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Research Article

The Correlation of Leaf SPAD Value at Different Part of Blade with Nitrogen Nutrient Status in Sugarbeet

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Abstract: In order to undersd and the distribution features of SPAD value at different part of blade and different leaf order of sugarbeet, both pot and field experiment with different sugarbet genotypes were conducted by using SPAD-502 chlorophyll meter. And the correlations between leaf SPAD value of different development stages and leaf chlorophyll content or the plant nitrogen content were analyzed in different genotypes of sugarbeet. The results were as follow: The leaves's SPAD values of sugarbeet were different; the blade tip of the highest leaf in sugarbeet plant should be taken as the optimum measured position of SPAD value. With the addition of nitrogen applying rate, the SPAD values increased. There were significant relationships between SPAD values and chlorophyll content, plant nitrogen. So the SPAD values were suitable for rapid diagnosis of nitrogen nutrition and used to monitor the development and photosynthetic efficiency in sugarbeet production.

Keywords: Nitrogen, nutrient diagnosis, sugarbeet, SPAD value

INTRODUCTION

Hand-held SPAD-502 chlorophyll meter (Soil Plant Analysis Development) (Minolta Co. Ltd., 1989) relies on LED of dual wavelength (650 nm red light and 940 nm near - IR light) as its light source. The SPAD value (Li *et al*., 2006) acquired by SPAD-502 chlorophyll meter, is obtained by difference of the ratio of two light densities between samples for testing and without sample. And then based on the SPAD value, nitrogen diagnosis and nitrogen fertilizer recommendation were estimated. Traditional technique of nitrogen diagnosis in the lab is dominated by chemical analysis with long testing period, high price and complex operation, while sample collection, testing and data analysis. So, it's difficult to detect or watch in real-time the nitrogen nutrition status in different growth stages of crop. As the rapid way to test the leaf's chlorophyll concentration of plant, the SPAD-502 chlorophyll meter has many advantages (Markwell *et al*., 1995; Chen and Lu, 1996; Jia *et al*., 2001; Tiancheng *et al*., 2000), such as easy to carry and operate, undamaged and real-time to detect and estimate crop chlorophyll status and nitrogen fertilizer recommendation, etc. In recent years, the SPAD-502 chlorophyll meter are growing concerned and adopted by many researchers and they have already studied in Triticum aestivuml (Zhu *et al*., 2005; Arregui *et al*., 2006; Hao *et al*., 2010; Wu *et al*., 2012), Oryza sativa (Turner and Jund, 1991; Yang *et al*., 2006; Li *et al*., 2007; Xie *et al*., 2010), Zea mays (Wood *et al*., 1992; Varvel *et al*., 1997; Yu *et al*., 2010; Zhao *et al*., 2011), Solanum tuberosum (Nie and Fan, 2009; Xiao and Guo, 2007), Gossypium spp (Luo *et al*., 2009; Sun *et al*., 2012), Brassica napus (Cao *et al*., 2012), Camellia oleifera (Zhang *et al*., 2011), Ramie (Li *et al*., 2011), Pepper (Yu *et al*., 2012), etc. SPAD value is different from leaf position and leaf arrangement (Li *et al*., 2007; Xiao and Guo, 2007; Luo *et al*., 2009; Yu *et al*., 2012). Otherwise, SPAD value , the chlorophyll concentration of leaves and plant nitrogen content are all significant correlation (Tiancheng *et al*., 2000; Zhu *et al*., 2005; Hao *et al*., 2010; Li *et al*., 2007; Yu *et al*., 2010; Zhao *et al*., 2011; Nie and Fan, 2009; Li *et al*., 2011).

