# Rapid diagnosis of nitrogen nutrition status in rice based on static scanning and extraction of leaf and sheath characteristics

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**Abstract:** According to the mechanism of rice growth, if nitrogen deficiency occurs, not only rice leaf but also sheath shows special symptoms: sheaths become short, stems appear light green, older sheath become lemon-yellowish. Nitrogen nutrition status of rice could be identified based on the differences of color and shape of leaf and sheath under different levels of nitrogen nutrition. Machine vision technology can be used to non-destructively and rapidly identify rice nutrition status, but image acquisition via digital camera is susceptible to external conditions, and the images are of poor quality. In this research, static scanning technology was used to collect images of rice leaf and sheath. From those images, 14 color and shape characteristic parameters of leaf and sheath were extracted by R, G, B mean value function and region props function in MATLAB. Based on the relationship between nitrogen content and the characteristics extracted from the images, the leaf R, leaf length, leaf area, leaf tip R, sheath G, and sheath length were chosen to identify nitrogen status of rice by using Support Vector Machine (SVM). The results showed that the overall identification accuracies of different nitrogen nutrition were 94%, 98%, 96% and 100% for the four growth stages, respectively. Different years of data were used for validation, identification accuracies were 88%, 98%, 90% and 100%, respectively. The results showed that additional sheath characteristics can effectively increase the identification accuracy of nitrogen nutrition status and the methodology developed in the study is capable of identifying nitrogen deficiency accurately in the rice.

**Keywords:** N deficiency, static scanning, leaf sheath, support vector machine (SVM), identification
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## 1 Introduction

Nitrogen (N) is one of the indispensable minerals for plant growth. Most of the world's agricultural soils are deficient in N supply. Using mineral N fertilizer can effectively overcome this problem, while the excessive use of N fertilizer may increase the risk of environmental pollution and increase production cost. Therefore, quick and accurate diagnosis of rice N nutrient status is necessary for guiding N fertilization.

Recently, the diagnosis of rice nutrition status is based on hyperspectral characteristics, which are determined using a hyper-spectrometer to measure the reflectance of rice canopies and leaves. Such as Shibayama researched the rice nutrition by using near-infrared and mid-infrared spectra of canopy[1] and the relation between yield and high spectral resolution reflectance[2]; Lin diagnosed[3] the phosphorus nutrition by using rice spectral information. Although reflectance is different under different nutrition conditions, the reflectance curve has a similar waveform that makes it difficult to discriminate the critical value. In addition, water stress[4], plant diseases and pests similarly influence the reflectance of canopies and leaves. Relying only on

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hyperspectral characteristics makes it difficult to build a single model to diagnose nutrition status for practical analysis.

Nitrogen deficiency most often results in stunted growth, slow growth, and chlorosis with small unit leaf area[5]. Because nitrogen is mobile, the older leaves appear chlorosis earlier than the younger leaves. The leaf color appears light green to yellow, especially older leaves[6]. Leaf color can indicate the plant nutrient and health status which is closely related to the amount and proportion of nitrogen content.

N deficiency rice shows obvious symptoms in the color and shape of leaf, which are important for identification of N nutrition status. How to get this information is very important. Static scanning is usually conducted in a closed environment, which could reduce the external disturbance during image acquisition process, and ensure the color and size reproduction of these micro-symptoms. Compared with common digital camera, the scanned image does not have complex background, multi-redundant information and image noise, thus can reduce errors in image analysis process. Hyperspectral image includes abundant information, and is mainly used to identify rice nutrition in lab research. But it always has many problems for field application, e.g., the equipment is expensive and portability is poor. Thus, in this research, static scanning was used to obtain digital images to capture the color and shape symptoms which appear on the leaf.

The analysis of plant leaf color has been proved useful in assessing plant nitrogen status. Extraction of leaf color was used to diagnose rice potassium stress[7], phosphorus stress[8] and nitrogen stress[9]. The anthocyanin was measured and evaluated by color information of lettuce leaf[10]. Based on the color information, the grapevine image was segmented to diagnose the potassium deficiency[11]. When rice suffers nutrition deficiency, the sheath will also appear specific symptoms[12]. Therefore, scanning images of rice leaf and sheath were analyzed to diagnose N nutrition status.

