PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol x(x): xx–xx
https://doi.org/10.14719/pst.2810

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RESEARCH ARTICLE

Correlation and regression studies of SPAD based leaf nitrogen estimation as impacted by nitrogen and zinc management under medium land rice in Alfisols of Odisha

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OPEN ACCESS

ARTICLE HISTORY

Received: 22 September 2023
Accepted: 09 February 2024

Available online
Version 1.0 : 04 September 2024

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE

Rath S, Mohapatra A K, Dash S. Correlation and regression studies of SPAD based leaf nitrogen estimation as impacted by nitrogen and zinc management under medium land rice in Alfisols of Odisha. Plant Science Today (Early Access). https://doi.org/10.14719/pst.2810

Abstract

One of the most significant staple crops in the world is rice (*Oryza sativa* L.), its productivity largely depends on various factors, including nutrient management. In particular, nitrogen and zinc management play crucial roles in rice cultivation as they significantly impact the leaf nitrogen content, which is closely associated with plant growth, photosynthesis and ultimately grain yield. Field tests were conducted at the Agricultural Research Station, Chhatabara, SOA (Deemed to be University), Bhubaneswar, Odisha, India during the *kharif* of 2021 and 2022 to measure the effect of nitrogen and zinc management on the productivity of rice crop yield. Split plot design was employed in this experiment in 3 replications The findings of the experiment revealed that 125 % higher the recommended dose of nitrogen (RDN) gave significantly the highest pooled, periodic SPAD values, leaf nitrogen contents, grain (5700 kg/ha) and straw yield (6880 kg/ha) respectively. This was at par with the treatment where 75 % of the RDN was supplied through Urea and the rest 25 % through FYM. Among the zinc management practices, one soil application and 3 foliar sprays of zinc sulphate @ 2.5 kg/ha and 0.5 % respectively, at the stages of active tillering, heading and panicle initiation yielded significantly than the other treatments. The SPAD value and leaf nitrogen content showed a positive correlation with grain and straw yields in rice crops across different growth stages and years.

Keywords

Rice; Nitrogen; zinc; SPAD value; leaf nitrogen content; correlation-regression

Introduction

Rice (*Oryza sativa* L.), is considered one of the most significant staple crops in the world, providing food security for a significant portion of the global population (1). Achieving sustainable and high-yielding rice production is of utmost importance to meet the increasing demands for food. Over 3.5 billion individuals get more than 20 % of their daily calories from rice (2). In India it is the staple food for more than 65 % of the population (3). Rice holds paramount significance in the context of sustainable development goals due to its role as a staple crop for a substantial portion of the global population. As a primary source of nutrition, it directly influences Goal 2 (Zero Hunger) and Goal 3 (Good Health and Well-being). Furthermore, the sustainable cultivation and management of rice fields can contribute to achieving Goal 13 (Climate Action) by mitigating greenhouse gas emissions associated with agriculture, fostering resilient ecosystems and sustainable water use (4). Fertilizer management practices, specifically soil test-based nitrogen management are of great importance for optimizing crop yield, improving nitrogen use efficiency and ensuring environmental sustainability. Various advancements have been made in this field, including the use of innovative methods such as deep placement of urea super granules, subsurface incorporation of urea and site-specific nitrogen management (5). Additionally, the use of leaf color charts, chlorophyll meters and optical sensors can help in accurately determining the nitrogen requirements of rice crops, thereby reducing excessive nitrogen application (1). Soil nitrate tests are also crucial for nutrient management in annual crops, as they aid in making field-specific nitrogen fertilization decisions and complying with regulatory programs (6). Furthermore, early-season soil nitrate levels can be used to determine the nitrogen requirement of corn and monitoring inorganic nitrogen content throughout the soil profile can help optimize nitrogen application methods (7). Overall, effective fertilizer management practices based on soil testing are essential for maximizing crop profitability, maintaining soil health and minimizing nutrient loss to water resources. Nitrogen (N) and zinc (Zn) are vital nutrients that play crucial roles in the growth, development and productivity of rice plants (8). Efficient management of these nutrients is vital for optimizing crop productivity while minimizing environmental impacts (9). Therefore, understanding the dynamics of N and Zn management and their impact on leaf nitrogen content estimation is imperative for sustainable rice production. Leaf nitrogen content serves as a reliable indicator of the nutritional status and photosynthetic capacity of plants (10). Measuring leaf nitrogen content accurately and non-destructively can provide valuable insights into the nutrient status of rice plants and guide farmers in implementing precise fertilizer management practices. The chlorophyll meter, also known as the Soil-Plant Analysis Development (SPAD) meter, has emerged as a widely used tool for estimating leaf nitrogen content rapidly and non-destructively. The relative chlorophyll content, which is strongly connected to leaf nitrogen concentration, is measured by the SPAD meter and can be used as a proxy for assessing plant nutrient status.

