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Seasonal Changes in Soluble and Non-Soluble Carbohydrates during and After Dormancy Release in Early and Late Varieties of Apple (Malus Sylvestris, Mill) Trees

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Abstract

The flower bud abortion is one of the main problems that limit apple varieties production in the Egypt. Insufficient chilling during the dormancy period is known as the main factor of this problem. One of the hypotheses to explain this problem is that the starch mobilization and carbohydrate fluxes to the buds are impeded when mild temperatures occurred during winter. In order to produce the physiological bases for choosing early- opining apple varieties that may avoid the depleted low winter temperatures, the terribly early and late- opining apple variety Barkhar, Local and Strakhan (*Malus domestica*) were used to study the relation between the seasonal changes and balance of water, dry weight and carbohydrates metabolism and opining time. This study investigated potential variations in chilling requirements, bud burst and development in early and late varieties of apple trees. The budburst and carbohydrates metabolism 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, there were increased in water, dry weight and soluble carbohydrates (sugars) at budburst. Although non-soluble carbohydrates levels was 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 in water content and carbohydrates metabolism throughout bud development compared to early varieties.

Keywords: Apple; Dormancy; Chilling Requirements; Water Content; Dry Weight; Non-Soluble Carbohydrates; Sugars.

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1. Introduction

Dormancy in deciduous fruit trees may be a physiological syndrome occurring annually to alter plants to survive cold winters. Deciduous trees need exposure to low winter temperatures is named a chilling requirement to beat dormancy and grow within the following spring (Howe et al., 1999 and Arora et al., 2003). Temperate zone fruit crops bear bud dormancy which may be represented as a mechanism for avoiding the exposure of tender flowers and leaves to low winter temperatures (Njuguna et al. (2004).

Plant growth and development are also influenced by nutrients like as sucrose, glucose and fructose; that represent the most assimilates of most plants (Katovich et al., 1998 and Pallardy, 2008). Sucrose is that the main sugar transported through the plant, and breakdown of sucrose could be a very important supply of energy and carbon skeletons required within the synthesis of amino acids, lipids and metabolites (Pallardy, 2008).Changes in sugar levels also are related to flowering, and alterations of soluble sugar contents regulate plant growth processes. Studies show that carbohydrates regulate dormancy standing, which sucrose and glucose not solely inhibit bud growth (Chao et al., 2006), however conjointly influence shoot emergence, bud formation and flowering (Katovich et al., 1998). Sugars are also thought to be concerned throughout the transition of vegetative buds from paradormancy to endodormancy (Arora et al., 2003; Anderson et al., 2005 and Chao et al., 2006) and are vital for maintaining paradormacy, probably moving cell cycle progression at the G1/S phase (Anderson et al., 2001; Horvath et al., 2002; Arora et al., 2003 and Horvath et al., 2003). In the initial phases of dormancy analysis, the supply of sugars was believed to be the crucial think about dormancy, associate degree short offer of sugars being the explanation for dormancy and therefore the unharness from dormancy by chilling being lead to by the promotion of process changing starch into sugar. In this respect, Benkeblia et al**.** (2004), Xu et al.(2006), Chao et al. (2006), Mornya and Cheng (2011), Mornaya et al.(2011), Marafon et al.(2011), Mornya and Cheng (2013), Rady and Seif El-Yazal (2013), Rady and Seif El-Yazal (2014),Seif El-Yazal et al.(2018), Zhang et al.(2018) who reported that total carbohydrates and total soluble sugars were at their minimum values during dormancy period and started to increase upon bud break with maximum values at full blooming. Whereas the insoluble carbohydrates showed an opposite trend.

