Metabolic Changes in Polyamines, Phenylethylamine, And Arginine During Bud Break in Apple Flower Buds Under Foliar-Applied Dormancy-Breaking Agents

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Abstract

The Environmental and climatic conditions affect the flower bud growth, flowering and yield performance of fruit species. Temperature appears to be important factors for temperate fruit trees in mild climates. The relationships between breaking bud dormancy and climatic influences on flowering, growth and metabolic changes in contents of biogenic amines (spermine, spermidine, putrescine, cadaverine and phenylethylamine), arginine and anthocyanin before and after bud break were evaluated for "Ain Shemer" apple (*Malus sylvestris*, Mill) trees under exogenously applied hydrogen cyanamide (dormex), calcium nitrate $Ca(NO_3)_2$ and thiourea. The studies have shown that breaking bud dormancy (as early date of bud break and percentage of bud break) by the above dormancy breaking agents were correlated with the high content of biogenic amines and arginine in buds. Seasonal variations in biogenic amines, arginine and anthocyanin level were present in buds during the research period (dormancy and dormancy release period). Levels of spermine, spermidine, putrescine, cadaverine, phenylethylamine, arginine and anthocyanin have simultaneously risen with bud burst and new growth. The most abundant polyamines in buds were cadaverine followed by spermine. The best results were obtained with Dormex after exposure the trees to about 274 natural chilling hours $\leq +7.2^{o}C$; therefore, we recommend using this compound to achieve bud break as early as possible in "Ain Shemer" apple variety by regulating the contents of biogenic amines and arginine in buds and subsequently the high percentages of bud break and maximum yield.

Keywords: Apple (*Malus sylvestris,* Mill); Dormancy; Dormex; Calcium nitrate Ca $(NO_3)_2$; Thiourea; Biogenic amines.

1. Introduction

Bud dormancy allows perennial plants of temperate and boreal zones to survive in low winter temperatures. Lateral buds are formed in early summer and enter a paradormant state that is mainly caused by apical dominance [1]. By mid- to late autumn, the timing varies depending on species, and the inhibitory control of bud growth shifts to the bud itself. These buds are referred to as being endodormant [2].

The major limiting factor for deciduous fruit production in Egypt is the lack of adequate winter-chilling in most areas of the country [3]. With appropriate management, the low chilling requirement for "Anna" and "Ain Shamer" cultivars is precocious and heavy-cropping. These cultivars flower, so profusely, early in spring with the use of dormancy breaking spraying compounds [4]. Hydrogen cyanamide (HC) is the most useful rest breaking treatment for apple and has been used effectively to supplement cold temperature, to achieve satisfactory bud break and to enhance cropping [3]. Several researchers justified the idea that dormancy breaking agents like Hydrogen cyanamide [5, 6, 7, 8], calcium nitrate [9, 10], and thiourea [11, 12] may spread within the plant.

Polyamines (PAs) that belong to major biogenic amines in plants are synthesized by the decarboxylation of either arginine or ornithine and catalyzed by arginine decarboxylase or ornithine decarboxylase, respectively [13]. Biogenic amines, namely spermine (Spm), spermidine (Spd), putrescine (Put), cadaverine (Cad), and phenylethylamine are found in different plant developmental process [14]. They modulate several growth and developmental processes viz., cell division, differentiation, flowering, fruit ripening, embryogenesis, rhizogenesis and are well known for their anti-senescence and anti-stress effects due to their acid neutralizing and antioxidant properties, as well as for their membrane and cell wall stabilizing abilities [15]. It has been suggested that PAs play important roles in modulating the defense response of plants to diverse environmental stresses, including metal toxicity [16], oxidative stress [17], drought [18], salinity [19], chilling stress [20] and dormancy [3]. In all these, PAs have been ascribed various roles such as that of a new class of plant growth regulators, hormonal second messengers and as one of the reserves of carbon and nitrogen at least in cultured tissues [21]. Aromatic monoamines occurring in plants are the products of enzymatic decarboxylation of L-phenylalanine (Phe), L-tyrosine, and L-histidine to phenylethylamine (PEA), tyramine and histamine, respectively [22]. Phenylethylamine (PEA) occurrence has been shown in some terrestrial plants [23]. The role of PEA in plants remains unclear. In common buckwheat PEA can be transformed into 2-phenylacetaldehyde which belongs to the compounds with the highest contribution to aroma of the plant [24].

