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Full Length Research Paper

Nitrogen determination in pepper (*Capsicum frutescens* L.) plants by color image analysis (RGB)

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In order to investigate the suitable index of the diagnosis of pepper plants nitrogen status by color image analysis, a field experiment was conducted. In this study, six levels of nitrogen fertilization were established so as to induce nitrogen status in pepper plants. At the flowering and fruiting stages, total nitrogen was evaluated by laboratory analysis. The chlorophyll index was determined using a soil plant analysis development (SPAD)-502 chlorophyll meter. Also, color images were taken with a digital camera; the color images were processed in order to determine the averages of the red (R), green (G) and blue (B) colors. The result show that there were significant negative relations between *G/(R+G+B)* ratio of coverage image and the indexes of pepper nitrogen status, for example inorganic nitrogen in soil, total nitrogen of plant, nitrate concentration of leafstalk and SPAD readings at flowering and fruiting stages. Color image analysis provides an accurate and quick way for nitrogen estimation in pepper plants.

Key words: Pepper, color image analysis (RGB), nitrogen status.

INTRODUCTION

There have been some assessing N status methods to predict nitrogen fertilizer need, such as soil inorganic nitrogen estimation, chlorophyll meter measurement, petiole sap nitrate measurement with reflex meter and estimation of N status by color image analysis (Blackmer et al., 1989; Fox et al., 1994; Minotti et al., 1994; Mercado-Luna et al., 2010; Westcott et al., 1993; Zhang et al., 1996). In these methods, the determination of plant's nutritional status by color image analysis is a quick and non-destructive method. In recent years, research focused on image analysis techniques for measuring growth and fertilization status is becoming increasing common. The color of vegetation coverage has a close correlation with its N status.

The inadequate N plant gives more reflex than fertilization N plant in the whole wave band of visible light (Al-Abbas et al., 1974; Blackmer and Schepers, 1994, Blackmer et al.,1996; Scharpf and Lory, 2002; Thomas and Oerther, 1972). That means that crop N status can be accessed from vegetation coverage reflex. Lukina et al. (1999) acquired vegetation coverage images of wheat from digital camera, computed vegetation coverage degree using digital image processing and estimated vegetation coverage biologic quantity of wheat. Adamsen et al. (2000) researched a method for using images from a color digital camera to estimate flower number fleetly.

Moreover, there were different color ratios or indexes in different plants and different environments. For reference, Adamsen et al. (1999) acquired vegetation coverage images of winter wheat from digital camera, computed the G/R ratio for green (G) and red (R) using digital images analysis and considered that there was significant relations between *G/B* ratio and SPAD readings. Jia and Cheng, (2004) obtained the relation model of vegetation coverage green degree and total N of overground wheat

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Abbreviations: SPAD, Soil plant analysis development; **R,** red color; **G,** green color; **B,** blue color.

Table 1. Total N application and ratios of base to topdressing N of each experimental treatment.

Treatment	Total N application (kg/hm^2)	Base fertilizer (kg/hm^2)	Topdressing N fertilizer	
			Topdressing of flowering stage (kg/hm^2)	Topdressing of fruiting stage (kg/hm^2)
Control	0	0	0	0
1	75	30	20	25
2	150	60	40	50
3	225	90	60	75
4	300	120	80	100
5	375	150	100	125

Base fertilizer was applied before field planting; P (140 kg/hm^2) in the form of P_2O_5 supplied as calcium superphosphate and K (300 kg/hm^2) in the form of K_2O supplied as sulphates.

winter and established recommendatory system of N fertilization of winter wheat at unsheathed stage availably on the basis of relative value of green color. Daughty et al. (2000) estimated corn leaf chlorophyll concentration by the *R/G* ratio.

Mercado-Luna et al. (2010) suggested that RC and BC values could be applied to estimate the N status on tomato seedlings.

However, we actually suggested that there were significant negative relations between *G/(R+G+B)* ratio of coverage image and the indexes of pepper nitrogen status in this experiment.

Hence, the suitable index of assessing pepper N status by color image analysis was discussed.

MATERIALS AND METHODS

Experimental design

Experiment was made at Vegetable Teaching Base of Jilin Agricultural University, China. The experimental soil was meadow blackland with the following characteristics: 3.68% organic matter, 117.6 mg/kg alkaline N and pH 6.56.

