MEYVECİLİK ARAŞTIRMA ENSTİTÜSÜ MÜDÜRLÜĞÜ
FRUIT RESEARCH INSTITUTE

ISSN: 2148-0036
Yıl /Year: 2018
Cilt(Sayı)/Vol.(Issue): 5(1)
Sayfa/Page: 12-17
Araştırma Makalesi
Research Article

Meyve Bilimi Fruit Science

Geliş Tarihi (date of arrival): 02.03.2018
Kabul Tarihi (date of acceptance): 12.10.2018

Effects of Nitrogen Fertilization on the Chlorophyll Content of Apple

Erdinç UYSAL[1,*]

[1]Department of Soil and Water Resources, Atatürk Central Horticultural Research Institute, Yalova, Turkey
*erdincuysal@yandex.com (Corresponding author)

Abstract

The experiment was carried out during 2009 - 2011 period in Yalova Atatürk Horticultural Central Research Institute. The aim of the experiment is to determine fertigation and postharvest foliar fertilization methods with different nitrogen doses (0, 30, 60, 90 g/tree) and application times (Application:1 starts at early spring before the buds burst and ends 40-45 days ago from harvest, Application:2 In addition to A1 postharvest foliar nitrogen application, Application:3 starts after blooming and ends 40-45 days ago from harvest, Application:4 In addition to A3 postharvest foliar nitrogen application) effects on leaf chlorophyll content of Golden Sel B apple variety grafted on M9 rootstock. The experiment was designed according to randomized block factorial experiment design with three replication. According to the results increasing doses of nitrogen had increased the chlorophyll content of the leaves, however no significant differences was observed due to the different nitrogen application times on the chlorophyll content.

Keywords: Apple, nitrogen, foliar fertilization, fertigation, chlorophyll

Azotlu Gübrelemenin Elmada Klorofil İçeriği Üzerine Etkileri

Özet

Bu çalışma 2009-2011 yılları arasında Yalova Atatürk Bahçe Kültürleri Merkez Araştırma Enstitüsünde yürütülmüştür. Çalışmanın amacı M9 anacı üzerine aşılı Golden Sel B elma çeşidinde, fertigasyon ve hasat sonrası yaprak gübrelemesi yöntemleri kullanılarak farklı dozlarda (0, 30, 60, 90 g/ağaç) ve farklı uygulama zamanlarında (Uygulama:1Erken ilkbaharda gözler uyanmadan önce başlayıp hasattan 40-45 gün önce biten, Uygulama:2 Erken ilkbaharda gözler uyanmadan önce başlayıp hasattan 40-45 gün önce biten ve hasat sonrası yaprak uygulaması olan, Uygulama:3 Çiçeklenme sonrası başlayıp hasattan 40-45 gün önce biten, Uygulama:4 Çiçeklenme sonrası başlayıp hasattan 40-45 gün önce biten ve hasat sonrası yaprak uygulaması olan) verilen azotun bitkilerin klorofil içeriği üzerine olan etkilerinin belirlenmesidir. Deneme, tesadüf bloklarında faktöriyel deneme desenine göre üç tekrarlamalı olarak kurulmuştur. Yapılan klorofil analiz sonuçlarına göre, yaprak klorofil içerikleri artan azot dozları ile artış göstermiş fakat farklı uygulama zamanları klorofil içerikleri üzerinde etkili olmamıştır.

Anahtar kelimeler: Elma, azot, yaprak gübrelemesi, fertigasyon, klorofil

1. Introduction

Apple is the 4th most important fruit crop after citrus, grapes and banana and one of the commercially most important horticultural crops grown in temperate parts of the world (O'Rourke, 2003). Turkey is one of the limited countries in the world in terms of both species and types of horticultures that is located within the mild temperate zone. Apple has the most important fruit in Turkey in the historical process (Ercisli, 2004). Cultivated apple is grown in almost every region of Turkey (Ozcagıran et al., 2004).

The world's apple production is around 85 million tonnes annually according to the latest data. Turkey is the 4th biggest apple producer after China, USA, Poland, and India in the world (FAO, 2017).

