The influence of various levels of ammonium to total nitrogen on post-harvest performance of three apple cultivars (Golab Kohans, Gala, and Granny Smith)

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Abstract

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Apple is one of the most popular fruit worldwide with a continuous demand for higher quality apples, especially storable varieties. Nitrogen is one of the most important elements in apple growth and production. Additionally, different nitrogen forms can have different effects on plant growth and fruit qualities. In this research, we investigated the effect of five types of nutrient solutions with different amounts of ammonium nitrogen to total nitrogen on post-harvest physiology of apples for two seasons (2016-2017 and 2017-2018). Apple seedlings of three cultivars (Granny Smith, Gala and Golab Kohans) were grown on a soil-based substrate in a factorial experiment of randomized complete block design. After harvesting, the fruit were stored in 0 °C and 90-95% humidity for 45 days for Golab, 90 days for Gala and 160 days for Granny Smith. Fruit traits were evaluated throughout storage period. The results showed that the increase in ammonium nitrate concentration significantly increased fruit weight, fruit dry weight, and titratable acidity. However, this increase in ammonium nitrate reduced firmness and the amount of total soluble solids with an increase in fruit weight loss index during storage. Furthermore, the results illustrated that the best fruit in terms of storability and postharvest performance were attributed to the nutrition solution without ammonium nitrate.

Abbreviations:

Gala (G), Golab Kohans (GK), Granny Smith (GS), Fruit Weight (FW), Fruit Weight Loss (FWL), Fruit Firmness (FF), Fruit Dry Matter (FDM), Fruit Color Changes (FCC), Soluble Solids Concentration (SSC), Titratable Acidity (TA)

1. Introduction

Apple (*Malus domestica* Brokh.) is one of the most important common fruit that is consumed to a considerable extent [1], and apple trees are one of the most widely cultivated fruit trees worldwide [2]. Apple production throughout the world is faced with many difficulties for various reasons such as the improper nutrition of trees, harvesting at an inappropriate stage, packing for global markets and the continuous demand for quality improvement [3].

The associated fruit production is a complex operation involving orchard, storage and marketing phases. Long-term storage of this important fruit has become possible due to the introduction of modern post-harvest technologies [4]. However, the quality of stored apples is dramatically influenced by pre-harvest factors such as weather conditions, nutrition [5], in addition to the fruit mineral composition and maturity phase at harvest [6][7][8].

Pre-harvest fruit nitrogen (N) concentrations are a particularly important factor that influences apple quality after harvest [9]. Insufficient nitrogen levels cause a weak vegetative growth and a cell division inhibition, which decreases the plant's potential to produce a high yield and leads to a loss in fruit quality. Accurate timing and the rate of N fertilizer application play an essential role in plant nutrition [10][11]. Furthermore, the form of nitrogen has a significant effect on the quality and postharvest life of apples, as it was reported that increasing ammonium in Gala and Golab Kohans trees nutrition program had positive effects on fruit size and dry matter percentage, while negatively affected fruit firmness with slight or no effects on total soluble solids, titratable acidity, and pH [12]. Other reports showed that ammonium-based nitrogen fertilizing resulted in the production of small fruit with poor quality, while nitrate-based fertilizer helped developing maturity indices in terms of color, sugar content, and juice acidity [13]. Therefore, finding the perfect balance between nitrogen sources in nutritional programs is crucial to introduce fruit of high qualities [14][15][16]. Therefore, the aim of this study was to investigate the effect of different ammonium content in nutrition solutions on fruit postharvest performance of three apple cultivars (Granny Smith, Gala and Golab Kohans).