In this research, the scanned images of rice leaf and sheath under different levels of nitrogen nutrition were compared, and the differences of characteristics between rice leaf and sheath under the different nutrition conditions were analyzed. SVM was used to develop the rules and construct the model for the identification of N deficiency. This result can provide theoretical evidences for the diagnosis of rice nitrogen deficiency and guiding fertilization in time.

## 2 Materials and methods

### 2.1 Experimental design

The experiment was designed to study rice under different levels of nitrogen nutrition. Rice seeds (cultivar ZheYou-NO.1) were pre-germinated in moist sand at 30°C for 3 d, and seedlings were individually transplanted 7 d after emergence into 5 L polyvinyl chloride (PVC) pots. Every pot contained clean, sieved, and thoroughly leached river sand to allow for precise nutrient control. After 35 d, the images of rice leaf and sheath were captured first time (August 4th of 2013 and July 29th of 2012). In 2012 and 2013, the experiment was carried out in a greenhouse located in the ZiJinGang campus of Zhejiang University (30°17'N, 120°05'E) in Hangzhou, China. The rice was planted under natural light conditions. The temperature of the greenhouse was maintained at 30°C/25°C (day/night), and the relative humidity was maintained at 50%. The nutrient solution was prepared with deionized water and contained 110.8 mg/L $CaCl_2$, 405 mg/L $MgSO_4·7H_2O$, and 16.5 mg/L $Na_2SiO_3·9H_2O$, respectively. The pots were arranged in five different nutrition level treatments (four N treatments and normal nutrition treatment), six replications for each level, and 10 rice plants in each pot. Five treatment levels ($NH_4NO_3$: 0, 28.60 mg/L, 57.20 mg/L, 85.70 mg/L, 114.30 mg/L) via nutriculture (hydroponic) solutions were added to different pots. The nutrient solutions in the pots were replaced every 14 d. Every five days, the pH of the nutrient solution in each pot was measured and adjusted to five using 1 mol/L NaOH.

### 2.2 Images acquisition

Leaf samples were taken respectively on August 4th, 18th, 27th and September 8th of 2013. The top-three leaves in 10 rice plants with five nutrition levels, totally 600 samples were collected for all growth stages. Four hundred and eighty rice leaf and sheath samples under four different N levels, and 120 rice leaf and sheath samples with normal nutrition were collected to build the diagnosis rule and the identification model. The rice leaf and sheath samples collected on July 29th, August 13th, 20th, and 31th of 2012 were used to validated the model and detected the applicability, which consisted of four different N levels (240 samples) and normal nutrition (60 samples). In this research, five treatment levels were represented by N1, N2, N3, N4 and N5 (normal nutrition), respectively.

All of the samples were analyzed in laboratory. First, leaves and sheaths were placed on a scanner (EPSON GT20000, Seiko Epson Corporation, Suwa, Nagano-ken, Japan) with a maximum scanning area of 11.7 inch × 17.0 inch and an R/G/B (the full color images consist of red (R), green (G), and blue (B) channels) and BK color CCD line sensor. The output image data were 16 bits per pixel per internal color and 1 to 8 bits per pixel per external color. The resolution was set to 300 dpi (dots per inch). Leaf area ($cm^2$) can be calculated by the sum of all of the pixels within the range of the leaf multiplied by $(2.54/300)^2$; length (cm) equals the number of pixels in the vein multiplied by 2.54/300, and width (cm) equals the number of pixels in the widest zone multiplied by 2.54/300.

### 2.3 Feature selection

As shown in Figure 1, the spatial distribution of color and shape of rice leaf in different N nutrient conditions were markedly different. When nitrogen deficiency occurred, the rice leaf appeared yellow and had smaller leaf area, length and width. The yellowing of rice leaf was more and more obviously, etiolated area of leaf tip was bigger and bigger from normal to extreme.