In field conditions, exact and scientific diagnosis of nitrogen nutrition has always been the foundation of rational fertilization and cultural regulation. Sugarbeet (*Beta Vulgaris* L.), as one of the main sugar crop in China and the world (Chen and Chen, 2010), is the major cash crop planted in northeast, northwest and north China (Wang *et al*., 2011) and its development, morphology, yield and quality has a great influence on nitrogen (Ni *et al*., 2008). Based on the relationship between SPAD value and chlorophyll concentration, the development and photosynthetic efficiency of sugarbeet could be detected or watched in real-time, in order to build the way of nitrogen nutrition diagnosis on SPAD

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Table 1: The list of different genotypes cultivated varieties of sugarbeet

No.	Name of cultivated varieties	Source	Ploidy	Graininess	No.	Name of cultivated varieties	Source	Ploidy	Graininess
1	KWS0113	KWS SAAT AG	Diploids	Monogerm	11	SR-411	SESvanderhave	Diploids	Monogerm
2	KWS1237	KWS SAAT AG	Diploids	Monogerm	12	PRESTIBEL	SESvanderhave	Triploids	Multigerm
3	KWS3148	KWS SAAT AG	Diploids	Monogerm	13	RIMA	SESvanderhave	Diploids	Monogerm
4	KWS3418	KWS SAAT AG	Diploids	Multigerm	14	Beta176	Beta USA	Diploids	Multigerm
5	KWS0120	KWS SAAT AG	Diploids	Multigerm	15	Beta240	Beta USA	Diploids	Multigerm
6	KWS8138	KWS SAAT AG	Diploids	Monogerm	16	Beta807	Beta USA	Diploids	Monogerm
7	KWS9149	KWS SAAT AG	Diploids	Monogerm	17	Beta812	Beta USA	Diploids	Monogerm
8	ACERO	SESvanderhave	Triploids	Monogerm	18	Beta866	Beta USA	Diploids	Monogerm
9	ADV0413	SESvanderhave	Triploids	Multigerm	19	HI0474	Syngenta AG	Diploids	Monogerm
10	CH0612	SESvanderhave	Diploids	Multigerm	20	HI1057	Syngenta AG	Diploids	Monogerm

value, to create the chance of nitrogen rational operation in the yield of sugarbeet and finally to provide the theory basis for highly effective cultivation and increasing yield.

MATERIALS AND METHODS

Material: 20 cultivated varieties of sugarbeet (with higher yield and sugars, better stability) were chosen according to their larger planting area in recent years and synthesis evaluation after testing on foreign cultivated varieties (Bai *et al*., 2011a; Li *et al*., 2012; Bai *et al*., 2011b), including 7 varieties from KWS SAAT AG (KWS), 6 from SESvanderhave, 5 from Beta USA and 2 from Syngenta AG. The details were in Table 1 and among these cultivated varieties, there were 17 diploids and 3 triploids; 14 monogerm and 6 multigerm.

Experimental design and method:

Pot experiment: Pot experiment was carried out at plant culture room of the main campus of Heilongjiang University in October 2013. Set two treatments of experiment: N_{150} (150mg/kg, for nitrogen supply, based on customary fertilizer rate in the field) and N_0 (0 mg/kg, without nitrogen supply, for control treatment). Three replications of each cultivated variety were used.

The test seeds with gauze wrap, soaked for 6 hours in flowing and clear water. After washing away the coat, put them into the 70% alcohol solution for 1 minute。 Then, washed and cleaned them using distilled water for 4 times; put them into 2‰ thiram solution for 12 h (passed the night). At last, washed them using distilled water for several times until cleaning.

Germinated the pretreated seeds on the flower pots (30 seeds per pot), by routine management of soil water. After the seedlings came out, final singling (10 seedlings per pot) was carried out.

When sowing about 50 days(4-6 euphylla of N_{150}, 2-4 euphylla of N_0), selected four close sugarbeet seedlings per pot, tested the SPAD value of the different positions on the leaf(tip, margin and lower part of the leaf) from the inside out using the SPAD-502 chlorophyll meter. After tested, the sugarbeet plant could be harvested. Divided the whole sugarbeet plant into three parts: rhizome, stem and leaf and weighed. Then, 70°C to dry, weighed, grinded and tested the total nitrogen of the whole plant including rhizome, stem and leaf. The total plant samples were used the heating digestion method adding concentrated sulphuric acid and catalyst and the routine Kjeldahl method (Bao, 2000) to measure the total nitrogen content.

