Pak. J. Bot., 53(5): 1859-1864, 2021. DOI: http://dx.doi.org/10.30848/PJB2021-5(39)

ASSESSING NITROGEN NUTRITION OF BANANA BASRAI CULTIVAR (DWARF CAVENDISH) THROUGH LEAF ANALYSIS AND CHLOROPHYLL DETERMINATION

BAGH ALI ABRO[1], MEHRUNISA MEMON[1*], ZIA-UL-HASSAN, MOHAMMAD YOUNIS ARAIN[2], ABDUL RAZAQ[1], DILDAR ALI ABRO[1] AND SAJAD ALI[1]

[1]Department of Soil Science, Sindh Agriculture University, Tandojam, Sindh, Pakistan
[2]Intitute of Plant Introduction, SARC-PARC Karachi
[*]Corresponding author's email: nisamemon@gmail.com

Abstract

Adequate supply of N nutrition is essential in banana production and is an indirect indicator of chlorophyll content, as considerable amount of it is incorporated in green pigment. Traditional methods of N determination are expensive and lengthy or require high technology instruments, affecting its timely determination. This study assessed N nutrition of banana leaf tissue through traditional Kjeldahl's method and regressed with chlorophyll content, each by spectrophotometry and SPAD-502. Fully grown suckers identical in appearance were randomly selected from thirty banana growing sites of district Naushehroferoze. Kjeldahl's N content was between 1.80 and 4.60 % with 60% samples falling under low, 3% under high and remaining were sufficient in N nutrition. There was strong, linear and significant relationship of chlorophyll determined by SPAD with chlorophyll "a" (r = 0.81) and total (r = 0.86) by spectrophotometry. In case of Kjeldahl's N, similar relationships were developed with chlorophyll content determined by SPAD meter (r = 0.85), chlorophyll "a" (r = 0.79) and total (r= 0.80) by spectrophotometry. Coefficient of determination explained 72% contribution of chlorophyll content by SPAD, and 63% and 64% ("a" and "total", respectively) by spectrophotometry to the total N content in leaf tissue. While, chlorophyll determination by SPAD meter explained 67%, 19% and 74% to spectrophotometric chlorophyll "a", "b" and "total", respectively. This study recommends that both the techniques of chlorophyll determination are closely associated with each other; however, spectrophotometric chlorophyll determinations have an edge over SPAD readings. Under spectrophotometric determination, chlorophyll "a" is the major portion of total chlorophyll associated with both N and SPAD values.

Key words: Spectrophotometric, Acetone extraction, SPAD-502, Kjeldahl's N

Introduction

Sindh is the main contributor of banana with 86% of the total production and 93% of the total area in Pakistan (Anon., 2015). Major area under banana cultivation in Pakistan is of single cultivar Dwarf Cavendish. District Naushehroferoze contributes significant share among banana cultivating zones. Nitrogen (N) being an essential nutrient is required by banana plant throughout its growth cycle i.e. starting from new plant tissue till fruit development (Shaahan *et al*., 1999). It is significant part of living cells and is present within amino acids which are the building blocks of proteins. It is main constituent of chlorophyll. Moreover, N being part of nucleic acid i.e. DNA is present in nucleus, mitochondria, and chloroplasts (Perchlik & Tegeder, 2018). Some proteins act as enzymes, and N provides energy to these enzymes to precede the biochemical reactions (Porra, 2002). Proteins break down to amino acids and N is released to move towards chlorophyll of leaf which increases the density of chlorophyll in plant cell (Terashima & Saeki, 1983). The intensity of green color measured directly determines leaf N content (Takebe *et al*., 1990).

Banana needs maximum quantity of N fertilizer during vegetative to fruiting stage as compared to phosphorus and potassium (Rajput *et al*., 2017). It contains lots of nutrients for human health such as sugar, polysaccharides, vitamin A, B6, C (Park *et al*., 20111) and micronutrients (Rajput *et al*., 2017) which are beneficial for good health and reduced risk for diseases (Lecerf, 2008). Leaves are the organs of banana which perform structural as well as functional acclimation of the photosynthetic apparatus to the intensity of light practiced during their growth (Prioul *et al*., 1980). Due to low N availability in plants, banana produces lesser number of leaves as compared to the number of leaves produced by banana plants supplied with adequate N (Adhikari *et al.*, 1999). Nitrogen deficient plants show poor growth and pale yellow leaves with reduced leaf area and rate of leaf production, therefore, N is the most preventive factor for the growth and development of banana (Tucker, 2004).

