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Impact of chilling requirements on metabolic changes in nitrogenous compounds in buds during and after dormancy releasing in early and late (*Malus sylvestris*, Mill) apple varieties

Abstract

In order to produce the physiological bases for choosing early- flowering varieties that may avoid the insufficient winter chilling requirements in Egypt. The early and late-opining apple variety Barkhar, Local and Strakhan (Malus sylvestris) were used to study the relation between seasonal changes in nitrogenous compounds and flower opining date according to chilling requirements for each variety. An improved understanding of the factors governing budburst and development and their underlying mechanisms is crucial for management of trees performance and yielding. This study investigated variations in chilling requirements, bud burst and development in early and late varieties of apple trees. Results showed less bud burst in late varieties than in early ones. In the former, there were increased in nitrogenous compounds (soluble nitrogen, total nitrogen, arginine and total free amino acids) at budburst in all varieties. As dormancy begins, storage proteins are synthesized, coinciding with a reduction in the levels of nitrogen and free amino acids. Consequently, as dormancy breaks, these storage proteins are degraded, and an increase in the concentrations of nitrogen and amino acids occurs, in order to support new growth. 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 nitrogenous compound pattern throughout bud development compared to early varieties.

Keywords: apple, chilling requirements, dormancy, nitrogenous compounds (arginine, soluble nitrogen, total nitrogen, total free amino acids)

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Introduction

Because of the winter in Egypt is short and does not meet the chilling requirements of buds, the delay in opening the buds of late-opining 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 late- opining apple productivity in Egypt.[1,2] Apple trees that do not receive adequate winter chill show poor bud break, uneven and delayed bloom that impact negatively on tree architecture and fruit production. Previous research indicates that the endodormancy progression of such trees differs from trees grown under adequate winter chill condition.[3] The majority of trees in temperate climates fulfill a chilling requirement (CR) in so as to overcome endodormancy.[4] Therefore, the CR could dissent by species, varieties, or growing regions.[5–8] Cultivars with low CR bloom and ripen earlier, whereas those with high CR bloom and ripen later.[9–13] For temperate-zone fruit species such as apricot, when winter cold requirements are not adequately satisfied, negative repercussions on productivity occur.[14] CR constrains the acceptable areas of cultivation of the various commercially important tree species and cultivars around the world. If chilling requirement don't appear to be met, irregular, delayed and asynchronous growth, flowering and fruit set are discovered inside the subsequent season.[15–17]

Amino acids play fundamental roles in a multitude of processes, including protein synthesis, hormone metabolism, cell growth, production of metabolic energy, and nucleobase and urea biosynthesis.[18] It is representing the principal long distance transport form of organic nitrogen (N) and is distributed through xylem and phloem to all plant organs.[19] Amino acids are the currency of nitrogen exchange between sources and sink tissues in plants and constitute a major source of the components used for cellular growth and differentiation.[20] Several studies that have examined the physiology of temperate woody species have focused on the cycling of N, and most have investigated carbon balance. However, there is increasing evidence regarding the importance of N storage and remobilization, which are necessary to meet the demands of early growth and development.[21] During autumn, deciduous species store N in the form of storage proteins that are deposited in aerial plant tissues, such as bark and bole wood, as well as in roots.[22] The main mechanism responsible for this storage is the redistribution nutrients, which occurs at the beginning of foliar senescence during autumn. In this process, the proteins of the leaves are hydrolyzed, and the resulting free amino acids are transported via the phloem to storage tissues, where they are converted into reserve proteins. In contrast, the inverse process occurs during the winter, when the hydrolysis of protein reserves (remobilization) produces free amino acids, which are used to meet the demands of new sprouts and inflorescences in the spring.[23]The contents of nitrogen modification among the bud dormancy inflicting and emotional processes was studied by many researchers.[24–31] They found that the incidence, termination, regulation and management of dormancy were regulated by seasonal changing in nitrogenous compound. Some vary of

studies are done on the modification of nitrogenous compounds from dormancy begin to bud break, but none on the relation between bud break and dynamic modification of nitrogenous compounds, likewise as a result of the equilibrium of early-opening apple varieties.

Additionally, this research may help further studies to be performed on how chilling requirement affect changes in the length to full bud break and help to express the effect of nitrogenous compounds on flowering and yield. So this work focuses mainly to explain the behavior of the nitrogenous compounds in buds and their reflections in the duration to full buds break, and the percentages of bud break and fruit-set.