Figure 1 Differences of symptoms of leaf under different N levels on Aug 27, 2013

As shown in Figure 2, under the same nitrogen condition, the color and shape of leaf of leaf positions are different. The symptoms of N deficiency are more obviously on the old leaf.


Figure 2 Differences of symptoms in different leaf positions under N2 on Aug 27, 2013

R, G, B mean value function and regionprops function in MATLAB (MathWorks Inc., USA) were used to determine the leaf color characteristics (LR, LG, LB) and shape characteristics (length (LL), width (LW), area (LA), perimeter(LP)), and sheath color characteristics (LSR, LSG, LSB) and length (LSL), which were used to identify N nutrition status.

Because N is very mobile in the plant and migrates to young leaves from old, senescing leaves, the symptoms of rice suffering from N deficiency often first appears on the tip of the leaf and then spread to the entire leaf. N deficiency leaf becomes light green at the tip, but the etiolated area of rice leaf is different under different degrees of N deficiencies[13]. Therefore, the color of leaf tip can be used to effectively identify the N status. In this research, color characteristic of leaf tip was the mean color value for 1/5 of the leaf length from the tip and is expressed as leaf tip R (LTR), leaf tip G (LTG), and leaf tip B (LTB). In total, 10 color and shape parameters were extracted from the scanned images of leaf (Table 1).

**Table 1 Characteristic parameters from the scanned images of leaf**

| No. | Parameter | No. | Parameter | No. | Parameter |
|---|---|---|---|---|---|
| 1 | LR | 5 | LW | 9 | LTG |
| 2 | LG | 6 | LP | 10 | LTB |
| 3 | LB | 7 | LA | | |
| 4 | LL | 8 | LTR | | |

As shown in Figure 3, the color and length of rice sheath in different N nutrient conditions also showed difference. According to the mechanism of plant nutrition, when N is deficient, rice stems become short and appear light green, older sheath become lemon-yellowish. From normal to extreme, the yellowing of sheath was more and more obviously, the sheath was shorter and shorter.


Figure 3 Difference of sheath symptom under different N levels on Aug 27, 2013

The R, G, B mean value function and the regionprops function in MATLAB, the sheath color characteristics (LSR, LSG, LSB) and length (LSL) under different levels of N nutrition were determined. The four additional parameters for sheath are shown in Table 2.

**Table 2 Characteristic parameters from the scanned images of sheath**

| No. | Parameter | No. | Parameter |
|---|---|---|---|
| 11 | LSR | 13 | LSB |
| 12 | LSG | 14 | LSL |

### 2.4 Research method

The levels of N nutrition can be identified by the color and shape characteristics of the leaf and sheath. But too more characteristics can result in redundant information, which increase the number of calculations and influence identification accuracy. It is difficult to identify N status of rice with so many sensitive characteristics. Therefore, to quickly diagnose N deficiency, it is necessary to choose the optimal set of characteristics using an effective feature-selection method.

The support vector feature selection (SVFS) method was used to select the optimal characteristic set to reduce the calculation burden and remove redundant information. This method makes full use of the main advantage of SVM (the generalizability from a small training sample)[14]. Additionally, this method can improve operational efficiency and ensure a rapid and stable screening process. The optimal characteristic set has maximum classification prediction ability. The method can remove the redundant characteristics of the subset by identifying high correlations between characteristics[15]. Therefore, the optimal characteristics subset can represent the set of N deficiency sensitive characteristics of. In this research, SVFS was used to screen the optimal characteristic set from the 14 characteristics in Libsvm-3.12.

