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S Kujur
Dept. of Agronomy, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

A Tandon
Dept. of Agronomy, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

A Poonam
ICAR- National Rice Research Institute, Cuttack, Odisha, India

BP Mallik
ICAR- National Rice Research Institute, Cuttack, Odisha, India

S Kumar
Dept. of Agronomy, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

D Das
Dept. of Agronomy, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

J Jena
Dept. of Agronomy, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Corresponding Author:
S Kujur
Dept. of Agronomy, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Effects of planting time and nitrogen management on microbial population, efficiency of nitrogen and yield of high protein rice during wet season

S Kujur, A Tandon, A Poonam, BP Mallik, S Kumar, D Das and J Jena

Abstract
Studies on effect of different planting time (July, July-August and August) and nitrogen management of 100% RDN and 150% RDN with 2 different splits (1/2+1/4+1/4 and or 1/3+1/3+1/3) on the growth, yield and quality of high protein rice, was conducted during wet season of 2017 and 2018 at the research farm, National Rice Research Institute, Cuttack, Odisha.
Significantly higher bacteria, fungus, actinomycetes and grain yield were recorded with late planting (August) after harvest in soil during 2017, 2018 and in pooled data. Crop fertilized with 150% RDN in 3 splits (1/2+1/4+1/4) recorded higher bacteria, fungus, actinomycetes population in soil after harvesting and grain yield during both years and in pooled data, however, it was at par with 150% RDN in 3 splits (1/3+1/3+1/3). Minimum bacteria, fungus, actinomycetes population and grain yield were recorded under control (No nitrogen) at 30, 60, 90 DAT and at harvest during 2017, 2018 and in pooled data. Significantly higher partial factor productivity was recorded with application of 100% RDN in 3 splits (1/2+1/4+1/4) during 2018 and in pooled data, however, it was at par with 100% RDN in 3 splits (1/3+1/3+1/3).

Keywords: Microbial population, efficiency of nitrogen and grain yield

1. Introduction
Fertilizer applications to the field have major impact on the activity of microbial population in soil. The biological attributes are responsible for determination and maintenance of physico-chemical properties of soil such as soil pH, EC, available nitrogen, phosphorus and potassium, which effect the growth and development of the crop. Nitrogen is one of the major plant nutrient required for plant growth for maximizing the yield and encourages above ground vegetative growth of plants. Nitrogen stimulates good supply of important compounds such as amino acid, protein, enzymes, nucleic acid, hormones, vitamin and chlorophyll (Dastan *et al*., 2012, and Yang *et al*., 2010) [1, 12]. Split application of nitrogen is one of the strategies for the efficient use of nitrogen application throughout the growing season by synchronizing N application with plant demands, denitrification and leaching losses can be reduced and N uptake improved resulting in maximum grain and straw yield (Lampayan *et al*., 2010) [7]. The objective of the present investigation was to find out the effect of nitrogen management on microbial population, use efficiency of nitrogen and yield of rice.

2. Material and Methods
The field experiment was conducted at National Rice Research Institute, Cuttack, Odisha, India (20°25/N, 85°55/E) at an elevation 24 m above mean sea level from 2017 and 2018. Total rainfall during wet season 2017 and 2018 was 1034 mm and 1326 mm, respectively. It was sufficient and favorably well distributed during 2018, but less rainfall was received during wet season 2017. The maximum temperature during wet season 2017 and 2018 was 20.2 to 35.8 ^{o}C and 24 to 35 ^{o}C and the minimum temperature of the respective years in wet season was 12.1 to 28.1 ^{0}C and 20 to 30 ^{0}C. During wet season 2017 the maximum relative humidity varied 61 to 98% and the minimum was 44 to 98% and the wet season 2018 was 80 to 98and 45 to 90%, respectively. The soil of the experiment field was sandy loam containing 0.512% organic carbon determined by Wakley and Black rapid titration method and 6.29 pH determined by Glass electrode method (pH meter) (Piper, 1967). The field experiment was conducted during wet season 2017 and 2018, laid out in split plot design. Treatment consisted of three planting time *i.e.* T_1- Early planting 15 days before normal planting (July or early planting), T_2 - Normal planting (July-August) and T_3- 15 days after normal planting (August or late planting) and five nitrogen management *i*.e. S_1- 100% RDN (100:50:50 kg ha^{-1}) in 3 splits (1/3+1/3+1/3), S_2- 100% RDN in 3 splits (1/2+1/4+1/4), S_3- 150% RDN in 3 splits