Chlorophyll is also used as an indicator of N content owing to the significant quantity of leaf N in pigment protein complexes (Bojovic & Markovic, 2009). Banana leaf chlorophyll serves as an integrator of environmental as well as nutritional variables, which ultimately affect the quality of a fruit and plant senescence (Moreira and Fageria, 2009). Nitrogen can be evaluated indirectly from chlorophyll content, because a significant amount of N in banana leaf is merged in that pigment (Filella *et al*., 1995). Some studies have shown positive relationship between total N and chlorophyll content of potato (Takebe *et al*., 1990), coffee (Guimaraes *et al*., 1999, Netto *et al.*, 2002) and cotton (Peterson *et al*., 1993) leaves due to significant amount of N present in chlorophyll molecule. Recent advancement show that chlorophyll determinations are normally carried out by using SPAD meters, which are relatively easy to handle (Uddling, *et al*., 2007). Studies highlighting the relationship between N content in leaf tissue, spectrophotometric chlorophyll and SPAD meter chlorophyll content are related to crops like wheat, corn and soybean (Daughtry *et al*., 2000; Bojovic & Markovic, 2009 and Haim *et al*., 2012), while those pertaining to banana are negligible (Melo *et al*.,

1914) and yet for the local variety of banana do not exist. The study by Melo *et al*., (2014) compared banana leaf tissue N with spectrophotometric chlorophyll only, while, SPAD meters are more commonly used for chlorophyll determinations due to their convenience and therefore, it is necessary to include chlorophyll assessment by both methods for local banana variety of Pakistan. The main objectives of this study were to evaluate the chlorophyll content of banana leaf tissue by SPAD-502 and spectrophotometry (acetone extraction) and develop relationship of each over traditional method of Kjeldahl's N determination and come up with the increase in N content associated with chlorophyll by each method.

Materials and Methods

Banana leaf sampling: Fully grown suckers identical in appearance were randomly selected from thirty banana growing sites of district Naushehroferoze. Leaf index tissue sampling was carried out in the month of July, 2016. One leaf (third leaf from top) was used to take SPAD readings from the center of leaf and either side of the mid rib. This followed the removal of 10 cm wide strip using stainless steel scissor from the center of leaf, perpendicular to the leaf length, including the SPAD meter reading spot. The leaves were placed in zip-lock plastic bags and kept in covered ice box during transportation to the laboratory. For chlorophyll determination by spectrophotometry, required portion of fresh leaf was preserved in acetone and placed in freezer (above 0^{o}C) (Arnon, 1949). The remaining leaf samples were air-dried in shade at room temperature, followed by oven-drying at 68^{o}C, ground to fine powder using stainless steel grinding machine and used for N determination.

Analytical methods: The dried samples were ground and N was determined by Kjeldahl's method (Bremner and Malvany, 1982). Samples were acid digested, followed by distillation using NaOH and toshiro indicator and finally the contents were titrated. The amount of HCl utilized was back calculated to determine the N% in leaf tissue.

For chlorophyll determination, SPAD meter (SPAD-502 plus, Konica Minolta Optics-2012) was calibrated without introducing leaf sample in the sample slot and pressing the measuring head with the fingers. The measuring head was closed (by pressing) until a beep sound heard and the screen displayed a message "calibration process completed". For leaf sample reading, the leaf sample was introduced into receptor window in such a way that it covered the window completely and the measuring head was closed using finger and kept on pressing the measuring head, till the beep sound and the measured values appeared on the display screen. Several readings were noted for each sample and mean values were recorded for each leaf sample by pressing the average button.

Spectrophotometric chlorophyll was carried out by extraction method as detailed by Arnon (1949). For this determination, the acetone dipped sample was taken out and cut into thin pieces using small stainless steel scissor. One gram sample along with 10 ml of 80% acetone was placed in pestle mortar and ground. The suspension was transferred into 25 ml graduated centrifuge tubes and the volume of the tube was raised to 20 ml by adding 80% acetone. The tubes were centrifuged for 10 minutes at 7000 rpm. Clear extract was run on spectrophotometer at two wavelengths i.e. 645 and 663 nm. The chlorophyll a, b and total was calculated by using relevant Arnon equations as presented here and converted to mg g^{-1} [extraction volume/(sample weight x1000)].

