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# EVALUATION OF RICE GENOTYPES FOR CHLOROPHYLL CONTENT, SPAD READINGS AND THEIR RELATIONSHIPS UNDER DIFFERENT NITROGEN FORMS AT FLOURISHING TILLERING STAGE

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**KEYWORDS**

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## ABSTRACT

The objective of the present study was to evaluate chlorophyll content of rice genotypes and conduct a comparative study between in vivo and in vitro data. 25 lines of $F_{15}$ generation of recombinant inbred lines (RILs) from the cross of Danteswari/Dagaddeshi along with few parents were tested with $NO_3^-$ and $NH_4^+$ form of nitrogen sources *i.e.* ammonical nitrogen from ammonium sulphate, nitrate nitrogen from calcium nitrate and control having no nitrogen fertilizer .The experimental design was laid out in randomized complete block design with factorial treatment structure. The present data revealed that SPAD value ranged from 32.25 to 24.23 while leaf chlorophyll a ranged from 2.104 to 1.690, leaf chlorophyll b ranged from 1.367 to 0.742 and total chlorophyll varied from 3.225 to 2.423 . This data shows the distinct pattern of leaf chlorophyll profile and SPAD values under variable nutrient levels. The positive and linear relationship between leaf chlorophyll profile and SPAD values is depicted from their $R^2$ values .670, .708, .705 under variable nutrient condition. This reflects their dependency on each other. Both in vivo and in vitro experiment gave comparable group of genotypes with low and high chlorophyll content under variable nutrient levels.

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## INTRODUCTION

Nitrogen is fundamental macronutrient in mineral nutrition of rice. Crop plants require nitrogen to thrive well and rapidly grow with flourishing green colour. Nitrogen fertilizers are not used efficiently because rice is grown in environment subjected to nitrogen losses through nitrification-denitrification, ammonia volatilization, run off and leaching. Therefore, finding efficiency level of nitrogen is important for growth, development, protein and yield (Gholizadeh *et al.*, 2009). Rice genotypes show significant variability for N uptake (external efficiency) and N utilization (internal efficiency). N-use efficient rice genotypes under low level of available soil nitrogen were identified by Pinto *et al.* (2001). These genotypes can be utilized efficiently in breeding program me for development of well-adapted and nutrient enriched varieties. Chlorophyll meter is portable diagnostic tool that measures greenness or relative chlorophyll content of leaves (Chung *et al.* 2000). There is strong positive relationship between SPAD (Soil Plant Analysis Development) values and leaf nitrogen concentration, but there relationship varies with crop growth stage and/ or variety (Hussain *et al.*, 2000). This relationship has led to the adaptation of SPAD meter to monitor crop N status. Thus the chlorophyll meter or SPAD offers a new strategy for synchronizing N application with actual crop demand in rice (Peng *et al.*, 1996). Real time N management on growth and yield of rice through LCC and SPAD meter are found to be effective as a decision tool for N management in rice (Duttarganvi *et al.*, 2014). Chlorophyll meter and leaf colour chart offers a wide perspective for enhancement of fertilizer nitrogen use efficiency in irrigated rice (Singh and Khind, 2015). Considering the above stated facts the overall objective of this research was framed. Rice genotypes were evaluated for leaf chlorophyll profile and SPAD values under variable nutrient levels.

## MATERIALS AND METHODS

### Plant material and experimental site

Field experiment was conducted on vertisol of research cum instructional farm of College of Agriculture, IGKV, Raipur. 25 lines of recombinant inbred lines (RILs) developed from the cross of Danteshwari /Dagaddeshi along with 7 parents were chosen as experimental materials and evaluated under irrigated and rainfed condition. The whole experiment was accomplished during growing season 2015.

### Fertilizer treatments

The experiment was laid out as factorial in randomized complete block design (RCBD) with two replications. Treatments involved in the experiment consisted of three nitrogen fertilizers levels.

$T_1$ = Ammonium sulphate providing ammonical nitrogen ($NH_4^+$-N)

$T_2$ = Calcium nitrate providing nitrate nitrogen ($NO_3^-$-N)

$T_3$ = Control having no nitrogen fertilizer ($N^0$-N)

### Statitical methods

All data was analyzed by analysis of variance, and F-test was used to determine

treatment significance. Duncan's multiple range test (DMRT) was used to compare treatment means at 5% probability level using M-STAT-C 14.2 software. Appropriate regression equations were also used for further analysis of relations between different parameters.

### Determination of leaf chlorophyll content

Leaf chlorophyll content was measured by in vivo procedure using Soil Plant Analysis Diagnostic Meter (SPAD-502) and by in vitro procedure using acetone as extraction solvent.

### In vivo assay

SPAD-502 was used to measure chlorophyll content of leaves in SPAD units. Readings were taken of young fully expanded leaves of five representative plants from each genotype and average reading was recorded. Takabae *et al.*, (1990) projected the importance of chlorophyll meter for providing simple, quick and non destructive method for estimation of leaf N concentration.

### In vitro assay

To obtain relationship between SPAD value and chlorophyll content of leaves, leaves were harvested just after taking the SPAD value and were stored at -20° C for further in vitro analysis. All steps were accomplished under dim light to prevent any degradation of photosynthetic pigments. Leaf samples was segmented, 20mg sample accurately weighted and then pulverize in a porcelain mortar and pestle containing 20ml 80% acetone. Leaf homogenate obtained was filtered through filter paper and extract obtained was used for chlorophyll measurement by a spectrophotometer following equation published by D.I. Arnon (1949).