2. Material and Methods

2.1. Plant material and nutrition program

The subjects of this experiment were six years old apple trees of the cultivars Golab Kohans (GK), Gala (G) and Granny Smith (GS) grafted on M9 rootstock and planted in 20-liter pots filled with a substrate of soil and perlite. The study was carried out at the Department of Horticulture (University of Tehran) and for two seasons (2016 - 2018). A completely randomized design was used. Overall, the experiment involved five treatments, 3 replicates and 3 views per cultivar for each screening period. For this experiment, five different nutrition solutions with different ratios of ammonium to total nitrogen [NH₄+/ (NH₄++NO₃-)] were used. Solution 1 S1 (Control), Solution 2 (S2), Solution 3 (S3), Solution 4 (S4), Solution 5 (S5) had an ammonium to total nitrogen ratio of 0, 0.08, 0.16, 0.19 and 0.27 meg/l respectively. Full nutrition solutions in addition to microelements compositions can be found in the Supplementary material file (Supplementary Tables 1-5 and 6). The base solutions for the macro elements, microelements, and iron were prepared separately. First stock solutions were prepared with a concentration of 1000 times the final irrigation solution. pH for nutrition solution was controlled used nitric acid (0.2 N) to a degree of 6.5±0.1. In springtime, trees were irrigated three times with an irrigation solution while the rest spring irrigations were pure water. In summer the trees were irrigated once a week with the irrigation solution and twice with pure water. The amount of solution added for each tree was two liters/pot. Fruit of each cultivar were harvested at commercial maturity. In the case of GK (29th June), G (21st July) and GS (18th September), then, they were transferred to cold storage and stored at 0 ± 1 °C, with 90 - 95 % relative humidity.

2.2. Fruit traits

Fruit weight (FW), fruit weight loss (FWL), fruit firmness (FF), fruit dry matter (FDM), fruit color changes (FCC), soluble solids concentration (SSC) and titratable acidity (TA) were evaluated directly after harvest and during storage phase. Overall, four screenings were conducted for GK (0, 15, 30, 45 days) and for G, (0, 30, 60 and 90 days), while five screenings for GS (0, 40, 80, 120 and 160 days) were carried out during storage. Three fruits were randomly selected from each replicate of each treatment per cultivar for each evaluation.

The weight of each fruit was measured after harvest with an electric balance and the average weight per fruit was calculated. Furthermore, fruit weight was measured for each evaluation and fruit weight loss was calculated using the following formula:

Fruit weight loss (%) = (Initial weight – Final weight)/Initial weight × 100

FF (kg/cm²) was measured by removing the skin at two positions of the fruit surface and applying a firmness tester (penetrometer) equipped with an 8 mm conical plunger. To measure dry matter, 4 - 5 g slices were prepared from a third midway part of the fruit. After weighing, the slices were placed in an oven at 70-80 °C for 72 hours until a constant weight was obtained. The dry matter content was calculated using the following formula:

Dry matter content = $(Dry weight)/(Wet weight) \times 100$

Fruit were juiced and SSC was measured using a drop of the extracted juice from each fruit on a hand-held refractometer (Atago N-32, Tokyo) at laboratory temperature. The results were expressed as a percentage. TA was determined by titration of 5 mL of filtered juice by 0.1 N NaOH up to a pH of 8.3. Fruit color was evaluated using a Colorimetric device (Minolta model CR-400). At every stage, readings were taken from two opposite points on the fruit, and the color parameters L *, a *, b * were measured in addition to Hue and Chroma. FCC was then calculated to determine differences in fruit color throughout storage.

2.3. Statistical analysis

The collected data were analyzed after averaging the two seasons' evaluations. Data analysis was carried out using GenStat statistical software. A two-way analysis of variance (ANOVA) test was used, and the means were compared using Duncan's multiple range test (DMRT). Comparison of means was carried out using the least significant difference test (LSD). Heat maps for fruit traits development were drawn using GraphPad Prism 7 software.

3. Results and Discussion

3.1. Fruit weight

Although S2, S3, S4, and S5 solutions induced fruit weight increase when compared to S1; it was found that S4 solution caused the highest significant increase in fruit weight for all cultivars with 66.26, 136.6, and 163.0 g for GK, G, and GS respectively when compared to S1 (control) which recorded 53, 76.2, and 133.4 for the same cultivars respectively (Table 1). All ammonium containing solutions increased GS fruit weight when compared to the control solution; however, only S4 solution increased weight significantly in comparison to S1 solution. The increased fruit weight with higher nitrogen input was previously reported by [17]; therefore, it can be concluded that higher doses of nitrogen (regardless of its origin) can induce an increase in apple fruit weight. However, our results showed that there are limitations to these effects when using an ammonium nitrogen source, as S5 solution recorded a significant decrease in fruit weight for all cultivars (59.22, 114.4, and 146.6 g for GK, G, and GS respectively) when compared to S4 solution (Table 1)

3.2. Weight loss

The results showed a significant fruit weight decrease in all cultivars between the first and last days of cold storage (Table 2) and (Fig 1 A). The reasons for this decrease were water loss and respiration [18].