Field experiment: Field experiment was carried at the test field of Hulan campus of Heilongjiang University in May 2014. Selected HI0474 (No.19) from Syngenta AG as tested cultivars and set one treatment of N_0 (0 mg/kg, Without nitrogen supply), five replications. Tested the SPAD value and the chlorophyll content of fresh leaves (Li, 2006) in different developmental stages (seedling, cluster rapid growth period and root sugar accumulation period). Divided the whole sugarbeet plant into three parts: rhizome, stem and leaf and weighed. Then, 70°C to dry, weighed, grinded and tested the total nitrogen of the whole plant including rhizome, stem and leaf. The total plant samples were used the heating digestion method adding concentrated sulphuric acid and catalyst and the routine Kjeldahl method (Bao, 2000) to measure the total nitrogen content.

Data processing: Test results were made the data statistics analysis and correlation analysis with EXCEL2007 and SPSS19.0.

RESULTS AND ANALYSIS

The leaf of Beta vulgar is oval and glossy (Fig. 1). Chose the tip, margin and lower part of the leaf as the testing positions by SPAD-502 chlorophyll meter and determinate of the same position for consecutive 15 times, then deleted the extreme, finally checked the average. Four close sugarbeet seedlings per pot were selected and tested the SPAD value of the different positions on the leaf (tip, margin and lower part of the leaf) from the inside out.

Fig. 1: The leaf of *Beta vulgaris* (4 euphylla stage)

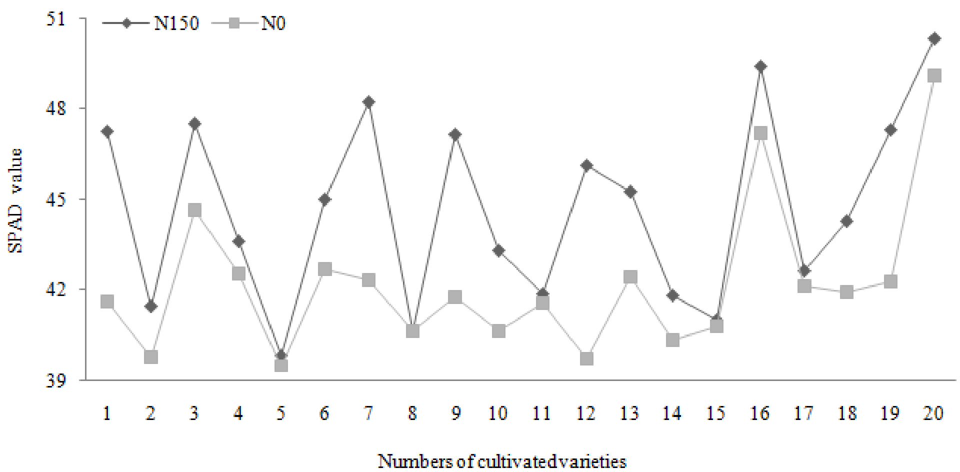

Fig. 2: The varies of SPAD value in the different cultivars under different nitrogen supply

Table 2.The repeated times of the biggest SPAD value in the different leaf position of the different genotype of sugarbeet

No.		1	2	3	4	5	6	7	8	9	10
N_0	Tip	15	17	17	18	15	15	18	16	20	22
	Margin	7	6	6	5	5	7	5	3	3	1
	Lower part	2	1	1	1	4	2	1	5	1	1
N_{150}	Tip	27	36	39	39	36	32	40	30	32	26
	Margin	10	8	4	8	2	8	6	12	14	11
	Lower part	11	4	5	1	10	8	2	6	2	11
No.		11	12	13	14	15	16	17	18	19	20
N_0	Tip	18	17	17	18	18	18	18	18	18	22
	Margin	3	4	4	4	4	4	3	2	5	1
	Lower part	3	3	3	2	2	2	3	4	1	1
N_{150}	Tip	42	41	41	38	27	27	24	30	38	36
	Margin	3	4	4	7	12	12	13	15	8	9	9
	Lower part	3	3	3	3	9	8	9	10	1	3