The idea that biogenic amines, arginine and aromatic monoamines are involved in the regulation of dormancy is justified in some plants [3, 6, 14].

Anthocyanin accumulation is stimulated by various environmental stresses, such as drought, sugar and nutrient deficiency [25] and chilling stress [26]. Anthocyanin is utilized as a precursor with cytoprotective function in the secondary metabolism [27]. Thus, the increased level of anthocyanin indicates an index for a good mechanism of plant resistance towards the changes in the environmental conditions [25]. The major function of anthocyanins seems to be their photoprotective role [28] and they play important roles as anti-oxidants that protect DNA and the photosynthetic apparatus from high radiation fluxes [29].

The objective of this study is to investigate the role of Polyamines, phenylethylamine, arginine and anthocyanine in the seasonal cycling of growth and dormancy by measuring Polyamines, phenylethylamine, arginine and anthocyanine levels in buds collected after spraying, in winter when buds are approaching dormancy and when endodormancy breaks down, but trees are still hardened and in spring when bud burst occurs. Also, to understand how HC, Ca $(NO_3)_2$ and thiourea influences dormancy and bud break in apple. Moreover, this work was considered as a trial for increasing the yield of "Ain Shemer" apple variety by hastening the beginning of flower bud break in the suitable time for fruit set. In addition, this work reports the dynamics of some biogenic amines and arginine pools in apple buds during and immediately after dormancy release with and without HC, Ca $(NO_3)_2$ and thiourea treatments.

2. Materials and Methods

This study was carried out during the two successive seasons of 2008 and 2009 in the orchard of the Horticulture Station in Aboksha Abshawi, Fayoum Governorate, Egypt. It is an attempt to break dormancy of "Ain-Shemer" apple variety (*Malus sylvestris*, Mill) grafted on Malling-Merton 106 (MM 106) root stock for increasing the yield in this variety. Chilling hr ≤ +7.2 ^{o}C from November 1 to treatment date during both 2016/2017 seasons had been determined (**Table 1**).

Table 1. Chilling accumulation (hours ≤ +7.2^{o}C from November 1to each application date

Application	Hours ≤ +7.2^{o}C		
	2015/2016	2016/2017	Mean
December, 10	216	220	2
December, 25	270	278	

Also, to study the seasonal changes in biogenic amines, arginine and anthocyanin in buds during the period of dormancy and dormancy release under foliar-applied dormancy-breaking agents. The trees were 12 years old when experiment started and were grown in loamy sand soil. Trees were selected in November, 2015 as uniform as possible for spray treatments. Trees were arranged in a complete randomized design. Each tree

was used as a replicate. Each treatment included six replicates, receiving only one of the following treatments.

2.1. The Experimental Design

1- Control (spraying with tap water)

2- Spraying with hydrogen cyanamide (Dormex) at a rate of 4%

3- Spraying with calcium nitrate at a rate of 6%

4- Spraying with thiourea at a rate of 2%

The control trees were sprayed with tap water, however hydrogen cyanamide is commercially known as "Dormex" (molecular weight 42.04g/mol and formulation 49% hydrogen cyanamide, density 1.065g/1), calcium nitrate (containing 15.5% nitrogen) and thiourea (molecular weight 76.12, Assay 99-101%, Sulphated ash 0.1%) All spray treatments were applied twice; on 10 December and 2 weeks later (Nearly 24th of December), with a volume of 4 L/tree for each of them. Triton B as a wetting agent, at 0.1%, was added to the spraying solutions.

The selected concentrations of the DBAs were found to be most significant for later bud growth in "Ain Shemer" apple trees (data not shown). Therefore, these treatment levels were used for this study.

