Impact of Chilling Requirement on Budburst, Floral Development and Hormonal Level in Buds of Early and Late Apple Varieties (*Malus sylvestris*, Mill) under Natural Conditions

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Abstract. In order to produce the physiological bases for choosing early- flowering varieties that may avoid the insufficiently low winter temperatures, the early and late- opening apple varieties Barkhar, Local and Strakhan (*Malus sylvestris*) were investigated. The relation between the seasonal changes in endogenous hormones and flower buds opening date were studied. An improved understanding of the factors maybe managing budburst and development, and their underlying mechanisms is crucial for management of trees performance and fruit yield. This study investigated variations in chilling requirements, bud burst and development in early and late varieties of apple trees. The budburst and hormonal profile of flower and vegetative buds of early and late varieties were additionally investigated. Results showed less bud burst in late varieties than in early ones. In the former, promoters (indole-3-acetic acid and gibberellins) were increased at budburst. Although endogenous inhibitors levels of abscisic acid were considerably reduced by bud development in all varieties. We conclude that late varieties (Strakhan) are less economical in manufacturing new growth, as indicated by less bud vigor at budburst than early varieties (Barkhar and local) and show a marked differential hormonal level throughout bud development compared to early varieties.

Introduction

The majority of trees in temperate climates fulfills a chilling demand (CR) to overcome endodormancy and has a requirement of heat for flowering (Campoy et al. 2012). Chilling Hours (CH) is that the easiest way to estimate chilling requirement. Therefore, the CR could dissent by species, varieties, or growing regions (Stino, 1995, Luedeling and Brown, 2011, Wang et al., 2012, Andreini et al., 2012). CR is one the major issue that determines the bloom date (Albuquerque et al., 2008). Cultivars with low CR bloom and ripen earlier, whereas those with high CR bloom and ripen later (Scorza and Okie, 1990, El-Shewy et al., 1999a). CR constrains the acceptable areas of cultivation of the various commercially important tree species and cultivars around the world. Previous studies have indicated that CR might be quantitative character controlled by minimum of one major sequence (Hauagge et al., 1991, El-Shewy et al., 1999b). Winter chill is associate agro environmental condition issue that integrates the length of cold periods also as prevailing temperature ranges. If chilling requirements don't appear to be met, irregular, delayed and asynchronous growth, flowering and fruit set can be found in subsequent season if chilling requirements don't appear (Luedeling et al., 2009, Campoy et al., 2011). One of the possible effects of temperature change can be a delay inside the beginning of chill accumulation; the fulfillment of chilling requirements and then the time at those trees become receptive to heat through spring. Since flower and leaf emergence result from a minimum of part consecutive fulfillments of cold ('chilling') and warm ('forcing') requirements, later and slower chilling accumulation should thus end in later flower and growth in spring (Luedeling et al., 2013).

Changes within the equilibrium of phytohormones are related to the onset, maintenance and removal of dormancy in seeds, buds, bulbs and tubers. At present, the molecular regulation of dormancy is poorly understood (Michalczuk, 2005). The relation of plant hormones and bud

dormancy in additionally as bud break was a major down aspect for every theories and production practices. Seasonal changes in endogenous hormone during and after bud dormancy release was studied by many researchers (Guevara et al., 2008, Dong et al., 2009, Mornya and Cheng, 2011, Okay et al., 2011, Seif El-Yazal and Rady, 2014, Seif El-Yazal et al., 2014, 2018a, b, c & d). They found that the incidence, termination, regulation and management of dormancy were regulated by hormones throughout sufficiently winter chilling requirement. Additionally, this research may help further studies to be performed on how chilling requirement affect changes in the length to full bud break, plant growth regulators and help to express the effect of endogenous growth regulators on flowering and yield

Materials and Methods

The 12-year-old trees of 'Barkhar, local and Strakhan' apple trees (*Malus sylvestris* Mill.) grafted on Malling-Merton 106 (MM 106) rootstock were designated randomized, for a preliminary study in 2015/2016 and for the most analysis studies within the 2016/2017 and 2017/2018 seasons. All trees were full-grown within the wood lot (newly reclaimed saline chalky soil) of the Horticultural Station at Aboksah in Abshawai, Fayoum, Egypt. For the most 2-seasons study, designated trees of every selection (n = 6) were tagged in November 2015 and 2016, and sampled from September–March 2016/2017 and 2017/ 2018. Trees chosen for the study within the 1st season were not the identical trees that were designated for the second season. Each tree was designed as one replicate, and each variety included six trees (total n = 18).

