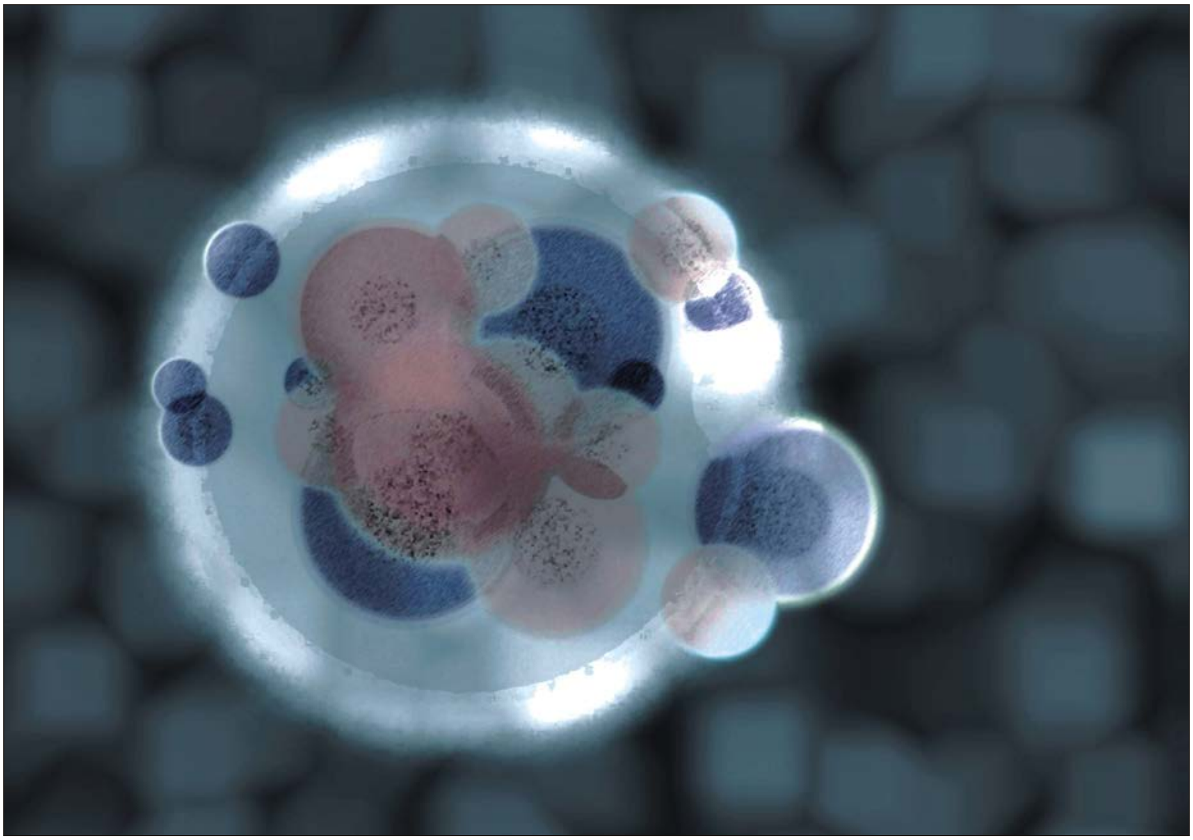

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Estimating Leaf Nitrogen Status of Strawberry by Using Chlorophyll Meter Reading

