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Dr. Vinita Zhodape
Department of Plant Physiology, Agricultural Biochemistry, Medicinal and Aromatic Plants, College of Agriculture, IGKV, Raipur, Chhattisgarh, India

Dr. Dharmendra Khokhar,
Department of Plant Physiology, Agricultural Biochemistry, Medicinal and Aromatic Plants, College of Agriculture, IGKV, Raipur, Chhattisgarh, India

Kavita Sahu
Department of Plant Physiology, Agricultural Biochemistry, Medicinal and Aromatic Plants, College of Agriculture, IGKV, Raipur, Chhattisgarh, India

Bhagawat Prasad
Department of Plant Physiology, Agricultural Biochemistry, Medicinal and Aromatic Plants, College of Agriculture, IGKV, Raipur, Chhattisgarh, India

Corresponding Author:
Dr. Vinita Zhodape
Department of Plant Physiology, Agricultural Biochemistry, Medicinal and Aromatic Plants, College of Agriculture, IGKV, Raipur, Chhattisgarh, India

Influence of nitrogen levels on nitrogen content, protein content, starch content and photosynthesis pigments (Chlorophyll) of different rice (*Oryza sativa* L.) genotypes

Dr. Vinita Zhodape, Dr. Dharmendra Khokhar, Kavita Sahu and Bhagawat Prasad

Abstract
Nitrogen is a critical input involved in plant metabolism growth and indifferent biochemical processes. Nitrogen participates directly in amino acid, protein and other cellular component syntheses, which are required for plant growth and development. Therefore, nitrogen application greatly influences starch and protein composition and, very little information is available on the effect of nitrogen fertilizer on protein content and starch components with little emphasis on quality characteristics. Therefore, field trial was carried out to investigate the impact of different nitrogen levels (40 kg ha^{-1}, 80 kg ha^{-1} and 120 kg ha^{-1}) was studied to assess their effects on biochemical attributes likes, nitrogen content, protein content, starch content, photosynthesis pigment – chlorophyll a, chlorophyll b, total chlorophyll and SPAD value contributing to yield of 20 Rice genotypes during 2016-2017 at experimental field of Department of Plant Physiology, IGKV, Raipur (C.G.). Nitrogen and other fertilizers (P and K) were applied in the form of Urea, SSP and MOP respectively. The experiment was carried out in a Split Plot Design with three main plot (N levels) and 20 sub plot (Rice genotypes) with two replications.
The objective of this study was to improve the understanding of interaction between the nitrogen doses and biochemical and molecular improvement of rice genotypes. Thus, recognition of such rice cultivar is essential in developing high yielding varieties that can survive in low nitrogen condition. In this experiment, results revealed that increasing nitrogen levels significantly enhanced the nitrogen content, protein content, starch content, SPAD value and photosynthetic pigment i.e. chlorophyll ‘a’ (mg gm^{-1}), chlorophyll ‘b’ (mg gm^{-1}) and total chlorophyll (mg gm^{-1}) of rice, due to improved biosynthesis of chlorophyll and enzymatic activity. Nitrogen have a efficiency, including biochemical attributes and photosynthetic ability which depends on the chlorophyll content of plant and offer significant role in realizing higher crop yield.

Keywords: Nitrogen content, protein content, starch content, photosynthetic pigment, chlorophyll

Introduction
Rice (*Oryza sativa* L.) is the staple food of more than half of the world population belonging to the family Poaceae (Hadiarto and Tran 2011). The cultivated rice (*Oryza sativa*) is divided into three subspecies - indica, javanica and japonica (Datta *et al*., 2003) [8]. In Asia, two main subspecies - indica and japonica are grown. Indica rice comprises 80% of cultivated rice in the world (Ramesh *et al.*, 2009) [30]. It is cultivated over an area of 158.8 m ha with a production of 473 Mt. (IGC, 2015-16) [16]. Total production of rice during 2015-16 is estimated 103.61 million tonnes, which is lower by 1.87 million tonnes than its production of 105.48 million tonnes during 2014-15 (in India). Rice consumption is estimated to the 581 Mt in 2015 as against a consumption of 531 Mt in 2005 (Prasad, 2011) [27]. To meet the global rice demand, it is estimated that about 114 Mt of additional milled rice need to be produced by 2035, which is equivalent to an overall increase of 26% in the next 25 years (Kumar and Ladha, 2011) [18].
The crop productions mostly depend upon the nutrients (Ananthi *et al*., 2010) [2]. After water stress, nutrients are recognized as the second most limiting factor in Asia. (Haefele and Hijmans *et al.*, 2007) [14]. Nitrogen is one of the most expensive though but an essential nutrient to plant as the commercial N fertilizers represent the major cost in plant production (Singh, 2005) [32]. Nitrogen represents 67% of fertilizer applications to rice in a world basis (Eagle *et al.*, 2001) [10]. Moreover, crop plants are able to utilize only 30–40% of applied nitrogen for food production and the remaining N is left over into environment leading to

hazardous environmental pollution by N contamination (Raun and Johnson, 1999) [31].

Nitrogen is the major nutrient added to increase crop yield (Camara *et. al.*, 2003) [6]. At a cellular level, N increases the cell number and cell volume; at the leaf level, it increases the photosynthetic rate and efficiency (Lawlor *et. al.*, 1988) [21]. Increases in crop growth rate are largely produced through an increase in leaf area index, and also by an increase in radiation use efficiency (RUE, dry matter produced per unit of either incident radiation or intercepted radiation) (Brown *et. al*., 1987; Lawlor, 1995) [5, 20].

According to Ladha *et al.* (1998) [19], desirable cultivars with high nitrogen use efficiency (NUE) should produce large yields at low and high N supply. Although N supply drives productivity, low nitrogen use efficiency (NUE) is a major problem of irrigated rice cultivation (Cassman *et al.*, 1998) [7]. Excessive nitrogen input of N application lead to the poor physiological nitrogen use efficiency in rice production and cause problems such as environmental pollution, increased production cost, grain yield reduction, and could even lead to global warming. Nitrogen use efficiency mostly base of the plant dry matter accumulation which is depends on the total C fixed during photosynthesis and the fraction of nitrogen converted into dry matter. Grain yield mostly depends on three main factors: light interception, nutrient management, and harvest index (Mitchell *et al*. 1998) [23].