2. Material and Methods

Trees selection Uniform, 12-year-old ‘Barkhar, local and Strakhan’ apple trees (Malus sylvestris Mill.) grafted on Malling-Merton 106 (MM 106) rootstock were selected at random, for a preliminary study in 2015/2016 and for the main research studies in the 2016/2017 and 2017/2018 seasons. All trees (n = 18) were grown in the Orchard (newly reclaimed saline calcareous soil) of the Horticultural Station at Aboksah in Abshawai, Fayoum, Egypt (29170N; 30530E). For the main 2-season study, selected trees of each variety (n = 6) were

labeled in November 2015 and 2016, and sampled from 1 September–15 March 2016/2017 and 2017/2018. Trees chosen for the study in the first season is not the same trees that were selected for the second season. Each tree was designed as one replicate, and each variety included six trees (total n = 18).

2.1. Quantification of Chilling Requirements

In this study, from November to March of next year, apple branches from each variety were collected every 15 days and cultivated in artificial lighting incubator with water to determine the bud dormancy releasing time (50% bud break). Moreover, the number of chilling hours (temperatures between 0 and 7.2 ℃) during this period until the time of opening buds in each variety has been calculated (by using Thermograph). The most common chilling model, and one that is used widely, is the Chilling Hours Model, also known as the Weinberger Model (Bennett 1949, Weinberger 1950). This model, which 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 each opining date in three apple varieties under study during both 2016/2017 and 2017/2018 seasons had been determined (Table 1).

Varietes	Hours under temperature7.2°C from1st November to 50% bud opining time			
	2016/2017		2017/2018	
	Date of 50% bud opining	Chilling Hours	Date of 50% bud opining	Chilling horse
Barkhar	2 st February	232	1st February	249
Local	23 th February	276	1st March	280
Strakhan	24th March	283	27th March	285

Table-1: Chilling accumulation (hours below 7.2°C) from1st November to each opining date for each variety during both 2016/2017 and 2017/2018 seasons

2.2. Morphological Characteristics and Yield Measurements

Bud count was made for each tree (n = 6) in each treatment. The dates on which floral and vegetative buds started to open were recorded. In addition, the dates at which flowering reached 25, 50, 75 and 100 % of the

total flowers were estimated in each variety. The dormant buds were also counted and were expressed, with opened buds, as a percentage of the total number of buds. The final fruit set was calculated 6 weeks after full bloom stage as a number of persisted fruits per hundred spur and lateral buds (Westwood, 1978). At harvest stage, apple fruits were harvested, counted and weighed for each examined tree.

2.3. Chemical Analyses

Samples of floral and vegetative buds were collected from each replicate of each treatment 15 days interval started from September, 2016 till March, 2017 in the first season and from September, 2017 till March, 2018 in the second season, for determining the changes in the contents of water, dry weight, sugars and total and non-soluble carbohydrates. Bud samples were taken at random and immediately transported to the laboratory for the following determinations:

2.4. Water Content

The water content of buds was determined by the methods described by A.O.A.C. (1995).

2.5. Dry Weight

The dry weight content of buds was determined by the methods described by A.O.A.C. (1995) and expressed as mg bud^{-1}.

2.6. Total Carbohydrates Determination

Total carbohydrates were extracted from floral and vegetative buds by placing 10 mg dry sample with 10 ml of H_2SO_4 (0.1 N) in test tubes and extracted on a boiling water bath for 30 min. Samples were filtered to remove the insoluble material. After filtration, the solution was completed to 100 ml with distilled water. Carbohydrate contents were determined by phenol-sulfuric acid method (Rao and Pattabiraman, 1989). Briefly, 50 µl 80% phenol was added to 1 ml of sugar solution and then 3 ml 98% sulfuric acid was added. The mixture was vortexed and kept at room temperature for 30 min and the absorbance was read at 490 nm and expressed as mg g^{-1} dry weight.