The current study aims to look into the influence of nitrogen and zinc management on leaf nitrogen content estimation using SPAD meter readings in medium land rice cultivation in the Alfisols of Odisha, India. The study area in Odisha represents a significant rice-growing region with Alfisols as the dominant soil type (11). By examining the relationships between SPAD values, leaf nitrogen content and rice yield, this research will provide valuable insights into optimizing nutrient management strategies to enhance rice productivity while minimizing environmental risks.

Correlating SPAD values with leaf nitrogen content and yield is crucial for validating the accuracy and reliability of the SPAD meter as a means for nutrient management decision-making. By establishing robust correlations and regression models, this study aims to develop a practical framework for estimating leaf nitrogen content using SPAD readings in the context of medium land rice cultivation. Such knowledge can aid farmers and agronomists in optimizing fertilizer applications based on real-time measurements of leaf nitrogen status, ultimately leading to improved rice productivity, resource-use efficiency and environmental sustainability.

Materials and Methods

Experimental Particulars and Crop Husbandry

The experiment was carried out during *kharif* season of 2021 and 2022 at the Agricultural Research Station, Chhatabara, Siksha 'O' Anusandhan (Deemed to be) University, Bhubaneswar, Odisha, located in the east and south eastern coastal plain Agro-Climatic Zone of Odisha (20˚15' N latitude and 85˚41'E longitude, 45 m above MSL) (Fig. 1). The average atmospheric conditions are represented in Table 1. The soil of the experimental site was sandy loam. The details of the soil conditions are expressed in Table 2.