Quannbao *et. al*. (2007) [29] studied on four rice cultivars under two soil conditions (sandy and clay soil) in soil culture pool and found that nitrogen utilization of rice differed with soil conditions observed that nitrogen harvest index (NHI) and physiological nitrogen use efficiency (PNUE) were higher in sandy soil than in clay soil. Apparent nitrogen recovery efficiency (ANUE), partial factor productivity for applied nitrogen (Pfp) and was higher in clay soil than sandy soil. Agronomic nitrogen use efficiency (ANUE) was varied in different cultivars under different soil condition. NHI, ANUE, PNUE, PEP and soil N depended rate were decreased significantly with the increment with the amount of nitrogen applied under two soil condition.

India stands second in N fertilizer consumption and production (FAI, 2013) [11]. Nitrogen is not only expensive but also an essential nutrient to plant as the commercial N fertilizers show the major cost in plant production (Singh, 2005) [32]. Where, crop yields are increased by application of N fertilizers, there are also some environment hazards due to increased use of N fertilizers like effects of global N cycle, ozone layer depletion and nitrate leaching problems in soil. Nitrogen use efficiency is found low in rice due to leaching which effects economic efficiency of applied N (Hakeem *et al.*, 2012) [15].

The most pressing target of improving agricultural NUE is to improve the recovery of N from fertilizer (Dawson *et al*., 2008) [9]. Nitrogen use efficiency had synergistic effect as RUE increased at higher leaf nitrogen contents (Fischer, 1993) [12]. Therefore, this present study was carried out in order to examine the nitrogen use efficiencies on nitrogen content, protein content, starch content, SPAD value and photosynthetic pigment of local rice particularly for Chhattisgarh.

Methods and Materials

Experimental Details

A field experiment was conducted during *Kharif* season of 2017-18 at College Farm, College of Agriculture, Indira Gandhi Krishi Vishwavidyalya, Raipur. The experiment was laid out in split plot design with three main plot (N levels) and twenty sub plot (Rice genotypes) in two replications. The experimental trials included various treatments of T1: 40 kg N ha^{-1}, T2: 80 kg N^{-1}, T3: 120 kg N^{-1} and twenty local rice. The rice genotypes were sown separately in raised bed nursery and thirty day old seedlings were transplanted into plots by adopting a spacing of 20 X 15 cm. The fertilizers were applied as per the recommended doses (40:60:40, 80:60:40 and 120:60:40 kg ha^{-1}).

N: P: K applied in the form of Urea, SSP and MOP, respectively. The half dose of N was applied as basal dose and the rest half N was applied in two equal splits at 30 and 50 days after transplanting. Irrigation and weed management was done in time to time. Data were collected from selected plants in the rows. The collected data includes nitrogen and protein content estimated by formula (AOAC, 1970), starch content (anthrone reagent method), SPAD value (Chlorophyll meter), chlorophyll a, chlorophyll b and total chlorophyll (Acetone method, Arnon 1949) [3].

The data was analyzed using analysis of variance (ANOVA) by software and mean separation was carried out at 5% probability level. (Fisher 1963) [13]

Treatments details of the experiment

S.N.	Entry no.	S.N.	Entry no.
1	DXD (124)-1-12	11	DXD (124)-5-72
2	DXD (124)-1-14	12	DXD (124)-6-74
3	DXD (124)-2-17	13	DXD (124)-9-89
4	DXD (124)-2-20	14	DXD (124)-9-91
5	DXD (124)-2-22	15	DXD (124)-11-133
6	DXD (124)-3-28	16	DXD (124)-15-164
7	DXD (124)-3-30	17	DXD (124)-17-192
8	DXD (124)-3-59	18	DXD (124)-17-193
9	DXD (124)-3-60	19	DXD (124)-17-210
10	DXD (124)-4-70	20	DXD (124)-17-211

1. Nitrogen and Protein content (%)

Plant samples collected at 50% flowering stages of rice were oven dried and powdered into fine powder and used for chemical analysis. Straw samples collected were first shade dried and then oven dried at 65°C. The dried straw was powdered through grinding machine properly and the finely grind material was used for chemical analysis. In these samples nitrogen content was analyzed by adopting the standard procedures (Piper, 1966) [28].

Weighing of 0.5 of powdered leaf sample was taken and placed in a digestion tube. Then 1.00 g of salt mixture ($CuSo_4$ + K_2So_4) and 5ml of concentration H_2So_4 added. The samples were digested using KEL Plus system (Pelican Equipments, Chennai) until the solution became colourless at 420^0C (approx. 40 to 60 min.). The digest samples were distilled with distillation unit of KEL Plus system. A 100 ml conical flask was taken containing 10 ml boric acid. Then 2 to 4 drops of mixed indicator dye was added and the flask was placed beneath the condenser with the delivery tip immersed in the solution.

The digest was transferred to distillation unit and 8 to 10 ml sodium hydroxide (NaOH) was added to the digest and steamed until about 20 ml of distilled was calculated in the conical flask. The tip was rinsed with water and titration was done against the standard HCL (0.01 N) solution until the first appearance of violet colour was observed, as the end point. A blank containing the same quantity of all the reagents but without sample for every set of nitrogen determination was used. For estimation of protein content the formula suggested by AOAC (1970) was used below:

% N = (ml HCL used in determination – ml HCL used in blank) x Normality of HCL x 14.00 x 100 mg sample.

Protein (%) = % nitrogen x (5.95) factor for a given sample

2. Chlorophyll content (a, b and total) (mg/g fresh weight)

Total chlorophyll content was estimated by the method described Arnon (1949) [3]. 25g of leaf samples (2^{nd} leaf) were dipped in 10 ml of 80% acetone in a graduated glass tube. The glass tubes were incubated in dark at 4ºC for 48 hrs. After 48 hrs absorbance were taken at 645 nm and 663 nm. Quantification of the total chlorophyll, chlorophyll a and chlorophyll b content in an 80% acetone extract was done by following equation:

Total chlorophyll (mg/g fresh weight) = 20.2 (A_{645}) - 8.02 (A_{663})* dilution factor

Chlorophyll a (mg/g fresh weight) = 12.7 (A_{663}) - 2.69 (A_{645})* dilution factor

Chlorophyll b (mg/g fresh weight) = 22.9 (A_{645}) - 4.68 (A_{663})* dilution factor

Where, A_{663} is the absorbance at 663 nm and A_{645} is the absorption at 645.