2.7. Total Sugars and Non-Soluble Carbohydrate Determinations

Total sugars were determined (mg g^{-1} dry weight) using phosphomolybdic acid reagent (A.O.A.C., 1995). Briefly, sample of 0.5 g frozen buds was crushed in a porcelain mortar and extracted with 50 ml of 80% (v/v) boiling ethanol for 5 min. Sample was filtered to remove the insoluble material. The extract was centrifuged at 10,000 rmp for 10 min. The volume of supernatant was adjusted to 100 ml with water. Protein was precipitated by adding 1.0 ml of ethanol extract with 3 ml of basic lead acetate (137 g l^{-1} distilled water) and the excess of lead acetate was precipitated with a solution of 1 M sodium phosphate monobasic (141.7 g l^{-1} distilled water). The mixture was centrifuged and the volume of the supernatant was completed to 10 ml. To

determine total sugars, 1 ml of the filtrate was mixed with 1 ml 1 N HCL then the mixture was neutralized with sodium bicarbonate solution (1 N) and the volume was completed to 5 ml. Total sugars were determined using a known volume as described in A.O.A.C. (1995), 1 ml of the filtrate was mixed with 1 ml of copper sulphate solution (13.2 g sodium sulphate and 6.0 g copper sulphate were dissolved in 1 l distilled water) and 1 ml of alkaline tartarate solution (12 g sodium potassium tartarate, 20 g anhydrous sodium carbonate, 20 g sodium bicarbonate and 18 g potassium oxalate were dissolved in 1 l distilled water). The mixture was heated in boiling water bath for 10 min. After cooling, 2 ml of phosphomolybdic acid reagent (23 g molybdic acid and 5 g sodium tungstate were dissolved in 200 ml of 10% sodium hydroxide and boiled for 20–30 min. After cooling, 125 ml of phosphoric acid were added and the volume was completed to 500 ml with water) were added. The developed blue colour was measured at 540 nm.

Non-soluble carbohydrates were obtained by subtracting total sugar content from total carbohydrate content and expresses as mg g^{-1} dry weight.

The values presented in the following results expressed the mean of the two seasons studied.

3-Result

3.1. Low Winter Chilling Hours Delayed Bloom Date

To confirm how the winter accumulated chilling hours affected the spring events; we investigated the full bloom date after different numbers of controlled chilling hours. In each case, we used the number of days that elapsed between moving into the greenhouse and reaching full bloom, also known as the days to full bloom date. Data in Table (1) clearly show that the dormancy releasing time of Barkhar, Local and Strakhan varieties were 2st of February, 23th February and 24th March, after the accumulation of 232, 276 and 283(CH) respectively in the first season and were1 st of February, 1th March and 27th March, after the accumulation of 249, 280 and 285(CH) respectively in the second season. Data also show that 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 requirement of Strakhan was higher than that of Local and Barkhar varieties**.** Strakhan Varity needed more of sum efficiently accumulative low temperature at (7.2°C) than Barkhar and Local for bud break. Moreover, the sum efficiently accumulative low temperature (chilling hours) of bud break were gradually satisfied in the two varieties, Barkhar and Local opened in February and first of March due to meeting the need of efficiently accumulative

low temperature (Ch7.2°C), while Strakhan still couldn't opened because the efficiently accumulative low temperature was less than needed for bud break (Ch7.2°C).

3.2. Date of Floral Bud Break

With the accumulation of low temperature, the chilling requirement for fruit trees was gradually satisfied. When the environment was suitable, the bud would open. However, if the environment temperature was high, buds could not open, which was called endodormancy. Data in Table (2) indicated that, the dates to full flowering (50% flowering) 15 February, 16 March and 16 April for Barkhar, Local and Strakhan apple varieties respectively. The earliness reached about 60 and 31 days for Barkhar and Local apple varieties respectively as comparison with Strakhan variety.

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

Table-2: Date of flower bud opening and flowering period in the three apple varieties

3.3. Percentage of Bud Break and Fruit Set

Data presented in Table (3) clearly show that all early- opining apple varieties gave a high percentage of flower bud break and fruit set as compared with the late-opining apple variety. The percentage 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.

3.4. Fruit Yield and its Components

Data in Table (3) also show that, all early- opining apple varieties have great number of apple fruits tree^{-1} and total fruit yield tree^{-1} when compared to the late- opining apple variety. It 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 the Barkhar and Local apple varieties respectively as comparison with Strakhan variety.