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Abstract: Measurement of leaf N through chlorophyll meter reading is of high importance to monitor leaf N status of plant throughout growing season. In present study, five short-day strawberry (*Fragaria x ananassa Duch*) cultivars including Sweet Charlie, Redlans Hope, Kabarla, Festival and Camarosa, were grown in 2003-2004 growing season to investigate the possibility of usage of Chlorophyll (CHL) meter reading to estimate leaf N content. Fully matured leaves were used for related measurements. Leaf CHL was measured by using SPAD 502 chlorophyll meter. There was significant correlation ($r = +0.84$, $p<0.001$) between leaf N and CHL reading regardless of the cultivars. The results indicated that leaf N and CHL contents of strawberry cultivars showed great variability. The cultivar containing highest leaf N also contained the highest CHL. Kabarla had the highest leaf N (2.46% N) and CHL (33.90 unit), followed by Redlans Hope (2.18%N, 31.65 unit). Sweet Charlie had the lowest leaf N (1.68% N) and CHL reading values (29.30 unit). Leaf N status of the cultivars used in this experiment can be estimated using the regression equations belong to each cultivar. However, due to high significant correlation between leaf N and CHL reading value irrespective of cultivar, leaf N content of any cultivar can be estimated by using the regression equation $y = -2.1309+0.1344x$, where x is CHL reading value and y is leaf N.

Key words: Chlorophyll, leaf nitrogen, strawberry (*Fragaria x ananassa Duch)*

INTRODUCTION

Strawberry is an important fruit crop for many countries as in the Turkey (Kaska, 1997). In 2005, 3.5 million tons strawberry was produced in 250.751 ha area in the world. Strawberry production was 160.000 tons at the 10,500 ha area in Turkey in 2005 (FAO, 2005). Nitrogen is the most important nutrient affecting strawberry yield and quality and its fertilizer-management is of vital importance. Nitrogen deficiency reduces leaf area, root mass and fruit size (Johanson and Walker, 1963; Ulrich *et al*., 1980) and excessive N content can leads soft fruit, delays ripening, decreases yield, increases powdery mildew and increases mite pressure (Voth *et al*., 1967; May and Pritt, 1990; Miner *et al*., 1997). Leaf N content has been used as a tool to monitor N status of the strawberry plants (John *et al*., 1975; Hochmuth *et al*., 1991; Pritts and Handley, 1998). Also, in fertigated crops, plant N requirement should be arranged in relation to plant growth stage and the environmental factors. This arrangement necessitates monitoring N status of plant as frequently as possible. However, conventional nitrogen determination technique is time consuming and expensive. Chlorophyll meter have been used for determination of requirements of many crops including corn (Schepers *et al*., 1992; Piekielek and Fox, 1992; Piekielek *et al*., 1995; Blackmer and Schepers, 1995; Varvel *et al*., 1997), wheat (Singh *et al*., 2002), rice (Turner and Jund, 1991; Singh *et al*., 2002) and potato (Shaaban and El-Bendary, 1999; Minotti *et al*., 1994; Gianquinto *et al*., 2003). Yadava (1986) working with 22 species representing 14 plant families including strawberry found significant correlations between CHL reading and CHL concentration determined by a conventional technique. However, there is limited information on relationship between leaf N and CHL reading value for strawberry and utilization of CHL meter as a tool to monitor leaf N status of strawberry. The aims of this study were to investigate the relationship between leaf N and CHL reading values of strawberry cultivars and the utilization of CHL meter reading to estimate leaf N status of strawberry.

MATERIALS AND METHODS

Five short-day strawberry (*Fragaria x ananassa Duch*) cultivars (Sweet Charlie, Redlans Hope, Kabarla, Festival and Camarosa) were grown in a raised bed on clay-loam soil in 2003-2004 growing season in the research field of The Black Sea Agricultural Research Institute,

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Table 1: Metrological data of experimental site