Quantification of chilling requirements

In this study, from Nov to March of next year in the orchard, apple branches from every variety were collected every 15 days and cultivated in artificial lighting setup with water to determine the bud dormancy releasing time (50% bud break). Moreover, the quantity of chilling hours in the orchard (temperatures between 0 and 7.2 °C) throughout this period till the time of gap buds in every variety has been calculated (by using Thermograph). The foremost common chilling model, and one that is used wide, is the Chilling Hours Model, additionally referred to as the Weinberger Model (Bennett 1949, Weinberger 1950). This model that was first developed for peaches in Georgia (United States), interprets all hours with temperatures between 0 and 7.2 °C as effective for chilling accumulation. These chilling hours are accumulated through the winter season. Chilling hour's below 7.2°C from 1st November to every opening date in three apple varieties beneath study throughout each 2016/2017 and 2017/2018 seasons had been determined (Table 1).

Morphological characteristics and yield measurements on trees

Bud count was created for every tree (n = 6) in each variety. The dates on which floral and vegetative buds started to open were recorded. Additionally, the dates at that flowering reached 25, 50, 75 and 100 % of the full flowers were calculated for every variety. The dormant buds were additionally counted and were expressed, with opened buds, as a proportion of the full number of buds. The ultimate fruit set was calculated 6 weeks after full bloom stage as a variety of persisted fruits per hundred spur and lateral buds (Westwood, 1978). At harvest stage, apple fruits were harvested, counted and weighed for every examined tree.

Extraction and estimation of endogenous hormones in apple buds

Bud samples were collected, 15-day intervals, starting from one September up to fifteen March for determining the hormonal content in buds. Buds were haphazardly sampled and right away transported to the laboratory. Vegetative and floral bud samples were taken from each tree of every variety and were analyzed for endogenous levels of indole-3-acetic acid (IAA), gibberellin (GA₃) and abscisic acid (ABA). The extraction and purification was conducted according to Kettner and Doerffling (1995). Samples (1.0 g fresh buds) were collected, from every variety, and ground, at 4°C, in 80% methanol alcohol containing 0.1 g l⁻¹ an antioxidant, butylated hydroxyl toluene (BHT). The hormones were extracted at 4°C in dark for 72 h with subsequent modification of solvent. The extracted samples were centrifuged and therefore the supernatant was reduced to

aqueous phase using exploitation rotary skinny film evaporator (RFE). The pH of aqueous phase was adjusted to 2.5–3.0 and divided three times with 1/3 volume of ethyl acetate. The ethyl acetate phase was dried completely using RFE. The dried sample was utterly exploited in 1 ml of methanol (100 %) and was analyzed on HPLC (Shimadzu, C-R4A Chromatopac; SCL-6B system controller) using UV detector and C18 column (39 9 300 mm). For identification of hormones, 100 µl samples filtered through 0.45 millipore filters were injected into column. Pure IAA and GA₃ (Sigma, USA) were used as standards for identification and quantification of those hormones. The identification was created on the idea of retention time and peak space of the standards. Methanol, acetic acid and water (30:1:70, respectively) were used as a mobile part. The rate of flow was adjusted at 0.5 ml min⁻¹ with a mean time for fifteen min sample⁻¹. The wavelength used for the detection of IAA was 280 nm, whereas for GA₃ was 254 nm. For ABA, samples were injected onto a C18 column and eluted with a linear gradient of ethanol (70%), containing 0.01 % acetic acid, at a flow rate of 0.8 ml min⁻¹. The retention time of ABA decided by using authentic standards, observance the extraction of normal at 254 nm.

Statistical analysis

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

The values presented in the results obtained in this investigation are the mean of the two seasons under the study.