Classification using SVM has two steps, training and classification[16]. The training process is: taking the feature vectors which reflecting the different categories as an input, use an appropriate kernel function, and introduce a non-negative Relaxation term and penalty coefficient C to map the feature vectors that reflecting the different categories from the low dimensional nonlinear space to high dimensional linear separable space; Then searching for the optimal separating hyperplane by solving linear equations in mapping space, to form the classifier. Inputting the unknown category data which has already been preprocessed into classifier to classify, we can get the classification results[17]. This research used SVM in Lib-SVM platform, and adopted RBF kernel function.

$$K(X,X_i)=\exp\left\{-\frac{\|X-X_i\|^2}{2\sigma^2}\right\} \qquad (1)$$

The classification function is:

$$f(x)=\mathrm{sgn}\left[\sum_{i=1}^{1}\alpha_i y_i K(X,X_i)+b\right] \qquad (2)$$

where, $\sigma$ and c are both kernel parameters which are calculated by grib.py of Lib-SVM.

## 3 Results and discussion

### 3.1 Screening of the optimal subsets

Under different N nutrition levels, the symptoms appeared on rice leaf had more obvious difference. The old leaves, and sometimes all leaves, become light green and chlorotic at the tip. Except for the greener primary leaves, deficient leaves are narrow, short and lemon-yellowish[13]. In this research, the optimal subsets of the characteristics represented the set of the most sensitive characteristics under different N nutrition levels. The results are shown in Table 3 (the numbers represent the characteristics).

As shown in Table 3, the characteristics optimal set of the different leaf positions included leaf color and shape in every growth stage, which means that every nutrition deficiency can affect two types of characteristics.

Rice requires less N nutrition in early growth period, in this time, the appearance mainly were the difference of leaf color, length, area and perimeter for different levels of N nutrition, the tip of leaf would not appear the obvious deficient symptoms. With sustainable growth, rice required more N nutrition, and the tip of leaf began to appear brown if N is deficient. So the spectral characteristics of leaf tip were added for identification of N nutrition levels.

**Table 3 Selected feature subsets of rice leaves in different positions**

| Growth Stage | Leaf Position | Subset |
|---|---|---|
| 4/8/13 | 1st leaf | 1, 2, 3, 4, 6, 7 |
| | 2nd leaf | 2, 3, 4, 6, 7 |
| | 3rd leaf | 2, 3, 4, 7 |
| 18/8/13 | 1st leaf | 2, 3, 4, 7, 9, 10 |
| | 2nd leaf | 2, 3,4, 7, 9 |
| | 3rd leaf | 2, 3,4, 7, 9 |
| 27/8/13 | 1st leaf | 2, 4, 7, 9, 10 |
| | 2nd leaf | 2, 4, 7, 9 |
| | 3rd leaf | 2, 7, 9 |
| 8/9/13 | 1st leaf | 2, 4, 7, 9 |
| | 2nd leaf | 2, 4, 7, 9 |
| | 3rd leaf | 2, 4, 7 |
| Universal characteristics | | 2, 4, 7, 9 |

Note: 4/8/13, 18/8/13, 27/8/13 and 8/9/13 indicate the four growth stages (August 4th, August 18th, August 27th and September 8th, respectively).

Taking the leaf and sheath samples of all growth stages together, four characteristics (LG, LL, LA, and LTG) were determined as universal characteristics for all growth stages for the identification of N nutrition status. The four characteristics can better reflect the differences showed on leaf under different levels of N nutrition, which is consistent with expression of growth mechanism of rice suffering from N deficiency.

### 3.2 Establishment of diagnosing model

After screening the characteristics, SVM was used to build the diagnostic model of N nutrition for the four growth periods. SVM in Lib-SVM platform was used in this research. For every growth stage, 150 samples were used to build the model. The results are shown in Table 4.

**Table 4 Overall identification accuracies of different N nutrition** unit: %

| Growth stage | 1st leaf | 2nd leaf | 3rd leaf |
|---|---|---|---|
| 4/8/2013 | 76 | 82 | 86 |
| 18/8/2013 | 78 | 84 | 88 |
| 27/8/2013 | 82 | 82 | 86 |
| 8/9/2013 | 80 | 86 | 90 |

Note: 4/8/13, 18/8/13, 27/8/13 and 8/9/13 indicate the four growth stages (August 4th, August 18th, August 27th, and September 8th, respectively).