The results revealed that there was a significant increase in FWL with the addition of ammonium nitrate in the nutrition solution. The highest FWL was found in fruit from trees fertilized with S5 solution which recorded 11.84, 6.09, and 4.23 % weight losses with GK, G, and GS respectively, while the lowest FWL appeared in fruit of trees fertilized with S1 (for GK with 6.43% and G with 4.04%) and S2 (for GS with 3.28%) as seen in (Table 1) and (Fig. 1 B). These results refer to the negative significant effect of the increased ammonium nitrate in nutrition solution on post-harvest performance by inducing an increased weight loss during cold storage.

3.3. Fruit firmness

FF is considered an important fruit quality trait that affects the value and marketing of apples [19][20]. The lack of FF is a serious issue in terms of fruit quality losses [21]. In this research, the data at the harvest stage showed the highest value of FF for all cultivars with GS being the firmest with 6.07 kg/cm² followed by G with 5.23 kg/cm² then GK with 4.21 kg/cm² (Table 2). The difference in the firmness of apple cultivars is mostly related to their pectin installation architecture [22].

All cultivars showed a significant decrease in FF during the cold storage period (Table 2). This firmness loss related to cell wall breakdown resulting from enzymatic activities which in order lead to the destruction of pectin [23][24], all of which weakens cell walls, leading to a significant decrease in FF [21][25].

The comparison between the different nutrition solutions showed that the maximum value of FF was found in apples from trees fertilized with S1 (control) with values of 3.39, 5.45, and 5.19 kg/cm² for GK, G, and GS respectively, while the minimum values were observed in the products of those fertilized with S5 which recorded 2.56, 3.58, and 4.7

kg/cm2 for the same cultivars respectively (Table 1). Similar results were obtained by [26] referring to the negative effect of ammonium on fruit firmness during storage.

Table 1. The effect of various levels of ammonium to total nitrogen in nutrition solutions on post-harvest traits of three apple cultivars (Golab Kohans, Gala and Granny Smith) during storage periods.

Cultivar	N treatment	Fruit weight (g)	Weight loss (%)	Fruit firmness (kg/cm ²)	Dry matter (%)	SSC (%)	TA (mg/100ml)
Golab kohans	S1	$53.00^{\rm b}$	6.43 ^b	3.39^{a}	14.46 ^c	10.10^{a}	0.29^{a}
	S2	52.41 ^b	8.10^{b}	3.06^{b}	14.74 ^c	10.13^{a}	0.28^{a}
	S3	60.69a	12.05a	3.00 ^b	15.61 ^b	10.42^{a}	0.28^{a}
	S4	66.26a	10.40a	2.85bc	16.76ª	9.97a	0.28a
	S5	59.22ab	11.84a	2.56 ^c	$15.40^{\rm b}$	9.50a	0.27a
	LSD	7.21**	1.72**	0.32**	0.28**	0.90ns	0.027ns
Gala	S1	76.2 ^d	4.04c	5.45a	15.95c	12.77a	0.56^{a}
	S2	91.5c	4.67b	4.35b	16.25b	12.31a	0.55ab
	S3	105.6 ^b	6.03a	4.10bc	17.42a	11.62ab	0.55^{ab}
	S4	136.6a	6.00a	3.86 ^{cd}	17.54a	10.90b	0.53ab
	S5	114.4 ^b	6.09a	3.58^{d}	16.28 ^b	10.46b	0.51 ^b
	LSD	11.03**	0.56**	0.33**	0.14**	1.18**	0.018ns
Granny smith	S1	133.4 ^b	3.54bc	5.19a	16.37^{d}	11.64a	1.01a
	S2	146.2 ^b	3.28c	5.00^{ab}	16.61 ^c	11.36ab	1.05ª
	S3	147.2 ^b	3.86ab	4.82b	17.78a	11.59a	1.06a
	S4	163.0a	4.04ab	4.85b	17.84a	10.64bc	1.12a
	S5	146.6b	4.23a	4.70b	16.99b	10.42c	1.12a
	LSD	14.34**	0.49**	0.31*	0.14**	0.73**	0.19 ^{ns}

Different letters in each column for each cultivar mean significant difference. LSD interpretation (ns: non-significant, *: significant at the 0.05 level and **: significant at the 0.01 level).