3. SPAD Value reading

Chlorophyll meter (model SPAD 502 of Minotta co., Japan) was used to measure SPAD values at tillering and 50% flowering. The leaf selected for measurement of SPAD reading was between fourth from base to leaf tip of the plant. The SPAD values were measured on 10 leaves in each treatment and then mean value was calculated.

4. Starch content

Estimation of Starch was done by Anthrone Reagent method.

Procedure:

To remove sugars from the sample of 0.5 g, it was homogenize with 80% ethanol and centrifuged and retain the residue. Washed the residue repeatedly with hot 80% ethanol till the washings did not give colour with anthrone reagent. Residue was dried well over a water bath. To the residue 5.0 ml of water was add and 6.5 ml of 52 percent of perchloric acid added than extracted at 0°C for 20 min. Sample was centrifuged and the supernatant was collected. Extraction was repeated using fresh perchloric acid. Centrifuged and pooled the supernatants and make up to 100 ml. 0.1 ml of sample pipette out of the supernatant and made up the volume to 1 ml with water. Standard was prepared by taking 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard and made up the volume to 1 ml in each tube with distilled water. 4 ml of anthrone reagent was added in each tube. Tubes were incubated for eight minutes in a boiling water bath and finally cooled rapidly and the observations were recorded for intensity of green to dark green colour at 630 nm.

Calculation: Multiply the value by a factor 0.9 to estimate the starch content.(Hedge and Hofreiter,1962).

Result and Discussion

Starch content (%) under nitrogen treatments

The data on starch content has been presented in table 1 and fig. 1. Significant differences were noticed among different nitrogen treatments in relation to starch content during year 2017-18. As per the result found that, Starch content, protein content and nitrogen content (leaf and grain) shows similar pattern they all were increased with higher levels of nitrogen. In 80 kg N, DXD (124)-17-193 (93%) was found maximum starch content followed by DXD (124)-17-211 and DXD (124)-17-211 (89%), similarly and minimum starch content was recorded in rice genotype DXD (124)-4-70 (71%) followed by DXD (124)-5-72 (76%). In 40 kg N, DXD (124)-11-133 and DXD (124)-9-91 (89%) similarly was found maximum starch content and minimum starch content was recorded in rice genotype DXD (124)-4-70 (70%) and DXD (124)-5-72 (73%) similarly. Under 120 kg N, maximum starch content was found in DXD (124)-17-193 (99%) followed by DXD (124)-17-211 (98%) and minimum starch content was recorded in DXD (124)-4-70 (72%) followed by DXD (124)-5-72 (73%).

In this study found that, 120 kg N recorded maximum starch content followed by 80 kg N and 40 kg N. Umemoto *et al.* (1976) [36] found that activities of ADPGP pase and starch branching enzymes were significantly correlated with starch accumulation rate and starch synthesis and they are key enzymes during starch synthesis course in rice grains. Bahmanyar and Ranjbar (2007) [4] found that nitrogen contributes to starch accumulation in culms and leaf sheaths during the pre-heading stage and in the grain during the ripening stage of rice.

Grain protein content (%) under different nitrogen treatments

The data on grain Protein content have been presented in Table 1 and fig. 2. Significant differences were noticed among different nitrogen treatments in relation to grain protein content during the year 2017-18. In 80 kg N, DXD (124)-3-28, DXD (124)-11-133 and DXD (124)-17-193 (13.1%), similarly was found maximum grain protein content and minimum grain protein content was recorded in rice genotype DXD (124)-5-72 and DXD (124)-3-30 (6.5%) similarly. In 40 kg N, DXD (124)-11-133, DXD (124)-9-89 and DXD (124)-2-17(11.3%), similarly was found maximum grain protein content and minimum grain protein content was recorded in rice genotype DXD (124)-3-30 (6.0%) followed by DXD (124)-5-72 (6.5%).Under 120 kg N, maximum grain protein content was found in DXD (124)-3-28 and DXD (124)-17-192 (14.3%) similarly and minimum grain protein content was recorded in DXD (124)-5-72 (7.1%) followed by DXD (124)-3-30 (7.7%).

In this result found that protein content of grain was significantly increased by elevated levels of N. This might be due to increased nitrogen assimilation (protein synthesis) in plants because nitrogen is a major component of amino acids and proteins. Similar results were reported by Singh and Bajpai (1990) [33].

Nitrogen content (%) in grain under different nitrogen treatments

The data on nitrogen content has been presented in Table 1 and fig. 3. Significant differences were noticed among different nitrogen treatments in relation to nitrogen content during the year 2017-18. As per the following data it is recorded that nitrogen content in grain and leaf significantly increases with increased level of nitrogen. In 80 kg N, DXD (124)-3-28,DXD (124)-11-133 and DXD (124)-17-193 (2.2%) similarly was found maximum nitrogen content and minimum nitrogen content was recorded in rice genotype DXD (124)-5-72 and DXD (124)-3-30 (1.1%) similarly. In case of 40 kg N, DXD (124)-11-133,DXD (124)-9-89, DXD (124)-2-17 and DXD (124)-6-74 (1.9%) similarly was found maximum nitrogen content and minimum nitrogen content was recorded in rice genotype DXD (124)-3-30 (1.0%) followed by DXD (124)-5-72 (1.1%). Under 120 kg N maximum nitrogen content was found in DXD (124)-3-28and DXD (124)-17-192(2.4%) and minimum nitrogen content was recorded in DXD (124)-5-72 (1.2%) followed by DXD (124)-3-30 (1.3%).

In the present investigation found that N content increases with the higher level of nitrogen. These results are confirmed by Kaushal *et al.* (2010) [17] who also reported that N content in grains increased significantly with the increase in N level from 90 to 120 kg N ha^{-1} through highest value was recorded at 150 kg N ha^{-1}. Highest N content in straw was recorded in rice hybrids as compared to rice varieties. Similar report was found by Pal *et al.* (2008) [25].

Nitrogen content (%) in leaf under different nitrogen treatments

The data on nitrogen content have been presented in Table 1 and fig. 4. Significant differences were noticed among different nitrogen treatments in relation to nitrogen content during the year 2017-18. In 80 kg N, rice genotype DXD (124)-1-14and DXD (124)-3-28 (0.9%) similarly found maximum nitrogen content and minimum nitrogen content was recorded in rice genotype DXD (124)-4-70 (0.1%) followed by DXD (124)-3-30 (0.3%). In case of 40 kg N, DXD (124)-3-28 and DXD (124)-1-14 (0.8%) found maximum nitrogen content and minimum nitrogen content was recorded in rice genotype DXD (124)-4-70 (0.1%) followed by DXD (124)-3-30 and DXD (124)-9-89 (0.3%) similarly.