Results

Impact of winter chilling hours on bloom date

To confirm however the winter accumulated chilling hours affected the spring events; we tend to investigate the total bloom date (50% bud break) once completely different numbers of controlled chilling hours. Data in Table (1) show that the dormancy releasing time of Barkhar, Local and Strakhan varieties were 2nd of February, 23rd February and 24th March, after the accumulation of 232, 276 and 283(CH) respectively in the first season and were 1st of February, 1th March and 27th March, after the accumulation of 249, 280 and 285 (CH) respectively in the second season. There were about 21, 29 and 50 days difference in the first season, i.e., the occurrence of opening buds of Strakhan was 29 days later than that of Local and 50 days later than that of Barkhar in the first season and 27, 26 and 55 days difference in the second season and also the occurrence of opening buds of Strakhan was later than that of Local 26 days later and 55 days later than that of Barkhar in the second season, indicating that the chilling hours of Strakhan was higher than that of Local and Barkhar varieties. Strakhan variety needed more of chilling hours accumulative at low temperature (7.2°C) than Barkhar and Local for bud break. Moreover, the sum accumulative low temperature (chilling hours) of bud break were step by step happy within the two varieties, Barkhar and Local opened in February and initial of March thanks to meeting the necessity of expeditiously accumulative low temperature (CH7.2°C), whereas Strakhan still couldn't opened as a result of the expeditiously accumulative low temperature was but required for bud break (CH7.2°C).

Table 1. Chilling accumulation (hours below 7.2°C) from 1 November to each break date for each variety during 2016/2017 and 2017/2018

Varieties	Hours under temperature 7.2°C from 1 November to 50% bud break			
	2016/2017		2017/2018	
	Date of 50% bud break	Chilling Hours	Date of 50% bud break	Chilling horse
Barkhar	2 nd February	232	1 st February	249
Local	23 rd February	276	1 st March	280
Strakhan	24 th March	283	27 th March	285

Date of floral bud break in the orchard

With the buildup of low temperature, the chilling demand for fruit trees was step by step happy. Data in Table (2) indicated the dates to flowering (50% flowering) 15 February, 16 March and 16 April for Barkhar, Local and Strakhan, respectively. The earliness reached about 60 and 31 days for Barkhar and Local apple varieties respectively as comparison with Strakhan variety.

Table 2. Date of flower bud opening and flowering period in apple varieties in the orchard

Varieties	Date of flower bud opening					Flowering period (day)
	Beginning	25% flowering	50% flowering	75% flowering	End of flowering	
Barkhar	10 Feb	13 Feb	15 Feb	17 Feb	24 Feb	14
Local	5March	11March	16March	19March	23March	19
Strakhan	9 April	11April	16April	21 April	26 April	18

Proportion of bud break and fruit set

Data presented in Table (3) indicated that early- opening apple varieties gave a high percentage of flower bud break and fruit set comparing with the late-opining apple variety. The proportion of flower bud break was 85.83 and 82.96% for Barkhar and Local apple varieties respectively as comparison with 72.17% for Strakhan variety. However, the percentage of fruit set was 58.53 and 37.05% for Barkhar and Local apple varieties respectively as comparison with 14.11% for Strakhan variety.

Table 3. Percentage of bud break, dormant buds and fruit set in three apple varieties

Varieties	Bud break (%)	Dormant buds (%)	Fruit set (%)
Barkhar	85.83a	14.17a	58.53a
Local	82.96b	17.04b	37.05b
Strakhan	72.14c	27.86c	14.11c

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

Number of fruit tree⁻¹ and fruit yield

Data in Table (4) also show that, early-flower opening apple varieties (Barkhar and Local) have great number of apple fruits tree⁻¹ and total fruit yield tree⁻¹ when compared to the late-opening apple variety (Strakhan). Which exceeded by 94.12 and 69.03% for number of fruits tree⁻¹ and 63.75 and 20.90 % for fruit yield tree⁻¹ in Barkhar and Local apple varieties respectively as comparison with Strakhan variety.

