

Evaluation of the SPAD Value in Faba Bean (*Vicia faba* L.) Leaves in Relation to Different Fertilizer Applications

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Abstract : The correlations of the reading of a portable chlorophyll meter (SPAD-502) with the chlorophyll and N contents of leaves of two faba bean (*Vicia faba* L.) cultivars, Japanese (Ryousai-issun) and Egyptian (Cairo 241), were examined. The SPAD readings positively correlated ($p < 0.01$) with the chlorophyll contents and the r^2 values were 0.99 and 1.00 for Ryousai-issun and Cairo 241, respectively. A close linear relationship ($p < 0.001$) was observed between SPAD reading and total leaf N content at the pod development stage of faba bean plants with $r^2 = 0.88$ and 0.99 for Ryousai-issun and Cairo 241, respectively. The SPAD reading was the highest in the 2nd to 4th leaves counted from the top (the youngest fully expanded leaves). The changes in leaf chlorophyll content of both cultivars from 3 weeks after transplanting to the ripening stage showed an incomplete “M” type curve. SPAD readings were significantly higher in Ryousai-issun than in Cairo 241 throughout the growth season. Organic fertilizers application improved faba bean plant growth. These results suggest that the SPAD-502 chlorophyll meter can be used to measure chlorophyll and nitrogen contents of faba bean leaves for quick screening faba bean genotypes.

Key words : Chlorophyll, Faba bean, Nitrogen, Nutrition, Organic materials, SPAD-502 meter.

Faba bean (*Vicia faba* L.) is an economically important crop with widespread commercial production and utilization. The measurement of leaf chlorophyll and leaf nitrogen (N) contents by destructive methods is laborious, time consuming and costly. The Soil Plant Analysis Development (SPAD) division of Minolta Co., Ltd. has developed the SPAD-502 chlorophyll meter (Minolta Co., Ltd., Japan), a portable, self-calibrating, convenient, and nondestructive device which can be used for measuring the amount of chlorophyll present in plant leaves (Yadava, 1986; Minolta, 1989). The SPAD meter has been developed primarily for N management in rice production and is being used for this purpose in Japan (Turner and Jund, 1991). This meter records optical density at two wave bands (600–700 and 400–500 nm), converts them into digital signals, and then into a SPAD value (Minolta, 1989). The SPAD values have been found to correlate positively ($r^2 = 0.97$) with the chlorophyll content of rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), and soybean [*Glycine max* (L.) Merr.] measured with a destructive chlorophyll meter (Monje and Bugbee, 1992) and with extractable chlorophyll content in 11 food crop species ($r^2 > 0.90$) (Marquard and Tipton, 1987). A close relationship between leaf N content and SPAD values was found in apple (*Malus domestica* Borkh.), but it varied with the growing season (Nielsen et al., 1995). After adjustment based on leaf weight, SPAD values were correlated ($r^2 = 0.97$) with leaf N content in corn (*Zea mays* L.) (Chapman and Barreto, 1997). However, these studies indicated the need to calculate relationships for each species and individual meter (Yadava, 1986; Marquard and Tipton,

1987) and for particular plant growth conditions (Campbell et al., 1990). Hitherto, no research has been reported to evaluate the ability to determine chlorophyll and nitrogen contents using SPAD values for faba bean. The objectives of this study were (1) to evaluate the possibility of using the SPAD-502 Chlorophyll Meter to measure the relative leaf chlorophyll and N contents in faba bean leaves, (2) to investigate the change with time in leaf chlorophyll content and difference in leaf chlorophyll content between two faba bean cultivars and between the leaves at different nodal position in the canopy and (3) to elucidate the influence of organic and inorganic fertilizers on faba bean plant.

Materials and Methods

1. Pot experiment

A factorial experiment with faba bean was conducted in a randomized complete block design with four replications in a polyvinyl house, Gifu University. The factors were two cultivars (Japanese large seeded, Ryousai-issun, and an Egyptian small seeded, Cairo 241) and five fertilization treatments. The application of chemical fertilizer (recommended dose), (60 mg N + 160 mg P₂O₅ + 200 mg K₂O) *pot*⁻¹ (CF), rice straw (16 g *pot*⁻¹) combined with oilseed rape residues (60 mg N *pot*⁻¹) (OM1), OM1 + 50% CF (OM2), and OM1 + 100% CF (OM3), and non-amended soil as a control. Faba bean seeds were sown on November 23, 2000, in plastic pots (500 g air dried soil capacity). One 30-day-old seedling of faba bean was transplanted to each pot (1/5000a Wagner's pot). Pots with a surface area of 0.02 m² were filled with 2.1 kg of dried and sieved clay loam soil. Some

