



Improving Growth and Performance of Young Almond Trees in Nursery by Optimizing Mineral Nutrition

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ABSTRACT

Short growing season restricts production of standard-sized fruit trees in nurseries at cold regions. Enhancing plant growth by optimizing program of mineral nutrition may solve the problem. This study evaluated efficiency of fertilizers [urea, sulfur coated urea (SCU), or foliar applications of a NPK compound fertilizer] for optimizing the growth of seedling rootstocks and grafted young almond trees at the nursery in a cold region. At the end of the first growing season, the growth and quality of the almond seedlings were evaluated and they were budded in September. At the following season, the treatments were repeated on the grafted trees and grafting success and performance of the young almond trees were evaluated. The result showed that application of 600 kg ha⁻¹ SCU and urea were the most successful treatments on enhancing the growth of the seedlings. Application of 400 and 600 kg ha⁻¹ urea and SCU, and 30 kg ha⁻¹ NPK resulted in the highest grafting success at the second season. The largest shoots of scion were found in the 400-600 kg ha⁻¹ urea and SCU treatments. The highest leaf greenness and chlorophyll concentration were found in the 600 kg SCU ha⁻¹ and 30 kg ha⁻¹ NPK treatments. The highest leaf potassium and phosphorus concentrations were found in 30 kg ha⁻¹ NPK treatment. The highest leaf nitrogen concentration was detected in treated plants with 400 and 600 kg SCU and 600 kg urea per hectare. According to the results, application of 400 kg ha⁻¹ SCU is recommended to obtain young standard-sized almond plants in the nurseries.

Introduction

Almond (*Prunus dulcis* Mill.) is of the most economically important nut crops of Iran (Ansari and Gharaghan, 2019), which is known for its drought tolerance (Karimi *et al.*, 2015). Moreover, almond seedlings are used as tolerant rootstocks for almond and stone fruits (Ansari and Gharaghan, 2019), especially in regions with dry alkaline calcareous soils (Tagliavini *et al.*, 2000). Therefore, almond

cultivation should be developed in a situation where the world is facing with climate change and water deficiency (Gutiérrez-Gordillo *et al.*, 2019). In order to expand the almond cultivations, firstly, seedling production and growth of the grafted trees should be optimized in nurseries. However, environmental conditions may restrict plant production in nurseries and availability of rootstocks for fruit trees in some

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areas. For instance, short growing season, due to incidence of late spring frosts and early autumn frosts, does not allow seedling or grafted almond trees to grow well at cold regions. Thus, the production of standard-sized trees is negatively affected in such regions and nurseries prefer not to produce almond trees. Therefore, the almond growers may be forced to provide these plants from nurseries at neighbor regions with warmer climates (Ghasemi *et al.*, 2010). This issue considerably increases orchard establishment costs. More importantly, the trees and cultivars, which are provided from warm areas, may not be completely adapted to the cold regions. These problems can restrict development of almond and similar species in cold regions.

Standard seedlings of almond have a height of 100-180 cm with three to five branches with 30-40 cm apart around the trunk (Ghasemi *et al.*, 2010). Optimizing the plant nutrition in nursery is the most practical approach to achieve this considerable growth. Among the essential nutrients, nitrogen has the most important impact on plant growth and development (Marschner, 2012). The positive effect of different forms of nitrogen on growth and performance of fruit trees (Dehghanipoodeh *et al.*, 2018; Rajaie and Motieallah, 2018; Akhlaghi *et al.*, 2016), ornamentals (Rutto *et al.*, 2018; Aboutalebi Jahromi and Farahi, 2016; Geshnizjani and Khosh-Khui, 2016; Afifipour and Khosh, 2015) and vegetables (Barzegar *et al.*, 2016) have been reported. Soils in arid and semi-arid regions (favorable conditions for almond production) are mostly poor of nitrogen because of low organic matter content (Batjes, 2014; Chaker *et al.*, 2019). Therefore, nitrogen application as organic and inorganic fertilizers is required to supply the plant needs. Almond trees need to receive considerable amounts of nitrogen for developing branches, flowering and fruiting (Muhammad *et al.*, 2015). Rahnamoun (2002) reported that application of 600 g nitrogen per tree in two parts –in March (at the beginning of growing season) and 45 days later in May– is required to obtain the highest yield and growth of almond ‘Azar’.

Muhammad *et al.* (2015) obtained the highest almond yield by application of 466 g nitrogen at different times (20% in late February, 30% in mid–April, 30% in late June and 20% in September after harvest). Neilsen *et al.* (2001) reported that fertilization at the beginning of spring, increased flowering, and improved growth of spur leaves and branches in the subsequent growing season. Zarata-Valdez *et al.* (2015) reported that the highest yield, number of fruits, leaf area, and mineral nutrients’ concentration were obtained by application of 300 kg ha⁻¹ nitrogen. Studies have shown that the highest almond yield was obtained in presence of 2.7% of nitrogen, 0.135% of phosphorus, 1.25% of potassium, 3.15% of calcium, 0.95% of magnesium and 100 mg kg⁻¹ of iron, 36 mg kg⁻¹ of zinc, and 57 mg kg⁻¹ of manganese in leaf dry matter (Rahnamoun, 2002). Improving the nitrogen availability to almond trees, increases leaf chlorophyll concentration and promotes its photosynthesis capacity (Mohammadi, 2017).