The experimental pepper variety was Jijiao No. 8 (*Capsicum frutescens* L.) seeded in the solar greenhouse on January 27th, 2010. The experimental design was a randomized complete block with six treatments (Table 1). Each treatment had four replicates in four individual plots of 3 × 4 m^2 wide. Each plot contained 96 plants.

Determination and analysis

Soil sample (0~40 cm under the soil surface) was analyzed for its basic characteristics before fertilization. When the pepper growth reached the flowering and fruiting stages, the indexes of pepper nitrogen status, for example inorganic nitrogen of mixed soil (0~40 cm under the soil surface), total nitrogen concentration of plant, nitrate concentration of leafstalk and SPAD readings of leaves were determined. The idiographic methods were as follows; NH_4^+-N and NO_3^--N levels were analyzed from fresh soil sample filtered with a 3-mm sieve and extracted in 2 N KCL solution. The NH_4^+-N and NO_3^--N concentration were measured by distillation and colorimetry respectively.

In addition, total nitrogen concentration of plant was determined using Kjeldahl method.

Nitrate concentration in the leafstalk (NO_3^--N) was measured by reflex meter from 15 leaves samples in each treatment. The leaf samples were taken only from plants with fully expanded fresh leaves and cut off from the leafstalk (1 cm) in the laboratory

Then, the juice was squeezed from leafstalks using juice extractor and diluted to measure the nitrate concentration by reflex meter.

Chlorophyll concentration was measured by Minolta SPAD-502 portable chlorophyll meter (Japan) from 30 fully expanded fresh leaves of each treatment.

The results were expressed as mean value of SPAD readings of 30 leaves samples.

Digital image acquiring and processing

Digital images of pepper coverage were acquired by Canon G3 digital camera from a height of 1.2 m at an angel of 60°C between 12:00 am and 13:00 pm at the key stage of flowering and fruiting stages.

The resulting images had a size of 1024 × 768 pixels at Auto shoot mode of digital camera, and the colorific equipoise of images could been controlled at automatic exposal mode during the time of exposure; so the color images acquired were true red, green and blue bands.

The digital images were stored on JPEG format into computer. Adobe Photoshop 7.0 program was used for processing and analysis of images color.

The mean value of the red, green and blue input bands of vegetation coverage images in Adobe Photoshop 7.0 program was calculated as the mean value of color intensity of vegetable coverage (absolute value of red, green and blue).

Statistical analysis

Statistics of data analysis were taken by the data processing system (DPS).

RESULTS AND DISCUSSION

Relationships between nitrogen concentration in soil and the indexes of color image analysis

The nitrogen applied to the pepper had a highly significant effect on the indexes of standardization of RGB

Table 2. The relationships between standardization RGB indexes of pepper canopy surface and inorganic nitrogen concentration of flowering stage in soil.

RGB index	*G*	*G/R*	*G/B*	*G/L*	*G/(R+G+B)*	*B/(R+G+B)*
Coefficient of regression (R2)	0.210*	0.624 **	0.657**	0.642**	0.763**	0.655**

*Significant correlation at $P<0.05$; **significant correlation at $P<0.01$.

($p<0.05$ or $p<0.01$).
The coefficient of regress equation of *G/(R+G+B)* ratio was the biggest (Table 2). Hence, *G/(R+G+B)* ratio was a favorable index to express pepper N status in this experiment.

Relationship between the *G/(R+G+B)* ratio of pepper canopy surface and the different variables evaluated

There was significant negative relationship between *G/(R+G+B)* ratio of pepper canopy surface and nitrogen concentration in soil (Figure 1a).

When the inorganic nitrogen concentration in soil was increased, the *G/(R+G+B)* ratio was decreased. The correlative coefficient between the inorganic nitrogen concentration in soil and the *G/(R+G+B)* ratio of pepper coverage image was 0.7628. Also, there was significant negative relation between *G/(R+G+B)* ratio of pepper coverage image and total nitrogen concentration of plant (Figure 1b).

The correlative coefficient between *G/(R+G+B)* ratio of pepper coverage image and total nitrogen concentration of plant were 0.5794.

There was also significant negative relation between *G/(R+G+B)* ratio of pepper coverage image and nitrate concentration of leafstalk (Figure 1c).