According to the mechanism, rice stems appear light green and older sheath become lemon-yellowish when N deficiency occurs[12]. So the color and length characteristics of sheath were introduced to increase identification accuracy of N nutrition levels.

After adding the four parameters, SVFS method was used to select the optimal characteristic set from 14 characteristics again. Finally six characteristics (LG, LL, LA, LTG, LSG and LSL) were determined as universal characteristics for the identification of N nutrition status of all growth stages. SVM was used to identify N nutrition status, and a universal model was built for four growth periods. The results are shown in Table 5. Comparing with Table 4, Table 5 indicates that adding sheath characteristics could effectively improve identification accuracy, and verified the importance of sheath in the identification of N deficiencies.

**Table 5 Overall training accuracy of identification with additional sheath characteristics** unit: %

| Growth stage | 1st leaf | 2nd leaf | 3rd leaf |
|---|---|---|---|
| 4/8/2013 | 86 | 88 | 94 |
| 18/8/2013 | 90 | 92 | 98 |
| 27/8/2013 | 80 | 88 | 96 |
| 8/9/2013 | 90 | 92 | 100 |

Note: 4/8/13, 18/8/13, 27/8/13 and 8/9/13 indicate the four growth stages (August 4th, August 18th, August 27th, and September 8th, respectively).

According to Figure 4, the leaf positions with the highest identification accuracy were the same for four growth stages (the third leaf), which coincided with the theory that N is very mobile in the rice. When N was deficient, the nutrients were transferred into the primary leaves from the old, and the symptoms first appeared on the older leaves.

To know the detailed identification results of each N nutrition level, further analysis was done focused on the samples of the third leaf with the highest overall identification accuracy. The results are shown in Table 6.

The research found that all samples could be identified in four growth periods under N1 and N2. In the early part of growth stage, the identification accuracies all were 90% under N3, N4 and N5 (10% N3 samples misdiagnosed to be N4, 10% N4 samples misdiagnosed to be N5, and 10% N5 samples misdiagnosed to be N4). In early-middle stage, 10% N4 samples were misdiagnosed to be N5. In middle stage, 10% N4 samples were misdiagnosed to be N5, 10% N5 samples were misdiagnosed to be N4.