Months	Precipitation (mm)			Temperature (°C)			Relative humidity (%)		
	Long term	2003	2004	Long term	2003	2004	Long term	2003	2004
January	58.4	28.1	84.2	6.9	9.3	8.1	68.0	72.2	61.3
February	48.8	77.8	43.9	6.6	4.8	7.5	70.4	74.0	66.3
March	52.7	73.5	66.2	7.8	5.0	8.5	75.8	75.4	75.4
April	58.3	45.0	101.1	11.1	8.7	11.4	79.5	79.6	77.5
May	50.6	54.7	68.6	15.3	16.2	14.9	80.6	78.4	82.4
June	47.9	3.3	53.4	20.0	20.7	19.8	76.3	68.8	81.5
July	31.3	37.2	68.1	23.1	23.7	21.7	73.4	72.5	80.4
August	50.9	94.0	14.6	23.2	24.1	22.9	73.7	72.9	76.5
September	87.4	194.7	66.2	19.8	19.5	18.9	74.7	75.5	78.8
October	78.6	64.0	83.4	15.9	17.5	15.6	75.8	69.3	81.2
November	73.3	104.0	233.4	11.9	11.5	11.1	70.4	79.7	71.3
December	55.7	61.2	109.8	9.3	9.3	7.6	66.8	64.6	68.8
Total	694.0	838.0	993.0	-	-	-	-	-	-
Mean	-	-	-	14.19	14.19	14.0	73.8	73.6	75.1

Samsun, Turkey (41° 21' N Latitude, 36° 15' E Longitude, 4 m elevation). Meteorological data of the experimental site were given in Table 1. The soil pH was 7.85 (1:5 soil: water), total salinity 0.07% (1:5 soil:water), $CaCO_3$ 5.85%, organic matter 3.58%, available P 53 kg ha^{-1}, exchangeable K 1080 kg ha^{-1}. Experimental design was Randomized Complete Block Design with four replications. Before planting each plot received 40 ton ha^{-1} farmyard manure (0.89% N, 1.65% P and 0.30% K, 2.94 dS m^{-1} EC), 150 kg P ha^{-1} as triple superphosphate (42% P_2O_5) and 200 kg K ha^{-1} as potassium sulphate (50% K_2O). Daughter plants were sown in July 18, 2003, each plot received 20 plants. After planting, total of 139.1 kg N ha^{-1} (200 mg L^{-1}) as ammonium nitrate was supplied with irrigation water through drip irrigation system once in a week, from April 2004 through September 2004. Leaf samples were taken for determination of total N and CHL reading in June 01, 2004. The leaf chlorophyll was measured using Minolta SPAD-502 chlorophyll-meter. Total of 30 chlorophyll meter readings were made on ten leaves in each plot, 3 reading on each leaf. Readings were taken from the same point of the leaves as much as possible. The same leaves were dried at 70° for 48 h for chemical analysis. Leaf N was determined by micro-kjeldahl method (Chapman and Pratt, 1961).

Analysis of variance (ANOVA), LSD Test and regression analysis were performed on each variable using MSTAT program. The Cate-Nelson's graph (Cate and Nelson, 1987) modified by Piekielek *et al.* (1995) was used to group data with N deficiency and N sufficiency. CHL reading was X-axis and leaf N was Y-axis. The mean leaf N and CHL reading values irrespective of cultivars were employed as the critical levels. Data located in the upper right and lower left quadrants corresponded to N sufficient and N deficient plants, respectively. Data positioned in the lower right and upper left quadrants represented errors of overestimation and underestimation of the plant CHL level, respectively.

RESULTS

Leaf nitrogen: There were statistically significant differences ($p<0.001$) among cultivars in terms of leaf N (Table 2). When the cultivars were taken into account, Kabarla had the highest leaf N (2.46% N), followed by Redlans Hope and Camarosa. Sweet Charlie cultivar had the lowest leaf N (1.68% N).

The mean leaf N content was 2.07% and mean leaf CHL reading was 31.23 unit regardless of the strawberry cultivars. When drawn a horizontal (leaf CHL) and vertical line (leaf N%) showing these mean values on leaf N vs CHL reading graph, 2 points out of 20 were identified as outliers (Fig. 1). This graph is a modified Cate-Nelson graph, which used to separate N-deficient from N-sufficient levels in plants (Piekielek *et al.*, 1995). Vertical and horizontal lines are to maximize the number of points in two groups: those in the lower left quadrant of the graph (N-deficient plant) and those in the upper right quadrant (N-sufficient plant).

