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PHOTOSYNTHESIS AND RELATED PHYSIOLOGICAL CHARACTERS OF RICE (*Oryza sativa* L.) GENOTYPES UNDER VARYING NITROGEN LEVEL AND REPRODUCTIVE STAGE MOISTURE STRESS CONDITION

O. P. Dhurve[1], I. M. Khan[2] and G. P. Ricchariya[3]

[1]Assistant Professor, [2]Principal Scientist Department of Plant Physiology, JNKVV, College of Agriculture, Rewa-486 001, MP, Mob: 09424975323, E-mail: op_dhurve@rediffmail.com,imkagc@gmail.com and [3]Professor, Department of Botany, Govt. P.G. College Satna (MP) Corresponding Author: O.P. Dhurve

Abstract: *Ten rice genotypes were subjected in to four nitrogen level under reproductive stage moisture stress condition to know the genotypic variations in the rate of photosynthesis, transpiration, inter cellular CO_2, stomatal conductance, chlorophyll content index and yield parameters. Higher mean photosynthesis (Pn) and transpiration rate (Trmmol) was found in genotype IET- 20924 (11.21µmol CO_2 m^2 s^{-1} and 2.26 mmol H_2O m^2 s^{-1} respectively) followed by IET- 20601 and IET- 21075. Mean stomatal conductance (0.18µ mol CO_2 m^{-2} s^{-1}), inter cellular CO_2 (204.38µ mol CO_2 m^2 s^{-1}), Chlorophyll content index (18.83) were superior in cv. IET- 20925 in two years indicated that the metabolic changes that occur as a result of drought are resistance of the photosynthetic apparatus to dehydration. Genotypic variability was observed in number of panicles/m2, number of spikelets/m^2, 1000 grain weight and in grain yield. Minimum values of these parameters were associated with IET- 21100 and Tulasi. Nitrogen treatment had significant influence on all characters during both the years and mean of all parameters increased with increasing level of nitrogen. The highest value of photosynthesis related characters and yield attributes was associated in genotype IET- 20924 and 120 kg N/ha under reproductive stage moisture stress condition. Coefficient of Correlation of Pn and Trmmol, Ci, Sc and CCi were significant to highly significant with grain yield at all levels of nitrogen.*

Keywords: *Photosynthesis, Transpiration, Intercellular CO_2, Stomatal Conductance, Chlorophyll content, Drought, Grain yield*

Introduction: Production of rice (*Oryza sativa*) is limited by the availability of water [1] and the tolerance of rice cultivars to drought stress [2]. As the rice is cultivated in a diverse range of climates and habitats and lowland varieties generally considered to be more productive, but less tolerant of drought, than the upland varieties [3]. Yield are closely related to photosynthesis under both irrigated and drought conditions [4,5]. The eflux of CO_2 for photosynthesis involves two major resistance steps at the stomata and at the surface of the mesophyll layer [6,7]. Drought stress results in various physiological changes in plants that may include, reduction in PAR, photosynthetic rate, transpiration rate, stomatal conductance, pigment degradation and relative water content (RWC) resulting in decreased water use efficiency (WUE) and growth reduction prior to plant senescence. Growth stage panicle initiation was the most sensitive one exhibiting more adverse effects on all the physiological and agronomic parameters [8]. Therefore, effective screening and analysis of the photosynthetic and gas exchange characteristics of rice genotypes under stress conditions has the potential to identify functional traits associated with optimization of water use efficiency and the maintenance of yield under drought. Rates of photosynthesis are not only determined by diffusive CO_2-uptake, but also by the activity of ribulose-1,5-bisphosphate (RubisCO) [9]. Rice yield is sensitive to water deficit at different developmental stages of plant growth. Early drought stress can affect leaf expansion and later drought stress during flowering or grain-filling stages can disrupt the formation and development of grain [3]. The photosynthetic and yield responses of ten *O. sativa* genotypes were analysed in relation to sustained drought.