Establishing the orchard in agreeable climate and soil conditions and applying all cultivation applications with sufficient and correct techniques at the right time, carries great importance in terms of increasing and preserving the productivity and quality of plants. Among these said applications, the matter of fertiliza-

tion for the sufficient nutrition of the plants very significant.

Pressurized irrigation systems in modern cultivation and establishments of intense fruit plantations, which have rapidly started to increase recently, has increased the interest in fertigation that enables a more controlled management of fertilizers. Compared to conventional fertilization, fertigation applications increase fertilizer and water efficiency by 20 - 50% (Gaskell, 2004). Fertigation applications are important in terms of providing the required amounts of nitrogen during the growth period. When the water and fertilizers to be used are not determined in accordance with the plants' needs, it is not possible to achieve the benefits expected from drip irrigation.

Active pigments, which play a role in photosynthesis, are chlorophylls that are the green pigments of plants. Chlorophylls, which are green pigments, are mostly located in the mesophyll cells of the plants' leaves. Therefore, photosynthesis appears in plants' leaves the most. Today, there are at least 9 different chlorophylls known and the chlorophylls that are the most abundant in all autotrophic organisms are chlorophyll a and chlorophyll b (Kacar et al., 2002).

The chlorophyll content of a leaf is the indicator of a plant's physiological condition. Chlorophylls are pigments which are necessary in luminous energy being converted into chemical energy. The amount of radiation absorbed from the sun depends on the amount of photosynthetic in the leaf. Therefore, the amount of chlorophyll depends on photosynthetic activity and primary production (Curran et al., 1990). In addition to this, the amount of chlorophyll approximately indicates the correlation between nitrogen, one of the plants' nutrition elements, and the pigment rate (Filella et al., 1995). Because nitrogen is one of the main elements of chlorophyll it is very important for photosynthesis in plants. Hence, chlorophyll molecules disperse when there is a lack of nitrogen (Turan and Horuz 2012).

This study examines the effects of different fertilizer amounts and fertilization times on the chlorophyll content of plants by means of mainly using the fertigation technique on Golden Sel B apple variety grafted on M9 rootstock.

2. Materials and Methods

This experiment was carried out between 2009 and 2011 for a period of 3 years in the institute research plot. Considering that the effect of fertilization applications would not be observed during the first year (2009) of the experiments, data collected from 2010 and 2011 was used.

Some soil characteristics of the experiment area are shown in the Table 1. The climate of the region where the research was executed has macro-climate type which is a transition climate between Mediterranean and Black Sea regions. The region where the study was carried out has an annual average temperature of 14,6 0C and an annual precipitation amount of 727 mm.

The experiment was conducted in the Golden Sel B apple orchard with 2 year-old trees in 2009, when the experiment was initiated, which were grafted on M9 rootstocks and planted with 1,5 x 4 m spaces. The experiment was conducted in 3 repetitions according to the randomized blocked factorial experiment design and 5 trees were used on each treatment. Four different doses of nitrogen were administered at 4 different application times during the experiment which lasted for three years. The doses of nitrogen were determined as below.

N0= 0 g/tree
N1= 30 g/tree
N2= 60 g/tree
N3= 90 g/tree

Table 1. Some physical and chemical soil properties of the experiment area

Soil properties		0-20 cm	20-40 cm
Soil texture	Silty (%)	16.08	16.44
	Clay (%)	22.91	23.65
	Sand (%)	61.01	59.91
EC 1:2,5 soil-water extract (µmhos/cm)		230	210
pH 1:2,5 soil-water extract		7.70	7.60
$CaCO_3$ (%)		1.62	0.61
Organic matter (%)		2.98	2.47
Total nitrogen (%)		0.112	0.104
Available phosphorus (mg/kg)		19	14
Exchangeable potassium (me/100 g)		0.52	0.38
Exchangeable calcium (me/100 g)		26.54	26.39
Exchangeable magnesium (me/100 g)		2.83	1.94
Available iron (mg/kg)		11.82	13.00
Available manganese (mg/kg)		28.83	28.02
Available zinc (mg/kg)		1.19	0.86
Available copper (mg/kg)		9.24	8.49

The application times for the fertilizer with nitrogen were performed as below:

Application (A1): Started in early spring before the buds were burst (March) and ended 40-45 days ago from harvest (August). Fertilization was performed using the fertigation method.