Furthermore, when the nitrate concentration of leafstalk was increased, the *G/(R+G+B)* ratio decreased. The correlative coefficient between the nitrate concentration of leafstalk and the *G/(R+G+B)* ratio of pepper coverage image were 0.6221.

There was significant negative relation between *G/(R+G+B)* ratio of pepper coverage image and SPAD reading (Figure 1d).

It was also observed that when the SPAD reading increased, the *G/(R+G+B)* ratio decreased. The correlative coefficient between the SPAD reading and the *G/(R+G+B)* ratio of pepper coverage image was 0.6022.

Effect of nitrogen applied in soil on dry weight content in pepper plant

The results observed in plant dry weight content, shoot dry weight content and fruit dry weight content in response to different amounts of nitrogen applied in soil is shown in Table 3. The dry weight of plant, shoot and fruit in pepper increased when the quantity of nitrogen fertilizer increased and the dry weight response of nitrogen fertilizer was strong on low N status. The dry weight changed slightly with the enhanced quantity of nitrogen fertilizer (>225 kg/hm^2). The dry weight of plant and shoot in pepper always increased when the quantity of nitrogen fertilizer was increased. However, the dry weight of fruit in pepper was not always increased when the quantity of nitrogen fertilizer was increased. Although the excess nitrogen fertilizer is beneficial to the growth of plant and shoot, it was not with the fruit.

Nitrogen recommendation on the basis of the *G/(R+G+B)* ratio of pepper canopy surface

The suitable *G/(R+G+B)* ratio at different growth stages of different N status in pepper can be derived according to the correlation relation of *G/(R+G+B)* and nitrate concentration of leafstalk and then the quantity of nitrogen fertilizer can be recommended. Table 4 shows the nitrogen recommendation and *G/(R+G+B)* at different growth stages in pepper. Therefore, we can assess pepper nitrogen status by using a digital camera to acquire color images from vegetation coverage in pepper.

Conclusion

Under this experimental conditions, there were significant negative relations between *G/(R+G+B)* ratio of coverage image and the indexes of pepper nitrogen status, such as inorganic nitrogen in soil, total nitrogen of plant, nitrate concentration of leafstalk and SPAD reading at flowering and fruiting stages. The correlative coefficients between *G/(R+G+B)* ratio of pepper coverage image and the indexes of pepper nitrogen status were from 0. 5794 to 0.6221, respectively. This means *G/(R+G+B)* ratio of coverage image can be used to express pepper N status. It will be a favorable method to assess pepper nitrogen status with color image analysis as similar results were also obtained by Thomas and Oerther, (1972).

Furthermore, it had been reported that some other digital indexes of vegetation coverage image could assess crop growth status (Adamsen et al., 1999; Daughtry et al., 2000; Jia and Cheng, 2004; Mercado-