Materials and Methods

The experiment was conducted at the JNKVV, College of Agriculture instructional farm Rewa, M.P. during the two successive Kharif season of 2012 and 2013. Low rainfall was (573.4 mm in 21 rainy days) was recorded during Kharif 2013 (Fig.1) however, terminal drought were experienced during both the years during vegetative and reproductive stages during the experimental period. The soil at the experimental site was a neutral in reaction (pH 7.1), low in available nitrogen (235 kg/ha) and phosphorus (9.6 kg/ha) and high in available potash (461 kg/ha). Direct seeding was done in rainfed condition. Nitrogen and phosphorus potash fertilizers were applied at the rate of 100:60:40 Kg/ha. Half dose of nitrogen + full doses of phosphorus and Potash fertilizers at the time of sowing and remaining half dose of nitrogen in two equal doses at the time of tillering and panicle initiation stages of crop growth were applied. All the recommended package of practices were adopted. Six IET-AVT breeding lines-genotypes and four released varieties were grown under rainfed conditions. Seeds were sown in dry soil in rows spaced ~20 cm apart in the 1.0 x 2.0 m plots. The experimental design was split plot with varieties as main plot and nitrogen as sub plot in three replications. Weeds were controlled by the application of standard agricultural herbicides and manual picking. During the drought stress period (reproductive phase), physiological observations was taken to know the physiological potential and efficiency in two years field experiments at flowering stage.

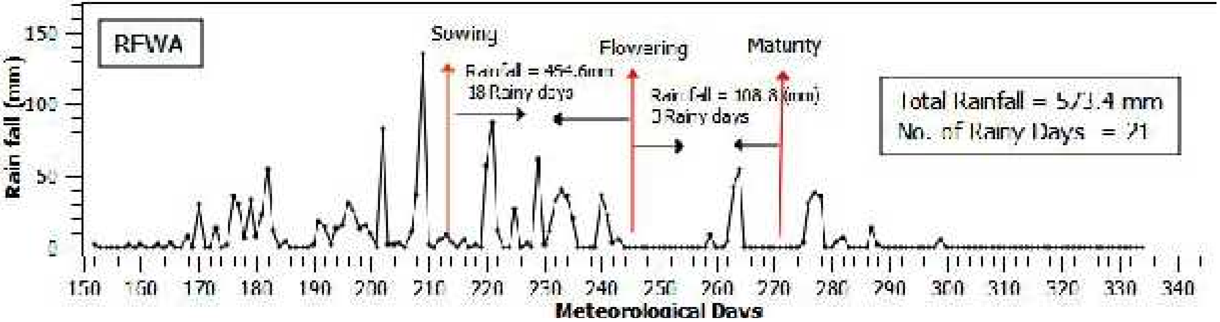

Fig. 1. Rainfall distribution and number of rainy days during crop growth period during Kharif-2013.

Leaf gas-exchange and fluorescence of plants under stress conditions were measured with a LI-6400 leaf chamber portable photosynthesis system (Li-Cor, Inc., Nebraska, USA) on flag leaves enclosed in a temperature, light and humidity controlled cuvette. The measurements were made in situ between 11.00 and 14.00, in saturating PPFD (1400 µmol $m^{-2}s^{-1}$), with leaf temperature of 32°C. The measurements of internal CO_2 (Ci), stomatal conductance (Sc, µmol $m^{-2}s^{-1}$), transpiration (Trmmol) caused the exit flow to be greater than the incoming flow rate was noted. The equation that the LI-COR-6400 used for transpiration was

$$E = \frac{F\,(W_s - W_r)}{100\,S\,(100 - W_s)}$$

When F is air flow rate (µmol s-1), WS and Wr was the sample and reference water mol fractions (mmol H2O/mol air^{-1}) and S= leaf area (cm^2), and chlorophyll content (CCi) was measured by opti-science chlorophyll meter, USA, which is a portable diagnostic tool that measured green ness or relative chlorophyll content of leaves. Chlorophyll has distinct optical absorbency characteristics and strong absorbency bands are present in the blue and red spectrum but net in the green or infra red band, an estimate of the amount of chlorophyll present in the tissue measured by the instrument. By using this infrared band to quantify bulk leaf absorbency, factors such as leaf thickness taken into account in the chlorophyll content index value. CCi was measured in the flag leaves of every treatment at the time of flowering between 11.00 to 14.00. Yield parameters and yield were recorded at harvest in ten rice genotypes. Coefficient of correlation was also worked out between yield and photosynthetic parameters as per the methods [10].

