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-flowering apple variety Barkhar, Local and Strakhan (Malus sylvestris) were used to study the relation between seasonal changes in nitrogenous compounds and flower opening 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, budburst and development in early and late varieties of apple trees. Results showed less budburst 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)

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-flowering 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-flowering 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.9-12 For temperate-zone fruit species such as apricot, when winter cold requirements are not adequately satisfied, negative repercussions on productivity occur.13 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.14-17

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 bale 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 conflicting and emotional processes was studied by many researchers.24-27 They found that the incidence, termination, regulation and management of dormancy were regulated by seasonal changing in nitrogenous compound. Some vary of
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

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Chilling hours

Date of 50% bud break

| Varieties     | Months   | 2016/2017 | 2017/2018 |
|---------------|----------|-----------|-----------|
| Barkhar       | 1st February | 249       | 25th 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. 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 15th March 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.19

Extraction and determination of arginine

Bud samples were collected at 15-day intervals, from 1st September to 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. 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). The solid residue was boiled gently for 10 min in 40 ml water to extract the residual amino acids. After cooling to room temperature and centrifugation at 10,000g, the aqueous extract was combined with the methanolic extract and made up to 100 ml. A 20 ml aliquot was loaded onto a cation-exchange column (Dowex-SOW 8%; 200–400 mesh) with a bed volume of 3 ml. The column was washed with 45 ml 0.01 M HCl, followed by 5 ml water, then eluted with 30 ml 2 M NH4OH to release the amino acids. The flow rate was maintained at approx. 1.0 ml min-1 using a small vacuum pump. Columns were regenerated by washing sequentially with 5 ml water.

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. Horticul Int J. 2019;3(5):230–238. DOI: 10.15406/hij.2019.03.00137
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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 4th of February, 1st March and 27th March, after the accumulation of 249, 280 and 285(CH) respectively in the first season and were 25th 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- opening apple varieties gave a high percentage of flower bud break and fruit set comparing with the late-opening 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 25% flowering | Date of 50% flowering | Date of 75% flowering | Date of flowering | Flowering period (day) |
|-------------|-----------------------|-----------------------|-----------------------|-------------------|------------------------|
| Barkhar     | 11 Feb                | 12 Feb                | 14 Feb                | 16 Feb            | 22 Feb                | 12                     |
| Local       | 4 March               | 13 March              | 17 March              | 20 March          | 21 March              | 18                     |
| Strakhan    | 7 April               | 10 April              | 12 April              | 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

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Number of fruit tree$^{-1}$ and 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 till 1st 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 till 1st 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$^{-1}$ and 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; $n=6$

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
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

|   | Varieties | Dates  | V.   | F.   | V.   | F.   | V.   | F.   |
|---|-----------|--------|------|------|------|------|------|------|
|   | “Barkhar” | 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

|   | Varieties | Dates  | V.   | F.   | V.   | F.   | V.   | F.   |
|---|-----------|--------|------|------|------|------|------|------|
|   | “Barkhar” | 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

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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 till 1st 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 till 1st 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

| Varieties   | Dates  | "Barkhar" | "Local" | "Strakhan" |
|-------------|--------|-----------|---------|------------|
|             |        | 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. 19 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

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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 & Rady24 and Seif El-Yazal et al.25 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.49 In addition, Wang and Faust50 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.51 found that arginine and glutamine, as well as abscistic 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, Durzan52 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 & Bunemann53 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 ‘Fujis’ 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.54 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.55 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.56

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.

References

1. Seif El-Yazal MA, Rady MM, Seif El-Yazal SA. Foliar-applied mineral oil enhanced hormones and phenols content and hastened breaking bud dormancy in “Astrachan” apple trees. International Journal for Empirical Education and Research. 2018a;1(2):57–73.
2. Seif El-Yazal MA. Seasonal changes in soluble and non-soluble carbohydrates during and after dormancy release in early and late varieties of apple (Malus Sylvestris, Mill) trees. International Journal for Empirical Education and Research. 2019;3(20):1–18.
3. Cook NC, Calitz FJ, Alderman LA, et al. Diverse patterns in dormancy progression of apple buds under variable winter conditions. Scientia Horticulturae. 2017;226(19):307–315.
4. Campoy JA, Ruiz D, Alderman L, et al. The fulfillment of chilling requirements and the adaptation of apricot (Prunus armeniaca L.) in warm winter climates: An approach in Murcia (Spain) and the Western Cape (South Africa). European Journal of Agronomy. 2012;37(1):43–55.
5. Stino GR. Aspects related to temperature zone fruit production in Egypt. Acta Hort. 1995;409:203–204.
6. Luedeling, E., Brown, PH. A global analysis of the comparability of winter chill models for fruit and nut trees. Int J Biometeorol. 2011;55(3):411–421.
7. Wang LR, Zhu GR, Fang WC. Peach genetic diversity, origin, and evolution. In: Wang LR & Zhu GR, et al, editors. Peach genetic resource in China. Beijing, China: Chinese Agriculture Press; 2012:263. (in Chinese)
8. Andreini L, Viti R, Bartolini S, et al. The relationship between xylem differentiation and dormancy evolution in apricot flower buds (Prunus armeniaca L.): the influence of environmental conditions in two Mediterranean areas. Trees. 2012;26(3):919–928.
9. Scozza R, Okie WR, Peaches (Prunus Persica L. Batsch). Acta Horticulturae. 1990;290:177–231.
10. El—Shewy AA, Ibrahim AA, Zeid FA, et al. Effect of some dormancy breaking components on blooming, fruit set, yield, yield components and physical and chemical properties of fruits of some apple cultivars. A. blooming and fruit set. Annals of Agric Sci Moshtohor. 1999a;37(4):2235–2246.
11. El—Shewy AA, Ibrahim AA, Zeid FA, et al. Effect of some dormancy breaking components on blooming, fruit set, yield, yield components and physical and chemical properties of fruits of some apple cultivars. B.—Yield, yield components and physical and chemical properties of fruits. Annals of Agric Sci Moshtohor. 1999b;37(4):2247–2267.
12. El—Shewy AA, Ibrahim AA, Zeid FA, et al. Effect of some dormancy breaking components on leaves and fruits chemical composition of some apple cultivars. 1. Chemical composition of leaves. Annals of Agric Sci Moshtohor. 1999c;37(4):2269–2278.