According to its low price, readily availability and high nitrogen content, urea is the most dominant nitrogen fertilizer which is used for growing plant crops (Rui *et al.*, 2019). Due to its high nitrogen content (46.7%), the transport, storage and application costs per nitrogen unit is lower than the other nitrogen fertilizers (Boncz *et al.*, 2012). More importantly, high acidification capability of this fertilizer may improve absorption of the other essential nutrients such as phosphorus, iron, zinc, copper and manganese in the alkaline calcareous soils of arid and semi-arid regions (Barker and Pilbeam, 2015). However, urea fertilizer is exposed to leaching due to its high solubility; therefore, farmers have to use more amounts of fertilizer to meet the plants' needs (Rui *et al.*, 2019). The loss of fertilizer due to leaching not only increases production costs, but also contributes to soil hardening and pollution of groundwater resources. Sulfur coated urea (SCU) can be used to solve this problem. SCU is a slow-release fertilizer, which has many benefits to the soil and plant (Azeem *et al.*, 2014, Da Costa *et al.*, 2019). Nourgholipour *et al.* (2008) reported that this fertilizer improved nitrogen use efficiency by reducing

nitrogen leaching and sublimation. SCU contains about 36% nitrogen and 20% sulfur. In addition to solving the problems of urea fertilizer, its sulfur can improve the absorption of micronutrients by amendment of the calcareous alkaline soils' reaction (Azeem *et al.*, 2014). Madani *et al.* (2009) in a study on sunflower showed that application of 100 kg-ha⁻¹ SCU resulted in higher fertilizer use efficiency than urea. However, Nourgholipour *et al.* (2008) stated that SCU was not as effective as ammonium sulfate in providing nitrogen. These results indicate that the amount and type of nitrogen fertilizer should be optimized according to crop and climatic conditions. Furthermore, optimizing fertilizer application rate, especially nitrogen, is more important for temperate zone fruit trees because excess fertilization can induce plant growth at the end of the growing season and enhance frost damage susceptibility in autumn (Mng'omba *et al.*, 2010).

This study was conducted to optimize growth of almond rootstock and grafted young-trees by application of different rates of urea and sulfur coated urea (SCU) fertilizers in a cold region nursery. Furthermore, the efficiency of the fertilizers was compared with foliar application of a 20-20-20 compound fertilizer at different doses. In addition to nitrogen, compound fertilizers provide phosphorous, potassium and the essential micronutrients. On the other hand, foliar application of compound fertilizers

may reduce fertilizer requirements and therefore is more environmentally friendly. As so, application of such fertilizers is interesting for nurserymen.

Material and Methods

This study was conducted in a commercial nursery at Fereydounshahr, Isfahan, Iran (32°56'9.88"N, 50°6'40.37"E) during 2015 - 2017. The experiment site was located in a mountainous region with elevation of 2550 m above sea level. Short growing season of this region does not allow the production of young almond trees. This region has a climate with moderate summers and cold winters, which is covered with snow for more than eight months per year (report of the meteorological office of Fereydounshahr, 2017). The number of frosty days of this region is 118 days (71 days in winter, 5 days in spring and 42 days in autumn).

The soil of the nursery was a clay loam. Physicochemical properties of top 50 cm layer of the soil are shown in Table 1. In autumn 2015, the field was ploughed in a form of ridge and furrow. Length of the furrows was 5 m with 40 cm width and 60 cm spacing. Almond kernels were disinfected by fungicide benomyl 0.1% for 1 h. The seeds were planted on the edge of furrows with 15 cm spacing. The furrows were irrigated with 8 days interval and no leaching was allowed.

Table 1. Physicochemical properties of top layer (0-50 cm) of the soil.

Parameter	Value
pH	7.9
EC (dS/m)	0.4
Organic carbon (%)	0.65
Neutralizing matters (%)	55
N (%)	0.07
P Av. (mg/kg)	16
K Av. (mg/kg)	320
Fe (mg/kg)	5.2
Zn (mg/kg)	3.5
Mn (mg/kg)	11
Cu (mg/kg)	2
Boron (mg/kg)	0.01

After germination at the following spring, the seedlings were subjected to 10 fertilizer treatments as described in Table 2. These treatments were applied at three times: May 5th, June 14th, and July 24th. One third of the amount of the fertilizers was applied in each time. Urea and sulfur-coated urea (SCU) were applied one hour before irrigation.

A compound fertilizer (Eurosolid™, the Netherlands) was used for foliar application of mineral nutrients.

The compound fertilizer was dissolved in 3 L distilled water and sprayed on the plants at evening. The compound fertilizer was a 20-20-20 supplemented with 5% sulfur, 2% magnesium oxide, 0.2% boron, 0.017% copper, 0.018% iron, 0.068% manganese, 0.005% molybdenum, and 0.002% zinc.

Table 2. The fertilizer treatments which applied to the almond rootstocks and budded trees.

	Treatment	Description	Method application
1	Control	No fertilizer was applied	-
2	Urea ₂₀₀	200 kg urea per hectare (20 g m ²)	Soil application
3	Urea ₄₀₀	400 kg urea per hectare (40 g m ²)	Soil application
4	Urea ₆₀₀	600 kg urea per hectare (60 g m ²)	Soil application
5	SCU ₂₀₀	200 kg SCU per hectare (20 g m ²)	Soil application
6	SCU ₄₀₀	400 kg SCU per hectare (40 g m ²)	Soil application
7	SCU ₆₀₀	600 kg SCU per hectare (60 g m ²)	Soil application
8	NPK ₁₀	10 kg compound fertilizer per hectare (1 g m ²)	Foliar application
9	NPK ₂₀	20 kg compound fertilizer per hectare (2 g m ²)	Foliar application
10	NPK ₃₀	30 kg compound fertilizer per hectare (3 g m ²)	Foliar application

Evaluating the performance of the rootstocks at the 1st growing season

The growth and quality of the seedling rootstocks were determined, before budding in September 1, 2016. From each replicate, 10 seedlings from the middle of the rows were selected and the following parameters were measured (Table 3): main stem diameter (at 25 cm above the soil surface), plant height, and number of lateral branches. Moreover, budding readiness of the rootstocks was scored by three budding experts. The budding readiness score was ranged between 0 for unfavorable to 20 for favorable seedlings for budding. Then, the plants were budded at 25 cm above the soil surface. T-budding, was the method which used for budding of the seedlings.

Evaluating the performance of the grafted trees at the 2nd growing season

The grafted trees were headed back at April 25, 2017. The fertilizer treatments and their application method were similar to the first growing season. The following measurements were made during October 6-12, 2017 (Table 3).