Application (A 2): Started in early spring before the buds were burst (March) and continued 40-45 days ago from harvest (August) with the fertigation method and another foliar nitrogen application was administered after harvest, before defoliation (November).

Application (A 3): Started after blooming (end of April-beginning of May) and ended 40-45 days ago from harvest (August). Fertilization was performed using the fertigation method.

Application (A 4): Started after blooming (end of April-beginning of May) and continued 40-45 days ago from harvest (August) with the fertigation method and another foliar nitrogen application was administered after harvest, before defoliation (November).

The postharvest nitrogen fertilizer applications were administered by means of spraying the 5 % urea solution on the leaves. Nitrogen was applied ammonium nitrate fertilizer containing 33% nitrogen during other periods. Ammonium nitrate fertilizer was dissolved in fertilizer tanks and divided into irrigation number during irrigation period and applied by means of the fertigation method.

Class A pan was used to determine the amount of applied irrigation water. The irrigation water was supplied as the cumulative water (100%) in the 5 days irrigation range for the open surface evaporation values measured from class A pan.

According to the results of the soil analysis, the irrigation water was distributed equally among the parcels for the required phosphor and potassium fertilizers, so as not to limit the growth. Potassium sulphate that contains 50% K2O and 17% S was used as potassium fertilizer while phosphoric acid that contains 85% P2O5 was used as phosphor fertilizer.

The chlorophyll analysis conducted on the foliage samples was performed according to (Witham et al., 1971). Foliage samples were collected from fully - developed leaves taken from the middle of the offshoot of every tree in mid-summer, towards the end of the fertilization season, for the chlorophyll analyses.

0.25 gram samples of fresh leaves were collected and grounded in acetone. This was drained with filter paper and was read in 663 nm wavelength in a UV spectrophotometer for chlorophyll a, 645 nm wavelength for chlorophyll b and 450 nm wavelength for total chlorophyll. These absorbance values were then later placed in their places according to the below equalities and the values of the chlorophyll a, chlorophyll b and total chlorophyll found in 1 gram was calculated in mg.

Chlorophyll a mg/g tissue =

[12.7 (D663)- 2.69 (D645)].(V/1000.A)

Chlorophyll b mg/g tissue =

[22.9 (D645)- 4.68 (D663)].(V/1000.A)

Total Chlorophyll mg/g tissue =

[27.8 (D652)].(V/1000A)

D: optical intensity of the plant extract at the specified wavelength, namely absorbance value,

V: 80% acetones latest volume,

A: fresh weight of the foliage tissue obtained in the extract as grams.

Variance analyses were performed on the results obtained from the experiment established according to randomized blocked factorial experiment design and the least significant differences (LSD) was calculated and the differences were indicated on the results.

3. Results and Discussion

Chlorophyll analyses were conducted on foliage collected from mid-summer shootings in order to determine the effects of the administered applications on the chlorophyll content of the trees and the results showing the chlorophyll a amounts have been presented in Table 2.

The times of the applications have had no effect on the amounts of chlorophyll a and no significant interaction connected to the nitrogen doses and application times has occurred. Nitrogen applications administered in different dosages, however, have created significant differences at a 1% level within 2 years. While the trees had the lowest chlorophyll a value with the N0 (control) dose, the highest chlorophyll a values were determined in N3 dose during both years.

The results of the chlorophyll b analyses conducted on the foliage samples have been presented in Table 3.

The application times of the nitrogen did not create a significant difference in the chlorophyll b amounts determined in both years. The effect of the increased doses of nitrogen led to the occurrence of differences which were 1% statistically significant for the results of both 2010 and 2011.

The lowest chlorophyll b value observed in 2010 occurred in the N0 (control) application and increases in

Table 2. Effect of nitrogen applied in different doses and at different times on the amount of chlorophyll a in Golden Sel B apples foliage.