3.4. Fruit dry matter

This study showed that there was a significant increase in DM throughout storage period (Table 2). This was due to water loss during storage with similar results noted by [27]. In addition, the data revealed a significant increase in DM with increased doses of ammonium nitrate in the nutrition solution for all cultivars with the maximum values in apples from trees fertilized with S4 solution resulting in 16.76, 17.54, and 17.84% DM for GK, G, and GS respectively), and minimum DM values in S1 (control) with 14.46, 15.95, and 16.37% for the same cultivars respectively (Table 1). We suggest that this increase in DM can be attributed to photosynthesis increase with ammonium nitrate increase in nutrition solution since it was reported that a relationship between fruit and leaf nitrogen and fruit quality factors such as size, color, and dry matter are present [28].

3.5. Soluble solids concentration

SSC is one of the most important criteria for the taste and quality determination of apples. The highest values of SSC were found in G (10.58% to 12.65%) while the lowest in GK (9.22% to 11.13%) throughout storage period (Table 2). SSC was found to increase during storage stage, with the highest value of SSC being found at the end of storage period for all cultivars which is similar to the results of [29], while other studies showed a decrease in SSC after 4 days of storage, after which the SSC remained constant [30].

Table 2. Post-harvest traits changes during cold storage period at 0±1 °C and 90 - 95 % relative humidity for three apple cultivars (Golab Kohans, Gala and Granny Smith).

Cultivar	Storage time (days)	Fruit weight (g)	Weight loss (%)	Fruit firmness (kg/cm2)	Dry matter (%)	SSC (%)	TA (mg/100ml)
Golab kohans	0	67.23a	0	4.21 ^a	15.18 ^c	9.22c	0.40a
	15	61.59^{ab}	5.77 ^c	$3.27^{\rm b}$	15.33bc	9.62bc	0.32^{b}
	30	55.86 ^b	9.88b	2.55c	15.47 ^{ab}	10.1 ^{2b}	0.24 ^c
	45	48.59c	13.64 ^a	1.85 ^d	15.60 ^a	11.13a	0.17 ^d
	LSD	6.45**	1.33**	0.28**	0.25**	0.80**	0.024**
	0	113.4a	0	5.23a	16.52 ^c	10.58c	0.74a
Gala	30	108.4^{ab}	4.43°	4.52^{b}	16.64bc	11.30bc	$0.57^{\rm b}$
	60	102.2bc	5.37b	4.08c	16.74 ^{ab}	11.89ab	0.48c
	90	95.6c	6.29a	3.25^{d}	16.85ª	12.65a	0.37 ^d
	LSD	9.86**	0.43**	0.29**	0.13**	1.06**	0.032**
Granny smith	0	154.9a	0	6.07a	17.01b	10.34c	1.71a
	40	152.7a	1.47 ^c	5.60 ^b	17.07 ^b	10.78bc	1.12 ^b
	80	150.2a	1.62 ^c	4.75 ^c	17.12ab	11.35 ^{ab}	1.02b
	120	146.0ab	2.75 ^b	4.31 ^d	17.16 ^{ab}	11.43ab	0.80c
	160	132.6b	9.33a	3.84e	17.23a	11.74a	0.72c
	LSD	14.34*	0.44**	0.31**	0.14*	0.73**	0.19**

Different letters in each column for each cultivar mean significant difference. LSD interpretation (ns: non-significant, *: significant at the 0.05 level and **: significant at the 0.01 level).

The data showed that the increasing ammonium content in nutrition solution treatment had no significant effect on SSC for GK; while on the other hand, it had a significantly negative effect on the SSC of G and GS cultivars (Table 1). The highest values of SSC appeared in apples from trees fertilized with S1 (control) with SSC values 10.1, 12.77, and 11.64% for GK, G, and GS respectively, while the lowest values of SSC was found in apples from trees fertilized with S5 with values of 9.5, 10.46, and 10.42% for the same cultivars respectively (Table 1). These results are similar to [31] reports of a deceased SSC by increasing nitrogen concentration in soil.