Table 4. Number of fruit tree⁻¹ and yield per tree (kg) in three apple varieties

Varieties	No. of Fruit tree ⁻¹	Yield per tree(Kg)
Barkhar	424.17a	25.02a
Local	369.34b	18.45b
Strakhan	218.50c	15.26c

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

Indole acetic acid (IAA)

Relative amounts of IAA in the studied flower and vegetative buds of Barkhar, Local and Strakhan varieties throughout the concerned period are shown in Table (5). It is clear from the present data that IAA content in flower buds of Barkhar, Local and Strakhan apple varieties decreased gradually from the first sample (1st of September) reaching its minimum content on the 15th of November for Barkhar, 1st of December in Local and 1st of January for Strakhan varieties with some fluctuation. Thereafter, IAA content increased sharply towards the last sample (maximum content) in the three studied apple varieties. It is clear from the results mentioned above that IAA content reached its minimum content during deep dormancy then started to accumulate

markedly during the early spring as buds opened. The same trend has been recorded in vegetative buds.

Table 5. Seasonal changes in the level of IAA ($\mu\text{g./g. D.W.}$) in vegetative (V) and flower buds (F) of three apple varieties during and after release from dormancy

Dates	Varieties					
	"Barkhar"		"Local"		"Strakhan"	
	V.	F.	V.	F.	V.	F.
1/9	4.15a	5.51a	6.85a	6.41a	8.04a	7.29a
15/9	3.95a	4.61b	6.35a	6.31a	7.85a	7.29a
1/10	2.18b	1.52c	6.24a	6.10a	6.14a	5.81b
15/10	1.98b	1.26c	6.01a	5.20b	4.02b	4.66b
1/11	1.25d	1.16d	4.18b	4.54b	2.99c	3.50c
15/11	1.49c	1.36c	2.02c	2.26c	2.15c	2.26c
1/12	1.10d	0.86d	0.78d	0.85d	1.45d	1.85d
15/12	1.84b	1.42c	1.54c	1.29d	1.09d	1.84d
1/1	1.95b	1.86c	1.66c	1.30d	0.85d	1.28d
15/1	3.99a	4.77a	3.04b	2.08c	3.89b	3.51c
1/2	4.15a	4.89a	3.25b	3.02c	4.15b	3.90b
15/2	-----	-----	6.17a	5.19b	4.91b	4.08b
1/3	-----	-----	7.56a	6.88a	7.18a	6.96a
15/3	-----	-----	-----	-----	7.25a	7.88a

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

Mean difference between dates of sampling

Gibberellic acid

Table (6) represent the fluctuation in the content of GA_3 in the flower and vegetative buds. It is evident that GA_3 content in Barkhar, Local and Strakhan apple varieties increased gradually from (1st of September) till 15th of October in Barkhar, 1st October in Local and 1st of November for Strakhan varieties, followed with marked decrease reaching its minimum content on the 15th of November for Barkhar, 1st of December in Local and 1st of January in Strakhan varieties. Thereafter it increased markedly reaching its most content within the last sample (maximum content) altogether the three studied apple varieties. It's worthy to notice that the goodish increase within the level of GA_3 was evident throughout the active growth period. On contrast they were poorly low through the period of retarded growth. The same trend was observed in vegetative buds.

Table 6. Seasonal changes in the level of GA_3 ($\mu\text{g./g. D.W.}$) in vegetative(V) and flower buds (F) of the three apple varieties during and after release from dormancy

Dates	Varieties					
	"Barkhar"		"Local"		"Strakhan"	
	V.	F.	V.	F.	V.	F.
1/9	1.74b	1.13c	4.02b	3.93b	2.47c	2.14c
15/9	1.84b	1.14c	5.09a	4.56a	2.98c	2.94c
1/10	1.95b	1.52b	5.21a	5.95a	4.55b	4.22b
15/10	2.01b	1.61b	4.14b	4.49a	5.38b	5.18a
1/11	1.18c	1.23c	3.81b	3.77b	6.21a	5.95a
15/11	0.94d	0.71d	1.54c	1.32c	3.18b	2.82c
1/12	1.11c	0.95d	1.01d	0.69d	1.97d	1.62d
15/12	1.25c	1.05c	1.86c	1.15c	1.84d	1.52d
1/1	1.30c	1.10c	2.48b	2.69b	1.25d	1.14d
15/1	1.52b	1.64b	2.98b	2.93b	1.47d	1.36d
1/2	3.93a	4.47a	6.87a	6.52a	3.98b	3.51b
15/2	-----	-----	7.01a	6.89a	4.75b	4.65b
1/3	-----	-----	7.33a	6.95a	7.04a	6.98a
15/3	-----	-----	-----	-----	7.23a	7.05a