Chlorophyll a (mg L^{-1}) = 12.7 (A 663) – 2.69 (A 645)
Chlorophyll b (mg L^{-1}) = 22.9 (A 645) – 4.68 (A 663)
Chlorophyll total (mg L^{-1}) = (Chlorophyll a + Chlorophyll b)

Results and Discussion

The Kjeldahl's N, chlorophyll by SPAD-502 and spectrophotometric chlorophyll ("a", "b" and total) in the form of range, mean, standard deviation, mode and coefficient of variability are presented in Table 1. Leaf tissue content of individual banana sites is presented in Figure 1. The relationship of Kjeldahl's N with chlorophyll by SPAD-502 and spectrophotometric chlorophyll ("a", "b" and total) is presented in Figure 2. While, relationship of SPAD-502 chlorophyll content with spectrophotometric chlorophyll ("a", "b" and total) in banana leaf tissue is presented in Figure 3.

Kjeldahl's leaf tissue N: The N content of banana leaf tissue was between 1.80% and 4.60%, with a mean value of 3.26% (Table 1). The coefficient of variability indicated 22.2% variation among thirty banana growing sites. The most frequently occurring Kjeldahl's N content among different sites of district Naushehroferoze was 3.18%. According to Jones *et al*., (1991), for N content to be sufficient, a 3rd leaf of 6-9 month banana plantation should have N content between 3.5-4.5%. Therefore, the standard critical value of N is actually 4.5%. Similarly, the values between 2.5-3.49% are low in N content. Therefore, any values <3.49% would be considered as low. (Fig. 1) illustrated that out of 30 banana sites, 11(37%) sites were falling under the sufficient range of 3.5-4.5%. Nonetheless, 6(20%) out of 11 (37%) sites were closer to the lower limit of 3.49% and those below 4% N were also at risk. This shows only 5 sites, rather 6(20%) being sufficient in N, as one value was slightly (i.e. 4.6%) above the sufficient range (4.5%). The N contents in leaf tissue (1.74-4.32% and 2.62-4.09%) of same banana cultivar as reported by Memon *et al*., (2010) and Rajput (2017) were similar to those obtained in this study. Literature related to N content of banana leaf tissue is scarce and depends on the leaf position, age and variety of banana plant (Rashid, 1996) in addition to fertilizer application and other management practices. The results of leaf tissue analysis from various studies can only be compared when similar sampling techniques have been used. In contrast, the N levels reported for Poovan banana cultivar were 3.24% at vegetative, 2.53% at flowering and 2.68% after the harvest (Selvamani *et al*., 2009).

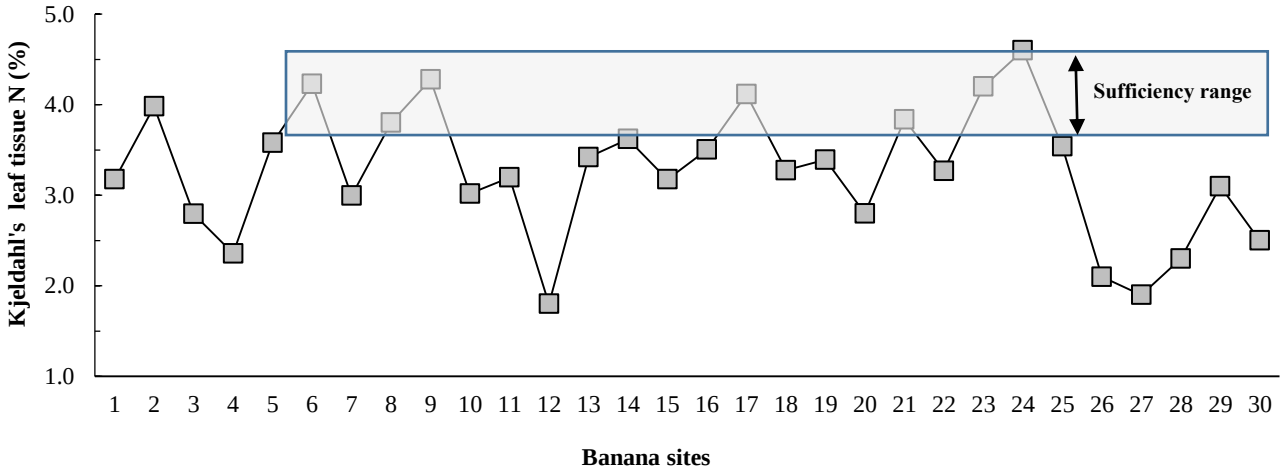

Fig. 1. Kjeldahl's N content in 6-9 month old banana leaf tissue with sufficiency range.

