Use of a Chlorophyll Meter to Diagnose Nitrogen Status of 'Fuyu' Persimmon Leaves

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Abstract. A possible relationship between leaf SPAD readings and nitrogen (N) concentrations was evaluated at different growing stages of 'Fuyu' persimmon trees under different N regimes in pots and in the field. When 5-year-old trees grown in pots received 0 to 40 g N over the growing season, leaf SPAD reading, N concentration, and specific leaf weight tended to increase with increasing N rate. The correlation between leaf SPAD reading and N concentration was statistically significant from late April to early November ($R^2 = 0.72$ to 0.97). It was noted that the slope of regression line decreased as the season progressed. Similar significant correlations were found from pot-grown 4- to 7-year-old trees grown under various nutrient regimes ($R^2 = 0.67$ to 0.96). Significant correlations were also confirmed with the leaves collected from 31 commercial orchards at different locations. The highest coefficient of determination was observed from the leaves collected at harvest time in both the pot and field experiments. Although the regression equations differed depending on the growing stage of trees, SPAD readings were found useful in estimating leaf N status at a specific time and in quickly judging the need for N application at that time. Seasonal changes of leaf SPAD readings from five well-managed orchards are presented as a reference guide for practical use.

The N status of persimmon trees is usually appraised by N concentration determined from midshoot leaves of late July to mid-August. Laboratory determination for leaf N concentration is time-consuming, and the results of such analyses are mostly used for N fertilization program of the next year in Korea. Under orchard situations in which tree vigor is less than desired, making a quick decision for supplemental N becomes more important throughout the current season. Because the traditional diagnostic techniques are useful mostly for the next season, the necessity arises for rapid N diagnosis for the current season. Growers often use leaf "greenness" as a visual indicator of the need for supplemental N. However, the degree of greenness is quite subjective; different people perceive it differently. A quicker and quantitative method is thus needed.

The SPAD-502 chlorophyll meter has been successfully used in assessing leaf N status of apple (Nielsen et al., 1995a, 1995b; Park et al., 2007; Porro et al., 2001), grapevine (Porro et al., 2001), and grapefruit (Li et al., 1998). It is easy, rapid, and non-destructive. However,

the correlation between the SPAD readings and N concentrations varied depending on growth stages of the tree (Nielsen et al., 1995b; Park et al., 2007) and environmental conditions (Nielsen et al., 1995b; Porro et al., 2001). Less is known for persimmon trees, although the readings were reported to be related to leaf N concentration (Choi et al., 2008). If SPAD readings are highly related to leaf N concentration of persimmon throughout the season, a standard curve could be developed. A SPAD meter would then be highly useful in estimating leaf N status to quickly determine whether N should be additionally applied at that time.

Many persimmon orchards are equipped with fertigation facilities in Korea. Combining fertigation with the chlorophyll meter could be extremely useful in managing N nutrition of the trees, thereby minimizing surplus N in the orchards. This experiment was conducted 1) to determine the changes in leaf SPAD readings and N concentrations as affected by different N fertigation rates; 2) to find the relationship between the two variables throughout the season; and 3) to provide seasonal SPAD readings obtained from some well-managed orchards producing premium-grade fruits.

Materials and Methods

Expt. 1 (in pots). Five-year-old 'Fuyu' persimmon trees (*Diospyros kaki*) were used to assess the effect of different N rates on

SPAD readings and N concentrations in 2008. Twenty-five trees were grown in 50-L pots filled with sandy loam soil and spaced at 1.5 m × 1 m. A total of 0, 10, 20, 30, and 40 g of N was applied in 20 equal proportions every week by fertigating 1% (w/v) urea solution, 0 to 450 mL per pot from 15 May to 25 Sept. Other fertilizers supplied to a pot included 3.5 g of phosphorus (P), 16.6 g of potassium (K), 8.6 g of calcium (Ca), and 3 g of magnesium (Mg); they were fertigated in five aliquots from early June to late September. Five single-tree replicates were assigned for each treatment in a completely randomized design. Flower buds were thinned to one or two a shoot in mid-May before flowering and the final fruit load was adjusted to a leaf–fruit ratio of 20 in early July. Trickle irrigator was adjusted to deliver 1 to 6 L of water per pot a day, starting at 1 L in early spring, gradually increasing to 6 L in midsummer, and then decreasing to 3 L at the end of the experiment. Tree management included routine chemical sprays to control diseases and pests. Five to 10 leaves per tree were sampled eight times from 28 Apr. (13 d after foliation) to 2 Nov. (harvest) to take SPAD readings with a chlorophyll meter (SPAD-502; Minolta Co., Tokyo, Japan). The measurements were made on the side of the midrib at the widest transverse section of leaves. After measuring leaf area with an area meter (Li-3100c; LI-COR, Lincoln, NE), the leaf samples were dried to calculate specific leaf weight (SLW).