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

Mean difference between dates of sampling

Abscicic acid

It is clear from the data in Table (7) that ABA content in flower and vegetative buds gradually decreased from the first sample (1st of September) till 1st October in Barkhar and till 1st of November in both Local and Strakhan variety followed with marked increase reaching its maximum content on the 15th of November in Barkhar, 1st of December for Local and 1st of January for Strakhan apple varieties. Thereafter it decreased in the three studied apple varieties reaching its minimum content in the last sample.

Table 7. Seasonal changes in the level of ABA ($\mu\text{g./g. D.W.}$) in vegetative (V) and flower buds (F) of three apple varieties during and after release from dormancy

Dates	Varieties					
	"Barkhar"		"Local"		"Strakhan"	
	V.	F.	V.	F.	V.	F.
1/9	0.35b	0.40c	0.95d	0.81c	0.30b	0.23b
15/9	0.30b	0.33b	0.89d	0.82c	0.25a	0.18a
1/10	0.21b	0.18a	0.66c	0.54b	0.18a	0.16a
15/10-	0.31b	0.34b	0.42b	0.30a	0.19a	0.14a
1/11	0.49c	0.48c	0.30b	0.25a	0.28b	0.24b
15/11	0.85d	0.91d	0.65c	0.79c	0.31b	0.28b
1/12	0.67c	0.71d	0.98d	0.96d	0.29b	0.27b
15/12	0.59c	0.46c	0.86d	0.91d	0.71d	0.82d
1/1	0.35b	0.29b	0.41b	0.31a	0.81d	0.84d
15/1	0.12a	0.18a	0.22a	0.27a	0.65c	0.58c
1/2	0.11a	0.17a	0.19a	0.23a	0.32b	0.43c
15/2	-----	-----	0.18a	0.21a	0.26a	0.27b
1/3	-----	-----	0.17a	0.20a	0.22a	0.18a
15/3	-----	-----	-----	-----	0.21a	0.20a

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

Mean difference between dates of sampling

Indole acetic acid /abscicic acid ratio

Data in Table (8) indicated the ratio of IAA/ABA in the studied flower and vegetative buds of Barkhar, Local and Strakhan varieties throughout the opening period. It is clear from the present data that IAA/ABA ratio in flower buds are decreased gradually from the first sample (1st of September) reaching its minimum content on the 1st of December for Barkhar and Local and 1st of January for Strakhan varieties with some fluctuation. Thereafter, IAA/ABA ratio increased sharply towards the last sample (maximum content) in the three studied apple varieties. It is clear from the results mentioned above that IAA/ABA ratio reached its minimum content during deep dormancy then started to accumulate markedly during the early spring as buds opened. Similar trend was recorded in vegetative buds.

Table 8. Seasonal changes in the level of IAA/ABA ratio in vegetative (V) and flower buds(F) of three apple varieties during and after release from dormancy

Dates	Varieties					
	"Barkhar"		"Local"		"Strakhan"	
	V.	F.	V.	F.	V.	F.
1/9	11.85b	13.77b	.692c	7.91c	26.80b	31.69a
15/9	13.16b	13.96b	7.13c	7.79c	31.40a	40.50a
1/10	10.38b	8.44c	9.45b	11.29b	34.11a	36.31a
15/10	6.38c	9.00c	14.30b	17.33b	21.15b	33.28a
1/11	2.55d	.136d	13.93b	1.80d	10.67b	14.58b
15/11	.175d	3.31d	3.10d	8.07c	6.93c	8.07c
1/12	.164d	1.21d	0.79d	1.07d	5.00c	6.85c
15/12	3.11d	1.40d	4.04d	1.41d	.153d	2.24d
1/1	5.57c	6.41c	1.79d	4.19d	1.04d	1.52d
15/1	33.25a	26.50a	13.81b	7.07c	5.98c	6.05c
1/2	37.72a	28.76a	17.10b	13.13b	12.96b	9.06b
15/2	-----	-----	34.27a	24.71a	18.88b	15.11b
1/3	-----	-----	44.47a	32.76a	32.63a	38.66a
15/3	-----	-----	-----	-----	34.52a	39.40a