Application times	Nitrogen doses				Average
	N0	N1	N2	N3	
	In 2010 the content of chlorophyll *a mg g^{-1}*				
A1	0.38	0.48	0.43	0.51	0.45
A2	0.38	0.45	0.45	0.47	0.44
A3	0.38	0.44	0.46	0.48	0.44
A4	0.38	0.41	0.43	0.48	0.42
Average	0.38 C**	0.44 B	0.44 B	0.49 A	
CV= 11.02					
	In 2011 the content of chlorophyll *a mg g^{-1}*				
A1	0.42	0.47	0.46	0.50	0.46
A2	0.42	0.45	0.51	0.49	0.47
A3	0.42	0.41	0.44	0.53	0.45
A4	0.42	0.46	0.50	0.52	0.48
Average	0.42 D**	0.45 C	0.48 B	0.51 A	
CV= 6.40					

The differences between the means indicated by different letters is important on a 1% and 5% level, (**) P<0.01, (*) P<0.05

the chlorophyll amounts were observed with increased nitrogen doses. While the value obtained from the N1 and N2 doses were higher than the control application, the highest chlorophyll dose was observed in N3 dose. While the lowest chlorophyll b in 2011 was found in the N0 (control) dose, it was followed by the N1 dose and the highest values were observed in the same groups of N2 and N3.

In the total chlorophyll amount obtained as a result of the analyses shows similar differences to the results obtained from chlorophyll a and chlorophyll b analyses. The analysis results for total chlorophyll amounts of both years are presented in Table 4.

Examining Table 4, which presents total chlorophyll amounts, we can see that the application times in both 2010 and 2011 did not create a significant different distance the chlorophyll content and there was no interaction and the application doses created significant differences at 1%.

While the lowest total amount of chlorophyll in 2010 and 2011 was found in the N0 (control) dose with no nitrogen, it was followed by in the same groups of N1 and N2 doses and the highest value was observed in N3 dose.

The results of the chlorophyll conducted on foliage samples collected in mid-summer in order to determine the performed applications on chlorophyll content were found to be considerably similar. During both years, no differences had occurred in the chlorophyll content with relation to the time of the application. Again, the application doses had affected the amounts of chlorophyll and created differences at 1% significance. It was determined that the chlorophyll amounts increased in connection with the doses of nitrogen, in general.

Table 3. Effect of nitrogen applied in different doses and at different times on the amount of chlorophyll b in Golden Sel B apples foliage.

Application times	Nitrogen doses				Average
	N0	N1	N2	N3	
	In 2010 the content of chlorophyll *b mg g^{-1}*				
A1	0.64	0.82	0.74	0.85	0.77
A2	0.64	0.76	0.77	0.80	0.74
A3	0.64	0.75	0.81	0.84	0.76
A4	0.64	0.69	0.74	0.85	0.73
Average	0.64 C**	0.76 B	0.77 B	0.84 A	
CV= 11.46					
	In 2011 the content of chlorophyll *b mg g^{-1}*				
A1	0.72	0.81	0.78	0.83	0.78
A2	0.72	0.76	0.92	0.85	0.81
A3	0.72	0.83	0.80	0.92	0.82
A4	0.72	0.77	0.85	0.94	0.82
Average	0.72 C**	0.79 B	0.84 AB	0.88 A	
CV= 8.52					

The differences between the means indicated by different letters is important on a 1% and 5% level, (**) P<0.01, (*) P<0.05

Table 4. Effect of nitrogen applied in different doses and at different times on the amount of total chlorophyll in Golden Sel B apples foliage.