1. The rootstock growth: Ten grafted trees at the middle of the rows were selected and number of suckers developed on the rootstock and rootstock diameter at 2 cm under the grafting point were recorded. Budding success percentage was calculated according to the number of developed scions. Callus quality was visually scored based on growth and uniform development of callus tissue at the grafting point. The callus quality score was ranged between 0 for poor to 20 for perfect tissue quality.
2. The scion growth: Diameter of scion stem at 2 cm above the grafting point, length of the scion main

stem, number and length of lateral branches of scion were measured. Scion leaf area was determined by using a leaf area meter (ADC AM100 leaf area meter, ADC Bioscientific, UK). Angle of lateral branches was measured by using a protractor. The leaves and stems of the scions were weighted (fresh mass- FM) and oven dried at 70°C till constant weight (dry mass - DM). Relative water content of scion shoot was determined according to the FM and DM of the scion shoot (Karimi *et al.*, 2017):

$$\text{Shoot relative water content} = \frac{\text{FM}-\text{DM}}{\text{DM}} \times 100$$

Eq. 1

3. Leaf greenness and chlorophyll concentration: Greenness and overall health of the leaves was determined by using a chlorophyll meter (SPAD 502). The measurements were performed on five leaves from middle of the scion shoot and at least five readings per leaf were made. Concentration of total chlorophylls (Chl a + Chl b) was determined in the leaves from middle of stem according to the method

described by Lichtenthaler (1987). 500 mg of leaf tissue was extracted twice by 25 ml of 80% cold acetone. After centrifuging, the absorption of the supernatant was measured at 647 nm (A_{647}) and 663 nm (A_{663}) by spectrophotometry (PerkinElmer, Lambda 25, USA). The concentration of total chlorophylls was calculated according to the following equation.

$$\text{Total chlorophylls} = 7.15 \times A_{663} + 18.71 \times A_{647}$$

Eq. 2

4. Mineral composition of leaves: Concentration of nitrogen (N), phosphorus (P) and potassium (K) was measured in the dried leaves. The elemental nitrogen content in the samples was determined using the Kjeldahl digestion. K content was determined in the samples by flame photometry (Jenway PFP7; Spectronic Analytical Instruments, UK). Concentration of P in the samples was determined by measuring absorbance in 860 nm by spectrophotometry (PerkinElmer, Lambda 25, USA).

Table 3. Characteristic, abbreviation (Symbol) and measurement method of the evaluated parameters.

Season	Characteristic	Abbreviation or Symbol	Unit	Measurement method
1 st growing season	Stem diameter	SD	Cm	Caliper
	Plant height	PH	Cm	Ruler
	Number of lateral branches	NLB	Number	Counting
	Budding readiness score	BRS	Code	Visual
2 st growing season	Stem diameter	SD	cm	Caliper
	Stem length	SL	cm	Ruler
	Number of lateral branches	NLB	Number	Counting
	Length of lateral branches	LLB	cm	Ruler
	Leaf area	LA	cm ²	Leaf area meter
	Angle of lateral branches	ALB	Degree	Protractor
	Leaf fresh mass	LFM	g	Scale
	Stem fresh mass	SFM	g	Scale
	Leaf dry mass	LDM	g	Scale
	Stem dry mass	SDM	g	Scale
	Shoot relative water content	SRWC		Scale
	Leaf greenness	LG	-	SPAD
	Leaf chlorophyll	LC	(mg kg FM ⁻¹)	Spectrophotometry
	Leaf nitrogen	LN	%	Kjeldal
	Leaf potassium	LK	%	Flame photometry
	Leaf phosphorous	LP	%	Spectrophotometry
Number of suckers	NS	Number	Counting	

Table 3. Continued.

Rootstock diameter	RD	cm	Caliper
Number of developed scions	NDS	Number	Counting
Callus quality score	CQ	Code	Visual
Budding success	BS	%	Counting

Statistical analyses

This study was conducted based on completely randomized design with three replications. Ten plants per replicate were used and each replicate was mean of these plants. Data was analyzed by SAS 9.1. Means were compared using Duncan's multiple range test (DMRT) at $P \leq 0.05$. Correlations between traits and draw Figs were carried out using SPSS software (version 18.0) and Excel, (2010) software.

Results

Rootstock growth in the first year

The growth of almond rootstock was measured at the end of the first growing season (Table 4). The highest

stem diameter was observed in the Urea₆₀₀ and SCU₄₀₀ and SCU₆₀₀ treatments. The control and NPK₁₀ treated plants had the lowest stem diameter. Application of urea and SCU increased stem length and by increasing these fertilizers amount, stem length was increased. The longest stems were found in the Urea₆₀₀ and SCU₄₀₀ and SCU₆₀₀ treatments. The 400 and 600 kg ha⁻¹ urea or SCU received plants had the lowest number of lateral branches, whereas the control plants and 10 and 20 kg ha⁻¹ NPK treated plants had the highest branch numbers. The highest score of budding readiness was observed in the Urea₆₀₀ and SCU₄₀₀ and SCU₆₀₀ treatments. The control plants had the lowest score of budding readiness.

Table 4. The effects of fertilizer treatments on growth of almond rootstocks prior to budding at the end of the first growing season.

