlová et al. 2008) in tobacco. This IAA upsurge might trigger other hormonal changes. For example, it has been recently demonstrated by kinetic and molecular studies that auxin herbicides and IAA by itself can initiate a "reaction chain" that starts with an increase in IAA, or related auxins, which induces ethylene biosynthesis, which in turn accelerates ABA biosynthesis and possibly jasmonic acid production (Grossman 2007).

The most of phenol compounds have been isolated from bud scales and have growth inhibitor role in buds. Literature reported by researchers indicate that phenol compounds increased during rest in peach flower buds, then decreased after rest and completely eliminated at blooming. The chilling period influences during disappear them. Therefore, the previous works were undertaken to determine if such a relationship exists between bud break and the phenol contents or not (Jindal and Mankotia 2004). Moreover, Codignola et al. (1988b) reported that, phenol composition has been increased from November to February and then, have been decreased in March and have been eliminated on blooming stage in peach buds. During dormant, number inhibitors such as naringenin, floridzin, caffeic acid and quercitrin are in all trees buds and gradually decrease companied with budbreak. Several studies have shown correlation between budbreak and seasonal variation of phenols in trees (Codignola et al. 1988a). The evaluation of dormant buds, has shown that, phenol

compounds rarely occur in a free state within the cell; rather they are commonly conjugated with other molecules. Many exist as glycosides linked to monosaccharides or disaccharides. Thus, the general biological role of phenolics in plants apparent (e.g., pigments, lignin) and some have been implicated as allelopathic agents, feeding deterrents, antifungal agents and phytoaloxin (Codignola et al. 1988a). Therefore, phenol contents exhibited seasonal subtraction in dubs of all apple cultivars investigated to spring. This finding could be taken some evidence, that, phenols could be play important role to protection buds during winter, dormant season and budbreak.

The decrease in free phenols after Dormex at different concentrations treatments may be due to that the reduction in free phenols contrasted with the increase in total indoles i.e. indogenous promoters increased and consequently indogenous inhibitors decreased in the buds which led to increasing in plant growth parameters. In this respect Sagi and Garay (1961) showed that phenolic effect on plant growth was contributed to either antagonism with I.A.A. activity. Also, Kefeli and Kutacek (1977) suggested that plant phenol may be decided into three groups, promotive, inhibitor and inactive. They added that promotion of plant growth by phenols may proceed through the modulation of either IAA biosynthesis or its destruction. Moreover, Wang et al. (1991) on apple found that dormant buds contained a high amount of phenolic substances which decreased after bud break then increased until the start of bud expansion. Phenolic compounds are found to be patent modifiers of catalase, peroxidase and polyophenol oxidase activity, as both inhibitors and stimulators in apple buds. Moreover, these substances may be stimulating the oxidation process of phenols by increasing the peroxidase activity.

5. Conclusion

The foliar application with hydrogen cyanamide (Dormex) at different concentrations for "Astrachan" apple trees hastened the bud break, shortened the period of flowering and improved bud growth and fruit set. Dormex at 3% was found to be the most effective, significantly improved the contents of endogenous total indoles, IAA, GA3 and conjugated phenols and reduced ABA and free phenols content. This led to an increase in the percentages of bud break and fruit set, and the reduction in the period of flowering, and finally the increase in the yield. Therefore, we recommend using Dormex at 3% for the increase in "Astrachan" apple trees productivity. It may provide a well strategy for the increase in the percentages of bud break and fruit set, and the reduction in the flowering period to protect the floral buds against the high temperature in late winter in Egypt.

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