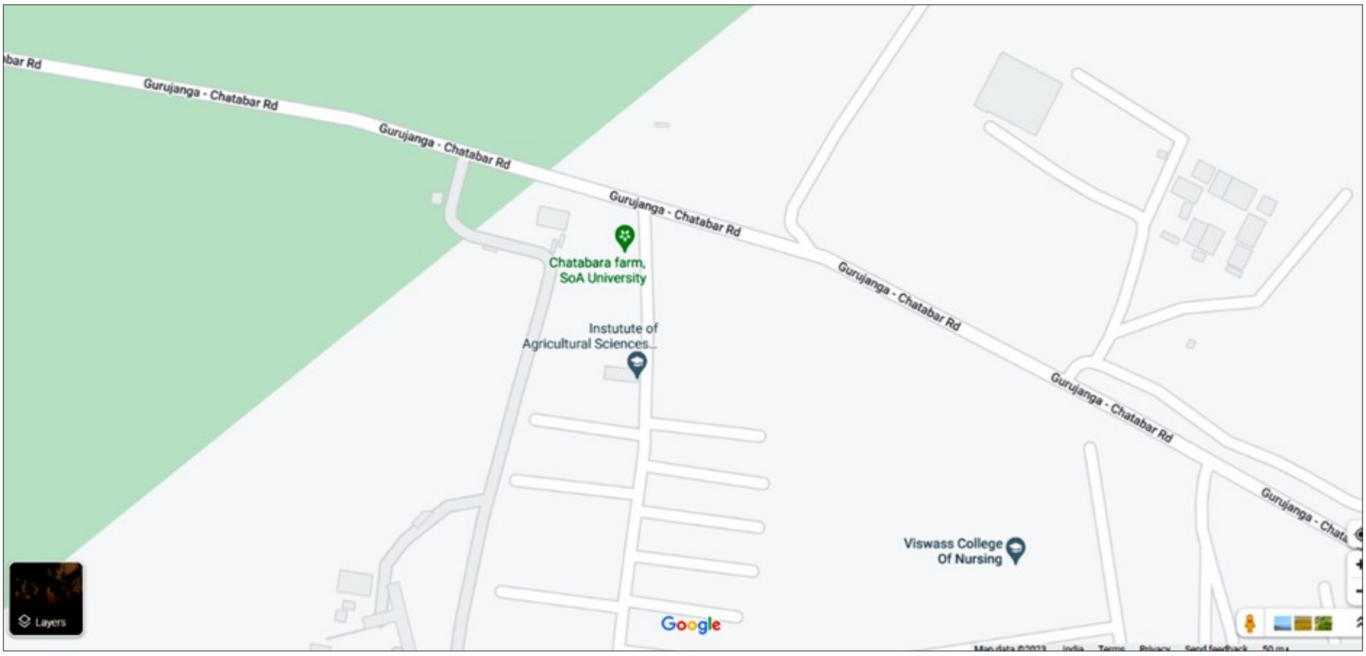

Fig. 1. Geographical location of the study area: Agricultural Research Station, SOA (Deemed to be University), Bhubaneswar, Odisha, India.

Table 1. Monthly weather parameters during the study period at the study site, Bhubaneswar, India.

Month	Tmax (˚C)		Tmin (˚C)		Rainfall (mm)		RH (%)	
	2021	2022	2021	2022	2021	2022	2021	2022
July	32.3	32.6	25.5	25.5	312.1	312.1	84	86
August	32.3	32.7	25.5	25.7	324.7	324.7	88	85
September	32.3	32.5	25.2	25.3	321.5	321.5	82	84
October	32.2	32.5	23.0	23.3	166.6	166.6	79	77

Table 2. Physicochemical properties of the soil.

Particulars	Composition	Method adopted
Organic carbon (%)	0.43	Walkley and Black chromic acid wet oxidation method (7)
pH	5.7	Buckman pH meter (7)
Electrical conductivity (dS/m at 250 C)	0.17	Digital electrical conductivity method (7)
Available N (kg/ha)	208	Kjeldahl method (7)
Available P_2O_5 (kg/ha)	21.3	Olsen"s extraction method (Reagent used 0.5 N NaHCO3 extractable) (8)
Available K_2O (kg/ha)	128.0	Flame photometric method (7)
DTPA extractable Zinc (mg kg-1)	0.41	Atomic absorption spectrophotometer method (9)

With 3 replications and a net plot size of 6 m x 5 m, the experiment was set up using a split-plot design. The main plot treatments were 4 in number, consisting of different doses and sources of nitrogen. Sub-plots were allotted with 4 zinc management practices. The experimental details of the treatments are represented in Table 3. Irrigated medium early variety 'Kalinga Dhan-1201' was used for the experiment. To grow the nursery, 10 kg of recommended seed per ha was used. The pre-soaked seeds were sown in the nursery on 26.07.21 and 29.07.22 respectively, after soaking overnight. It was transplanted in the main field on 28.08.21 and 01.08.22 respectively, at a spacing of 20 cm x 10 cm. The main plot size was 5 m x 4 m. The method of establishing the nursery using wet beds was employed. Rice seeds were manually scattered on the pulverized soil for this reason. At the time of the last land preparation, farm yard manure (FYM)@5 t/ha was applied uniformly to all the treatments (8). Before going for transplanting, at the time of puddling operation, one-fourth of the doses of fertilizers were administered according to the allocated treatments. Urea, DAP (Diammonium Phosphate), MOP (Muriate of Potash) and zinc sulphate were used as the nutrient sources. The soil test-based NPK recommendation was 100:50:50 kg/ha of N: P_2O_5: K_2O for the chosen cultivar as nitrogen, phosphorous and potassium were deficient in the test area and the recommended dose of fertilizer was 80:40:40 kg/ha of N: P_2O_5: K_2O (12). The crop was harvested on 28.11.21 and 30.11.22 respectively in both the years.