Chlorophyll meter reading: There were statistically significant differences ($p<0.001$) among cultivars in terms of CHL reading values (Table 2). CHL reading value ranged from 29.30 to 33.90, irrespective of the cultivars. Sweet Charlie had the lowest CHL (29.30 unit), Kabarla had the highest CHL (33.90 unit). The cultivars containing the highest and the lowest leaf N also contained the

Table 2: Leaf nitrogen content and chlorophyll reading of strawberry cultivars

Cultivars	Leaf N (%)	Chlorophyll reading
Sweet Charlie	1.68c***	29.300c***
R.Hope	2.18ab	31.650b
Kabarla	2.46a	33.900a
Festival	1.93bc	31.050bc
Camarosa	2.10b	30.250bc
Mean	2.07	31.230
LSD	0.3204	2.121
CV (%)	7.13	3.140

*** Significant at $p<0.001$, Values with same letter(s) are not significantly different

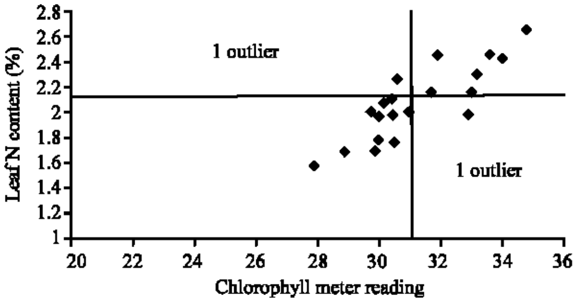

Fig. 1: Strawberry leaf N in relation to chlorophyll reading value

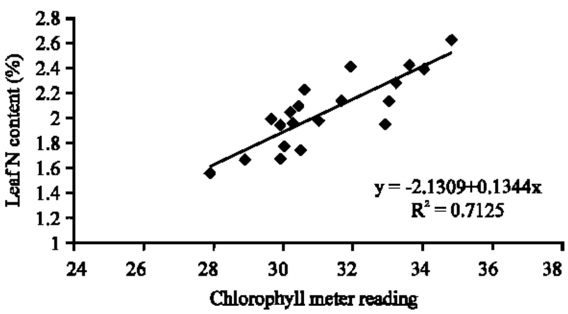

Fig. 2: Relationships between leaf N content and chlorophyll reading of strawberry regardless of cultivars

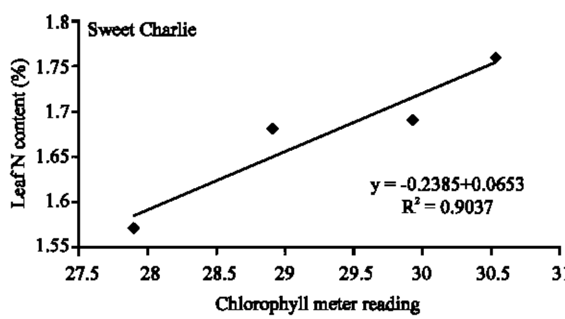

Fig. 3: Relationships between leaf N content and chlorophyll reading of Sweet Charlie

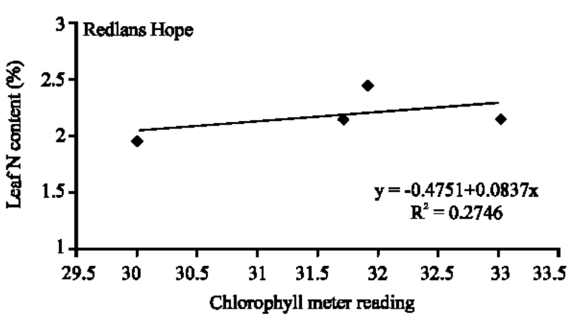

Fig. 4: Relationships between leaf N content and chlorophyll reading of Redlans Hope