Materials and methods

The 13-year-old trees of 'Barkhar, local and Strakhan' apple trees (*Malus sylvestris* Mill.) grafted on Malling-Merton 106 (MM 106) rootstock were designated willy-nilly, for a preliminary study in 2016/2017 and for the most analysis studies within the 2017/2018 and 2018/2019 seasons. All trees were full-grown within the wood let (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 2016 and 2017, and sampled from September–March 2017/2018 and 2018/ 2019. Trees chosen for the study within the 1st season were not the identical trees that were designated for the second season. Every tree was designed together replicate, and every selection enclosed 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 each fifteen days and cultivated in artificial lighting setup with water to see the bud dormancy emotional time (50% bud break). Moreover, the quantity of chilling hours (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 that the Chilling Hours Model, additionally referred to as the Weinberger Model.[32,33] This model, that was 1st 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 opining date in three apple varieties beneath study throughout each 2017/2018 and 2018/2019 seasons had been determined (Table 1).

Table1 Chilling accumulation (hours below 7.2°C) from1st November to each break date for each variety during 2017/2018 and 2018/2019.

Varietes	Hours under temperature7.2°C from1st November to 50% bud break 2016/2017		2018/2019	
	Date of 50% bud break	Chilling hours	Date of 50% bud break	Chilling horse
Barkhar	1st February	249	25st January	251
Local	1st March	280	15th February	278
Strakhan	27th March	285	20th March	281

Date of floral bud break

Morphological characteristics and yield measurements on trees

Bud count was created for every tree (n=6) in each variety. The dates on that floral and vegetative buds began to open were recorded. Additionally, the dates at that flowering reached 25, 50, 75 and 100 % of the full flowers were calculable in 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.[34] At harvest stage, apple fruits were harvested, counted and weighed for every examined tree.

Preparation of bud samples for chemical analyses

Bud samples were collected at 15-day intervals beginning from 1st September up to 15thMarch from each replicate of each treatment to determine the seasonal changes in bud contents from nitrogen, arginine and total free amino acids. Samples of vegetative and floral buds were randomly taken and immediately transported to the laboratory for the aforementioned determinations.

Determination of nitrogen (N)

Bud samples were collected 15-day intervals beginning from 1st September up to 15th March for determining the seasonal changes in bud components. Buds were randomly sampled and immediately transported to the laboratory for determining the nitrogen. Total N and soluble N (%) in dried material of buds were determined by using micro-Kjeldahl method described by the A.O.A.C.[35]

Extraction and determination of arginine

Bud samples were collected at 15 days intervals, from 1st September –15th March 2017/2018 and 2018/2019, to determine seasonal changes in arginine. Buds were sampled at random and immediately transported to the laboratory to determine their contents of arginine. Arginine was measured after each bud sample (2 g) had been freeze-dried and ground to a fine powder. Samples (0.5 g) were then extracted, at room temperature, by shaking for 24 h with 50 ml of a single-phase 12:5:3 (v/v/v) mixture of methanol: chloroform: water.[36] Norleucine (0.5 ml of a 4 mM solution in 0.01 M HCl) 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; Redgwell 1980).[37] The solid residue was boiled gently for 10 min in 40 ml water to extract the residual amino acids.[38] After cooling to room temperature and centrifugation at 10,0009g, 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 releasehe 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.[39] 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 Biochrom, Cambridge, UK). Analyses of amino acids was performed using an Alpha Plus amino acid analyzer (LKB Biochrom) fitted with a stainless steel column (200 mm 9 4 mm) filled with ion exchange resin (Ultropac 8; particle size 8 lm; LKB Biochrom). The content of arginine (in 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.[40]

Determination of total free amino acids

Total free amino acids were determined according to Jayarman[41] with some modifications.[42] A sample (500 mg) of frozen buds was extracted with 50 ml of 80% ethanol and filtered to remove insoluble materials, and then 1.0 ml of ethanol extract was added. Then, 0.5 ml of 0.07 mol l-1 phosphate buffer solutions (pH 8.04) and 0.5 ml of 2% ninhydrin solution containing 0.8 mg ml-^{1} of SnC_{12}–$2H_2O$ was added. The mixtures were then placed on a boiling water bath for 15 min, and then quickly cooled with cold water, and adjusted to 25 ml with water. After leaving to stand still for 10 min, the absorbance values of these blue-purple products were measured against a reagent blank at 550 nm.