Varieties	Bud break (%)	Dormant buds (%)	Fruit set (%)	No. of Fruit tree^{-1}	Yield per tree(Kg)
Barkhar	85.83	14.47	58.53	424.17	25.02
Local	82.96	17.04	37.05	369.34	18.45
Strakhan	72.14	27.86	14.11	218.50	15.26

Table-3: Percentage of bud break, dormant buds, fruit set, number of fruit tree^{-1}and yield per tree (kg) in the three apple varieties.

3.5. Water Content

Total water content of buds was measured at 15 days intervals from first of September till 15th of March as shown in Table (4).It is clear from the data that water content in the vegetative buds of the three studied varieties is generally decreased slightly from the first sample reaching its minimum content at 15th of December then increased gradually towards the last sample. As regards to water content of the flower buds of Barkhar variety, data in Table (4) show that it increase gradually from the first sample (1st September till 1st of November followed with slight decrease till 1st of January. Thereafter, water content increased markedly towards the last sample (1st of February). Water content of both Local and Strakhan varieties decreased slightly from the first sample (1st September till 1st of January in Local and 15th of December for Strakhan variety. Thereafter, it increased gradually towards the last sample.

Dates	Varieties					
	"Barkhar"		"Local"		"Strakhan"	
	V.	F.	V.	F.	V.	F.
1/9	48.01	45.81	46.58	50.00	45.98	46.59
15/9	47.91	45.40	47.19	50.00	47.50	46.50
1/10	47.01	46.57	52.76	48.27	50.31	47.85
15/10	46.90	50.80	52.80	47.41	49.93	47.55

1/11	45.08	53.85	48.91	45.28	50.06	45.69
15/11	45.32	51.08	47.26	45.90	50.40	43.04
1/12	44.17	45.53	45.71	46.04	48.89	42.35
15/12	42.80	44.01	39.18	46.68	36.81	42.03
1/1	42.94	42.50	44.77	42.49	43.79	46.49
15/1	47.10	53.97	47.18	38.54	49.25	26.68
½	48.58	58.35	49.78	54.33	53.93	47.91
15/2	48.58	61.70	50.56	59.66	53.68	49.86
1/3	-------	-------	-------	-------	56.86	49.91
15/3	-------	-------	-------	-------	56.95	50.50

V. =Vegetative buds F. =Flower buds

Table-4: Seasonal changes in the percentage of water content in buds of the three apple varieties during and after release from dormancy

3.6. Dry weight of Buds

The values of dry weight of either vegetative or flower buds are shown in Table (5). It is generally clear that dry weight of vegetative buds of the all studied varieties increased slightly from the first sample (1st September towards the last sample. Although, a period decrease occurred in the dry weight of different varieties till 1st of December in Barkhar, 15th of December in Local and 1st of January in Strakhan varieties, respectively (during rest period. As regards to the dry weight of the flower buds, data also show that the dry weight of the three studied varieties increased gradually from the first sample (1st September till 15th of November for Barkhar and Local and till last sample for Strakhan varieties, although with some periods of decrease at 15th of December and 1st of February and till1st of January for both Barkhar and Local varieties.

V. =Vegetative buds F. =Flower buds

Dates	Varieties					
	"Barkhar"		"Local"		"Strakhan"	
	V.	F.	V.	F.	V.	F.
1/9	8.00	50.00	9.00	44.50	8.00	60.00
15/9	8.00	50.00	9.50	45.00	8.50	62.00
1/10	8.00	53.00	10.00	46.50	9.00	65.00
15/10	12.00	54.21	10.50	47.00	9.00	69.50
1/11	13.00	64.71	12.50	50.50	10.00	73.00
15/11	11.00	66.00	15.00	57.50	12.00	82.57
1/12	10.00	55.00	14.00	55.00	14.00	84.00
15/12	15.00	50.50	13.00	53.00	14.00	78.00
1/1	17.00	47.00	14.00	48.00	12.50	81.50
15/1	18.00	50.00	14.00	54.00	17.00	83.00
1/2	22.00	56.00	14.00	55.00	16.00	75.00
15/2	22.10	60.01	16.00	55.00	16.00	77.00
1/3	------	------	------	------	16.00	98.00
15/3	------	------	------	------	16.50	98.50