In N_0 treatment of 2 euphylla period, 24 sets of data(4 plants per pot, 2 euphylla per plant, 3 replications per euphylla) per cultivated variety were measured; In N_{150} treatment of 4 euphylla period, 48 sets of data(4 plants per pot, 4 euphylla per plant, 3 replications per euphylla) per cultivated variety were measured

Variance analysis of SPAD value of the different position in the same leaf: SPAD value of the plant leaf has reflected the chlorophyll content of leaf and also indirectly reflected the nitrogen content of plant. The tip, margin and lower part of the leaf could be the testing position using SPAD-502 chlorophyll meter. The tested SPAD value of these three positions would decide the SPAD value of the leaf for such genotype of sugarbeet. According to the testing results, counted the repeated times of the biggest SPAD value in these three positions (tip, margin and lower part of the leaf) between different nitrogen treatments. In Table 2, the repeated times of the biggest SPAD value of leaf tip were obviously more than the values of margin and lower part of the leaf. In the N_0 treatment, the repeated times of the biggest SPAD value were 15-22 times in 24 times; in the N_0 treatment, the times were 26-42 times in 48 times. From these data, both treatments were all mixed up to 50%. So the SPAD value of leaf tip could be approximated the SPAD value of this leaf.

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Comparative analysis of SPAD value in different leaf order: In the condition of different nitrogen supply, the SPAD values of different leaf orders in sugarbeet varied. Organizing the data of SPAD values of leaf tip (2 euphylla per plant from the inside out) under the treatments of N_0 and N_{150}, compared their averages (Fig. 2). It was easy to see that the SPAD values of N_{150} were higher than those of N_0; even they varied in different cultivars under each treatment. The SPAD value of HI1057 from Syngenta AG (No. 20) was higher than that of other cultivars, 50.4 under N_{150}, 49.1 under N_0; KWS0120 from KWS SAAT AG (No. 5) was lower, 39.8 under $N_{150,}$ 39.5 under N_0.

The correlation was analyzed based on the SPAD value of the highest leaf of plant. Under the condition of nitrogen supply (N_{150}), the SPAD value of the highest leaf was significant correlation with that of other different leaf order in the same plant (Table 3). There were 19 among 20 cultivated varieties significant correlated at 0.05 level. The cultivated variety(No.20)of sugarbeet was different(the deviation between the SPAD value of the highest leaf and that of the total leaves was 3.45±0.24c). That is, after 4-6 euphylla period of sugarbeet, the SPAD value of the highest leaf could indicate the trends of the SPAD value among cultivated varieties. Thus, while using SPAD-502 chlorophyll meter in the field, we could choose the highest leaf of plant after 4-6 euphylla period as the tested leaf.

Correlation analysis between the SPAD value and chlorophyll content of leaf: Chlorophyll content of plant leaf positive correlated with the photosynthesis, might affect the synthesis of organic matter. Usually the more the chlorophyll content, the better the plant nutrition status. As the important element of chlorophyll, nitrogen deficiency of plant would depend on the correlation of the chlorophyll content and plant nitrogen content.

Table 3: Comparison with the deviation between the SPAD value of the highest leaf and that of the total leaves in sugarbeet

No.	N_{150}	No.	N_{150}
1	0.82±0.67ab	11	0.81±0.37ab
2	0.90±0.05ab	12	0.64±0.49ab
3	1.08±0.24ab	13	0.52±0.400a
4	0.91±0.49ab	14	0.53±0.400a
5	0.90±0.05ab	15	0.81±0.57ab
6	1.03±0.82ab	16	1.10±0.39ab
7	0.94±0.3ab	17	0.64±0.24ab
8	0.36±0.28a	18	1.42±0.260b
9	0.30±0.13a	19	0.94±0.36ab
10	1.09±0.69ab	20	3.45±0.240c

The data was average value±standard error. The different letters meaned the significance level of difference ($p<0.05$)