2.2. Data Recorded

2.2.1. Morphological Characteristics

In both seasons, bud counts were made for each tree. The dates on which flower and vegetative bud started to open were recorded. Number of vegetative and flower buds was counted when all buds were opened and the percentages were estimated. The dormant buds were also counted and were expressed as percentage from the total number of buds. The dates at which flowering reached 25, 50, 75 and 100 percent of the total flowers were estimated in each treatment. Flowers whose calyx began to extend were tagged in order to determine the percent of fruit set. The yield of fruits in kg/tree as well as the numbers of mature fruits /tree were recorded when fruits reached the commercial color to be picked.

2.2.2. Chemical Analyses

Bud samples were collected 15-day intervals beginning from 8th January up to 8th March from each replicate after spraying in the two studied seasons for determining the seasonal changes in bud components. Samples of buds were taken randomly and immediately transported to the laboratory for the following determination.

For extraction and measurement of biogenic amines and arginine, bud samples were collected at 15-d intervals, from 8th January up to 8th March 2008 and 2009, to determine seasonal changes in biogenic amines and arginine. Buds were sampled at random and immediately transported to the laboratory to determine their contents of biogenic amines and arginine. Biogenic amines and arginine were measured after each bud sample (2 g) had been lyophilized and ground to a fine powder. They were extracted from 0.5 g dry weight (DW) of each bud sample by shaking for 24 h at ambient temperature with 50 ml of a single-phase 12:5:3 (v/v/v) mixture of methanol: chloroform: water [30]. Norleucine (0.5 ml of a 4 mM solution in 0.01 M HCI) was added prior to extraction as an internal standard. After extraction, the colorless aqueous methanolic-phase (containing the amino acids) was separated from the chloroform-phase (containing pigments and lipids; **[31]**). The solid residue was boiled gently for 10 min in 40 ml water to extract the residual amino acids **[32]**. After cooling to room temperature and centrifugation at 10,000 × *g*, the aqueous extract was combined with the methanolic extract and made up to 100 ml. A 20 ml aliquot was loaded onto a cation-exchange column (Dowex-SOW 8%; 200 – 400 mesh) with a bed volume of 3 ml. The column was washed with 45 ml 0.01 M HCl, followed by 5 ml water, then eluted with 30 ml 2 M NH4OH to release the amino acids. The flow rate was maintained at approx 1.0 ml min–1 using a small vacuum pump. Columns were regenerated by washing sequentially with 5 ml water, 10 ml 1.0 M HCl, 5 ml water, 40 ml 0.2 M NaOH, 5 ml water, 10 ml 1.0 M HCl, and finally 5 ml water **[33]**. The ammoniacal eluates were lyophilized and stored at –10°C until analysis, at which time they were resolubilized in 0.2 M lithium citrate loading buffer (pH 2.2; LKB Biochrome, Cambridge, UK). Analyses of amino acids was performed using an Alpha Plus amino acid analyzer (LKB Biochrome) fitted with a stainless-steel column (200 mm × 4 mm) filled with ion exchange resin (Ultropac 8; particle size 8 μm; LKB Biochrome). The content of biogenic amines (mg kg^{-1} DW) and arginine (mg 100 g^{-1} DW) was measured using ninhydrin positive compounds. The reagent coil temperature was 135 °C. Data acquisition and peak integrations were evaluated using Baseline chromatography software (Waters Dynamic Solutions, Ventura, CA, USA) on an IBM 286 AT computer **[34]**.

For spectrophotometric determination of total anthocyanins content, a method described in **[35]**, with some modifications, was used. The apple buds were extracted with 1% HCl–MeOH for 24 h at room temperature, in darkness with occasional shaking. The extracts were carefully decanted and their absorbance was measured at 530 and 657 nm. The formula A530 - 0.25A657 was used to compensate for the absorption of chlorophyll degradation products **[35]**. Anthocyanin content was expressed as mg of cyaniding 3-glucoside in 100 g of dry matter, using 29,600 as molecular extinction coefficients.

2.3. Statistical Analysis

Results were statistically analyzed using the L.S.D. at probability level of 5% for comparisons [36].

3. Results

3.1. Date of Flower Bud Break

Data presented in **Table 2** clearly indicate that spraying apple trees with all the tested substances hastened the beginning of flower bud break as compared to the control. This earliness reached about 30 and 29 days for Dormex at 4%, 23 and 18 days for calcium nitrate at 6% and 20 and 19 days for thiourea at 2% respectively, if compared with the control trees in both seasons.