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

Mean difference between dates of sampling

Gibberellic acid/abscisic acid ratio

Table (9) represent the fluctuation in the ratio of GA₃/ABA in the flower and vegetative buds. It is evident that GA₃/ABA ratio in Barkhar, Local and Strakhan apple varieties increased gradually from (1st of September) till 1st of October in Barkhar, 1st November in Local, and Strakhan varieties, followed with marked decrease reaching its minimum content on the 15th of November for Barkhar, 1st of December in Local, and 15th of December in Strakhan varieties. Thereafter it increased markedly reaching its most content within the last sample (maximum content) altogether the three studied apple varieties. It's worthy to notice that the goodish increase within the level of GA₃/ABA ratio was evident throughout the active growth amount. Whereas they were poorly low through the period of retarded growth. Similar trend was recorded in vegetative buds.

Table 9. Seasonal changes in the level of GA₃/ABA ratio in vegetative (V) and flower buds (F) of the three apple varieties during and after release from dormancy

Dates	Varieties					
	"Barkhar"		"Local"		"Strakhan"	
	V.	F.	V.	F.	V.	F.
1/9	4.97c	2.82c	4.23c	4.85c	8.23c	9.30c
15/9	6.13c	3.45c	5.71c	5.62c	11.92b	16.33c
1/10	9.28b	8.44b	7.89c	11.04b	25.27a	33.16a
15/10	6.48c	7.14b	9.85b	14.96b	28.13a	37.00a
1/11	1.38d	1.35d	12.70b	15.02b	22.17b	24.75b
15/11	1.10d	0.78d	2.36d	.167d	10.25b	10.07c
1/12	1.65d	1.33d	1.03d	.071d	6.79c	6.00c
15/12	2.11d	2.28c	2.16d	3.48d	2.59d	1.85d
1/1	3.71c	3.79c	6.04c	8.67b	1.54d	4.75d
15/1	12.66b	9.11b	13.54b	10.85b	2.26d	7.55c
1/2	35.72a	26.29a	36.15a	28.34a	12.43b	27.00b
15/2	-----	-----	38.94a	32.80a	18.26b	25.83b
1/3	-----	-----	43.11a	34.75a	32.00a	25.83b
15/3	-----	-----	-----	-----	34.42a	44.00a