3.6. Titratable acidity

Acidity is considered one of the important taste and shelf-life factors in apple fruit [32]. In our investigation, a significant decrease in TA during storage period was observed in all cultivars. The maximum value of TA at harvest and during storage was found in GS with a TA of 1.71 mg/100ml, which is considered a trademark for this cultivar, while the minimum value was found in GK with 0.40 mg/100ml (Table 2). Our results contradict [30] results of no significant changes in TA during storage period.

The increased nitrogen content in our nutrition solutions had no significant effect on TA at harvest and throughout storage period (Table 1), which is similar to the reports of [31].

3.7. Fruit color changes

The results of this investigation showed that there was a significant decrease in L* during storage period in GK and GS cultivars with no significant differences for G cultivar while a significant decrease in Hue was observed in all cultivars was observed. Additionally, a significant increase in a*, b* and Chroma values throughout storage stages in the case of all the cultivars was observed (Table 3). These results might refer to cultivar related color controlling factors as [30] results also showed no change in L* and Hue with a significant increase in a* and b* during storage period.

Visually, it can be noticed that with the increase in nitrogen content in nutrition solution using ammonium-based nitrogen produced fruit with lighter skin color (Fig. 1). This result is in agreement with [33] stating that the lower

value of red color in apples was noticed in fruit from trees fertilized with 80 g N/tree compared with those fertilized with 40 g N/tree. Furthermore, slight visual changes can be noticed in each cultivar throughout storage period as all fruit became slightly darker in color. This might be due to storage pigmentation oxidization.

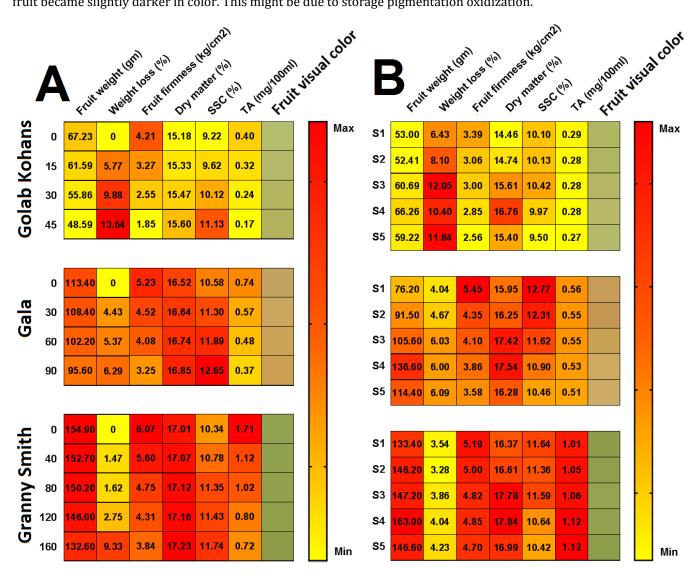


Figure 1. Mean values of the studied traits for the three cultivars of (Golab Kohans, Gala, and Granny Smith) against different storage evaluation stages (A) and the different used nutrition solutions (B). The scale to the right refers to the colors correlated with maximum and minimum values in each trait. Fruit visual color is a digital identification for colors obtained from Colorimetric device and has no relation to the scale.

4. Conclusion

The results of this investigation revealed that by increasing the nitrogen content in apple trees nutrition solutions using ammonium-based fertilizers can increase fruit weight and fruit titratable acidity. In addition, increasing nitrogen induces the production of lighter-skinned fruit. However, excess nitrogen significantly reduces soluble solid content and fruit firmness which leads to more weight losses and deterioration during cold storage in comparison to low nitrogen nutritional solutions. Therefore, it can be concluded that omitting ammonium-based nitrogen from the nutritional solution of apple trees can enhance fruit post-harvest performance.

Table 3. Fruit color changes during cold storage period at 0 ± 1 °C and 90 - 95 % relative humidity for three apple cultivars (Golab Kohans, Gala and Granny Smith).