Table 1. Kjeldahl's N, SPAD-502 and spectrophotometric chlorophyll in in 6-9 month old banana leaf tissue.

Parameters	Range	Mean ± standard deviation	Mode	Coefficient of variability (%)
Kjeldahl's N (%)	1.80-4.60	3.26 ± 0.72	3.18	22.20
SPAD-502-Chlorophyll	33.30-58.00	43.24 ± 7.14	56.00	16.50
Spectrophotometric Chlorophyll "a" (mg g^{-1})	0.72-0.90	0.81 ± 0.0496	-	6.09
Chlorophyll "b" (mg g^{-1})	0.18-0.32	0.24 ± 0.0366	-	15.26
Total chlorophyll (mg g^{-1})	0.90-1.22	1.05 ± 0.0718	-	6.81

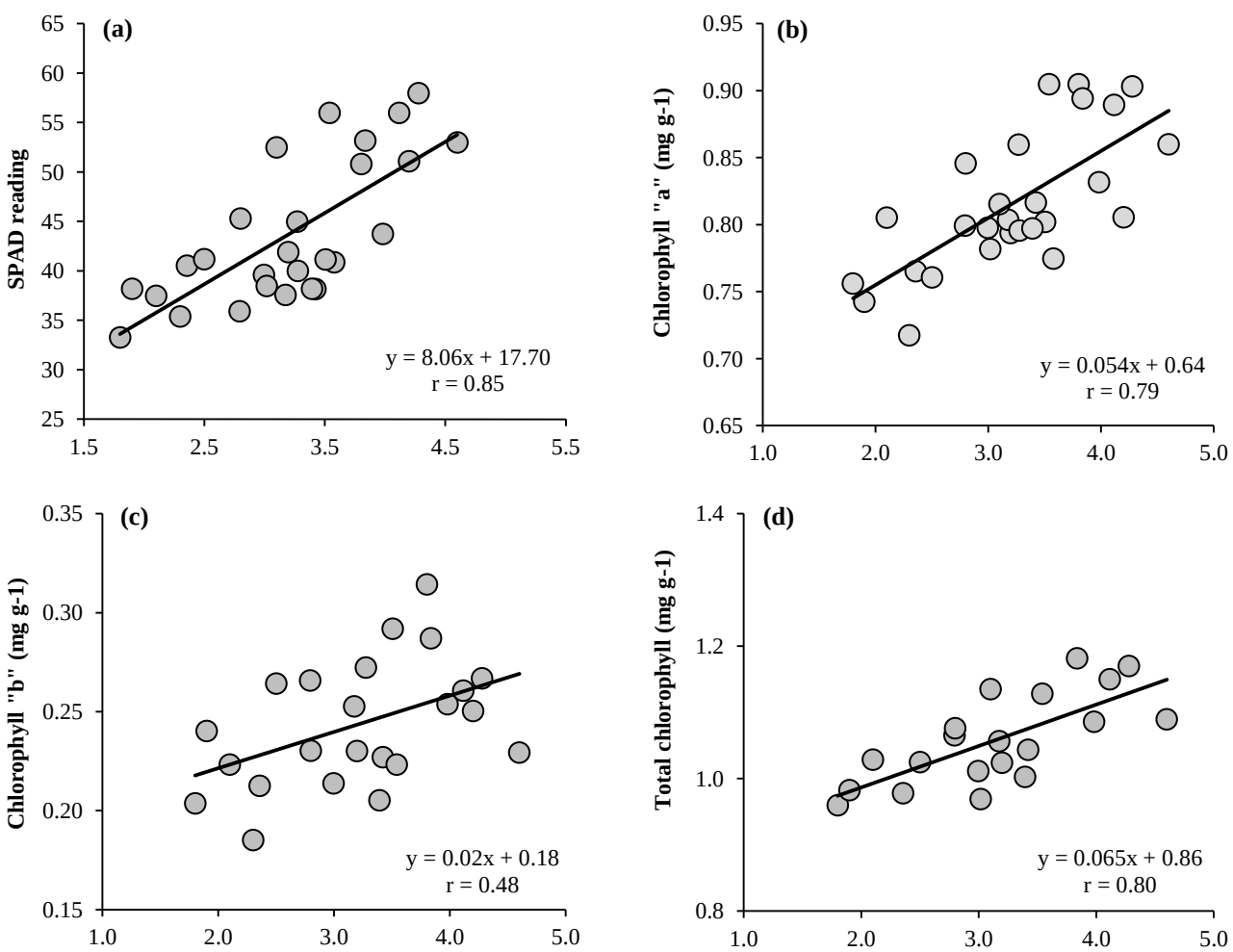

Fig. 2. Relationship of Kjeldahl's N with chlorophyll by portable SPAD-502 (a) and spectrophotometric chlorophyll "a" (b), "b" (c) and total (d) in 6-9 month old banana leaf tissue.