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characteristics of the soil, rice straw and oilseed rape cake determined before the start of the experiment are shown in Table 1. A range of N fertilizer was applied to establish the range of chlorophyll and N contents, and to investigate how N fertility affects the SPAD reading, total N per plant and dry weight of the whole plant. Organic materials were included in the fertilizer from the view of low input sustainable soil management. The organic and chemical fertilizers were applied just before transplanting and thoroughly mixed with the soil of each pot. The experiment was conducted until May 15, 2001, for one season. The pots were kept weed-free and maintained in an optimum soil moisture regime throughout the experimental period.

2. In situ measurement of chlorophyll content

The instrument used was a SPAD-502 model manufactured by Minolta Co., Ltd., Japan. Chlorophyll content of the fourth fully expanded leaves counted from the top of faba bean was measured at 2-week intervals from 3 weeks after transplanting (WAT) until the maturity. The SPAD value was read 15 times per leaf per plant. Thus, each SPAD value in this study represents a mean of at least 120 readings per treatment and 300 readings per cultivar.

3. Measurements of chlorophyll and nitrogen contents

After the SPAD measurements at the pod development stage (11 WAT), six leaf disks (total area = 17.0 cm²) were cut from each leaf to be examined and they were extracted with 85% aqueous solution of acetone to determine total chlorophyll content. The acetone solution was filtered through a sintered glass filter, and the filtrate made up to a known volume. The optical density of the filtrate was determined using Spectrophotometer (Hitachi180-60, Hitachi, Ltd., Japan) at wavelengths of 662 and 644 nm for chlorophyll *a* and *b*, respectively, and total chlorophyll (chlorophyll *a* + *b*) content was estimated using the extinction coefficient equations described by Wettstein (1957). Total chlorophyll content was expressed on a leaf area basis as mg m⁻². The plants used for chlorophyll measurement were carefully dug out, the roots were washed thoroughly to remove soil particles and plant debris, and separated to stems, leaves and reproductive organs. They were oven-dried at 60°C for 72 hrs then ground to pass a 0.5 mm sieve for estimating total N content by a CN analyzer, Sumigraph Model NC-800, Shimadzu Corporation, Japan.

4. Statistical analysis of data

Data shown in figures 4 and 5 were subjected to the analysis of variance appropriate to the design. Relationships between SPAD reading and extractable chlorophyll content were evaluated by polynomial regression, but the relationships between SPAD reading and total leaf nitrogen content and leaf position were evaluated using a

simple linear regression model.

Results and Discussion

Linear relationships were observed between the content of total extractable chlorophyll and SPAD readings, as in other plant species by, for example, Yadava (1986), and Campbell et al. (1990). Best fit was achieved using a second order polynomial equation as suggested by several authors (Markwell et al., 1995; Monje and Bugbee, 1992). Marquard and Tipton (1987) reported that the content of extractable chlorophyll expressed on a leaf area basis, showed a close correlation with SPAD reading than that expressed on a fresh weight basis due to the wide spread distribution of chlorophyll within the leaf. The regression models estimated for both faba bean cultivars for the relationship between SPAD reading and total chlorophyll content (expressed in mg m⁻²) were highly significant ($p < 0.01$) and the r^2 values were 0.99 and 1.00 for Ryouisai-issun and Cairo 241, respectively (Fig.1). A close linear relationship ($p < 0.001$) existed between SPAD reading and total leaf N content at the pod development stage of faba bean plants with $r^2 = 0.88$ and 0.99 for Ryouisai-issun and Cairo 241, respectively (Fig.2). This was expected from the SPAD-chlorophyll correlation in this study and SPAD-N relationships in other crops reported by Chapman and Barreto (1997). These results suggest that SPAD readings may be a rapid method of assessing the chlorophyll status of faba bean and that they are correlated with chlorophyll content and N status.