Result and Discussion

Photosynthesis Related Parameters: The rate of photosynthesis (Pn) and transpiration (Trmmol), cellular CO_2 (Ci), stomatal conductance (Sc) and chlorophyll content (CCi) of ten rice genotypes were measured at flowering stage in rainfed upland field condition. The mean rate of Pn varied significantly at flowering stages in rice genotypes. Rice breeding lines had higher Pn than released varieties. Higher Pn rate were found in the genotype IET- 20924 closely followed by IET- 21075 and IET- 20601 in two years (Fig.2) indicated the genetic efficiency that express under drought conditions. This indicated that the metabolic changes which occur as a result of drought are resistance of the photosynthetic apparatus to dehydration [6,11,5] as seems in the reversible decrease in nitrate reductase and sucrose phosphate synthesis activities [12, 13 & 8]. Nitrogen level influenced the mean rate of Pn which was 5.059 g/µmol CO_2 m^2 s^{-1} in N0 to 11.4725µmol CO_2 m^2 s^{-1} in N3 (120 kg N/ha). However, maximum increased was found between N1 to N2 followed by N2 and N3 level of nitrogen.

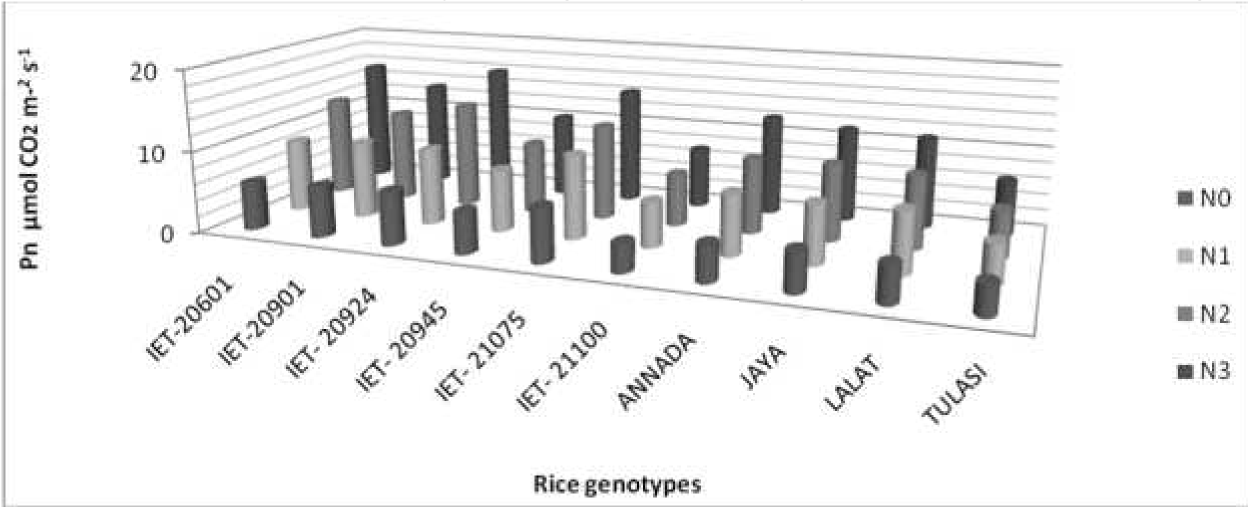

Fig. 2. Mean rate of photosynthesis *Pn* (µ mol CO_2 m^2 s^{-1}) at flowering stage in rice genotypes under reproductive stage drought and varying nitrogen level.