(1/3+1/3+1/3), S_4- 150% RDN in 3 splits (1/2+1/4+1/4) and S_5- Control (No nitrogen). The recommended dose of fertilizer (RDF) is 100:50:50:25 kg NPK ha^{-1} was applied in the form of neem coated urea, single super phosphate (SSP), muriate of potash (MOP) and $ZnSO_4$. One third or half dose of the nitrogen, entire dose of phosphorus, potash and zinc were applied at basal dose, remaining nitrogen was applied in during active tillering and panicle initiation stage. Neem coated urea and MOP was broadcasted along the field and SSP was incorporated in the root zone of plants. Test variety 'CR Dhan311' is high protein rice cultivar developed by National Rice Research Institute, cuttack, Odisha. It is a semi-dwarf, medium duration variety (120-126 days).

2.1. Bacteria, fungus and actinomycetes population

Total soil bacteria, fungus and actinomycetes count was made by serial dilution and plate count method. These populations were computed on the basis of $g^{-1}$of dry soil using the formula:

$$\text{Total population } g^{-1} \text{of oven dry soil} = \frac{\text{Number of colonies} \times \text{dilution factor}}{\text{Dry weight of one-gram moist soil}}$$

2.2 Efficiency of nitrogen

NHI, Nitrogen harvest index = N content in grain / N content in above ground dry matter

$$\text{NUE} = \frac{\text{(Nutrient uptake with N application – Nutrient uptake without N application (GYN0)}}{\text{Amount of fertilizer N applied (FN)}}$$

$$\text{PPFN} = \frac{\text{Grain yield}}{\text{Amount of fertilizer N applied}}$$

$$\text{AEN} = \frac{\text{(Grain yield with N application - Grain yield without N application)}}{\text{Amount of fertilizer N applied}}$$

$$\text{PEN} = \frac{\text{(Biological yield with fertilized plot – Biological yield without fertilized plot)}}{\text{(Nitrogen uptake with N application- Nitrogen uptake without N application)}}$$

Where, AEN = Agronomic efficiency of nitrogen, PEN = Physiological efficiency of nitrogen, PPFN = Partial factor productivity of nitrogen, ARE= Apparent recovery efficiency and NUE= Nitrogen use efficiency

3. Result and Discussion

3.1 Population of bacteria

Maximum population of bacteria (22.69, 20.50 and 21.59 cfu g^{-1} soil × 10^6) was recorded with late planting (P_3) during 2017, 2018 and in pooled data, respectively, which was at par with normal planting (July-August) (P_2) during 2017 (Table 1). Minimum population of bacteria was recorded in early planting (July) (P_1) during 2017, 2018 and in pooled data. Pittol *et al.* (2018) [11] reported that higher bacterial population recorded in flood water associated with vegetative plant may have been favored by the higher concentration of nutrients like phosphorus and potassium. Moreover, the reproductive stage demonstrated that the environment was favourable to microbes that can persist longer in paddy fields, where they are able to utilize the degradable fraction of organic materials and survive in waterlogged condition and dry conditions. Higher population of bacteria (25.89, 22.09 and 23.99 cfu g^{-1} soil × 10^6) was recorded with 150% RDN in 3 splits (1/2+1/4+1/4) (N_4) during 2017, 2018 and in pooled data, respectively (Table 1). However, it was statistically at par with 150% RDN in 3 splits (1/3+1/3+1/3) (N_3) and with 100% RDN applied in 3 splits (1/2+1/4+1/4) (N_2) during 2017, 2018 and in pooled data. Minimum bacteria population was recorded in control (No nitrogen) during 2017, 2018 and in pooled data. Patil *et al.* (2019) [10] reported that lower population of nitrobacter might be associated with the insecticidal property of neem seed but higher population of nitrosomonas in early stage of crop. In later stage, these compounds decomposed and increased nitrobacter population. The use of urea in fertilizers, which is applied mainly during the vegetative stage, can affect the concentrations of available nutrients, especially $NH4^+$, which is the main form of available N in the soil (Dubey and Singh 2000) [2]. Application of urea alone or in combination, may have helped to increase the bacterial population, increasing its diversity in the vegetative stage of crop. In the soil profile bacterial population are predominant followed by actinomycetes and fungi.