Table 2. Effect of Dormex at 4%, calcium nitrate at 6% and thiourea at 2% treatments on time of flower bud opening in "Ain Shemer" apple trees

Treatment	Date of flower bud opening											
	Beginning		25% flowering		50% flowering		75% flowering		End		Flowering duration (day)	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	201
Control	19 March	17 March	24 March	25 March	27 March	29 March	3 April	25 April	25 April	25 April	37	39
Dormex	18 Feb	16 Feb	25 Feb	26 Feb	26 Feb	28 Feb	10 March	9 March	16 March	17 March	27	29
$Ca(No_3)_2$	25 Feb	27 Feb	4 March	4 March	12 March	14 March	23 March	22 March	26 March	29 March	30	30
Thiourea	28 Feb	26 Feb	2 Feb	3 March	9 March	10 March	13 March	15 March	27 March	28 March	28	30

With regard to the effect of the tested substances on 50% bud break, the present results clearly show that all treatments hastened 50 % bud break as compared to the control. This earliness reached about 30 and 29 days for Dormex at 4%, 15 and 15days for calcium nitrate at 6% and18 and 19 days for thiourea at 2 % if compared with the control trees in both seasons, respectively.

3.2. Percentage of Bud Break and Fruit Set

Data presented in **Table 3** clearly show that all treatments gave a high percentage of flower bud break compared with the control. The maximum increases were recorded with Dormex at 4% which recorded 29.56 and 30.79 % in both seasons over the control, respectively. The data also show that all the tested substances increased apple fruit-setting as compared to the control trees. Such trend was true during the

two studied seasons. The maximum increases in fruit-setting were recorded with Dormex at 4% which recorded 83.90 % and 84.88 in both seasons over the control, respectively.

Table 3. Effect of dormex (4%), calcium nitrate (6%) and thiourea (2%) treatments on the percentage of bud break and fruit set in "Ein Shamer "apple trees

Treatments	Bud break (%)		Fruit-set (%)	
	2016	2017	2016	2017
Control	71.12c	71.32c	22.61c	23.88c
Dormex4%	92.15a	93.28a	41.58a	44.15a
Ca(No3)2 6%	81.90b	84.33b	35.55b	37.31b
Thiourea 2%	75.58b	77.28b	31.21bc	33.28bc

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

Table 4. Effect of dormex (4%), calcium nitrate (6%) and thiourea (2%) treatments on yield and total number of fruits /tree in "Ein Shamer "apple trees

Treatments	**Yield per tree (Kg)**		**Total number of fruits / tree**	
	2016	2017	2017	2017
Control	11.65c	11.77c	207.12c	217.69c
Dormex4%	20.00a	20.59a	320.79a	337.55a
Ca(No3)2 6%	17.99a	19.52a	256.15b	277.94b
Thiourea 2%	14.55b	15.10b	259.82b	269.64b

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

3.3. Yields and its Components

Data in **Table 4** indicated that all the tested substances increased apple yield and fruit number as compared to the control trees. Such trend was true during the two studied seasons. The maximum increases were recorded with Dormex at 4% which recorded 71.67 and 74.93 % for apple yield/tree and 54.88 and 54.93 % for fruit number in both seasons, respectively over the control trees.

3.4. ***Chemical Constituents of Buds***

3.4.1. The Contents of Polyamines (PAs) in Buds

The buds of "Ain Shemer" apple trees spraying with Dormex, Ca $(NO_3)_2$ and thiourea increased contents of PAs (spermine (Spm), spermi-dine (Spd), putrescine (Put) and cadaverine (Cad)) when compared to

the control **Table 5**. The PAs contents obtained with Dormex treatment were surpassed their contents found with all treatments in most of the sampled dates. The maximum contents of Spm, Spd, Cad and Put were obtained in the 7th February, 7th February, 22nd February and 8th March samples, respectively with Dormex application, while their minimum contents were obtained in the 23rd January, 22nd February, 8th January and 8th January samples with calcium nitrate, tap water, tap water and tap water treatments, respectively.