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
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

13. El-Shewy AA, Ibrahim AA, Zeid FA, et al. Effect of some dormancy breaking components on chemical composition of leaves and buds of some apple cultivars. 11. Chemical composition of vegetative and generative buds. *Annals of Agric Sci* Moasher. 1999d;37(4):2279–2306.

14. Viti R, Andreini L, Ruiz D, et al. Effect of climatic conditions on the overcoming of dormancy in apricot flower buds in two Mediterranean areas: Murcia (Spain) and Tuscany (Italy). *Scientia Horticulatae*. 2010;124(2):217–224.

15. Luedeling E, Blanke M, Gebauer J. Climate change effects on winter chill for fruit crops in Germany-Auswirkungen des Klimawandels auf die Verfügbarkeit von Kälteewirkung (Chilling) für Obstgärtnerei in Deutschland. *Erwerbs-Obsbaun*. 2009;51(3):81–94.

16. Campoy JA, Ruiz D, Egea J. Dormancy in temperate fruit trees in a global warming context: a review. *Sci Hortic*. 2011;130(2):357–372.

17. Yong Li, Wei-choa FANG, Geng-rui ZHU, et al. Accumulated chilling hours during endodormancy impact blooming and fruit shape development in peach (*Prunus persica* L.). *Journal of Integrative Agriculture*. 2016;15(6):1267–1274.

18. Coruzzi GM, Zhou L. Carbon and nitrogen sensing and signaling in plants: emerging ‘matrix effects’. *Curr Opin Plant Biol*. 2004;4(3):247–253.

19. Wipf D, Ludewig U, Tegeder M, et al. Conservation of amino acid transporters in fungi, plants, and animals. *Trends Biochem Sci*. 2002;27(3):139–147.

20. Couturier J, de Fay E, Fitz M, et al. PtAAP11, a high affinity amino acid transporter specifically expressed in differentiating xylem cells of poplar. *J Exp Bot*. 2010;61(6):1–12.

21. Millard P, Grelet GA. Nitrogen storage and remobilization by trees: ecophysiological relevance in a changing world. *Tree Physiology*. 2010;30(9):1083–1095.

22. Cooke JEK, Weih M. Nitrogen storage and seasonal nitrogen cycling in *Populus*: bridging molecular physiology and ecophysiology. *New Phytologist*. 2005;167(1):19–30.

23. Gomez L, Faurobert M. Contribution of vegetative storage proteins to seasonal nitrogen variations in the young shoots of peach trees (*Prunus persica* L. Batsch). *J Exp Bot*. 2002;53(379):2431–2439.

24. Seif El-Yazal MA, Rady MM. Changes in nitrogen and polyamines during breaking bud dormancy in ‘Anna’ apple trees with foliar application some compounds. *Scientia Horticulatae*. 2012;136(6):75–80.

25. Seif El-Yazal MA, Rady MM. Foliar-applied dormancy-breaking chemicals change the content of nitrogenous compounds in the buds of apple (*Malus sylvestris* Mill. cv. Anna) trees. *Journal of Horticultural Science & Biotechnology*. 2012;87(4):299–304.

26. Seif El-Yazal MA, Rady MM. Foliar-applied DormexTM or thiourea-enhanced proline and biogenic amino acids contents and hastened breaking bud dormancy in ‘An Shemer’ apple trees. *Trees*. 2013;27(1):161–169.

27. Seif El-Yazal MA, Rady MM. Exogenous onion extract hastens bud break, positively alters enzyme activity, hormone, amino acids and phenol contents, and improves fruit quality in ‘Anna’ apple trees. *Scientia Horticulatae*. 2014;169(6):154–160.