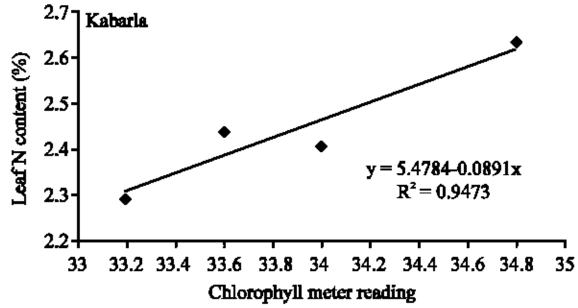

Fig. 5: Relationships between leaf N content and chlorophyll reading of Kabarla

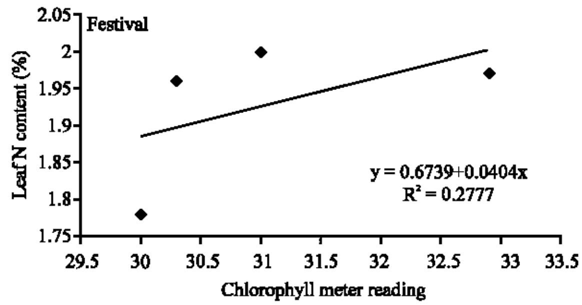

Fig. 6: Relationships between leaf N content and chlorophyll reading of Festival

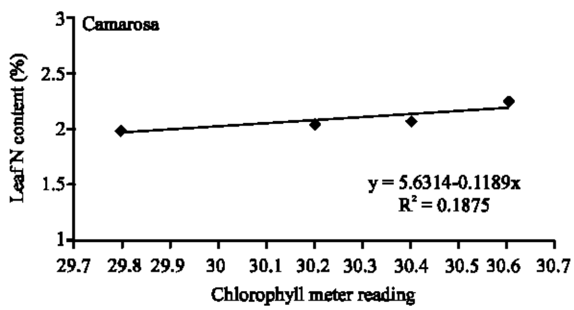

Fig. 7: Relationships between leaf N content and chlorophyll reading of Camarosa

highest and the lowest leaf CHL, respectively. The low variability between the CHL measurements indicates good accuracy.

Correlation between leaf N and chlorophyll meter reading: Regression equations, probabilities and the standard errors for regression analysis were given in Table 3. Average leaf N, CHL reading values, covariance and correlation coefficients were presented in Table 4. There was significant correlation ($r = +0.84$, $p<0.001$) between leaf N and CHL reading values of strawberry regardless of cultivar (Table 4). Cultivar having higher CHL reading value had the higher leaf N. Determination

Table 3: Regression equations, probabilities and standard errors for regression analysis

Cultivars	Regression equations	R^2	Probability	Standard error	Student's t-value
Sweet Charlie	y = - 0.2385+0.0653x	0.9037	0.049	0.015	4.33
R.hope	y = - 0.4751+0.0837x	0.2746	0.000	0.096	0.87
Kabarla	y = 5.4784-0.0891x	0.9473	0.053	0.048	4.18
Festival	y = 0.6739+0.0404x	0.2777	0.000	0.046	0.88
Camarosa	y = 5.6314-0.1189x	0.1875	0.083	0.087	3.25
Mean	y = - 2.1309+0.1344x	0.7125	0.001	0.020	6.68

Table 4: Average leaf N, CHL reading value, covariance and correlation values of cultivars

Cultivars	Average leaf nitrogen (%)	Average CHL reading	Covariance	Correlation
Sweet Charlie	1.68 (0.01)[y]	29.30 (1.31)	0.09	0.951
R.hope	2.18 (0.04)	31.65 (1.54)	0.13	0.524
Kabarla	2.46 (0.02)	33.90 (0.47)	0.09	0.947
Festival	1.93 (0.01)	31.05 (1.70)	0.07	0.527
Camarosa	2.10 (0.01)	30.25 (0.12)	0.03	0.917
Mean	2.07 (0.08)	31.23 (3.34)	0.45	0.844

[Y]Values in parenthesis show the variance of the variable

coefficient (R^2 = 0.71) of relationships between CHL reading value and leaf N was significantly higher (Fig. 2). Average leaf N and CHL reading values were 2.07% N and 31.23 unit, respectively. There were positive and significant correlations between leaf N and CHL in all cultivars. Correlation coefficients were significantly higher. Relationships between leaf N and CHL reading value of cultivars were shown in Fig. 3-7.