Statistical analysis

The values of the determined characters were subjected to statistical analysis according to the standard procedure described in.[43] 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 1st of February, 1st March and 27th March, after the accumulation of 249, 280 and 285(CH) respectively in the first season and were 25 th of January, 15th February and 20th March, after the accumulation of 251, 278 and 281(CH) respectively in the second season. There were about 27, 26 and 55 days difference in the first season, i.e., the occurrence of opening buds of Strakhan was 26 days later than that of Local and 55 days later than that of Barkhar in the first season and 20, 32 and 53 days difference in the second season and also the occurrence of opening buds of Strakhan was later than that of Local 32 days later and 53 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 varity 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 Janurary, 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).

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) 14 February, 17 March and 12 April for Barkhar, Local and Strakhan, respectively. The earliness reached about 57 and 26 days for Barkhar and Local apple varieties respectively as comparison with Strakhan variety.

Data presented in Table (3) indicated that early- opining 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 89.80 and 85.91% for Barkhar and Local apple varieties respectively as comparison with 76.16% for Strakhan variety. However, the percentage of fruit set was 55.50 and 39.01% for Barkhar and Local apple varieties respectively as comparison with 13.65% for Strakhan variety.

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

Varieties	Date of flower bud opening					
	Beginning	25% flowering	50% flowering	75% flowering	End of flowering	Flowering period (day)
Barkhar	11 Feb	12 Feb	14 Feb	16 Feb	22 Feb	12
Local	4March	13March	17March	20March	21March	18
Strakhan	7 April	10April	12April	19 April	25 April	19

Proportion of bud break and fruit set

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

Varieties	Bud break (%)	Dormant buds (%)	Fruit set (%)	
Barkhar	89.80a	10.20a		55.50a
Local	85.91b	14.09b		39.01b
Strakhan	76.16c	23.84c		13.65c

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

Citation: El-Yazal MAS, El-Yazal SAS. Impact of chilling requirements on metabolic changes in nitrogenous compounds in buds during and after dormancy releasing in early and late (Malus sylvestris, Mill) apple varieties. *Horticult Int J.* 2019;3(5):230–238. DOI: 10.15406/hij.2019.03.00137

Number of fruit tree–1and Fruit yield

Data in Table(4) also show that, early-flower 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 94.39 and 59.72% for number of fruits tree^{-1} and 61.43 and 22.37 % for fruit yield tree^{-1} in the Barkhar and Local apple varieties respectively as comparison with Strakhan variety.

Soluble nitrogen

Data in Table (5) indicated that the soluble nitrogen in vegetative buds gradually increased from the first sample till 1st October in all the studied varieties. Thereafter, a marked decrease in soluble nitrogen contents occurred for the studied varieties reaching its minimum value on that 1st of December for Barkhar variety and 15th of December in Local and Strakhan varieties followed with marked increase towards the last sample in all the studied varieties with some period of decrease just after bud burst. As regards to soluble nitrogen of the flower buds, it is clear from the present data that it generally behaved as similar to that exhibited by the vegetative ones. It increased gradually from the first sample till the first of October in both Strakhan and Local varieties and till1st November in Barkhar variety, followed by a marked decrease reaching its minimum value on the 1st of December in Barkhar variety and till 15th of December in both Strakhan and Local varieties. Thereafter, it increased markedly towards the last sample in all the studied varieties with some period of decrease just after bud burst.

Total nitrogen

Data in Table (6) indicated that the total nitrogen in vegetative buds gradually increased from the first sample till 1st November in all the studied varieties. Thereafter, a marked decrease in total nitrogen contents occurred for the studied varieties reaching its minimum value on that 1st of December for Barkhar variety and 15th of December in Local and Strakhan varieties followed with marked increase towards the last sample in all the studied varieties with some period of decrease just after bud burst. As regards to soluble nitrogen of the flower buds, it is clear from the present data that it generally behaved as similar to that exhibited by the vegetative ones. It increased gradually from the first sample till the first of October in both Strakhan and Local varieties and till1st November in Barkhar variety, followed by a marked decrease reaching its minimum value on the 1st of December in Barkhar variety and till 15th of December in both Strakhan and Local varieties. Thereafter, it increased markedly towards the last sample in all the studied varieties with some period of decrease just after bud burst.