Treatment	Rootstock diameter (mm)	Rootstock height (cm)	Lateral branches number	Budding readiness (0-20)
Control	7.1 ^e	66 ^e	4.3 ^a	8.33 ^f
Urea ₂₀₀	9.4 ^c	94 ^c	3.3 ^{bcd}	14.83 ^c
Urea ₄₀₀	10.9 ^b	125 ^b	2.7 ^{de}	18.50 ^b
Urea ₆₀₀	12.3 ^a	137 ^a	2.1 ^e	19.67 ^{ab}
SCU ₂₀₀	9.3 ^c	95 ^c	3.2 ^{cd}	16.00 ^c
SCU ₄₀₀	11.2 ^{ab}	119 ^b	2.5 ^{de}	18.83 ^{ab}
SCU ₆₀₀	12.6 ^a	133 ^a	2.0 ^e	20.00 ^a
NPK ₁₀	7.6 ^{de}	75 ^d	3.9 ^{ab}	10.5 ^e
NPK ₂₀	8.3 ^d	85 ^{cd}	3.7 ^{abc}	14.17 ^d
NPK ₃₀	9.9 ^c	99 ^c	3.1 ^{cd}	17.00 ^c

Means with at least one common letter are not significantly different according to DMRT ($P < 0.05$)

Evaluating the success of budding and the growth of rootstock in the second year

Fertilizer treatments did not affect number of suckers (Table 5). The highest rootstock diameter was observed in the Urea₆₀₀ and SCU₄₀₀ and SCU₆₀₀ treatments. The lowest diameter was found in the control plants which were not significantly different from the 10 and 20 kg ha⁻¹ NPK treated plants. The treated plans had higher budding success in

comparison to the control plants. Moreover, budding success was increased in parallel with increasing the amount of the fertilizers. Application of 200-600 kg ha⁻¹ urea or 600 kg ha⁻¹ SCU increased callus quality (Table 5). Callus quality in the other treatments was the same as the control treatment.

Table 5. The effects of fertilizer treatments on number of suckers, rootstock diameter, and budding success parameters.

Treatment	Sucker number	Rootstock diameter (cm)	Budding success	Callus quality (0-20)
Control	7.3 ^a	1.25 ^d	46.7 ^e	14.0 ^c
Urea ₂₀₀	7.3 ^a	1.43 ^c	70.0 ^{cd}	17.6 ^{ab}
Urea ₄₀₀	6.1 ^a	1.78 ^b	83.3 ^{abc}	18.1 ^{ab}
Urea ₆₀₀	5.0 ^a	1.98 ^a	93.3 ^a	19.6 ^a
SCU ₂₀₀	6.2 ^a	1.49 ^c	73.3 ^{bcd}	15.5 ^{bc}
SCU ₄₀₀	5.4 ^a	1.92 ^a	86.7 ^{ab}	17.0 ^{abc}
SCU ₆₀₀	7.0 ^a	1.99 ^a	93.3 ^a	18.5 ^{ab}
NPK ₁₀	8.2 ^a	1.32 ^d	63.3 ^d	15.4 ^{bc}
NPK ₂₀	7.1 ^a	1.34 ^d	70.0 ^{cd}	15.2 ^{bc}
NPK ₃₀	6.7 ^a	1.44 ^c	80.0 ^{abc}	15.9 ^{bc}

Means with at least one common letter are not significantly different according to DMRT ($P < 0.05$)

Evaluating the scion growth in the second growing season

Fertilizer application increased the diameter of scion stem. The highest diameter was obtained by application of 600 kg ha⁻¹ urea or SCU. The plants in the control and NPK₁₀ treatments had the lowest scion stem diameters (Table 6). The scions of control plants and the plants received 10 and 20 kg ha⁻¹ NPK was

significantly shorter than the other treatments. Moreover, by increasing fertilizer amount, the longitudinal growth of scion branch was enhanced. In sum, the longest branches were observed in 400 and 600 kg ha⁻¹ of urea or SCU treated plants (Table 6).

Table 6. The effects of fertilizer treatments on the growth of scion shoot in the second growing season.

Treatment	Stem diameter (cm)	Plant length (cm)	Lateral branches No.	Lateral branch length (cm)	Shoot dry weight (g)	Leaf area (cm ²)
Control	0.85 ^f	53 ^c	9.5 ^a	9.01 ^e	29.63 ^d	239 ^f
Urea ₂₀₀	1.07 ^{dc}	73 ^{cb}	8.3 ^a	12.7 ^{de}	35.70 ^d	369 ^{cde}
Urea ₄₀₀	1.44 ^b	106 ^a	7.7 ^a	19.3 ^{ab}	71.18 ^b	481 ^b
Urea ₆₀₀	1.58 ^a	123 ^a	7.3 ^a	22.8 ^a	93.19 ^a	514 ^{ab}
SCU ₂₀₀	1.13 ^c	85 ^b	8.1 ^a	16.8 ^{bc}	57.05 ^c	419 ^c
SCU ₄₀₀	1.43 ^b	119 ^a	7.7 ^a	20.0 ^{ab}	85.11 ^{ab}	511 ^{ab}
SCU ₆₀₀	1.63 ^a	125 ^a	6.7 ^a	23.1 ^a	100.02 ^a	541 ^a
NPK ₁₀	0.91 ^{ef}	65 ^{bc}	9.0 ^a	10.8 ^{de}	34.68 ^d	324 ^e
NPK ₂₀	0.98 ^{de}	68 ^{bc}	9.0 ^a	12.0 ^{de}	37.44 ^d	353 ^{de}
NPK ₃₀	1.12 ^c	79 ^b	8.3 ^a	14.8 ^{cd}	52.54 ^c	385 ^{cd}

Means with at least one common letter are not significantly different according to DMRT ($P < 0.05$).

Application of different fertilizers did not affect the number of scion lateral branches (data not shown). Each plant had an average of 8.1 lateral branches. The longest lateral branches of the scion were found in the 400 and 600 kg ha⁻¹ urea and SCU treated plants. Lateral branches in the control, NPK₁₀ and NPK₂₀, and Urea₂₀₀ treatments were significantly shorter than the other treatments (Table 6). The longest branches were found in the 400-600 kg ha⁻¹ urea or SCU treated

plants. The angle of lateral branches with the main stem in the 400-600 kg ha⁻¹ urea or 200-600 kg ha⁻¹ SCU treated plants were significantly sharper than the other treatments (Fig. 1). The highest branch dry mass was obtained by application of 600 kg ha⁻¹ urea or 400 and 600 kg ha⁻¹ SCU. The lowest dry mass of scion shoot was detected in the control and NPK₁₀ and NPK₂₀ treatments (Table 6).