Fig. 3 shows the SPAD reading of the leaf at each nodal position at the pod development stage of faba bean plants. Linear relationships between SPAD reading and nodal position of leaves were observed ($p < 0.001$). This relationship differed with the cultivar and r^2 was 0.77 and 0.48 for Ryouisai-issun and Cairo 241, respectively. The SPAD values of the first leaf from the apex

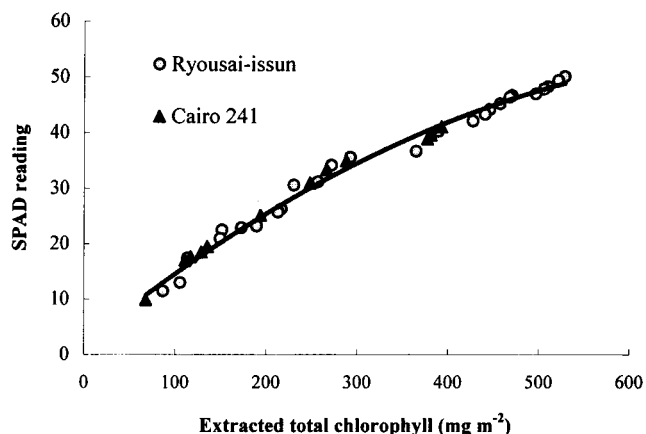


Fig. 1. Relationship between SPAD reading and extracted chlorophyll content in Ryouisai-issun and Cairo 241 faba bean cultivars. The solid line is the 2nd order polynomial function of all points, $y = 1.997 + 0.135x - 0.00009x^2$ ($r^2 = 0.99$; $n = 26$; $p < 0.01$) and $y = -0.284 + 0.169x - 0.0002x^2$ ($r^2 = 1.00$; $n = 12$; $p < 0.01$) for Ryouisai-issun and Cairo 241, respectively.

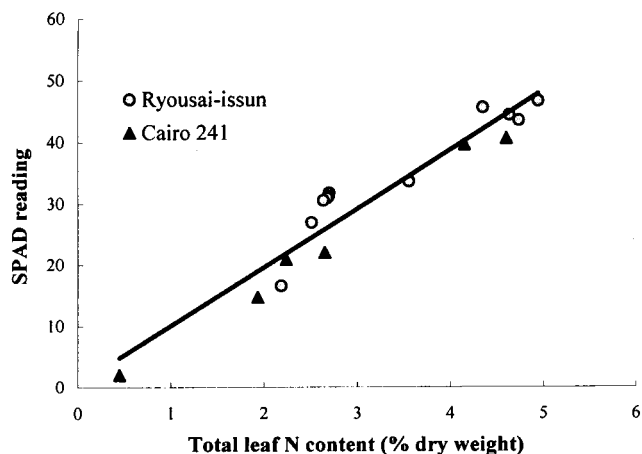


Fig. 2. Relationship between SPAD reading and total nitrogen content in Ryousai-issun and Cairo 241 faba bean cultivars at pod development stage. Fitted line is $y = 6.22 + 8.33x$ ($r^2 = 0.88$; $n = 11$; $p < 0.001$) and $y = -2.56 + 9.68x$ ($r^2 = 0.99$; $n = 6$; $p < 0.001$) in Ryousai-issun and Cairo 241, respectively.

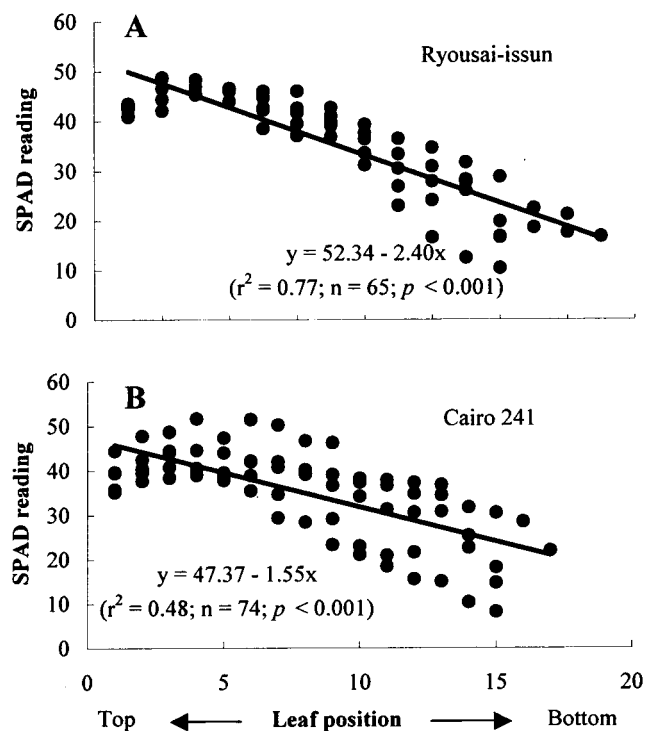


Fig. 3. Relationship between SPAD reading and nodal position of faba bean leaf (A) Ryousai-issun, and (B) Cairo 241 at pod development stage.