Rate of Trmmol H2O m^2 s^{-1} varied significantly among the genotypes with mean maximum in cv. IET- 20924 followed by cv. IET-20601 and mean minimum rate of transpiration was noted in the check cv. Tulasi and IET- 21100 in two years. However, the Trmmol was more variable during the year 2013 than that of 2012 (Fig. 3) may be due to stomatal control of water loss has been identified in all ten genotypes response to water deficit under field condition. The depression in gas exchange reduces daily carbon assimilation and water loss at the time of highest evaporation demand in the atmosphere and leads to a near optimization of carbons assimilation in relation to water supply [14, 15 & 16]. Nitrogen treatment had influenced Trmmol and maximum trmol was noted in cv. IET- 20924. Increased dose of nitrogen increased the Trmmol in two years which was 1.317 m mol H2O m^2 s^{-1} (N0) to 1.975 m mol H2O m^2 s^{-1} (N3) [17, 18].

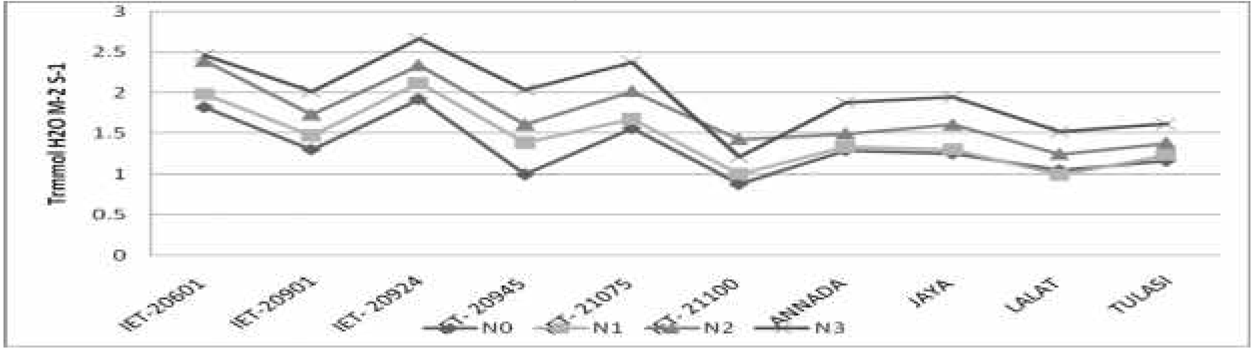

Fig. 3. Mean rate of transpiration (*Trmmol* H2O m^2 s^{-1}) at flowering stage in rice genotypes under reproductive stage drought and varying nitrogen level.

Mean Inter Cellular CO_2 level (Ci) was found superior in the genotype IET- 20924 closely followed by cv. IET- 20601 and lower value of Ci was found in cv. Lalat indicating genotypic variability in drought condition (Fig. 4) this indicated that the metabolic changes that occur as a result of drought [4, 6 & 9]. Nitrogen treatment also had significant variability with an increased Ci by increased N level in two years [18]. The Stomatal conductance (Sc) influenced by the leaf water potential particularly during drought was varied significantly among the rice genotypes. Mean maximum Sc was found in the genotype IET-20924 which was closely followed by cv. IET-20601 and 21075 while, lower value of Sc was found in the check variety Tulasi (Fig. 5). As the stomata are responded to chemical signals ie. ABA produced by dehydrating tissues while, leaf water status kept constant thus, changes in plant hydraulic conductivity have bean invoked as playing a major role in short term stomatal conductance. [14, 19, 7 & 9]. Nitrogen treatment had influenced the Sc significantly with maximum in N3 level of Nitrogen.