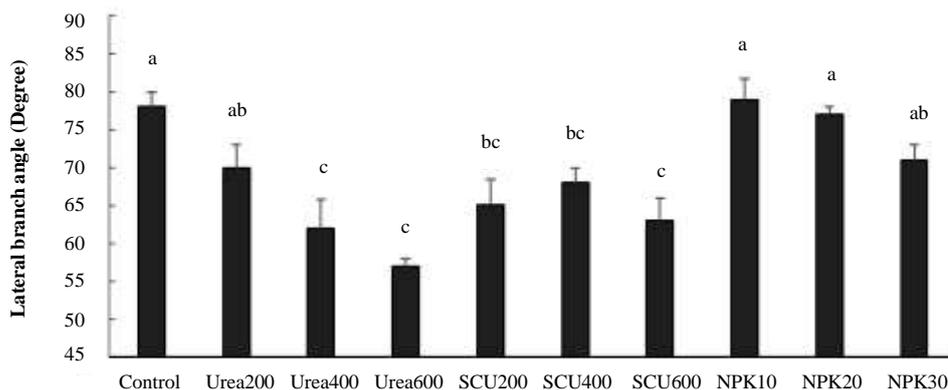


Fig 1. The effect of fertilizer treatments on lateral branch angle of scion. According to ANOVA, the effects of treatments was significant at $P < 0.05$.

Means with at least one common letter are not significantly different according to DMRT ($P < 0.05$).

The fertilizer treatments increased leaf area so that the highest leaf area of scion was found in the Urea₆₀₀ and SCU₄₀₀ and SCU₆₀₀ treatments. The control plants had a lower leaf area than other treatments (Table 6). Fig. 2 represents the relationships between the rootstock diameter in the first growing season and the budding

success indices in the following year. The scion relative water content was ranged between 62.3% in the control plants to 68.1% in the 600 kg ha⁻¹ urea treated plants. However, the effect of treatments on scion relative water content was not statistically significant.

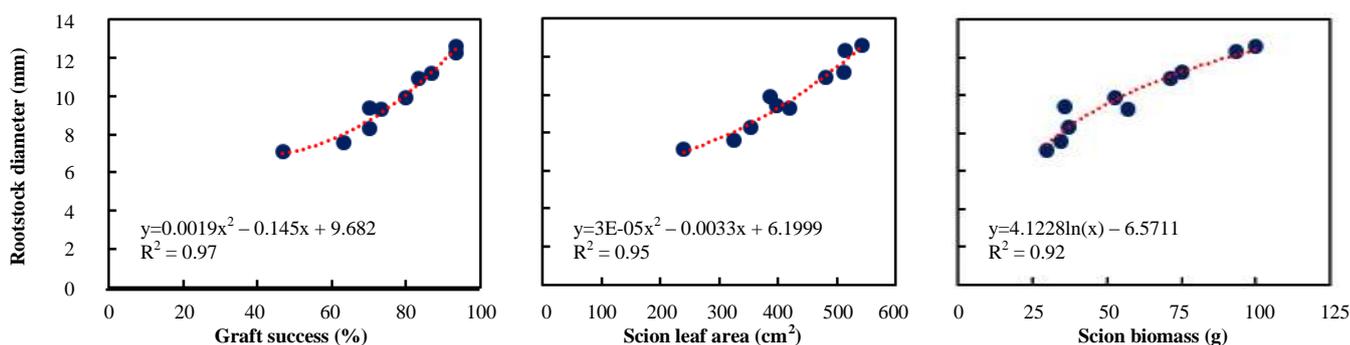


Fig.2. The correlations of rootstock diameter in the first year and graft success, scion leaf area and scion biomass in the second year of the experiment.

Concentrations of chlorophyll and minerals in scion leaf

In comparison to the control plants, fertilizer received plants had higher leaf greenness, and more concentrations of chlorophyll *a* (data not shown), *b* (data not shown) and total chlorophylls in their scion leaves. Leaf greenness in the NPK₃₀ and Urea₆₀₀ treatments was significantly higher than the other treatments. The highest concentration of chlorophylls was observed in the NPK₃₀ and SCU₆₀₀ treatments (Table 7). Fertilizer application increased the nitrogen (N) and phosphorus (P) contents of the leaves. The

highest leaf N content was found in the plants which received 400-600 kg ha⁻¹ SCU, or 600 kg ha⁻¹ urea. The control plants had the lowest N content in their leaves (Table 7). The highest leaf P content was found in the NPK₃₀ treatment and the control plants had the lowest P content (Table 7). The fertilizer treatments significantly increased leaf potassium (K) content. The highest leaf K content was found in the NPK₃₀ treatment (Table 7).

Table 7. The effects of fertilizer treatments on leaf greenness (SPAD), and concentrations of total chlorophylls (Chls), nitrogen (N), phosphorus (P) and potassium (K) in the scion leaves at the second growing season.