3.2 Population of fungus

Higher population of fungus (8.81, 6.97 and 7.89 cfu g^{-1} soil × 10^4) was recorded with late planting (P_3) during 2017, 2018 and in pooled data, respectively, which was at par with normal planting (P_2) during 2017 and in pooled data. Minimum population of fungus was recorded in early planting (July) (P_1) during 2017, 2018 and in pooled data. Higher population of fungus (9.03, 8.20 and 8.61 cfu g^{-1} soil × 10^4) was recorded with 150% RDN in 3 splits (1/3+1/3+1/3) (N_3) during 2017, 2018 and in pooled data, respectively. However, it was at par with 150% RDN in 3 splits (1/2+1/2+1/2) (N_4) during both the years and in pooled data, and with 100% RDN in 3 splits (1/3+1/3+1/3) (N_1) during 2017. Minimum fungus population was recorded in control (No nitrogen) during 2017, 2018 and in pooled data. Mallikarjun and Maity was (2018) [5] reported that application of 100% N as chemical fertilizer increased the population of bacteria, fungus and actinomycetes as compared to control (No nitrogen) treatments.

3.3 Population of actinomycetes

Maximum population of actinomycetes (3.75, 4.27 and 4.01 cfu g^{-1} soil × 10^3) was recorded with late planting (P_3) during 2017, 2018 and in pooled data, respectively followed by normal planting (July-August) (P_2) during 2017 and in pooled data, and early planting (July) (P_1) during 2018. Minimum population of actinomycetes was recorded in early planting (July) (P_1) during 2017 and in pooled data, and with normal planting (July-August) (P_2) during 2018. Maximum population of actinomycetes (4.44, 4.73 and 4.58 cfu g^{-1} soil × 10^3) was recorded with 150% RDN in 3 splits (1/2+1/4+1/4) (N_4) during 2017, 2018 and in pooled data, respectively followed by 150% RDN in 3 splits (1/3+1/3+1/3) (N_3). Minimum population of actinomycetes was recorded in control (No nitrogen) treatments during both the years and in pooled data. Gill *et al.* (2016) [3] reported that maximum population of bacteria, fungus and actinomycetes was recorded with 150 kg N ha^{-1} as compared to 120 kg N ha^{-1}, 90 kg N ha^{-1} and control (No nitrogen) treatment. Naher (2013) [9] a experiment at conducted Bangladesh Rice Research Institute, Gazipur reported that the effect of long term (24 years) higher soil microbial population (Total bacteria and actinomycetes) was found in the complete fertilizer plots (N or P or K) as compared to control treatment in wetland rice cultivation system. In the lowland rice ecosystem, biological nitrogen fixation is a spontaneous process, where adequate carbon sources are available (Kennedy *et al*., 2004) [4]. Besides biological nitrogen fixation, microbes have made significant contributions in plant growth promotion. Production of growth hormones by certain beneficial microbes induces extensive root system which enhanced nutrient uptake and photosynthesis in rice (Naher *et al*., 2009b) [8].

3.4 Nitrogen use efficiency, physiological efficiency of nitrogen and nitrogen harvest index

The range of NUE of high protein rice was from 35.39 to 37.72% during 2017, 32.37 to 34.76% during 2018 and from 34.33 to 35.07% in pooled data (Table 2). The range of PEN of high protein rice was from 45.21 to 47.03%, 50.92 to 83.04% and 48.07 to 64.79% during 2017, 2018 and in pooled data, respectively. While the range of NHI of high protein rice was from 0.61 to 0.62 during 2017, 2018 and in pooled data. The range value of NUE of high protein rice was from 31.10 to 42.19%, 30.01 to 34.39% and from 30.55 to 39.39 during 2017, 2018 and in pooled data, respectively. The PEN of high protein rice was from 31.10 to 63.28%, 50.39 to 91.12% and 45.34 to 61.11% during 2017, 2018 and in pooled data, respectively. The range of NHI of high protein rice was from 0.60 to 0.64 (2017), 0.60 to 0.62 (2018) and 0.61 to 0.62 (In pooled data).