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

Varieties	No. of Fruit tree^{-1}	Yield per tree(Kg)
Barkhar	432.20a	23.02a
Local	355.12b	17.45b
Strakhan	222.33c	14.26c

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

Table 5 Seasonal changes in soluble nitrogen content (%) in vegetative (V) and flower (F) buds of the three apple varieties during and after release from dormancy

Dates	Varieties					
	"Barkhar"		"Strakhan"		Local""	
	V	F.	V.	F.	V.	F.
01-Sep	0.45b	0.45b	0.44b	0.39b	0.33c	0.36c
15-Sep	0.48b	0.48b	0.45b	0.40b	0.38c	0.41b
01-Oct	0.55a	0.55a	0.48a	0.42b	0.45b	0.44b
15-Oct	0.54a	0.54a	0.47a	0.49a	0.41b	0.39b
01-Nov	0.50b	0.50b	0.46b	0.47b	0.32c	0.36c
15-Nov	0.40c	0.40c	0.44b	0.42b	0.30c	0.34c
01-Dec	0.32c	0.32c	0.40b	0.41b	0.25d	0.30d
15-Dec	0.35c	0.35c	0.23c	0.35c	0.22d	0.28d
01-Jan	0.50b	0.50b	0.29c	0.36c	0.33c	0.35c
15-Jan	0.58a	0.58a	0.36c	0.41b	0.36c	0.40c
01-Feb	0.55a	0.55a	0.45b	0.52a	0.42b	0.46b
15-Feb	------	------	0.48a	0.54a	0.45b	0.50b
01-Mar	------	------	0.47a	0.51a	0.52a	0.59a
15-Mar	------	------	------	--------	0.50a	0.56a

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

Table 6 Seasonal changes in total nitrogen content (%) in vegetative (V) and flower (F) buds of the three apple varieties during and after release from dormancy

Dates	Varieties					
	"Barkhar"		Local""		"Strakhan"	
	V.	F.	V.	F.	V.	F.
01-Sep	1.30b	1.23c	1.25c	1.36c	1.26c	1.30d
15-Sep	1.36b	1.35c	1.30c	1.39b	1.29c	1.32c
01-Oct	1.45a	1.47b	1.35c	1.47b	1.35c	1.35c
15-Oct	1.46a	1.50a	1.45b	1.46b	1.33c	1.32c
01-Nov	1.48a	1.52a	1.46b	1.45b	1.28c	1.30c.
15-Nov	1.25c	1.33c	1.40b	1.42b	1.25c	1.29d
01-Dec	1.23c	1.30c	1.38c	1.40b	1.23d	1.26d
15-Dec	1.36b	1.41b	1.30c	1.38b	1.22d	1.20d
01-Jan	1.44a	1.50a	1.41b	1.48a	1.35c	1.38c
15-Jan	1.48a	1.55a	1.55a	1.50a	1.47b	1.49b
01-Feb	1.46a	1.53a	1.58a	1.52a	1.49b	1.54a
15-Feb	------	-------	1.52a	1.49a	1.55a	1.58a
01-Mar	------	-------	1.53a	1.51a	1.52a	1.56a
15-Mar	------	-------	------	--------	1.56a	1.57a

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

Table 7 Seasonal changes in total arginine content mg/100g dry weight in vegetative (V) and flower (F) buds of the three apple varieties during and after release from dormancy

Dates	Varieties					
	"Barkhar"		Local""		"Strakhan"	
	V.	F.	V.	F.	V.	F.
01-Sep	319b	312b	354c	366c	320c	342b
15-Sep	321b	325b	362c	387c	330c	348b
01-Oct	331b	343b	459a	460b	356b	351b
15-Oct	325b	350b	452a	457b	325c	340b
01-Nov	314b	361a	453a	433b	314c	333c
15-Nov	155c	181c	425b	421b	309c	312c
01-Dec	123c	135c	412c	410b	300c	301c
15-Dec	148c	145c	390c	362c	298c	300c
01-Jan	352a	356a	410b	460b	350b	348b
15-Jan	365a	373a	455b	471b	366b	377b
01-Feb	357a	365a	468a	488a	374b	389b
15-Feb	------	-------	467a	485a	385a	401a
01-Mar	------	-------	483a	520a	381a	400a
15-Mar	------	-------	------	--------	398a	406a