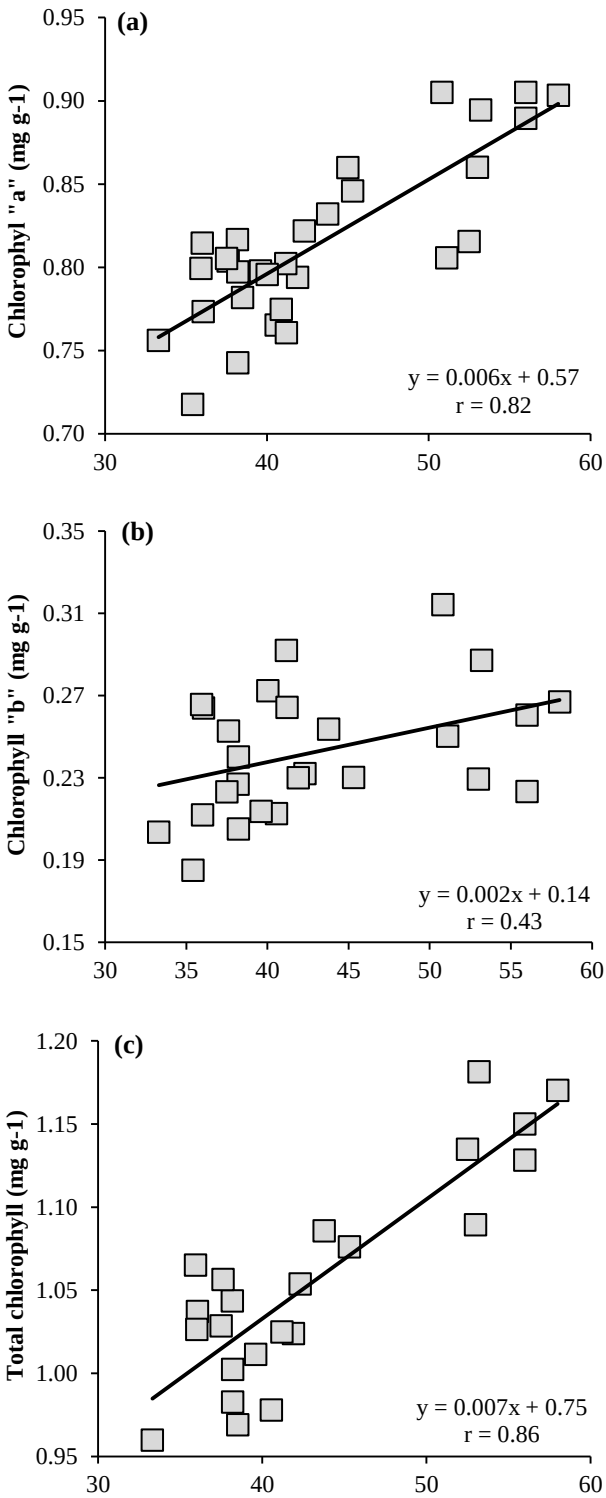

Fig. 3. Relationship of SPAD-502 chlorophyll content with spectrophotometric chlorophyll "a" (a), "b" (b) and total (c) in 6-9 month old banana leaf tissue.

SPAD-502 chlorophyll: The chlorophyll contents recorded with SPAD meter on the spot were 33.3-58.0 with a mean value of 43.24 (Table 1). There is different portable chlorophyll meters available in market. Being a quick test method, it is widely used for fruit and other crops (Schaper *et al*., 1991 and Ghasemi *et al*., 2011), however, only few studies report chlorophyll content of banana by this method. In this context, Hooks *et al*., (2009) using SPAD-502 (Minolta Corporation, Ramsey, NJ) reported chlorophyll readings of around 35-37.5 for Williams banana. Arantes *et al*., (2016) using Clorofilog CFL-1030 chlorophyll meter (Falker) reported mean values of 50.63-55.9 from banana leaf tissue of different cultivars (3rd leaf from apex). While the values by SPAD-501 were between 16.5 and 37.0 (Schaper *et al*., 1991). The variability in chlorophyll contents by these meters may be due to type of tree, position of leaf, under shade or exposed to sun, etc. (Bjorkman, 1981 and Schaper, 1991).