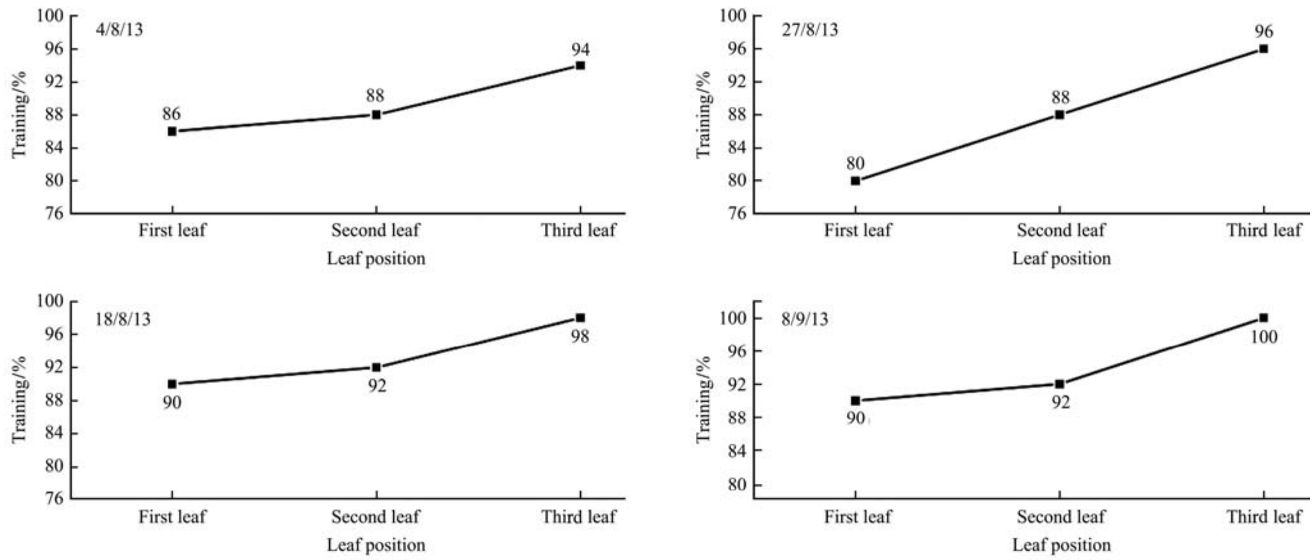


Figure 4 Overall training accuracies of different N nutrition with additional sheath characteristics

**Table 6 Training accuracies for the identification of different levels of nitrogen** unit: %

| Growth stage | N1 | N2 | N3 | N4 | N5 |
|---|---|---|---|---|---|
| 4/8/2013 | 100 | 100 | 90 | 90 | 90 |
| 18/8/2013 | 100 | 100 | 100 | 90 | 100 |
| 27/8/2013 | 100 | 100 | 100 | 90 | 90 |
| 8/9/2013 | 100 | 100 | 100 | 100 | 100 |

Note: 4/8/13, 18/8/13, 27/8/13 and 8/9/13 indicate the four growth stages (August 4th, August 18th, August 27th, and September 8th, respectively). N1, N2, N3, N4, and N5 indicate N nutrition levels (extreme shortage, severe shortage, moderate shortage, minor shortage and normal supply, respectively).

From early growth stage to early-middle growth stage, demand of N showed the increasing trend for rice, when rice suffered from N deficiency, the deficient symptoms gradually became obvious. So samples could be misjudged among N3, N4 and N5. In middle growth stage, rice suffered extended N deficiency, which led to the obvious difference among N1, N2 and N3. So the misjudgment mainly occurred between N4 and N5. In the later growth stage, the long-term N deficiency led the deficient symptoms to show obvious difference under different levels of N nutrition, so all samples can be identified correctly. From research results, it was known that the misjudgments mainly happened under N3-N4 and N4-N5. It showed that the identification of adjacent N nutrition had some difficulty.

### 3.3 Validation of model

Validation results of model are shown in Table 7. The results show that using SVM can effectively identify different degrees of N deficiencies, and the optimal leaf positions for identification conformed to the physiological characteristics of rice under different nutrition deficiencies in all growth stages.

**Table 7 Validation accuracies for identification of different nitrogen levels** unit: %

| Growth stage | 1st leaf | 2nd leaf | 3rd leaf |
|---|---|---|---|
| 29/7/2012 | 80 | 82 | 88 |
| 13/8/2012 | 80 | 88 | 98 |
| 20/8/2012 | 78 | 80 | 90 |
| 31/8/2012 | 88 | 90 | 100 |

Note: 29/7/12, 13/8/12, 20/8/12 and 31/8/12 indicate the four growth stages (July 29th, August 13th, August 20th, and August 31th, respectively).

## 4 Conclusions

Three most anterior leaves and sheaths of rice under different levels of N nutrition condition were chosen as the objects of the research. In laboratory condition, the color and shape parameters were acquired from the scanning image of rice leaves and sheaths. Then SVFS was used to select the optimal characteristic set for identification of different levels of N nutrition, the selected characteristics were LG, LL, LA, LTG, LSG and LSL. Finally SVM was used to build the diagnosis model.

The results showed that additional sheath characteristics could effectively improve the identification accuracy (94%, 98%, 96% and 100% for four growth stages). Data of different years were used for validation, validation accuracies were 88%, 98%, 90% and 100% for four growth stages, respectively.

The study provides evidences for quick diagnosis of rice N nutrient status, which makes it possible to accurately identify rice N status with scanning technology. Other crops such as maize, wheat suffering from N deficiency, usually have some special symptoms on leaf and sheath too, so this method could also be used to diagnose the N nutrition status for them.

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