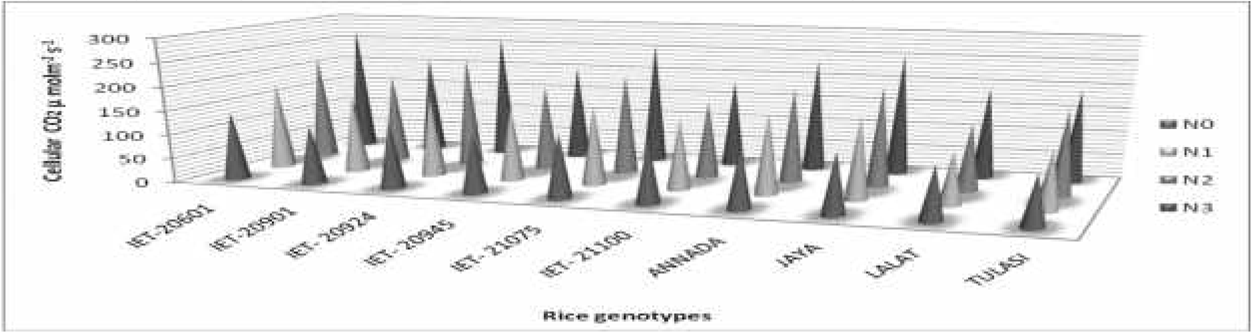

Fig. 4. Mean cellular CO_2 level (µ mol CO_2 m^2 s^{-1}) at flowering stage in rice genotypes under reproductive stage drought and varying nitrogen level in two years.

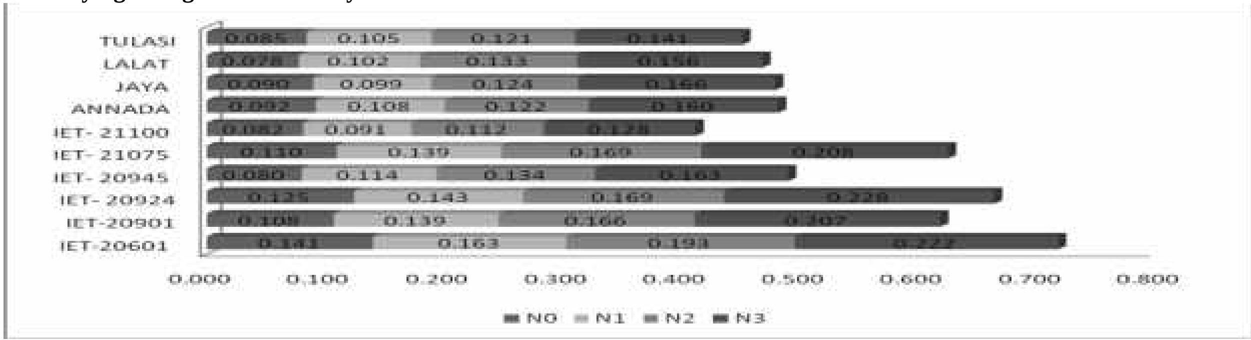

Fig. 5. Mean stomatal Conductance (µ mol m^2 s^{-1}) at flowering stage in rice genotypes under reproductive stage drought and varying nitrogen level.

Chlorophyll content index (CCi) differed significantly among the genotypes in two years and genotype IET- 20924 had maximum CCi which was closely followed by cv. IET- 20601 and IET-21075 while, mean minimum CCi was noted in the cv. Tulasi. CCi had increased significantly with the increased level of nitrogen and found maximum in N3 level of N during both years (Fig. 6) [19, 20 & 21].

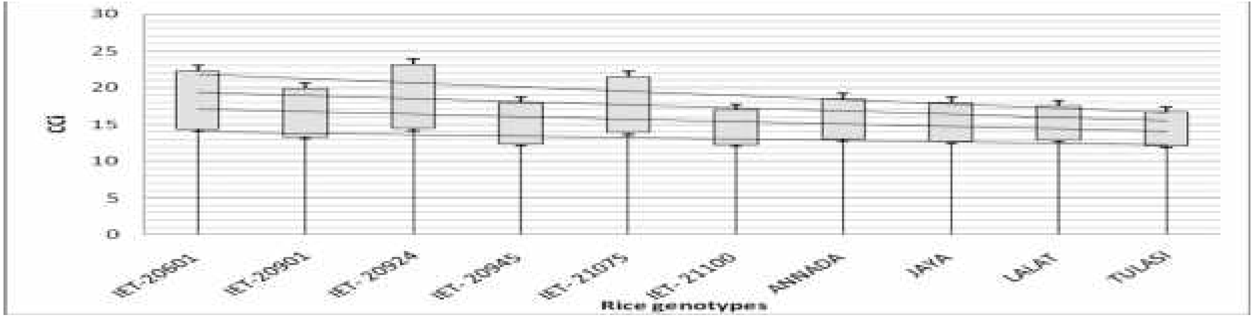

Fig. 6. Mean chlorophyll content index (*CCi*) at flowering stage in rice genotypes under reproductive stage drought and varying nitrogen level.