Spectrophotometric chlorophyll: This technique of chlorophyll determination has benefit over portable chlorophyll technique as it determines chlorophyll "a" and "b" separately instead of total. The chloroplast contain certain pigments (i.e. chlorophyll), categorized as "a" and "b" (or even c, d and e), and the former is the main pigment in plant cells having tendency to capture sunlight, and therefore, release electrons and regain them from various other sources (Ritchie, 2006). Chlorophyll "a" differs in both light intensity and spectral quality of light (Atwell *et al*., 1999). Banana possessing self-shading of leaves, a proportion of chlorophyll "b" is also important in capturing the energy from other wavelengths and its transfer to the specific molecule of chlorophyll "a" (Melo *et al*., 2014). Our results (Table 1) are in confirmation with above statements, where chlorophyll "a" was major part (0.72-0.90 mg g^{-1}) of total chlorophyll (0.90-1.22 mg g^{-1}), over chlorophyll "b" (0.18-0.32 mg g^{-1}). Chlorophyll "a" has been the major constituent (0.6-2.3 mg g^{-1}) in strawberry leaf tissue (Wood *et al*., 1993), and even other fruit crops. While, the total content ranged from 0.9 and 3.0 mg g^{-1}. The red maple leaves were found to have total chlorophyll content of 4.07 and 5.38 mg g^{-1} (Sibley *et al.*, 1996). Yet, there are disagreements among researchers regarding the role of chlorophyll "b" in the photosynthetic process (Bidwell, 1979).

Regression analysis: Kjeldahl's N content was regressed against chlorophyll content (SPAD-502 and spectrophotometric) to find out the nature of relationship between these parameters. Linear, positive and significant relationships were obtained for SPAD (r = 0.85) and spectrophotometric chlorophyll "a" (r = 0.79) and total (r = 0.80) with Kjeldahl's N content in leaf tissue (Fig. 2). Similar type of relationships were observed for spectrophotometric chlorophyll "a" (r = 0.81) and total (r = 0.86) when regressed with SPAD chlorophyll (Fig. 3). Considering chlorophyll "b" by spectrophotometry, it had relatively poor relationship with Kjeldahl's N (r = 0.48) and SPAD chlorophyll (r = 0.43). Similar relationships among chlorophyll by various portable meters and spectrophotometry have been reported in leaf tissue samples of various fruits with coefficient of correlation of 0.91 in mango, 0.95 in cashew, 0.96 in custard apple (Schaper *et al.*, *1*991), 0.91 in Asian pear (Ghasemi *et al.*, 2011) and 0.85 in muskmelon (Azia *et al*., 2014). In case of red maple, the relationship (r = 0.45) between portable and spectrophotometric chlorophyll was poor (Sibley *et al*., 1996). Linear, positive and strong relationships between portable chlorophyll and N content in leaf tissue

of Benjamin fig (r = 0.99) and Asian pear (r = 0.76) are also on the record (Loh *et al.*, 2002 and Ghasemi *et al.*, 2011). Yet, the relationships developed with regard to banana leaf tissue are a new addition with exception to few studies. Melo *et al.*, (2014) reported a strong relationship (R^2 = 0.88) between chlorophyll content by spectrophotometry and N content of banana leaf tissue.

Conclusion

Based on strong relationship of Kjeldahl's N to SPAD-502 and spectrophotometric chlorophyll, this study recommends that both the techniques of chlorophyll determination are closely associated with each other and support indirect estimation of N in six to nine month old banana Basrai leaf tissue. For each 1% increase in N, the associated chlorophyll increase is 8.06 by SPAD and 0.065 by spectrophotometry. Under spectrophotometric determination, chlorophyll "a" is the major portion of total chlorophyll associated with both Kjeldahl's N and SPAD values. Owing to separate determination of "a" and "b", spectrophotometric chlorophyll has an edge over the SPAD one.

References

Adhikari, C., K.F. Bronson, G.M. Panuallah, A.P. Regmi, P.K. Saha, A. Dobermann, D.C. Olk, P.R. Hobbs and E. Pasuquin. 1999. On-farm soil nitrogen supply and nitrogen nutrition in the rice-wheat system of Nepal and Bangladesh. *Field Crop Res.,* 64: 273-286.