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

Mean difference between dates of sampling

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 bud dormancy releasing to bud break, many seasonal changes in some chemical constituents of buds, especially, endogenous hormones played a vital role in regulating dormancy and bud break, but current studies mainly focused on the dormancy and bud break of buds (Dong et al., 2009). Low temperature accumulation in dormancy stage was indispensable to bud break for deciduous fruit trees (Table 1). Such models as (chilling hours Below 7.2°C) model, was the main dormancy releasing model (Weinberger, 1950), and their accuracy for determining the dormancy releasing time was probably influenced by specific environmental conditions due to lacking of the basis of physiological process. Throughout the method of bud dormancy cathartic to bud break, several physiological and biochemical indexes modified considerably in bud, like starch content, soluble sugar content, vital sign, metastasis pathways, protein content, amino acid content, bound water/free water quantitative relation, membrane consistence, etc. Especially, endogenous hormones played a vital role in regulating dormancy and bud break (Table 5, 6 & 7), but current studies mainly focused on the dormancy and bud break of buds. Hormone helped plants responding to environmental signals (Horvath et al. 2003). IAA might have a bearing on transcription of nuclear DNA that may contribute to cell enlargement, promote fruit development, and are concerned in apical dominance. The endogenous gibberellins additionally concerned the dormancy-releasing and early sprout growth in apple buds (Seif El-Yazal et al., 2018 c). 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 (El-Shewy et al., 1999 c&d, Horvath et al., 2003, Rohde et al., 2007). In this connection, Duan et al. (2005) reported that GA₃ concentration in cherry flower buds was reported to increase significantly inside the dormancy cathartic procedure and up to at the peak at terrible high speed before complete dormancy discharged. The rise within the endogenous content of bioactive GA₁ is not related to bud dormancy unharness, however with insurant bud sprouts growth (Suttle, 2004, Hartmann et al., 2011). Moreover, Sun (2006) found on *Lilium davidii* power unit Unicol that substances changes in mother scales were regulated by endogenous GA₃, whereas largely by endogenous ABA in female off spring scales. With the cathartic of dormancy, growth-promoting hormones like GA₃ and IAA accumulated stepwise, whereas growth-inhibiting hormones like ABA faded. GA₃ and ABA had the identical material substance MVA (Me-Valonic acid) for his or her synthesis and so the increasing lighting time promoted the synthesis of GA₃ in buds (Dong et al., 2009). Results in Table (6) indicated that GA₃ concentration in bud of Barkhar and Local when dormancy cathartic was on top of that of Strakhan. During the whole testing period, IAA, GA₃, IAA/ABA and GA₃/ABA concentrations in buds of three apple varieties at their release from dormancy was higher than those of before bud break. In contrast, ABA concentration in buds was higher before bud break than that of at dormancy releasing (Table 5, 6, 7 & 9). In this concern, Tu and Ding (2003) found that, within the process from dormancy to bud break, GA₃ /ABA ratios of *Toona sinensis* buds initial faded and so augmented. Also, gibberellic acid (GA₃) and ABA signaling as well as GA₃/ABA ratios are important as known for tree bud sprouting (Horvath et al. 2003; Rohde et al. 2007). GA₃ might promote the synthesis of sure mRNA and enzymes and increased the activity of certain enzymes like proteolytic enzyme, RNase and isocitrate lyase etc. Considering at the molecular level, it'd undoubtedly that there are existed associate unknown issue cooperating with GA₃ to alleviate the inhibiting state and guaranteed the transcribing methodology of sequence equine as promoted the synthesizing of mRNA and molecule. GA₃ might unleash dormancy and promote germinating for it might remove the inhibiting factors of sure mRNA (Suttle, 2004, Alexopoulos et al., 2008). With the alternative result, ABA might assembly of sure RNA indispensable to the synthesis of α -amylase, and so the most role of ABA at intervals the method of germination was restraining GA and inflicting the transforming of reserve substance (Gubler, 2005). Once Barkhar opened in mid-February, concentration of GA₃ in Barkhar was higher, because the total with efficiency

accumulative of low temperature (CH 7.2°C) meet the necessity of dormancy unleash, the concentration of ABA was lower, that resulted within the magnitude relation of GA₃ /ABA was higher (Table 9). Our results showed that when the dormancy released, IAA/ABA ratio had the same changing tendency to IAA content (Table 8). So, it could be presumed that IAA/ABA and GA₃/ABA ratio determined the metabolism direction. In this concern, Dong et al. (2009) suggested that the ratios of IAA/ABA and GA₃/ABA in apple buds were decreased in dormant buds, while increased in opening ones, which the balance of several hormones played a more important role than the level of certain single hormone in the procedure of dormancy releasing and opening of buds.

Conclusion

Accumulation of chilling hours in the winter was effect on dormancy release throughout the effect on the concentration of plant hormones. In late-opened variety Strakhan the chilling requirements was much higher than that in early-opened varieties Barkhar and Local at bud break. Strakhan needed high level of growth-promoting hormones to opened, such as GA₃ and IAA. The high levels of GA₃ and IAA concentration in Barkhar and Local at bud break led to significantly higher GA₃ /ABA and (GA₃+IAA)/ABA ratios than in Strakhan. Finally, from the results of the present investigation, it could be concluded that, under sufficient chilling requirements, growth-promoting hormones (IAA and GA₃) were increased from dormancy initiation to dormancy release which decreased during deep dormancy and increased with bud break. In contrast growth-inhibitor hormones ABA was decreased from dormancy initiation to dormancy release which increased during deep dormancy and decreased with bud break

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