Cultivar	storage time (days)	L*	a*	b*	Hue (°)	Chroma
Golab kohans	0	74.62a	-14.83c	$38.84^{\rm b}$	70.89a	41.73 ^b
	15	73.33 ^{ab}	-13.50 ^{bc}	39.51 ^b	68.86 ^b	41.86 ^b
	30	71.78 ^{bc}	-12.70 ^b	42.41 ^a	66.69°	44.29a
	45	70.69c	-10.84a	42.91a	64.22 ^d	44.32a
	LSD	1.72**	1.36**	1.36**	1.95**	1.26**
Gala	0	68.42a	2.66 ^c	38.56 ^c	45.85ª	38.90c
	30	69.93a	4.71 ^b	42.05b	43.60 ^b	42.43b
	60	69.49a	6.54 ^a	44.56ab	41.14 ^c	45.22a
	90	68.10a	7.77^{a}	44.98^{a}	40.23c	45.80a
	LSD	2.16 ^{ns}	1.46**	2.62**	2.15**	2.60**
Granny smith	0	63.90a	-19.02c	39.32 ^c	76.03a	43.77b
	40	63.24^{ab}	-18.77 ^{bc}	40.19bc	$75.04^{\rm b}$	44.37^{ab}
	80	63.05ab	-18.34 ^b	41.01ab	74.12 ^c	44.93ab
	120	62.96ab	-17.75a	41.70^{a}	73.14 ^d	45.27a
	160	61.44 ^b	-17.36a	41.99a	72.50 ^d	45.46a
	LSD	1.79*	0.54**	1.29**	0.66**	1.30*

Different letters in each column for each cultivar mean significant difference. LSD interpretation (ns: non-significant, *: significant at the 0.05 level and **: significant at the 0.01 level).

Table 4. The effect of various levels of ammonium to total nitrogen in nutrition solutions on apple fruit color of three apple cultivars (Golab Kohans, Gala and Granny Smith) during storage periods.

Cultivar	N treatment	L*	a*	b*	Hue(°)	Chroma
Golab kohans	S1	71.79 ^{ab}	-14.53b	42.26a	69.02a	44.84a
	S2	71.40 ^b	-12.94 ^a	41.44 ^{ab}	67.44a	43.53ab
	S3	72.20 ^{ab}	-12.17a	41.10 ^{abc}	66.69a	43.02bc
	S4	73.78 ^a	-12.43a	40.23bc	67.28a	42.18bc
	S5	73.67a	-12.75 ^a	39.57°	66.69a	41.67c
	LSD	1.93*	1.52*	1.52**	2.18 ^{ns}	1.41**
Gala	S1	67.10^{bc}	7.77^{a}	41.97 ^{ab}	39.70^{b}	42.87ab
	S2	65.52°	8.73a	42.32^{ab}	38.40 ^b	43.34 ^{ab}
	S3	68.75 ^b	4.43 ^b	45.01 ^a	44.21a	45.38a
	S4	71.74^{a}	3.43bc	43.09ab	45.62a	43.35ab
	S5	71.82a	2.52 ^c	40.29 ^b	45.59a	40.50 ^b
	LSD	2.42**	1.64**	2.93*	2.41**	2.91*
Granny smith	S1	61.52 ^c	-18.45 ^{bc}	41.71 ^a	73.90^{b}	45.63a
	S2	62.59 ^{abc}	-18.24b	39.72 ^b	74.71 ^a	43.71 ^b
	S3	64.36a	-17.10 ^a	41.10^{ab}	72.70 ^c	44.53ab
	S4	63.81 ^{ab}	-18.64 ^{bc}	40.77^{ab}	74.61a	44.82ab
	S5	62.33bc	-18.97 ^c	40.91 ^{ab}	74.91 ^a	45.10 ^{ab}
	LSD	1.79**	0.54**	1.29*	0.66**	1.30*

Different letters in each column for each cultivar mean significant difference. LSD interpretation (ns: non-significant, *: significant at the 0.05 level and **: significant at the 0.01 level).

Conflict of interest statement

The authors declared no conflict of interest.

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Data availability statement

The authors declared that all related data are included in the article. The nutritional compositions were provided as supplementary material.

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