Treatment	SPAD	Total Chls (mg kg FM ⁻¹)	N			P			K		
			(% of DM)			(% of DM)			(% of DM)		
Control	12.2 ^e	0.626 ^e	1.00 ^d	0.160 ^f	0.312 ^e	1.14 ^{cd}	0.163 ^e	0.375 ^d	1.52 ^b	0.167 ^e	0.525 ^{bc}
Urea ₂₀₀	13.7 ^d	0.773 ^f	1.52 ^b	0.168 ^{de}	0.537 ^b	1.80 ^a	0.176 ^d	0.425 ^d	1.69 ^{ab}	0.176 ^d	0.425 ^d
Urea ₄₀₀	13.9 ^d	0.959 ^{ef}	1.30 ^c	0.190 ^d	0.487 ^{cd}	1.81 ^a	0.190 ^d	0.487 ^{cd}	1.81 ^a	0.190 ^d	0.487 ^{cd}
Urea ₆₀₀	23.6 ^c	1.169 ^e	1.14 ^{cd}	0.232 ^c	0.550 ^b	1.30 ^c	0.270 ^b	0.575 ^b	1.30 ^c	0.270 ^b	0.575 ^b
SCU ₂₀₀	24.8 ^c	1.313 ^{cd}	1.36 ^{bc}	0.327 ^a	0.737 ^a	1.36 ^{bc}	0.327 ^a	0.737 ^a	1.36 ^{bc}	0.327 ^a	0.737 ^a
SCU ₄₀₀	30.3 ^b	1.283 ^{de}	1.36 ^{bc}	0.327 ^a	0.737 ^a	1.36 ^{bc}	0.327 ^a	0.737 ^a	1.36 ^{bc}	0.327 ^a	0.737 ^a
SCU ₆₀₀	32.3 ^{ab}	1.613 ^{ab}	1.36 ^{bc}	0.327 ^a	0.737 ^a	1.36 ^{bc}	0.327 ^a	0.737 ^a	1.36 ^{bc}	0.327 ^a	0.737 ^a
NPK ₁₀	17.0 ^{cd}	1.054 ^e	1.36 ^{bc}	0.327 ^a	0.737 ^a	1.36 ^{bc}	0.327 ^a	0.737 ^a	1.36 ^{bc}	0.327 ^a	0.737 ^a
NPK ₂₀	32.1 ^{ab}	1.378 ^{bc}	1.36 ^{bc}	0.327 ^a	0.737 ^a	1.36 ^{bc}	0.327 ^a	0.737 ^a	1.36 ^{bc}	0.327 ^a	0.737 ^a
NPK ₃₀	34.1 ^a	1.791 ^a	1.36 ^{bc}	0.327 ^a	0.737 ^a	1.36 ^{bc}	0.327 ^a	0.737 ^a	1.36 ^{bc}	0.327 ^a	0.737 ^a

Means with at least one common letter are not significantly different according to DMRT ($P < 0.05$).

Fig. 3 represents the relationships between concentration of minerals in leaf and leaf nutrients and biomass of scion in the second growing season. The chlorophyll content of the leaf was directly correlated to the concentration of elements in the leaves. Also,

biomass was directly related to the concentration of N in the leaves. However, no significant correlation was observed between leaf K and P concentration with biomass accumulation in scion shoot.

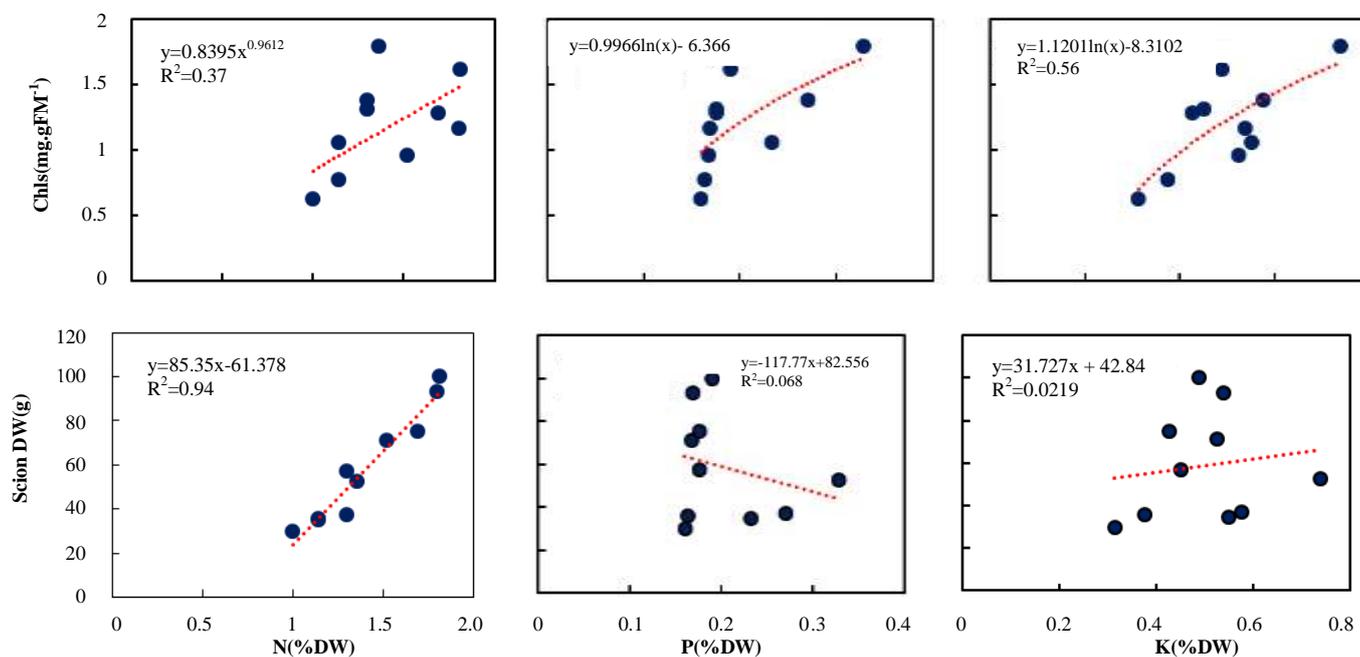


Fig. 3. The correlations of concentration of primary nutrients in the leaves with chlorophylls concentration and scion dry weight.

The relationships between leaf N concentration and shoot growth indices are presented in Fig. 4. Leaf area, stem diameter and also shoot height were

directly correlated to leaf N content but no significant relationships were found between the growth indices and leaf K or P content (data not shown).