which did not fully expand were 2–6 units lower than that of the most recently expanded (second) leaf. The SPAD readings of the 2nd to 4th leaves were the highest among those of all the leaves. SPAD readings of the youngest fully expanded leaves (2nd–4th leaves) were higher in Ryousai-issun than in Cairo 241 but those of the basal leaves were higher in Cairo 241 than in Ryousai-issun (Fig. 3). This may be because Ryousai-issun develops more branches than Cairo 241. Ryousai-issun has many branches (2.5 ± 0.7) and therefore many

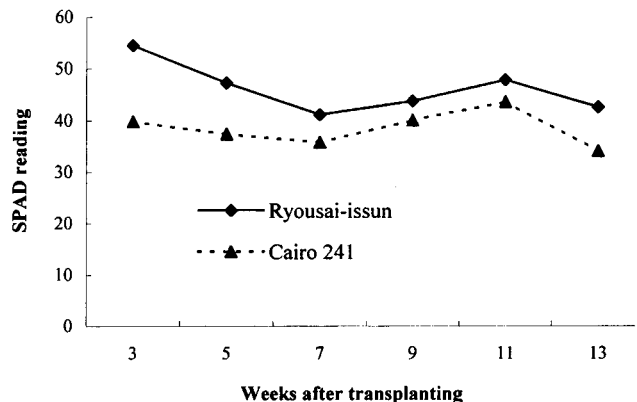


Fig. 4. Changes in SPAD reading with time of the fourth fully expanded leaves from the top of two faba bean cultivars.

leaves which may disturb more the light penetration into the basal leaves compared with Cairo 241 which has fewer branches (1.9 ± 0.5). However, the leaf number per stem was 13.0 ± 1.4 and 14.8 ± 1.8 in Ryousai-issun and Cairo 241, respectively.

The seasonal change in leaf chlorophyll content (measured by SPAD meter) from 3 weeks after transplanting to the ripening stage showed incomplete “M” type curve with peaks at full-bud and full-pod stages, in both cultivars. The content decreased sharply as pod ripening (Fig. 4). Faba bean had a genetic variability in SPAD reading when all other factors are held constant. Fig. 4 shows that Ryousai-issun had significantly higher SPAD readings than Cairo 241 from 3 to 13 WAT. Genetic effects on SPAD reading may be due to differences in leaf thickness or specific leaf weight as explained in rice plant by Peng et al. (1993).

The increase in SPAD reading, total N content per plant and dry matter weight of whole plant caused by applying organic materials (Fig. 5) can be attributed to the effect of mineral elements (Table 1) released from the decayed rice straw and oilseed rape residues. The nutrient elements in rice straw and oilseed rape residues, C, N, P, S, and K may promote the plant growth supporting the previous finding by Yoshimura and Tose (1926) and Abd-Alla and Omar (1998). The latter authors ascribed the increase in dry weight and N content of fenugreek plant mainly to improved N_2 fixation, which was reflected in the enhanced formation and growth of nodules. On the other hand, the favorable effect of straw and oilseed rape residues on growth and consequently on the yield of the plant might be due to the indirect effect of straw and oilseed rape residues. It has been reported that the incorporation of rice straw into soil caused a decrease in the amount of inorganic nitrogen in the soil available to the plant (Yoneyama and Yoshida, 1977; Aoyama and Nozawa, 1993; Nishio et al., 1993). Immobilization of N in soils due to incorporation of oilseed rape residues has also been reported (Jensen et al., 1997; Trinsoutrot et al., 2000). The immobilization of N may provide a suitable soil environment for faba bean nodula-