Under 120 kg N, maximum nitrogen content was found in DXD (124)-1-14 (1.8%) followed by DXD (124)-3-28 and DXD (124)-5-72 (1.7%) similarly and minimum nitrogen content was recorded in DXD (124)-4-70 (0.9%) followed by DXD (124)-3-30 (1.2%). In the present study 40 kg N recorded minimum nitrogen content in leaf in all the genotypes as compared to other nitrogen treatments.

Chlorophyll a content (mg g^{-1}) under nitrogen treatments

This experiment was conducted during 2016-17 and 2017-18 both years. The effects of different nitrogen levels on chlorophyll a content of rice genotypes at flowering stage has been presented in Table 2 and Fig. 5. Significant differences were noticed among different nitrogen treatments in relation to chlorophyll a during both the years (2016-17 and 2017-18) as well as in pooled data. As a result of nitrogen treatments, chlorophyll a, chlorophyll b, total chlorophyll and SPAD values were recorded in similar increasing pattern. All were higher in increased level of nitrogen. In 80 kg N rice genotype DXD (124)-9-91 (1.27 mg g^{-1}) and DXD (124)-17-193 (1.13mg g^{-1}) showed maximum chlorophyll a content followed by DXD (124)-5-72 (1.06 mg g^{-1}). Rice genotype DXD (124)-1-14 (0.57mg g^{-1}) found minimum chlorophyll a content. It's ranged from 0.57 to 1.27mg g^{-1}. Under 40 kg N rice genotype DXD (124)-9-91(1.19 mg g^{-1}), DXD (124)-5-72 and DXD (124)-17-193 (1.02 mg g^{-1}) similarly showed maximum chlorophyll a content, whereas DXD (124)-3-30 and DXD (124)-1-14 (0.45 mg g^{-1}), similarly followed by DXD (124)-4-70 (0.50 mg g^{-1}) showed minimum chlorophyll a content. It's ranged from 0.45 to 1.19 (mg g^{-1}).

Under 120 kg N rice genotype DXD (124)-9-91 (1.35 mg g^{-1}) and DXD (124)-17-192 (1.27 mg g^{-1}) showed maximum chlorophyll a content, whereas, DXD (124)-3-30 (0.73 mg g^{-1} fresh leaf weight) showed minimum chlorophyll a content followed by DXD (124)-1-12 (0.79 mg g^{-1} fresh leaf weight).It's ranged from 0.73 to 1.35 mg g^{-1}fresh leaf weight.

Chlorophyll b content (mg g^{-1}) under nitrogen treatments

This experiment was conducted during 2016-17 and 2017-18 both years. The effects of different nitrogen levels on chlorophyll b content of rice genotypes at flowering stage has been presented in Table 2 and Fig. 6. Significant differences were noticed among different nitrogen treatments in relation to chlorophyll b during both the years (2016-17 and 2017-18) as well as in pooled data. In 80 kg N, rice genotype DXD (124)-9-91 (0.87 mg g^{-1}) and DXD (124)-5-72 (0.76 mg g^{-1}) showed maximum chlorophyll b content followed by DXD (124)-3-60 (0.70 mg g^{-1}) and DXD (124)-3-30 (0.40 mg g^{-1}) found minimum chlorophyll b content followed by DXD (124)-9-89 (0.41 mg g^{-1}). It's ranged from 0.40 to 0.87 mg g^{-1}. Under 40 kg N, rice genotype DXD (124)-9-91(0.71 mg g^{-1}), DXD (124)-3-60 (0.68 mg g^{-1}) and DXD (124)-5-72 (0.66 mg/g) showed maximum chlorophyll b content, whereas DXD (124)-3-30 and DXD (124)-9-89 (0.33 mg g^{-1} fresh leaf weight) showed minimum chlorophyll b content. It's ranged from 0.33 to 0.71 (mg g^{-1}).

Under 120 kg N, rice genotype DXD (124)-9-91 (1.15 mg g^{-1}) and DXD (124)-11-133 (0.88 mg g^{-1}) showed maximum chlorophyll b content. Whereas, DXD (124)-3-59 (0.45 mg g^{-1} fresh leaf weight), showed minimum chlorophyll b content followed by DXD (124)-1-12 (0.47 mg g^{-1} fresh leaf weight). It's ranged from 0.45 to 1.15mg g^{-1} fresh leaf weight.

Total chlorophyll content (mg g^{-1}) under nitrogen treatments

This experiment was conducted during 2016-17 and 2017-18 both years. The effects of different nitrogen levels on total chlorophyll content of rice genotypes at flowering stage has been presented in Table 2 and Fig. 7. Significant differences were noticed among different nitrogen treatments in relation to total chlorophyll during both the years (2016-17 and 2017-18) as well as in pooled data. In 80 kg N, rice genotype DXD (124)-9-91 (2.24 mg g^{-1}) showed maximum total chlorophyll content followed by DXD (124)-17-193 (1.94 mg g^{-1}) and rice genotype DXD (124)-3-30 (1.0 mg g^{-1}) found minimum total chlorophyll content followed by DXD (124)-17-210 (1.04 mg g^{-1}). It's ranged from 1.00 to 2.24mg g^{-1}.Under 40 kg N, DXD (124)-9-91(1.54 mg g^{-1}) and DXD (124)-17-193 (1.37 mg g^{-1}) showed maximum total chlorophyll content, whereas rice genotype DXD (124)-3-30 (0.66 mg g^{-1} fresh leaf weight) followed by DXD (124)-1-14 (0.70 mg g^{-1}) showed minimum total chlorophyll content. Under 120 kg N rice genotype DXD (124)-9-91 (2.54 mg g^{-1}) and DXD (124)-5-72 (2.14 mg g^{-1})

showed maximum total chlorophyll content, whereas rice genotype DXD (124)-3-30 (1.26 mg g^{-1} fresh leaf weight), showed minimum chlorophyll b content followed by DXD (124)-1-12 (1.31 mg g^{-1} fresh leaf weight). It's ranged from 1.26 to 2.54 mg g^{-1}.