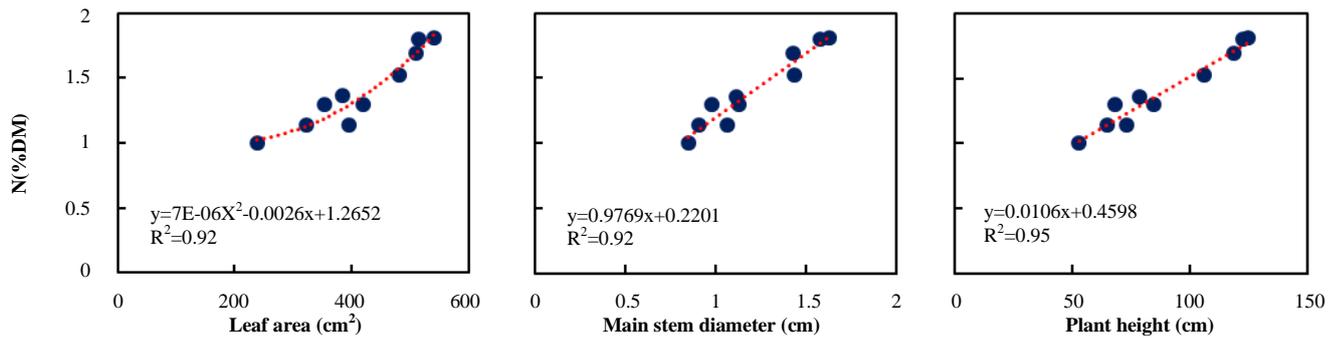


Fig. 4. The correlations between leaf nitrogen (N) concentration and the growth parameters of scion in the second growing season.

Discussion

The rootstock and scion growth

Nitrogen plays a critical role in growth and development of plants. This element exists in proteins, metabolites, and compounds that are used in the biosynthesis of materials, energy transferring and structure of the genetic material (Prasad and Power, 1997; Marschner, 2012). Therefore, nitrogen deficiency is the most important limiting factor for plant growth (Aboutalebi Jahromi and Farahi, 2016) and it should be applied extensively to agricultural lands every growing season (Semenov *et al.*, 2007). Restricted shoot and root growth, reduced flower size, biennial bearing, reduction in the fruit set and size of fruits and postponement of fruiting are the consequences of nitrogen deficiency in almond trees (Muhammad *et al.*, 2015).

Although nitrogen supply for trees in nursery can increase the quality and value of seedlings and production efficiency, and also improves the rooting potential in rootstocks (Izadi *et al.*, 2016), our knowledge about nitrogen requirements of almond rootstocks and trees in nursery is still quite limited. In the current study, stem and leaf growth of both rootstock and scion were enhanced by increasing soil nitrogen content. Moreover, increasing nitrogen supply directly enhanced biomass of grafted trees. These observations indicated that the nursery soil was nitrogen deficient and could not supply the plant needs. Dewald *et al.* (1992) also reported that nitrogen amendment increased growth of *Pinus elliotii* seedlings in nursery. Mackie-Dawson *et al.* (1995)

reported similar results about nitrogen application for *Acer pseudoplatanus* and *Picea sitchensis* seedlings. Ashrafi *et al.* (2013) demonstrated that increase in the growth of olive trees following application of filtered sewage was due to enhance soil nitrogen content. Mirabdolbaghi and Pishbin (2011) studied the effects of type and rate of nitrogen fertilizer on peach seedlings in nursery and reported that application of 200 kg ha⁻¹ of ammonium sulfate significantly increased the plant leaf area. However, our results indicated that almond positively responds to increasing urea content in the soil up to 600 kg ha⁻¹. The similar report on the positive effect of urea was reported on Thomson orang (Akhlaghi Amiri *et al.*, 2016). However, this growth could also be obtained by application of 400 kg ha⁻¹ SCU. These results show that the slow release fertilizer has a higher efficiency in enhancing the plant growth and may reduce production costs in the nurseries.

Evaluating the relationship between the primary elements and the plant biomass in the second growing season showed nitrogen is the most important size controller of the grafted trees. The plant growth enhancement could be a result of improving the plant photosynthesis capacity due to increased leaf area (Zhao *et al.*, 2005). Ahmadimotlagh *et al.* (2014) reported direct correlation between nitrogen availability and biomass production. Nitrogen may affect vegetative growth by changing the balance of phytohormones. In this regard, Fernández-Escobar *et*

al. (2009) stated that nitrogen application increases vegetative growth by reducing abscisic acid/gibberellin ratio. Zarata-Valdez *et al.* (2015) stated that necessary amount of nitrogen for maximizing the growth of mature trees was about 300 kg ha⁻¹.

Another possible explanation of the increased plant growth following application of the fertilizers, especially urea and SCU, was the increase in shoot relative water content. Water is one of the primary factors for the growth of plant cells and organs (Salisbury and Ross, 1992). Young and growing shoots generally have higher water content. The results indicate that application of fertilizer to the plants extend the growing period to the late season, which is not a good trait for deciduous trees. Young and growing branches during the late season period do not have enough time for maturation and preparation for dealing with freezing temperatures. In this regard, Villar-Salvador *et al.* (2013) and Zhu *et al.* (2001) stated that application of high doses of growth stimulating fertilizers enhances sensitivity of plant to drought or late season frost. In addition, the results suggest that application of high doses of N fertilizers, especially urea which is readily available to plant, should be avoided in cold regions.

Formation of lateral branches and suckers in nursery depends on rootstock genotype, the soil and environmental conditions, and cultivation practices (Kaplan and Barilla. 2006). Absences of lateral branches or suckers on the rootstock limits the number of growing points and provide more opportunity for the growth of main stem or scion. Budding or grafting on stems with fewer branches is easier and removing of suckers is costly. Although the treatments did not affect the number of suckers, application of 400 and 600 kg ha⁻¹ of urea or SCU in the first year reduced lateral branches on the rootstock. In contrast, Mirabdolbaghi and Pishbin (2011) found that nitrogen treatments did not affect lateral branches of peach seedlings in nursery. Ashrafi *et al.* (2013) stated that application of filtered sewage on olive trees did not affect the number of lateral branches, but the treated