Application times	Nitrogen doses				Average
	N0	N1	N2	N3	
	In 2010 the content of total chlorophyll *mg g^{-1}*				
A1	1.24	1.58	1.43	1.61	1.47
A2	1.24	1.47	1.51	1.56	1.44
A3	1.24	1.45	1.56	1.60	1.46
A4	1.24	1.34	1.41	1.63	1.41
Average	1.24 C**	1.46 B	1.48 B	1.60 A	
CV= 10.92					
	In 2011 the content of total chlorophyll *mg g^{-1}*				
A1	1.36	1.58	1.49	1.64	1.52
A2	1.36	1.51	1.80	1.65	1.58
A3	1.36	1.61	1.55	1.91	1.61
A4	1.36	1.46	1.61	1.78	1.55
Average	1.36 C**	1.54 B	1.61 B	1.74 A	
CV= 8.68					

The differences between the means indicated by different letters is important on a 1% and 5% level, (**) P<0.01, (*) P<0.05

The most active pigments that play a role in photosynthesis are chlorophylls, which are the green pigment of plants. Today there are at least 9 known chlorophylls and these are the chlorophylls that are the most abundant and well-known in all autotrophic organisms besides bacteria having chlorophyll a and chlorophyll b (Kacar et al., 2002).

The main reason why plants seem yellow when there is lack of nitrogen is that after the proteins are broken up, it is followed by the breaking up of the plastids and as a result chlorophyll synthesis either regresses or stops (Kacar et al., 2002). It is advised that there is a close relationship between the fertilization with nitrogen and chlorophyll content in the leaves (Odabas, 1981).

0, 80 and 250 kg ha-1 nitrogen dosages were used in a study carried out in order to specify the impacts of nitrogen applied in different ratios on the nitrogen and chlorophyll contents in the leaves of Golden Delicious apples and chlorophyll values were specified in 5 different periods (Prsa et al., 2007). The results showed that the highest chlorophyll content was identified in every period at the application of 250 kg ha-1. At the evaluation at the end of the season, high chlorophyll content value was reached at the application of 80 kg ha-1 nitrogen dosage as 250 kg N per hectare.

0, 10, 20 and 40 kg nitrogen dosages were applied per hectare in another study carried out on young Rocha pears yielding no fruits and chlorophyll contents were specified on leaf samples taken 160 days later after the full blooming. The study showed that the lowest chlorophyll content emerged during the application in which no nitrogen was applied and other 3 applications were evaluated in the same group and resulted in high chlorophyll content (Neto et al., 2011).

In a study to determine the effect of nitrogen fertilization in Hosui pears, it has been reported that nitrogen administered in appropriate amounts increase the chlorophyll a, chlorophyll b and carotene content (Lei et al., 2010). In another study which determine effects to chlorophyll content of fertigation and foliar nitrogen application on pear was carried out in Turkey (Uysal et al.; 2013). According to this study increasing doses of nitrogen had increased the chlorophyll content of the leaves, however no significant differences was observed due to the different nitrogen application times on the chlorophyll content.

It was specified in studies executed by various researchers on several plants such as tobacco leaves (Kowalczyk-Jusko and Koscik, 2002), sugar beets (Van den Berg and Perkins, 2004) mandarin and vine leaves (Shaahan et al 1999) that chlorophyll increased in plants in connection with the increase in nitrogen contents. The values we obtained in our study are in conformity with the results gotten in other studies and they show that chlorophyll contents increase parallel to the increases of nitrogen content.

4. Conclusion

We examined in this study the effects of nitrogen applications on the chlorophyll contents of the apple variety of Golden Sel B. To this end different nitrogen dosages were applied on plants in different periods. Chlorophyll values were specified as chlorophyll a and chlorophyll b in the sample leaves.

Application times had no difference on the results in both of the years and increases in nitrogen dosages

increased in parallel the chlorophyll contents in plants to a considerable extent.

It will be right to assert as a result of the study that nitrogen applied in different times doesn't affect the chlorophyll values, but increases in nitrogen dosages also have an increasing effect on chlorophyll values of plants.

References

Curran PJ, Dungan JL, Gholz HL, 1990. Exploring the Relationship Between Reflectance Red Edge and Chl Content in Slash Pine. Tree Physiol. 7: 33 - 48.

Ercisli S, 2004. A Short Review of the Fruit Germplasm Resources of Turkey. Genetic Resources and Crop Evolution 51: 419 - 435.