3.4.2. Phenylethylamine Concentrations in Buds

Phenylethylamine was increased with the foliar application with Dormex, Ca $(NO_3)_2$ and thiourea when compared to the control **Table 5**. Dormex was found to be more effective than the all other treatments. It hiccoughed the control by 4.95 %, 11.47 %, 19.63 %, 31.70 % and 23.07 % for the sampled dates of 8th January, 23rd January, 7th February, 22nd February and 8th March, respectively. In addition, data revealed that thiourea was less effective in this regard.

3.4.3. *Arginine Concentrations in Buds*

Floral buds of "Ain Shemer" apple trees sprayed with Dormex™, Ca $(NO_3)_2$ or thiourea had increased content of arginine when compared to the water-sprayed controls (**Table 5**). The Dormex™ treatment increased the arginine content to a greater extent than the other treatments on most sampling dates.

3.4.4. Anthocyanin Concentrations in Buds

Anthocyanin was increased with the foliar application with Dormex, $Ca(NO_3)_2$ and thiourea when compared to the control (**Table 5**). Dormex was found to be more effective than the all other treatments. It was superior to the control by 55.95 %, 58.40 %, 19.74 % 18.87 % and 51.07 % for the sampled dates of 8th January, 23rd January, 7th February, 22nd February and 8th March, respectively. In addition, data revealed that thiourea was less effective in this regard.

Table 5. Effect of Dormexat at 4%, Calcium nitrate at 6% and Thiourea at 2% treatments on biogenic amines content as mg/kg D.W., arginine and anthocyanine as mg/100g dry weight of buds in "Ain Shemer"apple trees

Compound	Treatment	Samples date				
		8 Jan	23 Jan	7 Feb	22 Feb	8 March
Spermine (mg/kg dry weight)	Control	603a	273b	955b	625c	623b
	Dormex	473b	298a	1350a	853a	851a
	$Ca(NO_3)_2$	310c	165c	601c	755b	655b
	Thiourea	520b	278a	507c	750b	650b

Spermidine (mg/kg dry weight)	Control	60c	51c	85b	23d	28d
	Dormex	104b	60b	178a	68a	75a
	$Ca(NO_3)_2$	123a	78a	81b	56b	61b
	Thiourea	117a	55c	59c	35c	43c
Putrescine (mg/kg dry weight)	Control	21c	54b	56c	68b	45c
	Dormex	37b	70a	95a	99a	66a
	$Ca(NO_3)_2$	36b	59b	71b	83a	60b
	Thiourea	45a	55b	60bc	69b	58b
Cadaverine (mg/kg dry weight)	Control	210b	2001b	2111c	2139b	2255b
	Dormex	995a	2141a	4351b	5865a	5988a
	$Ca(NO_3)_2$	956a	1761c	4942a	5811a	5866a
	Thiourea	225b	2247a	4182b	5790a	5811a
Phenylethyl-amine (mg/kg dry weight)	Control	101b	61b	82c	41b	39b
	Dormex	106a	68b	132a	54a	48a
	$Ca(NO_3)_2$	105a	80a	106b	48b	42b
	Thiourea	105a	78a	89c	45b	41b
Arginine (mg/100g dry weight)	Control	244c	216b	265c	221c	216c
	Dormex	335a	226a	339a	265a	232a
	$Ca(NO_3)_2$	311b	222b	327b	243b	230a
	Thiourea	317b	220b	323b	242b	225b

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

4. Discussion

Polyamines (PAs) are found in plant cells as free molecules; low-molecular weight, acid-soluble conjugates, bound, high-molecular weight and acid-insoluble conjugates [37]. PAs participate in a numerous physiological event in plants; they regulate cell division [38], cell enlargement [39], and stress tolerance [40]. PAs have been demonstrated to increase chilling tolerance of chilling-sensitive plants, probably by stabilizing cell membranes, thus minimizing permeability changes and leaking [41]. Phenylethylamine is a natural monoamine alkaloid, trace amine, and also the name of a class of chemicals with many members well known for psychoactive drug and stimulant effects [42].