Table-5: Seasonal changes in the average dry weight of buds (mg/Bud) of the three apple varieties during and after release from dormancy

3.7. Soluble Carbohydrates

Total sugars were found in the vegetative and flower buds at different intervals during the considered seasons are presented in Table (6).It can be seen that the values of sugars in the vegetative buds of the studied varieties generally decreased from the first sample reaching its minimum at 15th of November and 1st of November for

Strakhan Local and Barkhar varieties. Thereafter, total sugars content increased gradually till 1st of February in Barkhar and Local and till 15th February in Strakhan varieties which reached its maximum amount. As regards to the total sugars of the flower buds, data show that it increased gradually from the first sample till (15th October) followed by marked decrease till the 1st of December in Barkhar and Local and till 1st of January in Strakhan varieties. Henceforth, total sugars content markedly increased till the last sample with some period of decrease just before bud burst.

Dates	Varieties					
	"Barkhar"		"Local"		"Strakhan"	
	V.	F.	V.	F.	V.	F.
1/9	100.11	90.00	86.73	66.21	90.04	78.00
15/9	97.04	91.84	81.63	71.43	86.53	79.15
1/10	97.94	94.39	81.84	81.63	86.74	87.59
15/10	102.24	102.20	91.84	91.84	89.29	93.59
1/11	94.39	86.74	89.29	91.69	71.43	80.67
15/11	86.74	71.43	76.53	81.63	61.33	79.99
1/12	63.81	51.02	61.12	61.22	66.33	77.66
15/12	76.68	77.66	84.19	68.01	71.74	74.68
1/1	100.69	90.02	88.28	76.08	76.43	64.51
15/1	107.14	102.60	107.17	90.29	82.38	88.34
1/2	109.80	104.95	106.94	109.69	88.24	116.76
15/2	109.84	106.73	107.64	100.38	105.37	102.80
1/3	--------	--------	107.69	101.13	104.44	107.81
15/3	--------	--------	-------	105.07	107.93	111.19

V. =Vegetative buds F. =Flower buds

Table-6: Seasonal changes in the total sugars content in buds (mg./g. D.W.) of the three apple varieties during and after release from dormancy

3.8. Non-soluble Carbohydrates

Changes in the amount of non- soluble carbohydrates in all studied apple varieties throughout the period from 1st September till last sample are presented in Table (7). It is evident that the amount of non- soluble carbohydrates in the vegetative buds of all studied varieties were generally increased gradually from the first sample reaching its maximum content on the 1st of December in local and Strakhan and 15th of December in Barkhar varieties, although with some period of decreased differed from one variety to another ones. Thereafter, the content of non- soluble carbohydrates markedly decreased towards the last sample in all varieties. As regards to non- soluble carbohydrates content of the flower buds of the tested varieties, data generally show that it increased slightly (at the same time sugars were decreased) then decreased on the 1st of October in Strakhan, on the 15th October in Barkhar and Local varieties. Thereafter, it increased (at the same time sugars were decreased)reaching its maximum value on the 15th November in Local,1st of December in Barkhar and 15th of December in Strakhan varieties. Henceforth, the content of non- soluble carbohydrates markedly decreased towards the last sample with some fluctuation in Strakhan.

Table 7. Seasonal changes in non-soluble carbohydrates (mg. /g. D.W.) In buds of the three apple varieties during and after release from dormancy

Dates	Varieties					
	"Barkhar"		"Local"		"Strakhan"	
	V.	F.	V.	F.	V.	F.
1/9	14.10	14.68	14.81	11.74	19.75	14.68
15/9	15.77	19.07	19.65	14.05	26.29	23.10
1/10	17.17	19.72	20.29	11.34	27.59	22.14
15/10	16.00	18.45	20.34	11.31	26.32	24.36
1/11	16.51	33.14	27.38	18.57	27.29	29.43
15/11	17.75	33.71	36.93	32.47	34.83	29.63
1/12	27.86	37.37	39.50	24.31	35.74	30.55
15/12	34.22	35.20	23.51	20.57	32.11	32.76