Expt. 2 (in pots). The experiment in 2009 was to confirm the relationship between the SPAD readings and leaf N concentrations obtained in 2008. Twenty trees were randomly selected from a heterogeneous pool of 4- to 7-year-old 'Fuyu' trees. The trees were managed to have a wide range of apparent leaf greenness by fertigating different amount of N; the exact amount of N, or other elements, was out of concern in this experiment. A total of 0 to 40 g of N was distributed to a pot as 1% (w/v) urea solution, 0 to 400 mL per pot at 3- to 7-d intervals from 2 June to 29 Sept. To achieve the differences in both leaf greenness and N concentration. Based on the results of our preliminary experiments for other elements, different amounts of P, K, Ca, and Mg were surface-applied suitable for the trees of different sizes. Compound fertilizers, including 6 to 12 g of P, 11 to 20 g of K, and 5 to 10 g of Ca, and 3 to 5 g of Mg, were supplied several times from June to September. Flower buds were thinned to one or two a shoot in mid-May and fruit loads were adjusted to a leaf–fruit ratio of higher than 15 in early July; some trees bore only a few fruits as a result of little N supply or excessive fruit loads the previous year. The trees received similar irrigation and chemical sprays as those in 2008. Ten to 15 midshoot leaves per tree were sampled eight times from 30 Apr. (19 d after foliation) to 3 Nov. (harvest) in 2009 to measure leaf SPAD readings and N concentrations.

Expt. 3 (in the field). Leaf samples were collected from 31 commercial 'Fuyu' orchards in Gyeongnam Province in Korea. The orchards were 120 km apart at the farthest, and

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they had wide differences in tree ages, tree shapes, and the soil. Forty-five to 60 leaves from three trees per orchard were sampled from the midsection of the terminal shoots on the periphery of tree canopy on 2 to 5 June, 13 to 20 Aug., and 28 to 31 Oct. in 2008. SPAD readings were taken on the side of the midrib at the widest transverse section of the leaves.

To present a standard reference for practical use of SPAD readings throughout the season, we selected five most well-managed commercial orchards out of 31 in 2008 and 2009. The five orchards had been known to regularly produce more than 30 tons of fruits/ha; the fruits on average were greater than 240 g and 15.5 °Brix with no physiological disorders. Seasonal changes of SPAD readings and N concentrations were recorded from over 50 leaves of 20 trees at each orchard from 22 Apr. to 3 Nov. for both years. The time for foliation and flowering was ≈14 Apr. and 25 May in 2008, respectively, and it was earlier by ≈5 d in 2009. Leaves were sampled at 1-week intervals until early June and at 2-week intervals afterward until harvest. Procedures for taking SPAD readings and N determinations were the same as in pot experiments.

Leaf nitrogen determination and statistical analysis. All leaf samples were dried at 80 °C for 48 h and ground with a Wiley mill (3383-L10; Thomas Scientific, Swedesboro, NJ) to pass through a 20-mesh screen. To determine total N, 0.2-g subsamples were analyzed with a Kjeldahl instrument (Kjeltec 2300; Foss Co., Höganäs, Sweden) by using the micro-Kjeldahl method (Nelson and Sommers, 1973). Statistical analyses for regression and correlation were performed using the SAS program (SAS Institute, Inc., Cary, NC).

Results

Leaf SPAD reading, N concentration, and SLW of pot-grown trees tended to increase with increasing N rates (Fig. 1). SPAD readings increased until late August with all the N rates except for 0-g N treatment in which they gradually decreased from 23 July (Fig. 1A). Regardless of N rates, the readings decreased by 2 Nov. Leaf N concentration tended to decrease consistently after July (Fig. 1B). The SPAD reading and N were clearly low in those trees supplied with less than 10 g of N during the season; there were no significant differences among the trees when the N exceeded 20 g. SLW gradually decreased from 23 Aug. in 0-g N and from 25 Sept. in 10-g N trees (Fig. 1C). When the applied N rate was over 20 g, however, it did not decrease to lower than 12.8 mg·cm⁻² by 2 Nov.