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

Citation: El-Yazal MAS, El-Yazal SAS. Impact of chilling requirements on metabolic changes in nitrogenous compounds in buds during and after dormancy releasing in early and late (Malus sylvestris, Mill) apple varieties. *Horticult Int J.* 2019;3(5):230–238. DOI: 10.15406/hij.2019.03.00137

Total arginine

Data in Table (5) indicated that the total arginine in vegetative buds gradually increased from the first sample till 1st October in all the studied varieties. Thereafter, a marked decrease in total arginine contents occurred for the studied varieties reaching its minimum value on that 1st of December for Barkhar variety and 15th of December in Local and Strakhan varieties followed with marked increase towards the last sample in all the studied varieties with some period of decrease just after bud burst. As regards to total arginine of the flower buds, it is clear from the present data that it generally behaved as similar to that exhibited by the vegetative ones. It increased gradually from the first sample till the first of October in both Strakhan and Local varieties and till1st November in Barkhar variety, followed by a marked decrease reaching its minimum value on the 1st of December in Barkhar variety and till 15th of December in both Strakhan and Local varieties. Thereafter, it increased markedly towards the last sample in all the studied varieties with some period of decrease just after bud burst.

Total free amino acids

Data in Table (8) indicated that the total free amino acids in vegetative buds gradually increased from the first sample till 1st October in all the studied varieties. Thereafter, a marked decrease in total free amino acids contents occurred for the studied varieties reaching its minimum value on that 1st of December for Barkhar and Strakhan varieties and 15th of December in Local, variety followed with marked increase towards the last sample in all the studied varieties with some period of decrease just after bud burst. As regards to total free amino acids of the flower buds, it is clear from the present data that it generally behaved as similar to that exhibited by the vegetative ones. It increased gradually from the first sample till the first of October in both Strakhan and Local varieties and till1st November in Barkhar variety, followed by a marked decrease reaching its minimum value on the 1st of December in Barkhar variety and till 15th of December in both Strakhan and Local varieties. Thereafter, it increased markedly towards the last sample in all the studied varieties with some period of decrease just after bud burst.

Table 8 Seasonal changes in total free amino acids (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.
01-Sep	31.72c	36.36c	38.13c	40.67b	35.50c	31.64c
15-Sep	42.56b	43.20b	40.19b	45.88a	38.67c	36.15c
01-Oct	46.79a	45.38a	41.06b	46.00a	46.12a	48.67b
15-Oct	40.88b	40.11b	38.57c	40.54b	41.74b	44.34b
01-Nov	37.67c	39.70b	36.23c	38.85c	40.09b	40.28c
15-Nov	36.96c	37.27c	35.26c	37.83c	38.58c	39.55c
01-Dec	34.02c	35.47c	35.21c	33.02c	33.75c	37.18c
15-Dec	44.80a	41.95b	34.94c	30.33c	38.85c	44.13b
01-Jan	45.76a	45.76a	39.32c	33.58c	41.86b	51.99a
15-Jan	48.83a	47.12a	44.20b	40.79b	46.60a	53.28a
01-Feb	47.06a	45.03a	47.38a	43.06b	47.51a	54.75a
15-Feb	------	-------	40.71b	47.46a	52.04a	59.26a
01-Mar	------	-------	47.98a	45.73a	43.27b	54.83a
15-Mar	------	-------	------	--------	45.23a	54.92a

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

Discussion

It is clear nowadays that an outsized kind of factors can break dormancy. Throughout the methods of bud dormancy cathartic to bud break, several seasonal changes in some chemical constituents of buds, especially, nitrogenous compound which have a significant role in regulation dormancy and bud break. The increase in soluble and total nitrogen in buds of all varieties used after dormancy release (Table 5 and 6) was attributed to the movement of N-compounds from the bark and wood to the developing floral buds and growing points and also to hydrolyzed storage proteins to amino acids. In agreement with these results, El-Shewy et al.[13] reported that the relatively highest levels of total and soluble nitrogen in apple buds were observed subsequent to dormancy release in buds. Moreover, Wermelinger & Koblet [44] found that nitrogen reserves were found mainly to be in protein fractions of both wood and bark. These reserves were hydrolyzed in mid-late March resulting in a rapid increase in the soluble nitrogen level for use in growth. Also, Hill-Cottingham[45] and Tromp[46] reported that there was a reduction in N concentration in woody tissues in spring, particularly in bark tissues of shoots. This was attributed to the movement of N-compounds from the bark and wood to the developing floral buds and growing points. Relatively low levels of total protein in apple buds were observed following bud break.[25] Moreover, Kuroi[47] 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. In this connection, Marafon et al.[29] reported that storage and remobilization are considered key processes for the