1/1	21.76	31.64	19.12	11.87	28.07	22.67
15/1	14.66	17.28	19.12	11.53	26.28	16.07
1/2	11.15	12.37	18.48	10.70	26.00	11.60
15/2	8.70	8.65	15.20	10.01	16.81	14.05
1/3	-----	-----	14.08	7.84	16.47	13.95
15/3	-----	------	----	7.10	16.65	11.50

V. =Vegetative buds F. =Flower buds

3.9. Total Carbohydrates

Total carbohydrates were found in the vegetative and flower buds at different intervals during the considered seasons are presented in Table (8). It can be seen that the values of carbohydrates in the vegetative buds of the studied varieties generally increased from the first sample reaching its maximum at 15th of October in both Barkhar and Strakhan varieties and till 1st of November for Local and variety. Thereafter, total carbohydrates content decreased gradually in all varieties reaching its minimum values on the 15th November for Strakhan, 1st of December in Barkhar and Local varieties followed with a marked increase reaching its maximum value on the 1st of January in Barkhar, 1st of February in Local and till 15th February in Strakhan variety. A marked decrease occurred just before the bud burst followed with an increase in the last sample. As regards to the total carbohydrates of the flower buds, data show that it increased gradually from the first sample till (15th October) in Barkhar and Strakhan varieties and till15th November for Local variety followed by marked decrease till the 1st of December in Barkhar and Local and till 1st of January in Strakhan varieties. Thereafter, it increased sharply towards the last sample except those decreased which occurred on 15th of February and 1st of March in both Local and Strakhan varieties, respectively.

Table 8. Seasonal changes in total carbohydrates (mg. /g. D.W.) In buds of the three apple varieties during and after release from dormancy

Dates	Varieties					
	"Barkhar"		"Local"		"Strakhan"	
	V.	F.	V.	F.	V.	F.

1/9	114.21	104.68	14.81	101.54	77.95	92.68
15/9	112.81	110.91	101.28	85.48	112.82	102.25
1/10	115.11	114.11	102.13	92.97	114.33	109.70
15/10	118.24	120.65	112.18	103.15	115.61	117.95
1/11	110.90	119.88	116.67	110.26	98.72	110.10
15/11	104.49	105.14	113.46	114.10	96.16	109.62
1/12	91.67	88.39	100.62	85.53	102.07	108.21
15/12	110.90	112.86	107.70	88.58	103.85	107.44
1/1	122.45	121.66	108.14	87.95	104.50	87.18
15/1	121.80	119.88	126.29	101.82	108.66	104.41
1/2	120.95	117.32	125.42	120.39	114.24	128.36
15/2	118.54	115.38	122.84	110.39	122.18	116.85
1/3	--------	-------	121.77	108.97	120.91	121.76
15/3	--------	-------	-------	112.17	124.58	122.69

V. =Vegetative buds F. =Flower buds

4. Discussion

It is clear from the current results that the accumulation of low temperature normally enhances chilling demand needed for bud growth. Moreover, the results conjointly show that water content and dry weight of buds recorded the very cheap values at deep dormancy stage. On the opposite hand, the transition of buds from the dormant stage to the detonating processes was coincided with a gradual increase in water content and dry weight of the burst buds. In this concern, George et al. (1990) suggested that water and nutrients might also be mobilized to the growing points at the expense of the developing fruits. Moreover, Borkowska (1980) found that the transition of buds from the dormant stage to the detonating process is expounded to a rise within the water content in the tissue, mobilization of nutrients, and activation of hydrolytic enzymes and intensification of respiration. Moreover, the present results agreed with those obtained by Bachelard and Wightman (1973) who ended that the amount of increase in dry weight of each vegetative or flower buds seem to flow from the movement of