Yield Parameters and Yield: Significant variability among genotypes in all the yield attributing characters were found in two years. The number of panicles/m^2 (282, 307.5 & 321.5), Spikelets number/m^2 x 1000 (421.5, 441.5 & 461.5), test weight (25.85, 26.2 & 26.6 g) and grain yield (565.3, 644.5 & 773.9 g^{-1} m^{-2}) in all three level of nitrogen respectively were significantly superior in the genotype IET- 20924 while, genotype IET-21075 and IET- 20601 were at par. All other genotypes were intermediate in nature in two years under reproductive phase moisture stress condition. The level of nitrogen had increased all these yield attributing character significantly and maximum values was observed in N3 (120 kg N/ha) in two year of study (Table 1). The higher yield in these genotypes were associated with the higher value of photosynthesis related characters and yield attributing characters.

Correlation Coefficient between Photosynthetic Parameters and Grain Yield: Coefficient of correlation between photosynthetic parameters i.e. Pn, Trmmol, Ci, Sc, Cci and grain yield were calculated and presented in Table 2. Pn exhibited highly significant +ve correlation with Trmmol in all four level of nitrogen at flowering stage (r = 0.760**, 0.721**, 0.853** and 0.882**) indicated the strong association with the increasing level of nitrogen. Pn also had significant +ve association with Ci and the r value was 0.650*, 0.614*, 0.729**, 0.821** in N0, N1, N2 and N3 level of nitrogen treatment respectively. Pn exhibited highly significant +ve correlation with Sc where r = 0.739**, 0.809**, 0.896** and 0.943** for N0, N1, N2 and N3 level of nitrogen indicating strong association with increased nitrogen level. Highly significant correlation were also found between Pn and CCi which became strongest with increased level of nitrogen (r = 0.840**, 0.848**, 0.910** and 0.924** respectively). Pn had significant to highly significant +ve association with grain yield (r = 0.632*, 0.806**, 0.942 and 0.946**) in N0, N1, N2 and N3 level of nitrogen (Table 2).

Trmmol exhibited highly significant +ve correlation with Ci, Sc, Cci and with grain yield (r = 0.588**, 0.766**, 0.800** and 0.834**) in N0, N1, N2 and N3 level of nitrogen respectively. Ci exhibited significant to highly significant +ve association with Sc, Cci and with grain yield (r = 0.717**, 0.628**, 0.733** and 0.800**) in N0, N1, N2 and N3 respectively. Similar results was reported [22,8]. Similarly, Sc also exhibited significant +ve correlation with Cci (r = 0.900**, 0.912**, 0.912** and 0.965**) and with grain yield (r = 0.641*, 0.965**, 0.765** and 0.888**) in increasing level of nitrogen respectively. Cci had highly significant +ve association with grain yield [22 & 20] which became strongest with increased level of nitrogen (r = 0.707**, 0.764**, 0.851** and 0.931) in N0, N1, N2 and N3 level respectively.

Conclusion: It is concluded from the study that under reproductive stage moisture stress condition genotypic variations were recorded in the rate of photosynthetic parameters. Higher mean photosynthesis (Pn) and transpiration rate (Trmmol) was found in genotype IET- 20924 followed by IET- 20601 and IET- 21075. Mean Sc, Ci and CCi were superior in cv. IET- 20925 in two years indicated resistance to reproductive stage drought. Genotypic variability was observed in number of panicles /m2, number of spikelets /m2, 1000 grain weight and in grain yield. Nitrogen treatment had significant influence on all characters during both the years and mean of all parameters increased with increasing level of nitrogen. The highest value was associated 120 kg N/ha. Coefficient of Correlation of Pn and Trmmol, Ci, Sc and CCi were significant to highly significant with grain yield at all levels of nitrogen.

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Table 1. Yield attributing characters and yield of rice genotypes under reproductive stage drought and varying Nitrogen level (Mean of *Kharif* 2012 and 2013).