Selected HI0474 (No.19) from Syngenta AG as tested cultivars and set one treatment of N_0(0 mg/kg, Without nitrogen supply), five replications, three parallel samples for each replications. Built the linear equation (SPAD values of different development stages for x, total chlorophyll content (mg/g) for y) (Table 4). The results showed that the relationship between SPAD value of leaf and total chlorophyll content of plant in different developmental stages (seedling, cluster rapid growth period and root sugar accumulation period) was very significant correlation. The linear equation of seedling was $y = 2.196x-75.19$ ($r = 0.933^{**}$); in cluster rapid growth period, the linear equation was $y = 0.234x+2.546$($r = 0.943^{**}$); and in root sugar accumulation period, the linear equation was $y = 0.644x-23.99$ ($r = 0.933^{**}$). Therefore, trends of the SPAD value of leaf and total chlorophyll content of plant shared the same; we could estimate chlorophyll content of plant by the SPAD value of leaf.

Correlation analysis between the SPAD value and nitrogen content of plant:

Pot experiment: Table 5 showed the SPAD value of leaf and nitrogen content of plant in different nitrogen supply of sugarbeet. The SPAD value of leaf and nitrogen content of plant in N_{150} were more than that in N_0. The level of nitrogen supply and nitrogen content of plant could be indirectly compared by the SPAD value. Built the linear equation (SPAD values of different development stages for x, total nitrogen content (g/kg) for y) (Table 5). The results showed that the relationship between SPAD value of leaf and total eitrogen content of plant in different developmental stages (seedling, cluster rapid growth period and root sugar accumulation period) was significant correlation. The linear equation of N_{150} was $y = 1.257x+16.37$($r = 0.837^{*}$); that of $N_0$was $y = 1.272x +16.88$ ($r = 0.804^{*}$).

Table 4: The linear equation between SPAD value of leaf and total chlorophyll content of plant in different developmental stages of sugarbeet

Seedling	Cluster rapid growth period	Root sugar accumulation period
$y = 2.196-75.19$	$y = 0.234x+2.546$	$y = 0.644x-23.99$
$R^2 = 0.871$	$R^2 = 0.890$	$R^2 = 0.873$
$r = 0.933^{**}$	$r = 0.943^{**}$	$r = 0.934^{**}$

n = 15, * Significant, ** Very significant, the below was same

Table 5: The correlation of the SPAD value of leaf and total nitrogen content of plant in pot experiment of sugarbeet

	N_{150}		N_0	
No.	Nitrogen content of plant(g/kg)	SPAD value	Nitrogen content of plant (g/kg)	SPAD value
1	23.69	46.9	19.94	40.4
2	21.02	40.6	17.55	38.7
3	23.91	47.6	21.20	43.5
4	21.21	42.8	20.09	41.2
5	19.82	38.9	17.59	37.7
6	23.04	45.1	21.13	42.1
7	23.25	47.1	20.07	40.9
8	20.28	39.7	17.68	38.5
9	23.38	46.3	19.93	41.2
10	21.01	42.1	18.41	39.0
11	20.60	41.3	19.77	40.0
12	23.26	45.7	17.60	38.0
13	23.41	44.2	20.32	41.5
14	21.70	41.5	20.26	39.1
15	22.40	39.5	19.83	39.6
16	25.59	49.1	23.61	46.9
17	21.14	42.0	20.45	41.4
18	19.06	42.7	20.41	40.8
19	26.26	46.0	20.39	41.0
20	26.46	49.9	21.43	48.1
The linear equation	y = 1.257x+16.37 r = 0.837*		y = 1.272x+16.88 r = 0.804*	

Table 6: The correlation of the SPAD value of leaf and total nitrogen content of plant in field experiment of sugarbeet

Seedling	Cluster rapid growth period	Root sugar accumulation period
y = 0.322x-9.090 r = 0.782*	y = 0.128x–1.932 r = 871*	y = 0.659x-26.53 r = 878*

Field experiment: In Field experiment, trends of the SPAD value of leaf and total nitrogen content of plant in different developmental stages(seedling, cluster rapid growth period and root sugar accumulation period) was generally in good agreement with significant correlation (Table 6). The linear equation of seedling was y = 0.322x-9.090 (r = 0.782*); in cluster rapid growth period, the linear equation was y = 0.128x-1.932 (r = 0.871*); and in root sugar accumulation period, the linear equation was y = 0.659x - 26.53(r = 0.878*).