Arantes, A.D.M., S.L. Donato, D.L.D. Siqueira, E.M. Amorim and V.A. Rodrigues Filho. 2016. Chlorophyll index for real-time prediction of nutritional status of Prata banana. R. Bras. *Eng. Agric. Ambiental.,* 20(2): 99-106.

Arnon, D.I. 1949. Copper enzymes in isolated chloroplast. Polyphenol oxidase in Beta vulgaris. *J. Plant. Physiol.,* 24(1-15).

Atwell, B.J., P.E. Kriedemann and C. Eds. Turnbull. 1999. Plants in Action: Adaptation in Nature, Performance in Cultivation. Macmillan.

Azia, F. and K.A Stewart. 2014. Relationship between extractable chlorophyll and SPAD values in muskmelon leaves. *J. Pant. Nutr*., 24(6): 961-966.

Bidwell, R.G.S. 1979. Plant chlorophyll. In: Plant physiology. 2nd ed. New York: Macmillan.

Bjorkman, O. 1981. Responses to different quantum flux densities. In: *Physiological Plant Ecology I. Responses to the Physical Environment*. Encyclopedia of Plant Physiology, Vol. 12A. Eds. O.L. Lange, P.S. Nobel, C.B. Osmund and H. Ziegler. Springer-Verlag, Berlin, pp 57-107.

Bojovic, B. and A. Markovic. 2009. Correlation between nitrogen and chlorophyll content in wheat (*Triticum aestivum* L.). Kragujevac. *J. Sci.,* 31: 69-74.

Bremner, J.M. and C.S. Mulvaney. 1982. Nitrogen total. In: *Methods and Soil Analysis*: part 2. Chemical and microbiological properties. ASA Page, A.L. *et al*., Eds. Monograph., Number 9: 595-624.

Daughtry, C.S.T., C.I. Walthall, M.S. Kim, E. Brown De Colstoun and J.E. Mcmurtney. 2000. Estimating corn leaf chlorophyll concentration from leaf and canopy reflectance. *Rem. Sens. Environ.,* 74: 229-239.

Filella, I., I. Serrano, J. Serra and J. Penuelas. 1995. Evaluating wheat nitrogen status with canopy reflectance indices and discriminant analysis. *Crop. Sci.,* 35: 1400-1405.

Ghasemi, M., K. Arzani, A. Yadollahi, S. Ghasemi and S. Sarikhani. 2011. Estimate of leaf chlorophyll and nitrogen content in Asian pear (*Pyrus serotina* Rehd.) by CCM-200. Not. *Sci., Biol.,* 3(1): 91-94.

Anonymous. 2015. Economic Survey of Pakistan 2014-2015. Government of Pakistan, Finance Division, Economic Advisor Wing, Islamabad, Pakistan.

Guimaraes, T.G., P.C.R. Fontes, P.R.G. Pereira, V.H. Alvarez and P.H. Monnerat. 1999. Teores de clorofila determinados por medidor portatil e sua correlacao com formas de nitrogenio em folhas de tomateiro cultivados em dois tipos de solo. *Bragantia.,* 58(1): 209-216.

Haim, P.G., B.C. Zoffoli, E. Zonta and A.P. Araujo. 2012. Diagnose nutritional de nitrogenio em folhas de feijeiro pela analise digital de imagens. Pesq. *Agropec. Bras.,* 47: 1546-1549.

Hooks, C.R.R., R. Manandhar, E.P. Perez, K.H. Wang and R.P.P. Almeida. 2009. Comparative susceptibility of two banana cultivars to banana bunchy top virus under laboratory and field environments. *J. Econ. Ento.,* 102(3): 897-904.

Jones, Jr. J.B., B. Wolf and H.A. Mills.1991. Plant analysis handbook. Micro – Macro Publishing Inc., Athens, GA, USA.

Loh, F.C.W., J.C. Grabosky and N.L. Bassuk. 2002. Using the SPAD-502 meter to assess chlorophyll and nitrogen content of Benjamin Fig and Cottonwood leaves. *Hort. Techn.,* 12: 682.

Melo, A.S., C.D.S. Junior, P.D. Fernandes, M.E.B. Brito, L.F. Sobral, J.F. Suassuna, P.R.A. Viegas and R.L.D.S. Ferraz. 2014. Chlorophyll and macronutrients content in leaf tissue of Musa sp Prata-Ana under fertigation. *Afri. J. Agri. Res.,* 9(22): 1714-1720.

Memon, N., K.S. Memon, R. Anwar, S. Ahmad and M. Nafees. 2010. Status and response to improved NPK fertilization practices in banana. *Pak. J. Bot.,* 42(4): 2369-2381.