3.5 Partial factor productivity of nitrogen

Higher partial factor productivity of nitrogen (56.04 and 57.91%) was recorded with application of 100% RDN in 3 splits (1/2+1/4+1/4) (N_2) during 2018 and in pooled data, respectively. However, it was at par with 100% RDN in 3 splits (1/3+1/3+1/3) (N_1). Minimum partial factor productivity of nitrogen (32.38 and 34.04%) was recorded with 150% RDN in 3 splits (1/2+1/4+1/4) (N_4) during 2018 and in pooled data.

3.6 Grain yield

The grain yield of rice is a function of total number of panicles, number of grains panicle^{-1} and the test weight, which was significantly influenced by planting time and nitrogen management (Fig. 1 and 2). Late planting (August) registered maximum grain yield followed by normal planting (July-August) during 2017 and in pooled data, and early planting (July) during 2018. Minimum grain yield was recorded with early planting during 2017 and in pooled data, and normal planting during 2018. Application of 150% RDN in 3 splits (1/2+1/4+1/4) produced maximum grain yield, which was at par with 150% RDN in 3 splits (1/3+1/3+1/3). Minimum grain yield was recorded under control (No nitrogen) during 2017, 2018 and in pooled data. Maqsood (2000) [6] also reported that grain yield increased significantly with the application of nitrogen fertilizer.

4. Conclusion

It may be concluded that, higher bacteria, fungus, actinomycetes population and grain yield was recorded with late planting (August). Higher bacteria, fungus, actinomycetes population and grain yield was recorded with application of 150% RDN in 3 splits (1/2+1/4+1/4).

Table 1: Bacteria, fungus and actinomycetes content in soil of high protein rice as influenced by planting time and nitrogen management during wet season

Treatments	Bacteria (cfu g^{-1} soil × 10^6)			Fungus (cfu g^{-1} soil × 10^4)			Actinomycetes (cfu g^{-1} soil ×10^3)		
	2017	2018	Pooled	2017	2018	Pooled	2017	2018	Pooled
Planting time									
P_1	19.61	17.85	18.73	6.76	5.90	6.33	3.15	3.45	3.30
P_2	21.53	18.26	19.89	7.90	6.40	7.15	3.33	3.39	3.36
P_3	22.69	20.50	21.59	8.81	6.97	7.89	3.75	4.27	4.01
SEm±	0.39	0.38	0.27	0.35	0.16	0.18	0.12	0.13	0.03
CD(P=0.05)	1.55	1.51	1.06	1.39	0.64	0.72	0.45	0.50	0.12
Nitrogen management									
N_1	21.10	18.43	19.77	8.30	6.79	7.55	3.38	3.61	3.50
N_2	23.68	21.21	22.44	7.67	6.40	7.55	3.56	3.78	3.67
N_3	24.53	21.77	23.15	9.03	8.20	8.61	3.77	4.37	4.07
N_4	25.89	22.09	23.99	8.98	7.35	8.16	4.44	4.73	4.58
N_5	11.17	10.84	11.00	5.14	3.39	4.27	1.90	2.03	1.97
SEm±	0.784	0.489	0.449	0.265	0.333	0.189	0.208	0.161	0.150
CD(P=0.05)	2.29	1.43	1.31	0.77	0.97	0.55	0.61	0.47	0.44

P_1- Early planting 15 days before normal planting (July), P_2 - Normal planting (July-August), P_3- 15 days after normal planting (August)
N_1- 100% RDN (100:50:50 kg ha^{-1}) 3 splits (1/3+1/3+1/3) , N_2- 100% RDN in 3 splits (1/2+1/4+1/4) , N_3- 150% RDN in 3 splits (1/3+1/3+1/3) , N_4- 150% RDN in 3 splits (1/2+1/4+1/4) , N_5- Control (No nitrogen)