The correlation between leaf SPAD readings and N concentrations was statistically significant from late April until harvest in pot-grown trees in 2008 ($R^2 = 0.72$ to 0.97) (Table 1). There were highly positive correlations between SPAD reading and SLW from 23 June in 2008 ($R^2 = 0.35$ to 0.89). High correlations were also found between the SPAD reading and N concentration in 2009 when the trees were grown under different conditions including the N rates and application

times ($R^2 = 0.67$ to 0.96) (Table 2). Coefficient of determination (R^2) was the highest at harvest at 0.97 in 2008 and 0.96 in 2009. The re-

gression equation differed depending on sampling dates, but the difference became less as the season progressed. It was noted that the

Fig. 1A–C. Effect of N fertilization rates on seasonal changes in leaf SPAD readings (A), N concentrations (B), and specific leaf weight (SLW) (C) of pot-grown ‘Fuyu’ persimmon. Nitrogen was fertiligated to the trees as 1% (w/v) urea solution from 15 May to 25 Sept. in 2008. Data are means of five trees. Bars indicate SD.

Table 1. Regression equations and coefficients of determination (R^2) among leaf SPAD reading, nitrogen (N) concentration, and specific leaf weight (SLW) at different growing stages of 5-year-old ‘Fuyu’ persimmon supplied with different amounts of N ($n = 25$).^z

Date	Regression equation	R^2	Date	Regression equation	R^2
<i>SPAD reading (X) and N concentration (Y)</i>					
28 Apr.	$Y = 1.608 + 0.081X$	0.72**	23 July	$Y = -0.37 + 0.051X$	0.8**
28 May	$Y = 0.521 + 0.043X$	0.87**	23 Aug.	$Y = -0.187 + 0.043X$	0.9**
23 June	$Y = -0.082 + 0.05X$	0.81**	25 Sept.	$Y = 0.212 + 0.035X$	0.91**
8 July	$Y = 0.116 + 0.045X$	0.88**	2 Nov.	$Y = 0.542 + 0.03X$	0.97**
<i>SPAD reading (X) and SLW (Y)</i>					
28 Apr.	$Y = 6.917 - 0.059X$	0.42**	23 July	$Y = 7.909 + 0.071X$	0.35**
28 May	$Y = 8.632 - 0.002X$	0.00 NS	23 Aug.	$Y = 7.989 + 0.084X$	0.48**
23 June	$Y = 6.875 + 0.084X$	0.52**	25 Sept.	$Y = 6.727 + 0.114X$	0.61**
8 July	$Y = 6.134 + 0.094X$	0.63**	2 Nov.	$Y = 8.077 + 0.092X$	0.89**

^zNitrogen was fertiligated to the trees as 1% (w/v) urea solution from 15 May to 25 Sept. in 2008.

NS, **Non-significant or significant at $P \leq 0.05$ and $P \leq 0.01$.

Table 2. Regression equations and coefficients of determination (R^2) for leaf SPAD reading (X) and nitrogen concentration (Y) at different growing stages of 4- to 7-year-old ‘Fuyu’ persimmon ($n = 20$).^z

Date	Regression equation	R^2	Date	Regression equation	R^2
30 Apr.	$Y = 0.117 + 0.132X$	0.84**	25 July	$Y = -0.251 + 0.45X$	0.82**
27 May	$Y = 0.297 + 0.052X$	0.88**	25 Aug.	$Y = -0.11 + 0.038X$	0.9**
17 June	$Y = 0.11 + 0.043X$	0.84**	26 Sept.	$Y = 0.2 + 0.032X$	0.82**
7 July	$Y = 0.13 + 0.037X$	0.67**	3 Nov.	$Y = 0.425 + 0.029X$	0.96**

^zDifferent amounts of phosphorus, potassium, calcium, and magnesium were surface-applied to match the requirements of trees with different sizes. Nitrogen was fertiligated to the trees as 1% (w/v) urea from 2 June to 29 Sept. in 2009.

**Significant at $P \leq 0.01$.

slope of regression line decreased in both years as the season advanced.