Calculations: Use Arnon's equation (below) to convert absorbance measurements to mg Chl g-1 leaf tissue

Chl a (mg g-1) = [(12.7 × A663) - (2.6 × A645)] × ml acetone / mg leaf tissue

Chl b (mg g-1) = [(22.9 × A645) - (4.68 × A663)] × ml acetone / mg leaf tissue

Total Chl = Chl a + Chl b.

Where, A663, A645 = Absorbance at 663nm and 645nm, respectively.

## RESULTS AND DISCUSSION

### SPAD value and Leaf chlorophyll profile

Results showed that there are significant differences in leaf chlorophyll profile and SPAD value under variable nutrient levels at flourishing tillering stage. Results revealed that SPAD value ranged from 32.25 to 24.23 as presented in Table 1. NH4+-N treatment recorded significantly higher SPAD value as compared to $NO_3^-$-N and $N^0$-N treatment. This finding is in compliance with the research of Jinwen *et al.* (2009) who investigated response of rice SPAD readings to different nitrogen supply rates. Gholizadeh *et al.* (2009) also evaluated SPAD chlorophyll meter readings in two different rice growth stages to establish the fact that SPAD readings indicate the plant nitrogen status and physiological nitrogen requirement of crops at different growth stages. Results also revealed that rice genotypes showed considerably low level of leaf chlorophyll a, chlorophyll b and total chlorophyll under $NO_3^-$-N and $N^0$-N treatment. As opposed to this, leaf chlorophyll a, chlorophyll b and total chlorophyll of rice genotypes were higher under NH4+-N treatment. Chlorophyll profile showed significant differences under variable nutrient conditions. These results synchronize with findings of Hassan *et al.*, (2009). They confirmed genotypic variation in traditional rice varieties for chlorophyll content and SPAD values. The varieties evaluated by them showed consistent level of leaf chlorophyll profile under variable nutrient levels.

### Relationship between chlorophyll profile and SPAD value under variable nutrient levels

The relationships between leaf total chlorophyll and SPAD readings of rice genotypes under NH4+-N, $NO_3^-$-N, $N^0$-N treatment are depicted in Figure 1. This study shows that leaf chlorophyll profile shows linear and positive relationship with SPAD values at the variable nutrient levels at flourishing tillering stage. The corresponding $R^2$ values of NH4+-N, $NO_3^-$-N, $N^0$-

**Table 1: Effect of variable nutrient levels on leaf chlorophyll profile and SPAD value of rice genotypes at the flourishing tillering stage**

| | Chlorophyll a (mg $g^{-1}$) | Chlorophyll b (mg $g^{-1}$) | Total chlorophyll ((mg $g^{-1}$) | SPAD value |
|---|---|---|---|---|
| NH4+-N | 2.104 a | 1.367 a | 3.225 a | 32.72 a |
| $NO_3^-$-N | 1.825 b | 1.113 b | 3.194 b | 31.50 b |
| $N^0$-N | 1.690 c | 0.742 c | 2.423 c | 30.25 c |

Means followed by different letter within column indicate significant differences at $p < 0.05$ by DMRT

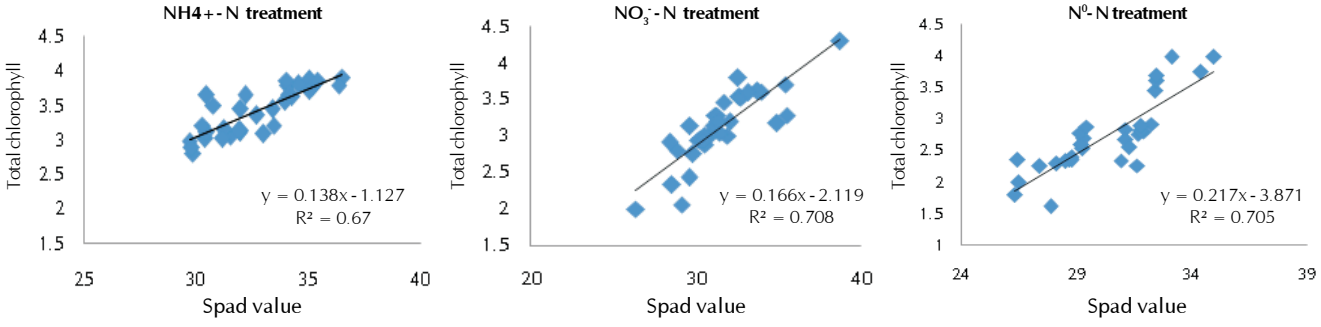


**Figure 1: Relationship between Total chlorophyll and SPAD values under variable nutrient levels at flourishing tillering stage**

N treatment are .670, .708, .705. There are studies that show high correlation between chlorophyll content and SPAD readings. A study conducted by Islam *et al.,* 2009 evaluated SPAD and LCC based N management in rice. There results indicated higher chlorophyll content in rice leaves showing higher SPAD readings. This relationship depicts the dependency of SPAD values on chlorophyll profile. Mamin (2003) reported a strong, linear and positive relationship between SPAD values and chlorophyll a and b contents.

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