Table 2: Nitrogen use efficiency (NUE), physiological efficiency of nitrogen (PEN) and nitrogen harvest index (NHI) of high protein rice as influenced by planting time and nitrogen management during wet season

Treatments	NUE (%)			PEN (%)			NHI		
	2017	2018	Pooled	2017	2018	Pooled	2017	2018	Pooled
Planting time									
P_1	37.72	32.37	35.04	47.03	52.03	49.53	0.61	0.61	0.61
P_2	36.01	32.65	34.33	46.54	83.04	64.79	0.62	0.61	0.61
P_3	35.39	34.76	35.07	45.21	50.92	48.07	0.62	0.62	0.62
SEm±	3.06	3.22	3.12	4.39	24.86	10.35	0.008	0.009	0.006
CD (P=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS
Nitrogen management									
N_1	31.10	30.01	30.55	31.10	91.12	61.11	0.61	0.60	0.61
N_2	35.30	34.39	34.85	35.30	55.37	45.34	0.64	0.61	0.62

N_3	36.90	32.04	34.47	55.36	51.10	53.23	0.61	0.61	0.61
N_4	42.19	36.60	39.39	63.28	50.39	56.83	0.62	0.62	0.62
N_5	-	-	-	-	-	0.70	0.60	0.62	0.61
SEm±	4.24	3.76	2.24	5.06	21.00	11.88	0.01	0.008	0.007
CD (P=0.05)	NS	NS	NS	15.16	NS	NS	NS	NS	NS

P_1- Early planting 15 days before normal planting (July), P_2 - Normal planting (July-August), P_3- 15 days after normal planting (August)
N_1- 100% RDN (100:50:50 kg ha^{-1}) 3 splits (1/3+1/3+1/3) , N_2- 100% RDN in 3 splits (1/2+1/4+1/4) , N_3- 150% RDN in 3 splits (1/3+1/3+1/3) , N_4- 150% RDN in 3 splits (1/2+1/4+1/4) , N_5- Control (No nitrogen)

Table 3: Partial factor productivity of nitrogen (PPFN), agronomic efficiency of nitrogen (AEN) and grain yield of high protein rice as influenced by planting time and nitrogen management during wet season

Treatments	**PPFN (kg kg^{-1})**			**AEN (kg kg^{-1})**			**Grain yield (q ha^{-1})**		
	2017	**2018**	**Pooled**	**2017**	**2018**	**Pooled**	**2017**	**2018**	**Pooled**
Planting time									
P_1	37.02	38.85	37.94	8.27	9.30	8.78	42.83	44.43	43.63
P_2	46.03	49.80	47.92	8.97	7.27	8.12	48.66	41.33	45.00
P_3	45.21	41.96	43.58	13.32	8.12	10.72	51.71	49.23	50.47
SEm±	7.15	9.30	4.93	2.23	2.24	1.28	1.26	0.87	0.93
CD (P=0.05)	NS	NS	NS	NS	NS	NS	4.95	3.43	3.64
Nitrogen management									
N_1	38.14	51.93	45.04	7.69	7.38	7.53	44.93	44.07	44.50
N_2	59.78	56.04	57.91	9.62	8.29	8.96	46.87	44.98	45.92
N_3	35.70	32.38	34.04	10.87	7.93	9.40	53.54	48.58	51.06
N_4	37.39	33.79	35.59	12.56	9.33	10.94	56.09	50.68	53.38
N_5	-	-	-	-	-	-	37.24	36.69	36.97
SEm±	9.91	6.41	4.98	1.81	1.29	0.96	2.03	1.40	1.09
CD (P=0.05)	NS	19.18	14.90	NS	NS	NS	5.93	4.08	3.17

P_1- Early planting 15 days before normal planting (July), P_2 - Normal planting (July-August), P_3- 15 days after normal planting (August)
N_1- 100% RDN (100:50:50 kg ha^{-1}) 3 splits (1/3+1/3+1/3) , N_2- 100% RDN in 3 splits (1/2+1/4+1/4) , N_3- 150% RDN in 3 splits (1/3+1/3+1/3) , N_4- 150% RDN in 3 splits (1/2+1/4+1/4) , N_5- Control (No nitrogen)

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