Significant correlations were also found between SPAD readings and N concentrations of the leaves collected from 31 commercial orchards, the R^2 being in the range from 0.61 to 0.86 (Fig. 2). The slopes of regression lines in early June, mid-August, and early November were similar to those in June, August, and November in the pot experiments (Tables 1 and 2), respectively. As were observed in the pot experiments, the SPAD readings varied widely toward harvest time with the highest R^2 values.

SPAD readings taken from the five well-managed orchards showed very similar changes in 2008 and 2009 (Fig. 3A). A slight difference was observed only in May when the reading was higher in 2009 than in 2008. The readings rapidly increased by early June followed by a gradual increase by mid-July; they did not significantly change from late July to late September. The readings steadily declined from October to harvest. In contrast to SPAD, leaf N concentration was the highest in late April followed by a rapid decrease during the period of active leaf expansion until mid-May (Fig. 3B). Leaf N concentration was higher in 2008 than in 2009 until mid-May. After a gradual decrease by mid-June, a slight decline in N concentration continued between early July and early October. N concentration decreased again with decreasing SPAD readings after mid-October.

Discussion

Seasonal changes in leaf N concentrations (Fig. 3B) were similar to those previously reported for persimmon leaves (George et al., 1994; Park, 2002). Higher SPAD readings with increasing N rates indicated an increase in leaf N and chlorophyll formation as was found in a turfgrass (Rodriguez and Miller, 2000). However, both SPAD readings and leaf N of the trees differed little when N rates exceeded 20 g per tree (Fig. 1). It is likely that some of the N applied at high fertilization rates was used for the growth of other vegetative organs or leached out of the pots. The SPAD readings by the end of May did not reflect leaf N concentrations during that period (Fig. 3). It was the time when the thin, pale-green leaves underwent active expansion growth. The rapid increase of SPAD reading after May (Fig. 3A) could be related to the increase in leaf thickness as shown in the positive relation between SPAD reading and SLW (Table 1). SPAD readings and chlorophyll concentrations increased with leaf thickness in apple leaves (Campbell et al., 1990). On the other hand, the decline of SPAD readings or N in later part of the season would be the result of the translocation of leaf N to other organs during senescence (Park et al., 2003; Titus and Kang, 1982).

Our results for significant correlations between SPAD readings and leaf N concentrations in persimmon are in general agreement with the previous reports of other fruit crops in that the SPAD readings do represent

Fig. 2. Relationships between the SPAD readings and N concentrations of the leaves collected from 31 commercial 'Fuyu' persimmon orchards at different sampling times in 2008. Each data point represents the mean of 3-tree determinations.

Fig. 3A–B. Seasonal changes of the SPAD readings (A) and N concentrations (B) of the leaves collected from five well-managed 'Fuyu' persimmon orchards. Bars indicate SD.

leaf N status (Li et al., 1998; Neilsen et al., 1995a; Porro et al., 2001). However, such a high correlation between the two parameters throughout the season for persimmon contrasted to the results of Neilsen et al. (1995b) for apple; they found a strong correlation only by mid-July but not thereafter. Despite high correlation coefficients at every sampling date, regression equations were not the same at every growth stage attributable mainly to the changes in leaf thickness as shown in SLW of this study (Fig. 1C; Table 1) and as has been reported in apple (Neilsen et al., 1995b). This suggests that SPAD readings

should be calibrated for a specific growth stage to represent leaf N status more accurately (Neilsen et al., 1995b; Porro et al., 2001). Lower R^2 values for field-grown trees than the pot-grown counterparts may be related to the differences in cultural and environment conditions affecting the growth stage within a sampling area. Higher R^2 values at harvest than at any other times would indicate that SPAD readings taken late in the season may be highly useful in planning fertilization program for the next season. From the pot and field experiments, it was concluded that the chlorophyll meter could be used to assess leaf N

status in persimmon throughout the season, a clear advantage over apple in which its usefulness may be limited only to a certain growth stage.

From this study, we aimed to provide seasonal changes in SPAD readings as a standard indicator whether supplemental N is needed. Comparing leaf SPAD reading (Fig. 3A) and N concentration (Fig. 3B), growers could get the necessary information about N status of their trees. However, the SPAD readings should be taken with care whether supplemental N is warranted, especially during the early part of the season when leaves are actively expanding. Also, the time of foliation and flowering should be taken into consideration as was clearly indicated by the differences of SPAD reading and N by mid-June of 2008 and 2009.

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