effective use of nitrogen in temperate fruit trees. As dormancy begins, storage proteins are synthesized, coinciding with a reduction in the levels of free amino acids. Consequently, as dormancy breaks, these storage proteins are degraded, and an increase in the concentrations of amino acids occurs, in order to support new growth. The data in (Table 7 and 8) also show that free arginine and total free amino acids increased during bud break. In this concern, El-Shewy et al.[13] and Seif et al.[48] reported that free arginine and total free amino acids increased during bud break and this play a role in dormancy release. Moreover, Seif El-Yazal & Rady[24] and Seif El-Yazal et al.[24] reported from their analyses of apple buds that a marked increase in total nitrogen, soluble nitrogen, soluble nitrogen / total nitrogen ratio, total free amino acids, free arginine, polyamines and biogenic amines were occurred and reaching its maximum values during bud break. Moreover, Seif El-Yazal et al.[24] found that total free amino acids in buds of 'Anna' apple trees were relatively in reduced levels during deep dormancy and increased gradually from the initiation of dormancy to the release of buds from dormancy. The release of buds from dormancy and the resumption of growth have been accredited to mechanisms regulating changes in metabolic activity and amino acids.[24] In addition, Wang and Faust[29] on apple found that arginine levels increased during March and April reaching the maximum at the end of April. From the onset of May, they decreased gradually until they reached a steady level at the end of July and beginning of August. Then the level increased until September when growth stopped. Bagni et al.[50] found that arginine and glutamine, as well as abscisic acid, decreased during the last phase of dormancy; however, the corresponding increases in polyamines appeared to be strictly related to bud break. In this connection, Durzan[51] reported that light intensity, photoperiod, and temperature changes were trophic factors contributing to the redistribution of amino acids from spruce needles to organs with meristems entering winter dormancy. During bud break in spring, the removal of inhibitory guanidine compounds provides sources of nitrogen (N) for the renewed synthesis of arginine. Arginine-N and guanidino-compounds may be useful as physiological biomarkers for tree improvement. Moreover, Oh & Bunemann[52] reported that asparagine and arginine contents were considerably higher in leaves and terminal buds of shoots. Floral buds that differentiated on Summer-pruned shoots had higher contents of asparagine and arginine compared with weaker spur buds. It has been suggested that irregular spur size and the poor development of spur buds in the apple cultivar 'Fuji' might be caused by poor translocation of amino acids and N-compounds from shoots and other vegetative organs. Recent research has shown that changes in amino acid profiles are associated with the release of buds from dormancy.[53] This increase in amino acid concentration, along with the process of sprouting, occurs as a result of N remobilization and is dependent on the occurrence of freezing temperatures during dormancy. Low temperatures induce the activity of an endopeptidase, which promotes degradation of storage proteins, producing free amino acids that are then transported via xylem to areas of growth.[54] In this manner, the initial growth of buds becomes almost entirely dependent on the N reserves of vegetative tissues, since the root system is only activated after the initiation of growth by new sprouts. Although few studies have described the dynamics of the process in detail, it is evident that the remobilization of N occurs before absorption by roots, at least in some species, such as apple.[55]

Conclusion

The hypothesis tested by this study was that the content of nitrogenous compounds such as soluble nitrogen, arginine and total free amino acids in apple trees increase prior to the buds sprouting during winter and spring in the region of Egypt. Therefore, the objective was to determine the nitrogenous compounds in early and late varieties of apple trees (Barkhar, Local and Strakhan) during and after dormancy. Finally, from the results of the current investigation, it may well be all over that, nitrogenous compounds (nitrogen and amino acids) were exaggerated from dormancy initiation to dormancy release that slashed throughout deep dormancy and increased with bud break.

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Conflicts of interests

Authors declare no conflict of interest exists.

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