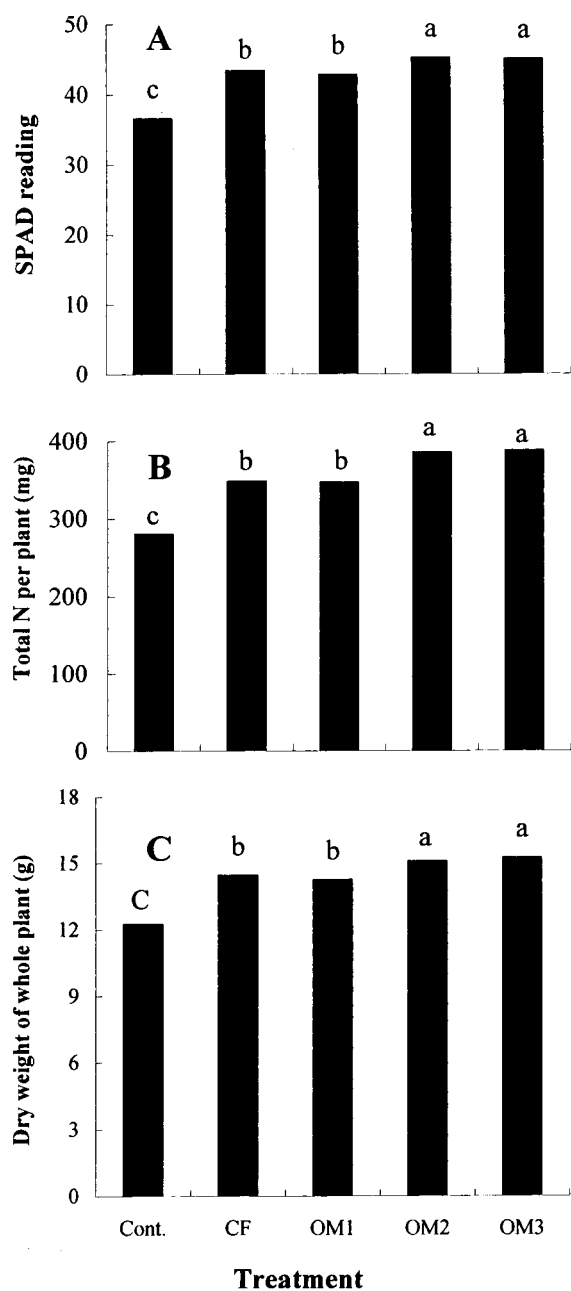


Fig. 5. Effect of fertilization on (A) SPAD reading of the fourth fully expanded leaves from the top, (B) total N per plant, and (C) dry weight of the whole plant over (average of two) faba bean cultivars at pod development stage. Bars with no common letter are significantly different as determined by Duncan's test ($p < 0.05$).

tion, which results in higher total N content in the plants, and consequently higher growth and dry matter yield in faba bean.

There was a linear relationship between SPAD reading and total N per plant, and the relationship was best described by the equation $y = 13.69 + 0.08x$ with $r^2 = 0.92$ ($p < 0.001$) (y and x , are total N per plant and SPAD reading, respectively). A linear relationship was also found between SPAD reading and dry weight of the whole plant, which was shown by the equation $y = -$

Table 1. Characteristics of clay loam soil, rice straw and oilseed rape cake before starting the experiment.

Characteristic	Clay loam soil	Rice straw	Oilseed rape cake
pH (H_2O)	4.9	7.2	5.9
EC ($dS m^{-1}$)	0.074	3.38	2.05
Total N ($g kg^{-1}$)	4.4	4.7	67.1
Total C ($g kg^{-1}$)	90.4	371.7	310.2
C/N	20.5	79.1	4.6
P_2O_5 ($g kg^{-1}$)	0.1	2.5	45.6
Na_2O ($mg kg^{-1}$)	34	495	396
MgO ($mg kg^{-1}$)	23	784	548
CaO ($mg kg^{-1}$)	175	364	1446
K_2O ($mg kg^{-1}$)	31	3154	3127
Organic matter ($g kg^{-1}$)	254.4	846.4	905.2
CEC ($cmol kg^{-1}$)	28.2	36.2	53.7
NH_4-N ($mg kg^{-1}$)	-	18	217

$1.32 + 0.37x$ with $r^2 = 0.96$ ($p < 0.001$). Furthermore, a linear relationship existed between total N per plant and dry weight of the whole plant, which is shown by $y = -57.2 + 28.52x$ with $r^2 = 0.84$ ($p < 0.001$).

It is well known that a high photosynthetic activity improves the yield. Ma et al. (1995) reported that photosynthetic rate in soybean leaf was consistently correlated with SPAD-502 meter readings in 16 genotypes. Hence, our results suggest that chlorophyll meter readings are quick, reliable and repeatable indicators of chlorophyll and nitrogen contents and leaf photosynthetic rate that can be used for screening faba bean genotypes.

In summary, SPAD readings were correlated well with chlorophyll and total leaf nitrogen contents in faba bean grown in a polyvinyl house. The SPAD reading was significantly higher in Ryousai-issun than in Cairo 241 throughout the growth season. Organic fertilizer application increased the SPAD reading and improved the plant growth of faba bean.

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