Whatever pot experiment or field experiment, the SPAD value of leaf and total nitrogen content of plant would be significant correlation. So, we could estimate total nitrogen content of plant by the SPAD value of leaf.

DISCUSSION

In this research, the SPAD value of leaf was measured using SPAD -502 chlorophyll meter in different genotypes of sugarbeet. Magnitude, stability and representative of the value would vary with the different testing position. The biggest SPAD value of leaf tip could occur more often than that of any other positions, so the leaf tip could be the suitable position in a leaf. This observation coincided with the results of Chen *et al*. (2014), the SPAD value of leaf tip was the bigger than that of other leaf positions in 5 forage grass leaves. The results of Ke *et al*. (2010) also confirmed it. But this result was not exactly the same in any other crops. The bigger SPAD value of winter wheat (*Triticum aestivum*) (Hao *et al*., 2010) with better stability occurred in the middle of leaf and the Coefficient Variation (CV) of the SPAD value was smaller inside the zone of 40~60% from the base of leaf. The suitable position of testing in *Camellia oleifera* (Zhang *et al*., 2011) was the zone of about 50% from the base of leaf. The SPAD value in maize was bigger and less deviation in the zone of 55% from the base of leaf (Wood *et al*., 1992; Zhao *et al*., 2011). One possible reason might be the more shining and better stability in the leaf tip of sugarbeet than margin and lower part of the leaf, resulting in the higher of leaf and the bigger SPAD value. But while measuring by SPAD-502 chlorophyll meter, we might try to avoid the venation(less photic) through the testing window in order to obtain the proper SPAD value (Yu *et al*., 2012).

In addition, the SPAD value of leaf was influenced by many factors, such as different cultivated varieties, developmental stages, growing environment, etc. (Markwell *et al*., 1995; Jia *et al*., 2001) So while testing, compared with the experience value in order to increase the accuracy and precision of the SPAD value. If you want to get the exact chlorophyll content, the common method (such as Spectrophotometry) in the lab should be used.

Besides all, correlation analysis among the SPAD value, chlorophyll content of leaf and nitrogen content of plant was carried out and fit the linear equation. It turned out that the SPAD value was positive interrelationships with both chlorophyll content of leaf and nitrogen content of plant. So it is possible to estimate indirectly chlorophyll content of leaf and nitrogen content of plant by the SPAD value. SPAD-

502 chlorophyll meter is easy to carry and operate, undamaged and real-time to detect and estimate crop chlorophyll status and nitrogen fertilizer recommendation. The conventional method in the lab needs long testing period, high price and complex operation, while sample collection, testing and data analysis. SPAD-502 chlorophyll meters should be widely promoted and used in the future.

CONCLUSION

The distribution regularity of SPAD value on different leaf position and leaf order was analyzed by pot experiment or field experiment in different geno types of sugarbeet. And the correlations were counted between leaf's SPAD value of different development stages and its chlorophyll content or the plant nitrogen content. The highest leaf tip of sugarbeet should be taken as the measured position of SPAD value. The changing trend of SPAD values was accord with chlorophyll content or the plant nitrogen content in different development stages. So the development and photosynthetic efficiency of sugarbeet could be constantly monitored by SPAD value and SPAD values of sugarbeet were suitable for rapid diagnosis of nitrogen nutrition.

Based on the relationship between SPAD value and chlorophyll concentration, the development and photosynthetic efficiency of sugarbeet could be detected or watched in real-time, in order to build the way of nitrogen nutrition diagnosis on SPAD value, to create the chance of nitrogen rational operation in the yield of sugarbeet and finally to provide the theory basis for highly effective cultivation and increasing yield.

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