FAO, (2017). FAOSTAT production data [online]. Available at http://www.fao.org/faostat/en/#data/QC (Acces data: 15.06.2017)

Filella I, Serrano L, Serra J, Penuelas J, 1995. Evaluating Wheat Nitrogen Status with Canopy Reflectance Indices and Discriminant Analysis. Crop Sci. 35: 1400 - 1405.

Gaskell M, 2004. Acid injection in irrigation water improwing pH adjustment for blueberries. Available from URL: http://www.sbceo.k12.ca.us/~uccesb1/sf1002.htm (Acces data: 02.07.2008)

Kacar B, Katkat AV, Öztürk Ş, 2002. Bitki Fizyolojisi. Uludağ Üniversitesi Güçlendirme Vakfı Yayın No: 198, Vipas .S. Yayın No: 74, s. 224-292, İstanbul.

Kowalczyk-Jusko A, Koscik B, 2002. Possible Use of the Chlorophyll Meter (SPAD-502) for Evaluation Nitrogen Nutrition of the Virginia Tobacco. Electronic Journal of Polish Agricultural Universities 5 (1): 05.

Lei C Tao, ShaoLing W, GaiFang Z, ShuTian Y, Bing T, YongChun J, HuiLian MC, 2010. Effects of Nitrogen Fertilizer on Fruit Quality and Leaf Physiological Metabolism of Hosui Pear. Journal of Fruit Science 27 (6): 871 - 876.

Neto CB, Carranca C, Clemente J, Varennes A, 2011. Assessing the Nitrogen Nutritional Status of Young Non-Bearing 'Rocha' Pear Trees Grown in a Mediterranean Region by Using c Chlorophyll Meter. Journal of Plant Nutrition 34: 627 - 639.

Odabas F, 1981. Effects to Chlorophyll Content of Nitrogen Fertilization on Bacchus Variety (Vitis Vinifera L.).Atatürk University Journal of the FacultyofAgriculture 12(2): 39-50.

O'Rourke D, 2003. World Production, Trade, Consumption and Economic Outlook for Apples In: Ferree DC, Warrington IJ, editors. Apples: Botany, Production and Uses. CABI Publishing, Cab International UK, pp. 15 - 28

Ozcagıran R, Ünal A, Ozeker E, Isfendiyaroglu M, 2004. Ilıman İklim Meyve türleri (Yumuşak Çekirdekli Meyveler Cilt-II) Ege Üniv. Zir. Fak. Yayın 556, İzmir, 200 s.

Prsa I, Stampar F, Vodnik D, Veberic R, 2007. Influence of Nitrogen on Leaf Chlorophyll Content and Photosynthesis of 'Golden Delicious' Apple. Acta Agriculturae Scandinavica Section B-Soil and Plant Science 57: 283 - 289.

Shaahan MM, El-Sayed AA, Abou El-Nour EAA, 1999. Predicting Nitrogen, Magnesium and Iron Nutritional Status in Some Perennial Crops Using a Portable Chlorophyll Meter. Scientia Horticulture 82: 339 - 348.

Turan M, Horuz A, 2012. Bitki Besleme. Bitki Beslemenin Temel İlkeri (Ed: M. R. Karaman), Gübretaş Rehber Kitaplar Dizisi: 2, s. 123-345

Uysal E, Sağlam MT, Büyükyılmaz M, 2013. Effects to Chlorophyll Content of Fertigation and Foliar Nitrogen Application on Deveci Pear Variety. Soil-Water Journal Special Issue for AGRICASIA'2013 1st Central Asia Congress on Modern Agricultural Techniques and Plant Nutrition 1-3 October 2013, Bishkek, Kyrgyzstan, p. 67-74

Van den Berg AK, Perkins TD, 2004. Evaluation of Portable Chlorophyll Meter to Estimate Chlorophyll and Nitrogen Contents in Sugar Maple (Acer saccharum Marsh.) leaves. Forest Ecology and Management 200:113 - 117.

Witham FH, Blaydes DF, Devlin RM, 1971. Experiments in Plant Physiology. Van Nostrand Reinhold Company, New York, USA.