Moreira, A. and N.K. Fageria. 2009. Reparticao e remobilizacao de nutrients na bananeira. *Rev. Bras. Frut.,* 31(2): 574-581.

Netto, T.A., E. Campostrini, J.G. de Oleveira and O.K. Yamanishi. 2002. Portable chlorophyll meter for all quantification of photosynthetic pigments, nitrogen and the possible use for assessment of the photochemical process in *Carica papaya*. *Braz. J. Plant Physiol.,* 14(3): 203-210.

Perchlik, M. and M. Tegeder. 2018. Leaf amino acid supply affects photosynthetic and plant nitrogen use efficiency under nitrogen stress. *Plant Physiology*, 178: 174-188.

Peterson, T.A., T.M. Blackmer, D.D. Francis and J.S. Scheppers. 1993. Using a chlorophyll meter to improve N management. A Webguide in Soil Resource Management: D-13 Fertility. Cooperative Extension, Institute of Agriculture and Natural Resources, University of Nebraska, Lincoln, NE, USA.

Porra, R.J. 2002. The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls a and b. *Photosyn. Res.*, 73: 149-156.

Prioul, J.L., J. Brangeon and A. Reyss. 1980. Interaction between external and internal conditions in the development of photosynthetic features in a grass leaf II. Reversibility of light-induced responses as a function of developmental stages. *Plant Physiol*., 66(4): 770-774.

Rajput, A., M. Memon, K.S. Memon, S. Tunio, T.A. Sial and M.A. Khan. 2017. Nutrient composition of banana fruit as affected by farm manure, composted pressmud and mineral fertilizers. *Pak. J. Bot*., 49(1): 101-108.

Rashid, A. 1996. Soils: Basic Concepts and Principles. National Book Foundation, Islamabad, Pakistan.

Ritchie, R.J. 2006. Consistent sets of spectrophotometric chlorophyll equations for acetone, methanol and ethanol solvents. *Photosyn. Res.,* 89: 27-41.

Santos, V.P., P.D. Fernandes, A.S. Melo, L.F. Sobral, M.E.B. Brito, J.D.M. Dantas and L.V. Bonfim. 2009. Fertirrigacao da bananeira cv. Prata-Ana com N e K em um Argissolo Vermelho-Amarelo. *Rev. Bras. Frut.,* 31(2): 567-573.

Schaper, H. and E.K. Chacko. 1991. Relation between extractable chlorophyll and portable chlorophyll meter reading in leaves of eight tropical and subtropical fruit- tree species. *J. Plant. Physiol.,* 138: 674-677.

Selvamani, P., K. Manivannan and J. Mohan. 2009. Impact of organic manures, inorganic fertilizers and biofertilizers on the nutrient concentration in soil at different growth stages of banana cv. Poovan Mysore (AAB) (*Musa* sp. L.). *Plant Arch.,* 11(1): 165-168.

Shaahan, M.M., A.A. EL-Syed and E.A.A. Abou EL-Nour. 1999. Predicting nitrogen, magnesium and Iron nutritional status in some perennial crops using a portable chlorophyll meter. *Sci. Hort.,* 82: 339-348.

Sibley, J.L., D.J. Eakes, C.H. Gilliam, G.J. Keever, W.A. Dozier, Jr and D.G. Himelrick. 1996. Foliar SPAD – 502 meter values, nitrogen levels and extractable chlorophyll for red maple selection. *Hort. Sci.,* 31(3): 468-470.

Takebe, M., T. Yoneyama, K. lnada and T. Murakami. 1990. Spectral reflectance ratio of rice canopy for estimating crop nitrogen status. *Plant & Soil,* 122: 295-297.

Terashima, L. and T. Saeki. 1983. Light environment within a leaf. *Plant & Cell Physiol.,* 24: 1493-1501.

Tucker, M. 2004. Primary nutrients and plant growth. - In: *Essential plant nutrients* (SCRIBD, Ed.). North Carolina Department of Agriculturae.

Udding, J., J. Gelang-Alfredsson, K. Piikki and H. Pleijel. 2007. Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. *Photosyn. Res.,* 91: 37-46.

Wood, C.W., D.W. Reeves and D.G. Himelrick. 1993. Relationship between chlorophyll meter readings and leaf chlorophyll concentration, N status and crop yield in variety of crops. A Review, Proc. *Agron. Soc. N.Z.*, vol. 23, pp. 1-9.

(Received for publication 15 September 2019)