Table 3. Treatments followed in the experiment.

Treatments denotation	Treatment details
Main-plot (4) nitrogen management practices	
N1	75% soil test based nitrogen (STN)
N2	100 % STN
N3	75% STN + 25% N through FYM
N4	125% STN
Sub-plot (4) levels of zinc	
Z0	Control (No Zinc)
Z1	Zn 5kg/ha soil application
Z2	Zn 2.5 kg/ha soil application + $ZnSO_4$ 0.5% spray at active tillering
Z3	Zn 2.5 kg/ha soil application + $ZnSO_4$ 0.5% spray at active tillering+PI+heading stage

Table 4. Details of nitrogen doses as per treatment.

Treatments denotation	Dose of nitrogen applied as per STN
N1	75 kg/ha N
N2	100 kg/ha N
N3	75 kg/ha N + 5 t/ha FYM
N4	125 kg/ha N

Soil Sample Collection

Two days prior to transplanting operation, on a cloud-free day, surface soil (0–20 cm) samples were taken from random 5 places in the field using a soil auger. To ascertain the soil's physicochemical properties, the composite soil samples were air-dried, pulverised and put through one mm and 0.149 mm sieves. Following Bao (2000)'s method, the soil's pH, organic matter, total nitrogen, accessible phosphorus and exchangeable potassium were measured (9). The soils' physical and chemical characteristics are presented in Table 2.

SPAD Reading

The SPAD value (chlorophyll content) was estimated by the use of SPAD-502 (Minolta Camera Co. Ltd, Osaka, Japan). A fully grown leaf from the top of the plant was selected for recording the SPAD values and the mean of 5 measurements per plant was recorded. For the mean SPAD value for each treatment, 5 plants were chosen at random. Observations were recorded at 20 DAS, till harvesting of the crop at 20 days interval.

Leaf Nitrogen Content

The plants selected for SPAD readings were suitably tagged. These plant samples were split into stems and leaves, including sheaths, then dried at 105 °C for 30 min, then at 70 °C until constant weight. The samples were then measured for dry weight and subjected to Kjeldahl wet oxidation technique analysis to determine leaf N concentration (LNC) (13).

Grain Yield, Straw Yield and Harvest Index

After the crop had fully matured and 80–90 % of the grains had turned straw-colored, the crop was harvested. The crop was ground level cut. The crop was transported to the threshing floor in individual bundles according to plot. Each unit plot's grain and straw yields were properly sun-dried (72 h) and weighed. The final grain yield per plot and grain yield per plant were calculated by adding the dry weight of the grains and straw from 5 sample plants to the corresponding unit plot yield. The total biological yield included both grain and straw yield. The rice grain and straw yield for each plot was used to construct the harvest index, which was then reported as a percentage (Eqn 1).

$$\text{Harvest Index (\%)} = \frac{\text{Economic Yield}}{\text{Biological yield}} \times 100 \quad \text{...........(Eqn. 1)}$$

Statistical Analysis

The data gathered from the experiment on various rice crop observations were pooled across 2 years and subjected to analysis using the conventional ANOVA method (13).