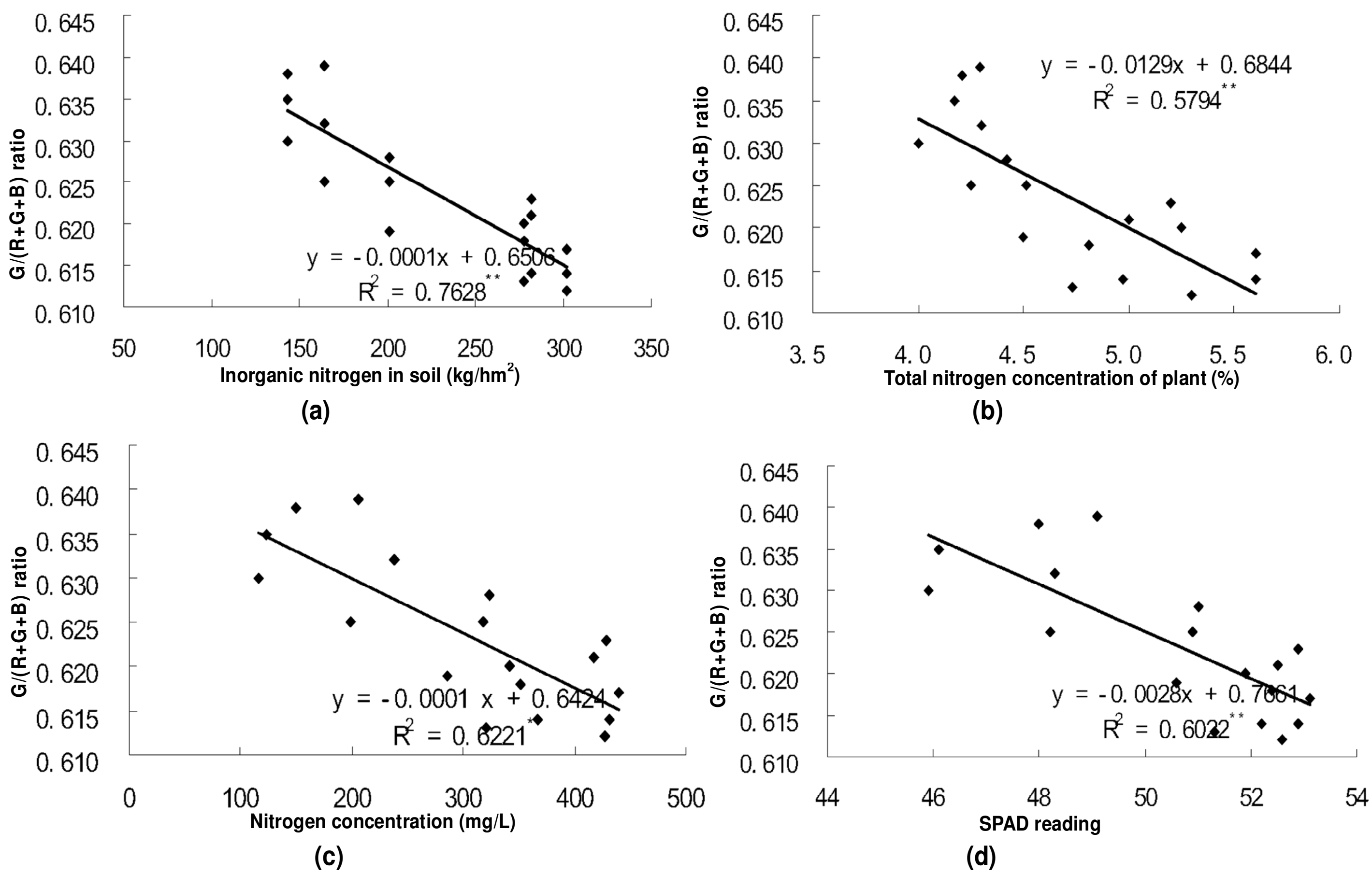

Figure 1. The correlation between *G/(R+G+B)* ratio of pepper canopy surface by color image analysis and inorganic nitrogen concentration in soil, total nitrogen concentration of plant, nitrate concentration of leafstalk and SPAD reading.**Significant correlation (P<0.01).

Table 3. Effect of nitrogen levels on dry weight of plant, shoot and fruit in pepper.

Treatment	Plant dry weight (kg/hm^2)	Shoot dry weight (kg/hm^2)	Fruit dry weight (kg/hm^2)
Control	2821.35^a	2150.68^a	1845.67^a
1	4232.52^b	2942.77^{ab}	2464.31^b
2	4972.61^{bc}	3418.56^{bc}	2729.45^{bc}
3	5349.22^c	3530.14^c	2993.11^c
4	5588.43^c	3618.32^c	3144.29^c
5	6221.74^c	4269.25^d	3123.82^c

Levels not connected by same letter are significantly different (P<0.05).

Table 4. Nitrogen recommendation on the basis of *G/(R+G+B)* ratio at different growth stages in pepper.

Growth stage	N status	G/(R+G+B) ratio	G/(R+G+B) ratio	Recommended N application (kg/hm^2)
	Deficiency	0.625 ~ 0.639	>0.628	80 ~ 100
Flowering	Normal	0.613 ~ 0.628	0.613 ~ 0.625	40 ~ 80
	Excess	0.612 ~ 0.617	<0.613	20 ~ 40
	Deficiency	0.657 ~ 0.672	>0.657	100 ~ 125
Fruiting	Normal	0.641 ~ 0.655	0.641 ~ 0.655	50 ~ 100
	Excess	0.632 ~ 0.641	<0.641	25 ~ 50

Luna et al., 2010). Also, relative value of correctional green band was considered a measure in the research of digital image acquired form general method and negative scan technique, which is the most appropriate index to reflect color message of vegetable coverage of different crops in the different geographical environment. This result, however, needs further verification with more tests and practices.

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