Variations in the levels of biogenic amines especially, (Spermine (Spm), Spermidine (Spd), Putrescine (Put), Cadaverine (Cad), phenylethylamine (PEA)), arginine (Ar), and anthocyanin (An) (**Table 5**) have been observed in apple buds during dormancy and subsequent to its release. These variations correlate well with those observed in the present study in relation to dormancy and bud break in apple buds. In general, high levels of 'Spm', 'Spd', 'PEA' and 'Ar' and low levels of Put, 'Cad' and 'An' seem to accumulate at the

beginning of dormancy and opposite situation was noticed during dormancy release. PAs frequently accumulate in plants in response to abiotic and biotic stresses [13]. The highest relative levels of 'Spm', 'Spd', Put, Cad, PEA, Ar and An in apple buds were observed subsequent to dormancy release (**Table 5**). Thus, the role of Spm, Spd, Put, Cad, PEA, Ar and An could be more important subsequent to dormancy release, i.e. during active growth. This result coincides with the earlier observations that within a plant the highest PAs concentration is associated with meristematic zones [38]. In this connection, it has been reported that spermidine was found to be the main PA in buds of mature peach (*Prunus persica*) at the beginning of spring growth [43]. The relative high accumulation of Spm and Spd during early phase of dormancy, **Table 5** indicates that it may play an important role in this process. Moreover, the increase in Put, Cad and PEA from the first sample till bud break indicates that it may play an important role in chilling tolerance. In this concern, it has been reported that Spd plays an important role in chilling tolerance of cucumber probably through prevention of chill-induced activation of superoxide-generating NADPH oxidases in microsomes [44]. PA conjugation with phenolic acids is usually associated with flowering [45] and our results (**Table 5**). In hazel buds, high levels of free PAs (spermidine and spermine) and also bound spermidine, correlated positively with bud burst in spring and their levels decreased in autumn [46]. It has been emphasized that PAs level is regulated to a large extent by conjugation, compartmentation and by oxidative enzymes [45]. Also, Sood and Nagar [47] found, in tuberose bulbs, that an inverse relationship between the concentration of free and conjugated PAs forms can be observed only for Put whereas conjugated and bound Spd and Spm show the same increase pattern with the start and release of dormancy. This indicates that the bound form of PAs has at least to some extent physio regulatory functions. In this connection, Altamura et al. [48] reported that, PAs affect many processes regulated by cytokinins and auxins and, in co-operation with these plant hormones, modulate various morphogenic processes.

On the other hand, it has been reported that a significant reduction in Put and Spm is involved in the early floral initiation in *P. tuberosa* [49]. These data along with the observed decrease in the ratio of Put to the rest of PAs during dormancy release of tuberose bulbs and vice-versa supports the hypothesis that the process relating to release of dormancy involves an interaction or balance between endogenous growth promoters and inhibitors as well as changes in the tissue sensitivity. The high increase of PEA level with dormancy release **Table 5** could have an impact on the synthesis of phenylpropanoids, including anthocyanins. In plants PEA can be transformed into 2-phenylacetaldehyde, and further converted to 2-phenylethanol [22]. The role of PEA in plants remains unclear. In common buckwheat PEA can be transformed into 2-phenylacetaldehyde which belongs to the compounds with the highest contribution to aroma of the plant [24].

Variations in the levels of arginine have been observed in apple buds during dormancy and subsequent to its release. These variations correlate well with those observed in the present study in relation to dormancy and bud break in apple buds. In general, a low level of arginine seems to accumulate at the beginning of dormancy and opposite situation was noticed during dormancy release (**Table 5**). Thus, the role of arginine could be more important subsequent to dormancy release, i.e. during active growth. This result coincides with the earlier observations that within a plant the highest amino acids concentrations are associated with bud break [50]. Moreover, it has been indicated that N (including amino acids) was present at low levels in buds or roots during the dormant stage and reached a maximum level just prior to bud break **[51]**.

Variations in the levels of anthocyanin have been observed in apple buds during dormancy and subsequent to its release. These variations correlate well with those observed in the present study in relation to dormancy and bud break in apple buds (**Table 5**), although anthocyanin do not see to be causally involved in dormancy release and probably was not involved in the dormancy-breaking mechanism.