Rice	Number of panicles/m^2				No. of Spikelets/m^2 x 1000				1000 grain weight (g)				Grain Yield(g/m^2)			
Genotypes	**N_0**	**N_1**	**N_2**	**N_3**	**N_0**	**N_1**	**N_2**	**N_3**	**N_0**	**N_1**	**N_2**	**N_3**	**N_0**	**N_1**	**N_2**	**N_3**
IET-20601	221	258.5	281.5	294	376.5	397	420.5	443	22.55	23.35	23.95	24.5	380.6	456.5	600.7	737.6
IET-20901	202.5	230.5	247	267	356	385.5	399.5	416.5	22.25	23.2	23.9	24.1	363.4	455	589.4	647
IET- 20924	246.5	282	307.5	321.5	393	421.5	441.5	461.5	25.45	25.85	26.2	26.6	376.2	565.3	644.5	773.9
IET- 20945	182	214.5	227.5	243	295	323.5	338.5	357	21	21.15	22.15	22.7	311.5	429.7	507.4	586.7
IET- 21075	214	248	253.5	271.5	370.5	396.5	418	431.5	23.55	24.05	24.6	24.95	398.7	528.5	614	675.5
IET- 21100	224	200	221.5	232.5	297	315	329.5	347	20.8	21.3	21.6	21.95	365.9	420.2	492.8	565.6
ANNADA	199.5	228.5	244.5	261.5	346	361.5	388	405.5	23.45	23.95	24.25	24.5	330.4	422.8	570.8	631.9
JAYA	188.5	223.5	233	252.5	328	346.5	361	387	24.2	24.7	24.8	25.25	373.5	462.1	530.4	610.9
LALAT	185	216.5	225.5	244	316.5	330.5	354	369.5	22.25	22.55	23	23.35	334.9	409.1	499.1	573
TULASI	171	185.5	203	223.5	265	284	301	330.5	21.85	22.2	22.55	23.1	302.6	366.4	410.6	455.7
Mean	205	231.45	247.81	264.09	334.35	356.15	375.15	394.9	22.73	23.23	23.7	24.1	353.7	451.5	545.9	625.8
CD (V)	11.31	13.29	14.78	15.32	8.86	9.13	10.41	11.67	0.48	0.59	0.89	0.91	12.06	18.54	22.94	55.11
CD (N)	NS	15.17	16.33	16.4	NS	11.77	12.32	13.82	NS	0.36	0.47	0.52	NS	12.36	15.31	19.82
CD (VxN)	NS	24.32	26.81	27.05	NS	18.42	20.17	21.18	NS	NS	1.02	1.13	NS	28.31	36.22	48.62

Table 2. Correlation coefficient between physiological parameters and grain yield of rice varieties under varying levels of nitrogen under rainfed upland condition.

	N level	***Trmmol***	***Ci***	***Sc***	***CCi***	***Grain Yield***
Pn	N0	0.760**	0.650*	0.739**	0.840**	0.632*
	N1	0.721**	0.614*	0.809**	0.848**	0.806**
	N2	0.853**	0.729**	0.896**	0.910**	0.942**
	N3	0.882**	0.821**	0.943**	0.924**	0.946**
Trmmol	N0	1.000	0.714**	0.917**	0.933**	0.588*
	N1	1.000	0.722**	0.906**	0.866**	0.766**
	N2	1.000	0.875**	0.887**	0.894**	0.800**
	N3	1.000	0.851**	0.929**	0.912**	0.834**
Ci	N0		1.000	0.763**	0.775**	0.717**
	N1		1.000	0.629*	0.602*	0.628*
	N2		1.000	0.647*	0.786**	0.733**
	N3		1.000	0.765**	0.802**	0.800**
Sc	N0			1.000	0.900**	0.641*
	N1			1.000	0.912**	0.965**
	N2			1.000	0.912**	0.765**
	N3			1.000	0.965**	0.888**
Cci	N0				1.000	0.707**
	N1				1.000	0.764**
	N2				1.000	0.851**
	N3				1.000	0.931**

** and ** = Significant at 5 % & 1% level of significance, N_0 = Control, N_1 = 40 kg, N_2 = 80 kg and N_3 = 120 kg N/ha.*