metabolites into the buds, throughout this period. These metabolites might have come back twigs, branches and roots of trees. The slight loss in dry weight of buds occurred before bud break, is also because of the rise within the respiration rate of the buds coincided the bud burst. Respiration losses in these buds might is sufficiently massive to mask the movement of materials into them. The information additionally show that, the reduction in non-soluble carbohydrates and also the increase within the total sugars by the beginning of bud break is also because of the magnified GA3 content throughout this period which caused the formation or activation of hydrolytic enzymes which hydrolyze enzymes which hydrolyze the insoluble carbohydrates leading to production of simple reducing and non-reducing sugars, therefore increasing the soluble carbohydrates and total carbohydrate content that are used for build up the new cell contents ensuing from the speedy cellular division. These results are in agreement with those reported by Robert and Francis (1985). In this concern, several workers reported that, carbohydrates are the main source of energy for the metabolic changes that occurred during the dormant release period (Sherson et al., 2003, Bonhomme et al., 2010 and Mornya and Cheng, 2011). Carbohydrate availableness is presumptively of major connexion to the management of bud growth and development throughout dormancy and dormancy unharness (Sugar comes from starch accumulated in reserve tissues throughout the preceding summer is reborn to sucrose during winter and therefore the enzymes concerned during this conversion are iatrogenic by the low temperatures. Chilling impact on the changes in starch and sugar concentrations is also explained as a result of amylase activity is iatrogenic by cold temperature, increasing starch hydrolysis and, consequently, sugar concentration (Elle and Sauter, 2000). During winter, starch degraded by amylases is employed to the sucrose synthesis by the sucrose-6-phosphate synthase (SPS) in response to decreasing temperature. The sucrose created in reserve tissue is transported by the vascular tissue pathway to the bud and hydrolyzed to glucose and fructose to produce energy and carbonaceous precursors (Yoshioka et al., 1988).Moreover, Sauter et al. (1998) found a high correlation between the low temperature, the starch degradation and therefore the increasing within the content of sugars in *Populus* stems. The nature of the sugars free from exposure of the shoots to cold showed that it absolutely was basically the alpha-amylase enzyme answerable for starch reduction at low temperatures. Also, varied changes within the level of carbohydrates occurred in buds as they supported the non-growing stage and starting of growth. Starch accumulated throughout the period of photosynthetic activity and was used for regeneration of growth within the spring (Gemma, 1995a&b).The reserve carbohydrate provides energy and substrates (glucans, maltose, and others) for future plant metabolism throughout sprouting. The previous sink so turns into a source: starch is diminished by numerous enzymes and accustomed support growth of the new plant (Viola et al., 2007). High starch and low soluble sugars concentrations coincide with the onset of endodormancy within the fall (Ben Mohamed *et al.*, 2010a). Increasing soluble sugars and high starch depletion were related to chilling acclimation and therefore the unleash of endodormancy (Vergara and Pérez, 2010), whereas constant starch concentrations

and fast soluble sugars consumption reveal the increasing in metabolic activity and a presprouting state (Ben Mohamed *et al*., 2012b). In distinction, Mornya and Cheng (2011) according that carbohydrates might not considerably influence the flowering pattern of tree peonies. Autumn flowering in tree peonies might so achieved by regulating GA_3, IAA and cytokinin levels, significantly at the induction and initiation stages of bud development to facilitate the completion of the floral formation cycle, well beforehand of bud dormancy period.

5. Conclusion

Accumulation of low temperature (below7.2°C) of late-opened variety "Strakhan" was much higher than that in early-opened variety "Barkhar" and "Local" at bud break. "Strakhan" needed high level of chilling requirements (Chr7.2°C) to open. The high levels of sugars concentration in "Barkhar" and "Local" at bud break led to significantly higher bud opining and yield than in "Strakhan" apple variety. Moreover, the results of the current investigation indicated that, water content and soluble carbohydrates such as (sugars) were increased from dormancy initiation to dormancy release which decreased through deep dormancy and increased with bud break. In contrast, non-soluble carbohydrates such as (starch) were decreased from dormancy initiation to dormancy release which increased throughout deep dormancy and decreased with bud break.

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