Results and Discussion

SPAD Value and Leaf Nitrogen Content

Results showed that there were significant differences between SPAD value and nitrogen content (%). among different treatments (Table 5). The SPAD value can also be used to directly monitor the N status of rice leaves and choose when to apply top dressing (14). Results showed that SPAD value increase with the increase in the age of the plants, but gradually decreases when it approaches maturity. Application of higher nitrogen (125 % Soil test-based nitrogen (STN)) recorded significantly higher SPAD value compared to other N environments but was at par where 75 % of the STN was supplied through Urea and the rest through FYM. Among the zinc management practices, the significantly highest SPAD value was obtained from Z3 treatment where there was 2.5 kg/ha soil application of zinc sulphate along with 3 foliar sprays of 0.5 % zinc sulphate at active tillering, PI and heading stage. A similar trend was also found in the case of leaf nitrogen content. When nitrogen is applied in both organic and inorganic forms at the appropriate times, the chlorophyll content is increased, leading to greater SPAD values. According to a study, nitrogen is a component of the enzymes involved in the synthesis of chlorophyll (15) and in another study, it was found that the chlorophyll concentration reflects the relative level of crop nitrogen status and yield (11). In case of zinc management, the decrease in chlorophyll concentration and the irregular structure of chloroplasts may have contributed to the observed drop in photosynthesis in zinc-deficient plants. According to a study, lack of zinc affects the ability of plants to produce chlorophyll (16). Zinc's function in proteins and enzymes as a structural and catalytic component as well as a co-factor for the development of pigment biosynthesis is responsible for the increased chlorophyll concentrations (16).

Grain Yield, Straw Yield and Harvest Index

The experimental results revealed that the significantly highest grain (5700 kg/ha) and straw yields (6880 kg/ha) were obtained from the application of 125 % higher of fertilizer than RDN treatment which was at par with 75 % of the RDN supplied by inorganic fertilizer and rest 25 % of RDN by FYM treatment among the nitrogen management practices (Table 6). Table 7 and Table 8 showed the two-way interaction between the nitrogen and zinc management practices and revealed that the treatment combination of 125 % higher nitrogen application along with Zn 2.5 kg/ha soil application + ZnSO4 0.5 % spray at active tillering + PI + heading stage gave better yield than the other treatments. When more nitrogen is available, the rice plants can produce more chlorophyll, leading to improved

Table 5. Periodic SPAD values and leaf nitrogen content of leaves under different nitrogen and zinc management practices in rice (pooled).

Treatments	**SPAD value**						**Leaf nitrogen content (%)**					
	20 DAS	**40 DAS**	**60 DAS**	**80 DAS**	**100 DAS**	**At harvest**	**20 DAS**	**40 DAS**	**60 DAS**	**80 DAS**	**100 DAS**	**At harvest**
N1	30.06	30.60	33.43	22.63	6.73	5.65	1.72	1.94	2.23	2.02	1.84	1.68
N2	30.87	31.47	33.93	23.17	7.15	5.88	1.78	2.02	2.31	2.09	1.89	1.66
N3	31.27	32.19	34.24	23.45	7.68	6.31	1.84	2.15	2.39	2.18	1.99	1.7
N4	32.08	32.40	34.98	23.54	8.15	6.45	2.21	2.22	2.52	2.29	2.08	1.75
SE(m)±	0.29	0.23	0.26	0.10	0.28	0.04	0.14	0.06	0.07	0.05	0.04	0.03
CD (5%)	0.89	0.75	0.85	0.33	0.91	0.18	0.42	0.18	0.20	0.15	0.11	0.08
Z0	30.58	30.98	33.68	22.42	7.05	5.85	1.78	1.91	2.12	1.98	1.84	1.64
Z1	30.86	31.47	34.03	22.86	7.43	5.88	1.81	1.99	2.24	2.01	1.88	1.68
Z2	31.33	31.85	34.26	23.25	7.60	6.05	1.88	2.08	2.38	2.15	1.92	1.71
Z3	31.51	32.36	34.63	23.63	7.81	6.18	1.96	2.25	2.42	2.16	1.94	1.79
SE(m)±	0.05	0.06	0.04	0.03	0.03	0.01	0.05	0.08	0.03	0.02	0.01	0.04
CD (5%)	0.15	0.18	0.12	0.10	0.11	0.04	0.14	0.23	0.10	0.05	0.04	0.09

Table 6. Yield of rice under various nitrogen and zinc management practices (pooled).