Total chlorophyll content increases with high nitrogen treatments, the results observed in the present study are in conformity with the results of Sudhakar *et al.* (2006) [34]. Li *et al*. (2012) [22] found that the total chlorophyll content was higher with higher level of nitrogen application. It was also showed that chlorophyll content decreased by 8% when fed with low N and increased by 12% when fed with high N in seedlings stage. Similarly, Amaliotis *et al.* (2004) [1] reported that there were very close relation between chlorophyll and nitrogen content.

SPAD value under nitrogen treatments

The effects of different nitrogen levels on SPAD values of rice genotypes at flowering stage have been shown in Table 2. Significant differences were noticed among different N treatments in relation to SPAD value during year 2017-18. In 80 kg N, rice genotype DXD (124)-3-60 (40.8) showed maximum SPAD value followed by DXD (124)-3-30 (40.5), whereas DXD (124)-11-133 (28.8) found minimum SPAD value followed by DXD (124)-15-164 (29.7). It's ranged from 28.8 to 40.8. Under 40 kg N, rice genotypes DXD (124)-1-14 (40.8), DXD (124)-2-22 (83.3) and DXD (124)-1-12 (38.3) showed maximum SPAD values, whereas DXD (124)-2-20 (29.9) followed by DXD (124)-17-210 (30.0) and DXD (124)-15-164 (30.6) showed minimum SPAD values. It's ranged from 29.9 to 40.8.

Under 120 kg N, rice genotypes DXD (124)-1-14 (45.6) and DXD (124)-1-12 (45.3) showed maximum SPAD value, whereas DXD (124)-6-74 (34.3), showed minimum SPAD value followed by DXD (124)-15-164 (34.8). It's ranged from 34.3 to 45.6. Highest SPAD values was recorded under 120 kg N followed by 80 kg N and 40 kg N. Critical or threshold SPAD value is important and it indicates the leaf area based critical nitrogen content in rice leaves. In this result, SPAD value increases with the higher nitrogen treatments. These results are in agreement with Swain *et al.* (2006) [35], who found highly significant and positive relation between total chlorophyll content at all the growth stages and grain number m^{-2}, indicating the role of chlorophyll content in sink development and grain filling, leading to higher productivity.

Table: The data on starch content has been presented

S.N.	Name of rice genotypes	Starch (%) in grain			Protein (%) in grain			N content in grain (%)			N content in leaf (%)		
		T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
1	DXD (124)-1-12	91	81	81	11.3	10.7	10.7	1.9	1.8	1.8	1.5	0.7	0.6
2	DXD (124)-1-14	92	81	80	11.9	10.7	10.1	2.0	1.8	1.7	1.8	0.9	0.8
3	DXD (124)-2-17	81	81	79	12.5	11.3	11.3	2.1	1.9	1.9	1.6	0.8	0.7
4	DXD (124)-2-20	93	82	80	12.5	10.1	7.7	2.1	1.7	1.3	1.5	0.6	0.5
5	DXD (124)-2-22	94	82	81	11.3	10.1	8.9	1.9	1.7	1.5	1.6	0.7	0.6
6	DXD (124)-3-28	91	83	82	14.3	13.1	10.1	2.4	2.2	1.7	1.7	0.9	0.8
7	DXD (124)-3-30	92	84	79	7.7	6.5	6.0	1.3	1.1	1.0	1.2	0.3	0.3
8	DXD (124)-3-59	83	83	79	9.5	8.9	7.7	1.6	1.5	1.3	1.5	0.6	0.5
9	DXD (124)-3-60	95	82	76	10.1	10.1	8.3	1.7	1.7	1.4	1.3	0.4	0.4
10	DXD (124)-4-70	72	71	70	11.9	11.3	9.5	2.0	1.9	1.6	0.9	0.1	0.1
11	DXD (124)-5-72	73	76	73	7.1	6.5	6.5	1.2	1.1	1.1	1.7	0.8	0.7
12	DXD (124)-6-74	97	87	87	11.9	10.7	10.3	2.0	1.8	1.9	1.4	0.6	0.5
13	DXD (124)-9-89	96	85	87	12.5	11.9	11.3	2.1	2.0	1.9	1.3	0.4	0.3
14	DXD (124)-9-91	94	87	89	11.9	10.1	9.5	2.0	1.7	1.6	1.4	0.4	0.5
15	DXD (124)-11-133	89	89	89	13.7	13.1	11.3	2.3	2.2	1.9	1.6	0.8	0.7
16	DXD (124)-15-164	97	88	86	11.9	11.3	9.5	2.0	1.9	1.6	1.5	0.6	0.5
17	DXD (124)-17-192	83	86	88	14.3	12.5	8.9	2.4	2.1	1.5	1.3	0.5	0.5
18	DXD (124)-17-193	99	93	88	13.7	13.1	10.1	2.3	2.2	1.7	1.4	0.5	0.5
19	DXD (124)-17-210	82	79	77	13.7	12.5	10.1	2.3	2.1	1.7	1.3	0.4	0.5
20	DXD (124)-17-211	98	89	88	12.5	11.3	9.5	2.1	1.9	1.6	1.6	0.7	0.6
	Treatment mean	98	88	85	11.8	10.8	9.4	2.0	1.8	1.6	1.5	0.6	0.5
	Factors	CD	SE(d)	SE(m)	CD	SE(d)	SE(m)	CD	SE(d)	SE(m)	CD	SE(d)	SE(m)
	Factor(N)	0.12	0.03	0.02	0.76	0.16	0.12	1.31	0.89	0.63	0.19	0.04	0.03
	Factor(G)	0.03	0.02	0.01	0.10	0.05	0.03	0.19	0.09	0.07	0.04	0.02	0.01
	G X N	0.09	0.03	0.01	0.34	0.08	0.02	0.25	0.16	0.08	0.10	0.04	0.03
	N X G	0.12	0.04	0.03	0.75	0.18	0.13	1.50	0.90	0.64	0.16	0.05	0.04