28. Seif El-Yazal MA, Seif El-Yazal SA, Rady MM. Exogenous dormancy-breaking substances positively change endogenous phytohormones and amino acids during dormancy release in ‘Anna’ apple trees. *Plant Growth Regul*. 2014;72(3):211–220.

29. Marafon AC, Herter FG, Hauwertho FJ, et al. Free amino acids in the xylem sap of pear trees during dormancy. *Ciência Rural Santa Maria*. 2016;46(7):1136–1141.

30. Seif El-Yazal MA, Rady MM, Seif El-Yazal SA. Metabolic changes in polyamines, phenylethylamine, and arginine during bud break in apple flower buds under foliar-applied dormancy-breaking. *International Journal for Empirical Education and Research*. 2018b;12(2):1–18.

31. Seif El-Yazal MA, Rady MM, Seif El-Yazal SA, et al. Changes in metabolic processes during break dormancy in apple buds under foliar-applied garlic extract. *International Journal for Empirical Education and Research*. 2018c;1(4):36–58.

32. Bennett JP. Temperature and bud rest period. *Calif Agric*. 1949;3(11):9–12.

33. Weinberger JH. Chilling requirements of peach varieties. *Proc Am Soc Hortic Sci*. 1950;56:122–128.

34. Westwood MN. Dormancy and plant hardiness. In: Temperate zone pomology. Freeman, San Francisco; 1978:299–319.

35. Official Methods of Analysis of the Association of Official Agricultural Chemists. 16th ed. Washington DC, USA: AOAC International; 1995.

36. Bielecki RL, Turner NA. Separation and estimation of amino acids in crude plant extracts by thin-layer electrophoresis and chromatography. *Anal Biochem*. 1966;17(2):278–293.

37. Redgwell RJ. Fractionation of plant extracts using ion exchange Sephadex. *Anal Biochem*. 1980;107(1):44–50.

38. Oland K. Nitrogenous reserves in apple trees. *Physiol Plant*. 1959;12:594–648.

39. Lazarus W. Purification of plant extracts for ion-exchange chromatography of free amino acids. *J Chromatogr*. 1973;87(1):169–178.

40. Henle T, Walter HW, Krause I, et al. Efficient determination of individual Maillard compounds in heat-treated milk products by amino acid analysis. *Dairy J*. 1991;1(2):125–135.

41. Jayarman J. Laboratory Manual in Biochemistry. New York: Wiley Eastern Limited; 1981:61–73.

42. Chen L, Chen Q, Zhang Z, et al. A novel colorimetric determination of free amino acids content in tea infusions with 2,4-dinitrofluorobenzene. *Journal of Food Composition and Analysis*. 2009;22(2):137–141.

43. Gomez KA, Gomez AA. Statistical analysis procedure of agricultural research. New York: John Wiley and Sons; 1984:25–30.

44. Wermelinger B, Koblet W. Seasonal growth and nitrogen distribution in grapevine leaves. *Vitis*. 1990;29:15–26.

45. Hill-Cottingham DG. The effect of climate and time of application of fertilizer on the development and crop performance of fruit trees. In: EJ Hewitt, CV Cutting, editors. Recent aspects of nitrogen metabolism in grapevine leaves. *Scientia Horticulturae*. 1974;12:1–17.

46. Seif SA, Abd El-Samad GA, et al. Response of ‘Orleans’ apple trees to hydrogen cyanamide application. *Fayoum J Agric Res & Dev*. 2003;17(1):33–47.

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
49. Wang SX, Faust M. Comparison of seasonal growth and polyamine content in shoots of orchard grown standard and genetic dwarf apple trees. *Physiologia Plantarum*. 1993;89:376–380.

50. Bagni N, Malacelli B, Torrigiani P. Polyamines, storage substances and abscisic acid-like inhibitors during dormancy and very early activation of *Helianthus tuberosus* tuber tissues. *Physiologia Plantarum*. 1980;49:341–350.

51. Durzan DJ. Arginine and the shade tolerance of white spruce saplings entering winter dormancy. *Journal of Forest Science*. 2010;56(2):77–83.

52. Oh S, Bunemann G. Seasonal changes of asparagine and arginine contents in spur buds, leaf buds, and flower buds induced by summer running in 'Fuji' and 'Jonagold' apple tree. *Hort Science*. 1992;27(6):75–80.

53. Judd MJ, Meyer DH, Meekings JS, et al. An FTIR study of the induction and release of kiwifruit buds from dormancy. *J Sci Food Agric*. 2010;90(6):1071–1080.

54. Sauter JJ, Van Cleve B. Seasonal variation of amino acids in the xylem sap of *Populus x canadensis* and its relation to protein body mobilization. *Trees*. 1992;7(1):26–32.

55. Dong S, Scagel CF, Gheng L, et al. Soil temperature and plant growth stage influence nitrogen uptake and amino acid concentration of apple during early spring growth. *Tree Physiol*. 2001;21(8):541–547.

**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