Spraying apple trees with any of the tested materials (Dormex at 4%, calcium nitrate at 6% and thiourea at 2%) resulted in early bud opening in "Ain Shemer" apple variety. The favorable effect of the used substances on date of flower bud opening Table 2 may be due to their stimulation effect of natural gibberellin. In this connection, Subha-drabandhu **[52]**, concluded that the induction of flowering could be correlated with a natural rise in gibberellin which promotes flower formation in plants by either facilitating the formation of flowering hormone in the leaves or expressing it in the growing buds. The increase in percentage of bud break Table 3 may be attributed to their stimulation effect of buds opening on formation of sink in shoot tip. In this concern, Skene **[53]** reported that when a bud opens and attains the shape of shoot, its tip acts as a strong sink for metabolites and thus being interception center for photosynthates and nutrient results in earlier start of the bloom. In this concern, Kuroi **[54]** and Yang et al. **[55]** concluded that, cyanide ion may play a role in inducing the enzyme activity, promoting the retranslation of stored reserves and the uptake of nitrogen with water for bud break. 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 **[56]**. The favorable effect of the used substances on yield (**Table 4**) was mainly attributed to its positive action on enhancing growth parameters and photosynthetic pigments. In this concern, George et al. **[57]** suggested that water and nutrients may also be mobilized to the growing points at the expense of the developing fruits. Moreover, George et al. **[58]** reported that cyanamide application on grapes doubled yields

by increasing bud burst on cordons and number of spurs, numbers of shoot per spur and higher numbers bunches per shoot on cordons and spurs.

Regarding the effect of hydrogen cyanamide and other substances used on polyamines, PEA, arginine and anthocyanin content **Table 5**, it is clear from the present data, that buds of apple trees contained higher concentrations of polyamines, PEA, arginine and anthocyanin under foliar spray with any of the treatments than the control. These findings agreed with the suggestion in [59] that hydrogen cyanamide is directly involved in nitrogen metabolism and the production of protein. The degradation of cyanamide was found to occur through urea to other compounds and both are utilized in production of amino acids. Also, Foott [60] found that hydrogen cyanamide penetrates the bud scales, gets absorbed in the buds and initiates the processes leading to bud break. It is rapidly metabolized in the plant and helps in the synthesis of amino acids. Moreover, even though calcium nitrate gives a similar effect on bloom timing, we would suspect that it works in a different way. We would guess that calcium nitrate increases the number of solutes within the tree's cells, triggering cell expansion through changing the osmotic potential, and thus triggering the tree to bloom by causing the required hormone imbalance. Treatment with calcium nitrate or thiourea hastened the beginning of flower bud break and increased biogenic amines (spermine, spermidine, putrescine, cadaverine and phenylethylamine), arginine and anthocyanin in buds. This was due to the fact that these treatments resulted in more availability of macronutrients (N and/or Ca) to plants. Nitrogen is a major macro-element limiting the growth and development of plants in agriculture. It is a constituent of many important substances within plant cells such as protoplasm, in addition to amino acids, nucleic acids, protein and chlorophylls. There are other nitrogen forms, which include amino acids, nitrite and urea, that are metabolized in plants. Amino acids in cells take part in the synthesis of protein and other nitrogenous compounds that help in body building [61]. Calcium is an essential plant nutrient, participating in enzymatic and hormonal invasion. It also participates in metabolic processes of other nutrients uptake and promotes proper plant cell elongation (cellular division and extension) [62].

5. Conclusions

From the results of the present investigation, it could be concluded that, biogenic amine (phenylethylamine, spermine, spermidine, putrescine and cadaverine), arginine and anthocyanin were increased from dormancy initiation to dormancy release which decreased during deep dormancy and increased with bud break. Moreover, the application of hydrogen cyanamide (Dormex), calcium nitrate and thiourea greatly hastened bud break and increased these chemical constituents. The constituents of these substances participate in the different metabolic processes which increase the syntheses of metabolites and absorption of essential

nutrients, so that the use of hydrogen cyanamide (Dormex), calcium nitrate and thiourea could increase buds opining and consequently apple productivity.

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