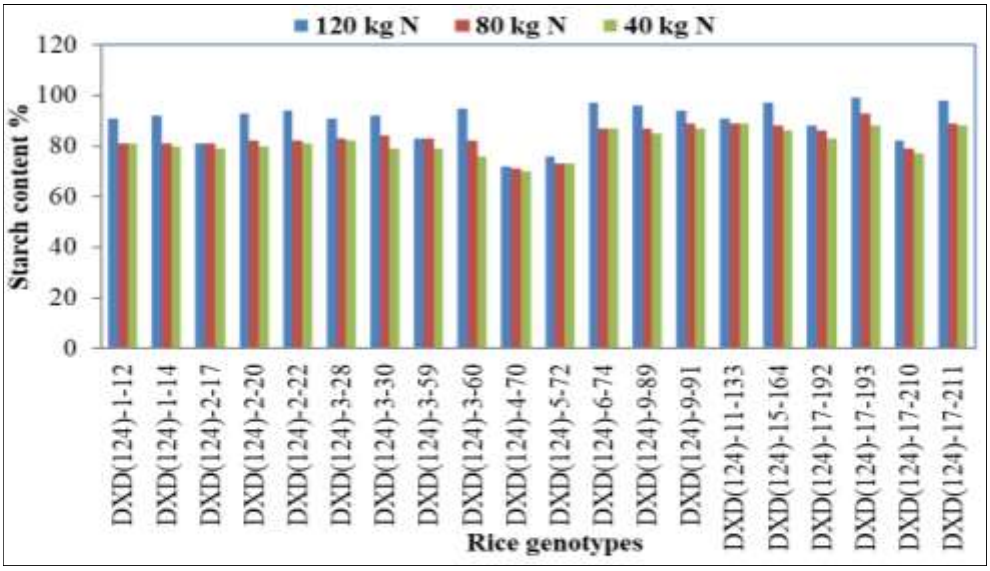

Fig 1: Effect of nitrogen levels on starch (%) of Rice genotypes

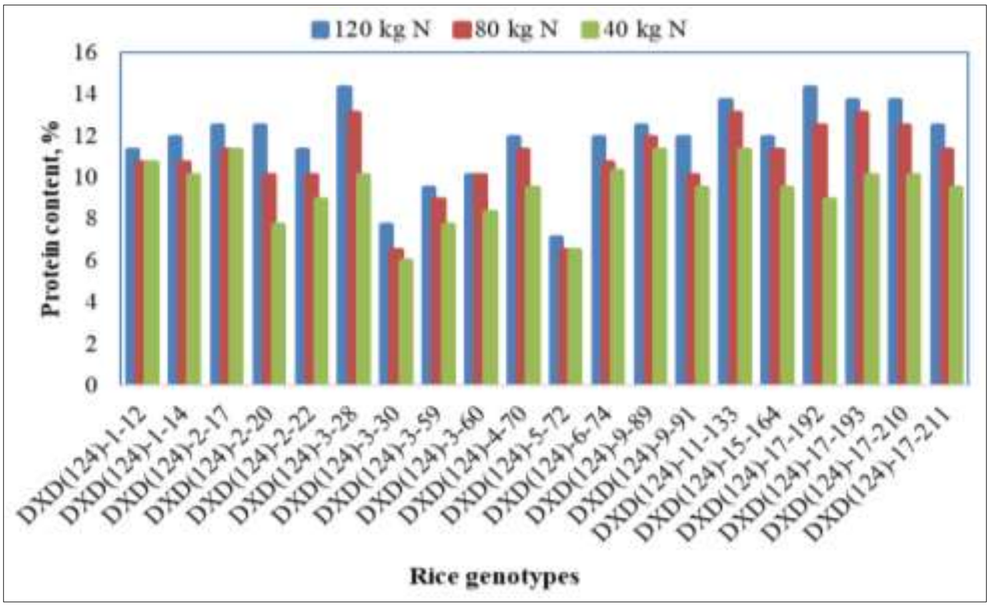

Fig 2: Effect of nitrogen levels on protein (%) of Rice genotypes

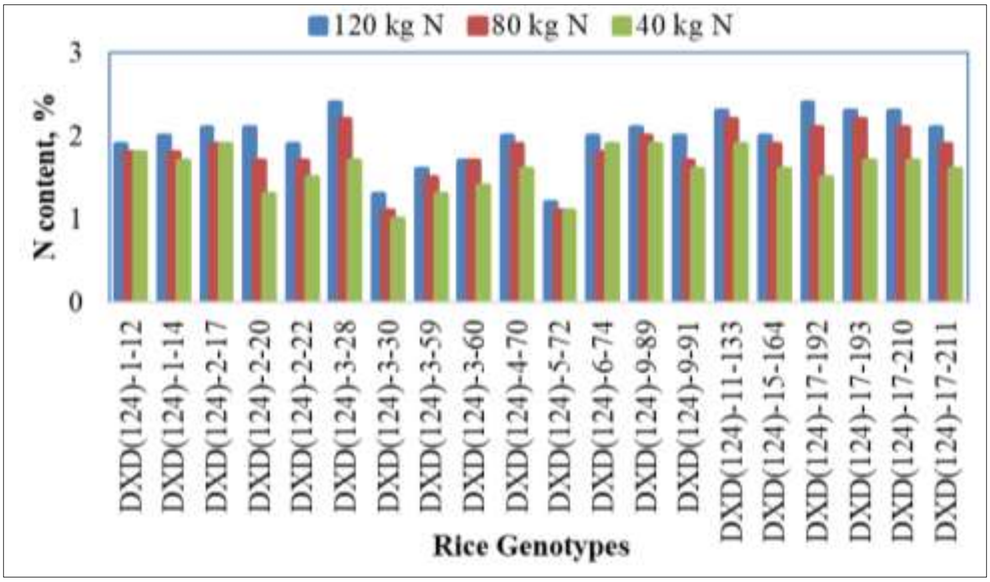

Fig 3: Effect of nitrogen levels on N content (%) of Rice grain

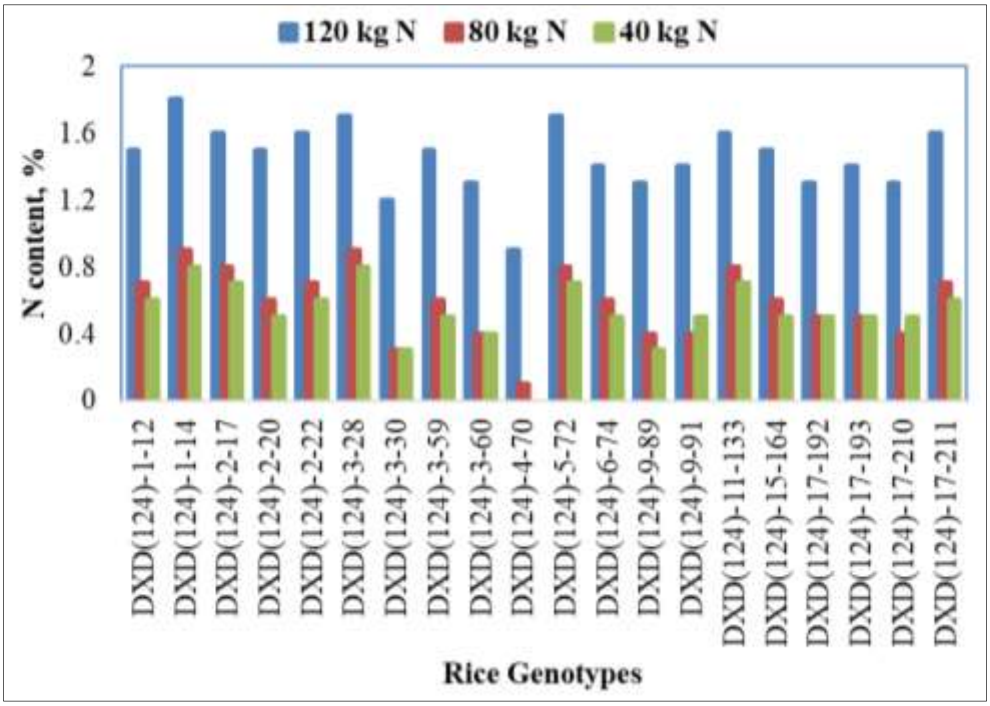

Fig 4: Effect of nitrogen levels on N content (%) of Rice leaf

Table 2: Effect of nitrogen levels on chlorophyll a and chlorophyll b, total chlorophyll content (mg g^{-1}) and SPAD value of Rice (*Oryza sativa* L.) genotypes in leaves at 50% flowering stage

S.N.	Name of rice genotypes	Chlorophyll a			Chlorophyll b			Total Chlorophyll			SPAD value		
		Pooled											
		T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
1	DXD (124)-1-12	0.79	0.65	0.52	0.48	0.44	0.37	1.31	1.08	0.73	45.3	36.7	38.3
2	DXD (124)-1-14	0.88	0.57	0.45	0.62	0.51	0.4	1.46	1.1	0.7	45.6	35.5	40.8
3	DXD (124)-2-17	1.10	0.59	0.50	0.81	0.47	0.37	1.76	1.06	0.71	43.2	32.6	36.4
4	DXD (124)-2-20	1.10	0.75	0.66	0.67	0.55	0.5	1.75	1.33	0.96	41.3	33.5	29.9
5	DXD (124)-2-22	1.05	0.88	0.68	0.57	0.49	0.43	1.72	1.44	0.93	36.7	37.9	38.3
6	DXD (124)-3-28	1.09	0.72	0.62	0.71	0.6	0.46	1.75	1.32	0.91	41.9	38.5	35.8
7	DXD (124)-3-30	0.73	0.59	0.45	0.55	0.4	0.33	1.26	1	0.66	35.7	40.5	36.0
8	DXD (124)-3-59	0.83	0.61	0.51	0.45	0.44	0.36	1.31	1.13	0.72	42.3	41.8	33.3