plants had longer branches and made narrower angles with the main stem. Shoots with narrow angles are more vigorous than them with wide angles (Faust, 1989). Formation of less lateral branches on the nitrogen treated rootstocks was an important desired trait of plant, because, such plants could be easily grafted or budded. At the end of the first growing season, rootstock diameter was ranged between 7.1 mm in the control to 12.6 mm in the SCU₆₀₀ treatment. Enhancement of rootstock growth, especially the main stem diameter increase, considerably improves plant quality and readiness for grafting. Bally (2006) described that the optimal diameter of the rootstock for budding is 6-15 mm; but, Hartmann *et al.* (2010) believed that a minimum diameter of 10 mm is essential for budding. The positive correlation of rootstock diameter in the first year and graft success in the second year (Fig. 2), revealed that diameter of almond rootstock must be more than 12 mm to achieve a 90% of budding success. Furthermore, budding on rootstocks with larger diameters at grafting point improved the scion growth in the following year (Fig. 2). Mhango *et al.* (2008) and Tyree *et al.* (2009) emphasized on stem growth for increasing success of budding and subsequent growth of plants. The results emphasize on the importance of proper nutrition to prepare rootstocks for budding and increasing the production efficiency (Mng'omba *et al.*, 2010). Application of the fertilizers increased readiness of almond seedling for budding. Moreover, the fertilizer treatments improved callus quality at the grafting point. The highest callus quality was found in the urea and SCU treated plants. Formation of callus tissue with active growth is essential for the success of the budding (Hartmann *et al.*, 2010; Ráufi *et al.*, 2017). This result, describes the higher budding success of fertilizer treated plants in comparison to the control plants. Callus quality is directly related to rootstock vigor which influences the growth and division capability of cells at grafting point (Hartmann *et al.*, 2010).

Leaf pigments and plant health

The fertilizer treatments increased leaf greenness and chlorophyll concentration in the scion leaves. So that, the highest SPAD index was obtained by application of 30 kg ha⁻¹ NPK or 600 kg ha⁻¹ SCU. This index represents the leaves' health and is related to concentration of chlorophylls in the leaves. The increase in concentration of chlorophylls was in line with increase in N, P and K contents in the leaves. Nitrogen application also increased leaf chlorophyll content of woody plants such as peach (Mirabdolbaghi and Pishbin, 2011), walnut (Shamsoddini *et al.*, 2016), olive (Ashrafi *et al.*, 2013) and herbaceous species such as wheat (Bojovic and Stojanovic, 2005), sunflower (Dordas and Sioulas, 2008) and tomato (Khavari-Nejad *et al.*, 2009). Nitrogen is of essential components of the chlorophylls and its deficiency reduces chlorophyll biosynthesis and enhances chlorophyll destruction by limiting biosynthesis of proteins (Marschner, 2012). Furthermore, nitrogen deficiency triggers catabolism of macromolecules such a proteins and chlorophylls by accelerating aging and promoting formation of free radicals in cell (Bernardo *et al.*, 1999). By increasing leaf chlorophylls' content, the capacity of light harvesting and photosynthesis are promoted and the plant biomass production is increased (Salisbury and Ross, 1992).

Application of 30 kg ha⁻¹ NPK resulted in the highest SPAD value and chlorophylls' content in the leaves. This was due to providing magnesium, iron, and the other micronutrients to the plant for biosynthesis of chlorophylls. The high pH of the soil was a restricting factor of availability of these elements to the plants. Therefore, application of the compound fertilizer provided iron for chlorophylls' biosynthesis and zinc for proteins' biosynthesis for protecting chlorophylls (Marschner, 2012). These results suggest that foliar application of a compound fertilizer plus nitrogen fertilizer is required to meets plant nutrient requirements. Increase in the P and K contents of leaves also increased total chlorophyll concentration

in the leaves. These elements are required for protein biosynthesis and protecting chlorophylls in the leaves and their availability to plant improves chlorophylls' content in the leaves (Marschner, 2012). However, their increases were mainly found in the NPK treated plants, and therefore the relationships between concentrations of these elements and leaf chlorophyll content are probably due to providing the micronutrients to the plants. In other world, spraying the compound fertilizer by providing magnesium, and micronutrients improved the chlorophylls' content of the leaves.

Mineral concentrations in leaves

Application of urea and SCU increased nitrogen contents of leaves. The highest leaf nitrogen content was obtained by application of 600 kg ha⁻¹ urea or 400-600 kg ha⁻¹ SCU. These observations suggest that SCU is more effective than urea in providing nitrogen to the plant. Nitrogen content in the leaves was directly related to the scion growth and plant health. Moreover, plants with high nitrogen content can support new root and shoot growth after transplanting through nutrient remobilization (Salifu and Timmer, 2003), which is particularly important for plant establishment and growth on poor and dry soils (Oliet *et al.*, 2009; Villar-Salvador *et al.*, 2012).

The highest leaf P and K concentrations were detected in the NPK 30 treatment, whereas the control plants had the lowest amounts. Phosphorus and potassium are the most important elements for primary growth of plant and shown to increase fruit yield (Norozzi *et al.*, 2019) Foliar application makes them more available to plant, since they are immobile elements in the soil. Silspour and Mollahosseini (2005) stated that application of phosphorus in soils with phosphorus level less than 15 mg kg⁻¹, caused significant increase in growth and yield of plant. Potassium affects photosynthesis through regulating stomatal movements, gas exchange and photosynthesis

(Marschner, 2012). In addition to the compound fertilizer treatments, application of urea and SCU also increased leaf P and K contents. It was probably due to increasing the root efficiency in water and minerals absorption (Marschner, 2012) and reducing soil reaction (Barker and Pilbeam, 2015). Urea is an acidifying fertilizer, which can reduce P and K fixation on the clay particles and improves their mobility in the soil (Prasad and Power, 1997). SCU was more effective in improving the absorption of P and K, which could be related to its higher acidification ability due to releasing sulfur in the rhizosphere.

Conclusions

The results indicated that, standard-sized almond trees can be produced in cold area nurseries if appropriate nutrition is provided. Application of 600 kg ha⁻¹ urea and 400-600 kg.ha⁻¹ sulfur-coated urea (SCU) improved the plant growth and budding success. Considering that SCU 400 treatment was not different from SCU 600 treatment in many traits, this level is proposed to reach the best growth and development of almond trees in nursery. No sign of enhanced sensitivity to drought or late season frost were observed in the treated plants. Evaluation of chlorophylls and minerals in the leaves showed that, although foliar application of compound fertilizers may not provide plant nutritional needs, it can be considered in nutritional program of nurseries to maximize the plant growth.

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