DISCUSSION

Cultivars used in this study showed great variability in relation to leaf N which varied from 1.68 (Sweet Charlie) to 2.46% (Kabarla). In three cultivars, R. hope, Kabarla and Camarosa, the leaf N were above 2%. These values were close to the values that reported by Kwong and Boynton (1959), Bould (1961), Hochmuth *et al.* (1996) and Stanisavljevic *et al.* (1997). Leaf N in strawberry shows great variability depending on the growing season and cultivars. It reaches to its highest level at flowering and fruiting stages and its lowest value at the end of the harvest. Leaf N content of strawberry ranges from 2.4 to 3.5% at the beginning of fruiting stage (Kwong and Boynton, 1959; Bould 1961; John *et al.*, 1975; Stanisavljevic *et al.*, 1997). In present study, leaf samples were taken at the beginning of fruiting stage (June 01, 2004). Hochmuth *et al.* (1996) fertigated strawberry cv Oso Grande and Sweet Charlie with increasing rate of N varying from 0.28 to 1.40 kg ha^{-1} day^{-1} and measured leaf N content from November to April. They found that leaf N content was above 2% in all growing stages and in all treatments. Our result is in agreement with the result of Hochmuth *et al.* (1996). In another study, greenhouse-grown strawberry cv Tufts fertigated with increasing level of N from 50 to 150 mg L^{-1} and found that leaf N content of plant was above 3% in June (Papadopoulos, 1987). This value was well above the value found by present study. These differences may be attributed to cultivar, nutrition and environmental differences. Because each cultivar has unique N response and performance and N performance diversity reflects the differences in N uptake capacity of the roots, or growth habit under given environmental conditions.

There were highly significant correlations between leaf N and CHL reading value. There have been many studies showing significant positive correlations between yield and CHL reading in many crops including rice (Turner and Jund, 1991; Singh *et al.*, 2002), wheat (Singh *et al.*, 2002; Denuit *et al.*, 2002), maize (Schepers *et al.*, 1992; Blackmer and Schepers, 1995; Piekielek and Fox, 1992; Piekielek *et al.*, 1995; Varvel *et al.*, 1997), potatoes (Minotti *et al.*, 1994; Shaaban and El-Bendary, 1999; Denuit *et al.*, 2002; Gianquinto *et al.*, 2003, tomatoes and cucumber (Güler and Büyük, 2004). Present results are in agreement with the results of above-mentioned authors. The cultivar containing the highest CHL reading value had the highest leaf N and also the highest yield. The yield per plant was 501 g for Kabarla and 374 g for Camarosa, followed by Festival (261 g), Sweet Charlie (261 g) and R.hope (187 g).

A wide range of N and CHL reading values of cultivars used in this experiment increases the representative power of the mean N (2.07%) and CHL value (31.23 unit) when used as reference values. In addition, positive and high correlation coefficients ($r = +0.84$, $p<0.001$) between leaf N and CHL reading values irrespective of cultivar support this result. In Cate-Nelson graph, 2 outliers out of 20 points indicated that reference value for CHL reading corresponding to leaf N can be used with 90% confidence. Therefore, with utilization of the general regression equation ($y = -2.1309+0.1344x$, where x is CHL reading value and y is leaf N), it is possible to predict the N status of any strawberry cultivar throughout the growing season by using CHL reading value. At the flowering and fruiting stages, N status of the strawberry plants can be monitored through maintaining the leaf CHL reading values above 31 unit. In the other stages (beginning at the end of the harvest) leaf CHL should be maintained closer to this value.

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