9	DXD (124)-3-60	1.07	0.99	0.96	0.71	0.7	0.68	1.83	1.74	1.36	41.5	40.8	34.2
10	DXD (124)-4-70	1.06	0.61	0.50	0.51	0.49	0.35	1.57	1.12	0.71	42.8	35.4	34.7
11	DXD (124)-5-72	1.22	1.06	1.02	0.79	0.76	0.66	2.14	1.93	1.36	42.1	31.0	31.2
12	DXD (124)-6-74	0.96	0.78	0.66	0.69	0.64	0.59	1.69	1.39	0.99	34.3	32.4	37.4
13	DXD (124)-9-89	0.88	0.65	0.54	0.48	0.41	0.33	1.42	1.08	0.72	40.4	35.8	33.4
14	DXD (124)-9-91	1.35	1.27	1.19	1.15	0.87	0.71	2.54	2.24	1.54	41.2	38.7	34.8
15	DXD (124)-11-133	1.22	0.94	0.90	0.88	0.64	0.6	2.04	1.67	1.25	39.7	28.8	30.8
16	DXD (124)-15-164	0.94	0.81	0.77	0.78	0.67	0.45	1.73	1.49	0.96	34.8	29.8	30.7
17	DXD (124)-17-192	1.27	0.99	0.68	0.82	0.5	0.52	2.11	1.55	0.99	36.1	36.4	33.3
18	DXD (124)-17-193	1.18	1.13	1.02	0.64	0.63	0.65	1.95	1.94	1.37	39.4	32.7	32.3
19	DXD (124)-17-210	0.98	0.59	0.54	0.84	0.44	0.34	1.65	1.04	0.75	35.9	32.8	30.1
20	DXD (124)-17-211	1.07	0.92	0.85	0.78	0.53	0.39	1.89	1.49	1.04	35.3	33.9	32.4
	Treatment mean	1.04	0.80	0.70	0.69	0.56	0.47	1.74	1.41	0.97	39.8	35.3	34.2
	Factors	CD	SE (d)	SE m)	CD	SE (d)	SE (m)	CD	SE (d)	SE (m)	CD	SE (d)	SE (m)
	Factor(N)	0.16	0.03	0.02	0.02	0.01	0.00	0.21	0.07	0.05	1.1	0.2	0.2
	Factor(G)	0.12	0.06	0.04	0.08	0.04	0.03	0.33	0.16	0.12	3.0	1.5	1.1
	GX N	0.22	0.11	0.11	0.14	0.07	0.01	0.69	0.23	0.12	5.3	2.6	0.8
	NX G	0.30	0.11	0.08	0.14	0.07	0.05	0.71	0.24	0.17	5.2	2.6	1.8

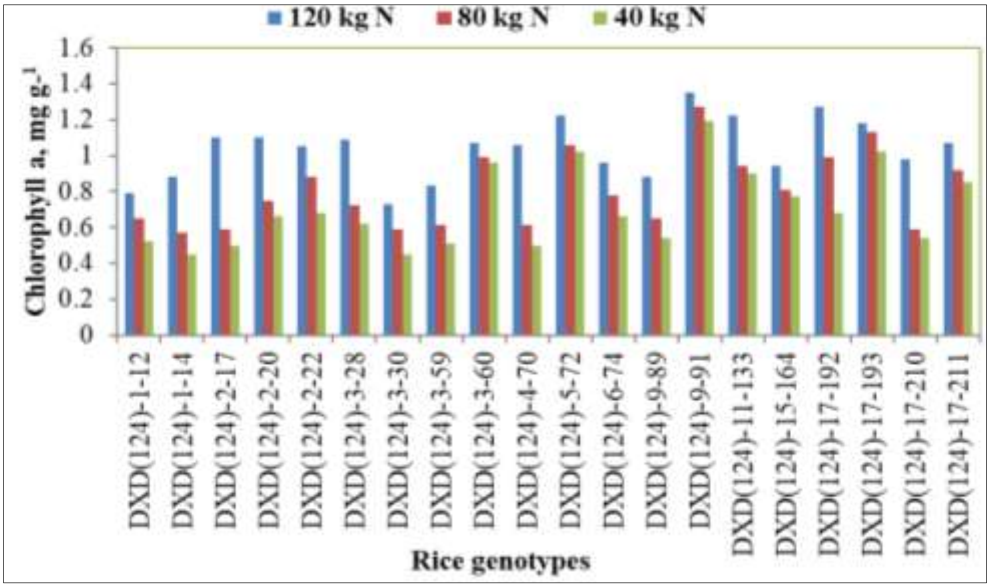

Fig 5: Effect of nitrogen levels on chlorophyll a content of Rice leaf

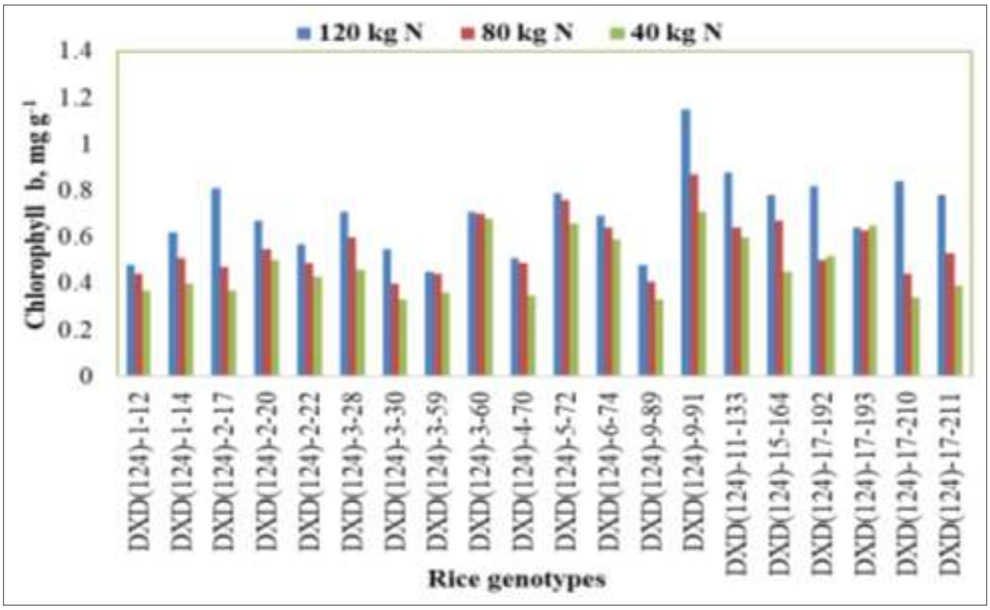

Fig 6: Effect of nitrogen levels on chlorophyll b content of Rice leaf

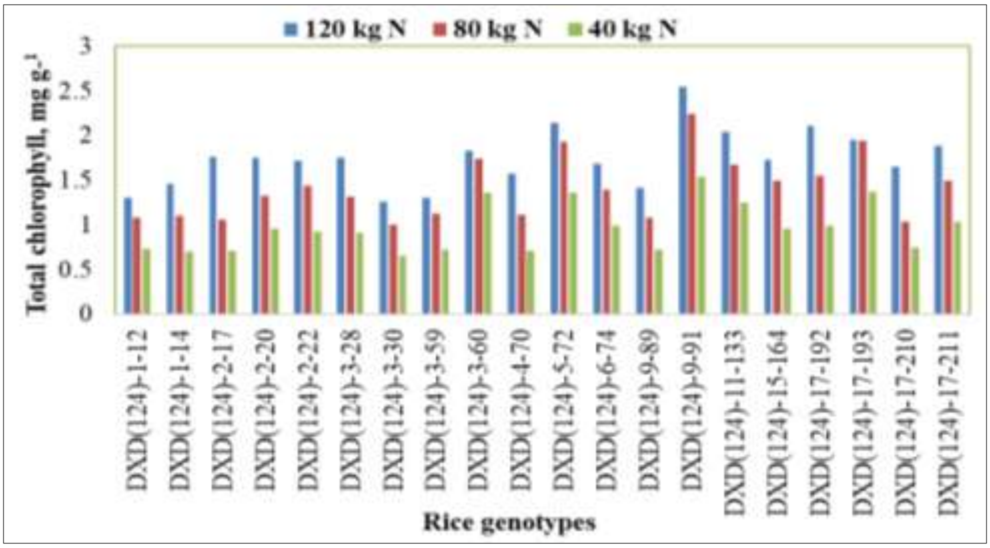

Fig 7: Effect of nitrogen levels on total chlorophyll content of Rice leaf

Conclusions

On the basis of experiment, results showed that nitrogen doses increases the starch content, nitrogen content, protein content, SPAD value and chlorophyll a, chlorophyll b and total chlorophyll content showed variation with all nitrogen levels. The significant difference among the treatments with respect to chlorophyll was noticed at 50% flowering stage. 120 kg N ha^{-1} recorded higher chlorophyll over the other treatments but there is a most important thing found that 80 kg N ha^{-1} also give similar result in all the genotypes as compared to 40 kg N ha^{-1}. So we suggested that 80 kg N ha^{-1} fertilizer should be apply to the field to control the excess use of fertilizer because 120 kg N ha^{-1} and 80 kg N ha^{-1} approximately give same result, there is no major variation observed in all the genotypes. Photosynthetic pigments like chlorophyll a, chlorophyll b, total chlorophyll and SPAD value and other biochemical parameters like starch content, protein and nitrogen content were also recorded better in similar genotypes which perform excellent in above all the parameters that is in DXD (124)-9-91, DXD (124)-17-192, DXD (124)-17-193, DXD (124)-11-133, DXD (124)-17-210, DXD (124)-15-164, DXD (124)-9-89, DXD (124)-3-28 and DXD (124)-1-14 at all nitrogen levels. So these genotypes are also recommended for high NUE genotypes which can perform better in low nitrogen dose also.

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