Treatments	Grain yield (Kg/ha)	Straw yield (kg/ha)	Harvest index (%)
N1	4120	4940	0.45
N2	5060	6090	0.45
N3	5560	6730	0.44
N4	5700	6880	0.45
SE(m)±	64	760	0.003
CD (5%)	220	264	0.011
Z0	4240	5760	0.45
Z1	5130	6040	0.45
Z2	5410	6270	0.45
Z3	5650	6560	0.45
SE(m)±	27	320	0.003
CD (5%)	77	920	0.009

Table 7. Effect of nitrogen and zincmanagement practices on rice grain yield (2-way table).

Nitrogen management	Zinc management practices				
	Z0	**Z1**	**Z2**	**Z3**	**Mean**
N1	3.72	3.92	4.34	4.49	4.12
N2	3.89	5.05	5.65	5.66	5.06
N3	4.49	5.65	5.85	6.23	5.56
N4	4.88	5.90	5.82	6.21	5.70
Mean	4.24	5.13	5.41	5.65	
	N	**Z**	**N within Z**	**Z within N**	
Sem	0.064	0.027	0.087	0.054	**Sem**
CD 5%	0.220	0.077	0.247	0.154	**CD 5%**
CV %	4.14	1.75			**CV %**

Table 8. Effect of nitrogen and zinc management practices on rice straw yield (2-way table).

Nitrogen management	Zinc management practices				
	Z0	**Z1**	**Z2**	**Z3**	**Mean**
N1	4.46	4.71	5.21	5.38	4.94
N2	5.67	6.06	6.18	6.45	6.09
N3	6.38	6.63	6.71	7.21	6.73
N4	6.55	6.78	6.99	7.20	6.88
Mean	5.76	6.04	6.27	6.56	
	N	**Z**	**N within Z**	**Z within N**	
Sem	0.076	0.042	0.120	0.083	**Sem**
CD 5%	0.264	0.119	0.340	0.238	**CD 5%**
CV %	4.14	2.26			**CV %**

photosynthetic activity and enhanced energy production for growth and grain development (17, 18). Similar trends were found in studies conducted by various recent literatures (6, 19, 20). Among the zinc management practices, significantly highest yield was obtained from Z3 treatment with 3 foliar sprays and one soil application of zinc sulphate (Table 6). This can be due to the fact that zinc is a vital constituent of many hormones, enzymes, required for protein synthesis, photosynthesis and chlorophyll formation, gives disease resistance, which in turn aided to uplift the yield of the rice crop. Similar results were obtained through experiments conducted in various reports (3, 7, 12).

The SPAD value and the leaf nitrogen content (%) were positively correlated with the grain and straw yields at each stage of growth of the rice crop in both of the years (Table 9 a-f; Fig. 2 a-f). They were also positively correlated with each other. It implies that as the SPAD value and leaf nitrogen content increase, the grain and straw yields of the rice crop also increase. This suggests that higher chlorophyll and nitrogen concentrations in the leaves are

Table 9. Pearson's correlation matrix among the SPAD value, Leaf Nitrogen content (%), straw yield (kg/ha), grain yield (kg/ha) and Harvest index (%) of rice under different nitrogen and zinc management practices at (**a**) 20 DAS; (**b**) 40 DAS; (**c**) 60 DAS; (**d**) 80 DAS; (**e**) 100 DAS and (**f**) At harvest.

a

	SPAD	Leaf N content (%)	Grain yield (kg/ha)	Straw yield(kg/ha)	Harvest index (%)
SPAD	1				
Leaf N content (%)	0.90811	1			
Grain yield (kg/ha)	0.911819	0.701927428	1		
Straw yield(kg/ha)	0.93975	0.743069887	0.928893172	1	
Harvest index (%)	-0.13096	0.08503273	-0.295112667	-0.372824779	1

b

	SPAD	Leaf N content (%)	Grain yield (kg/ha)	Straw yield(kg/ha)	Harvest index (%)
SPAD	1				
Leaf N content (%)	0.94795	1			
Grain yield (kg/ha)	0.971666	0.898891491	1		
Straw yield (kg/ha)	0.962743	0.840515753	0.928893172	1	
Harvest index (%)	-0.32336	-0.254642806	-0.295112667	-0.372824779	1

c

	SPAD	Leaf N content (%)	Grain yield (kg/ha)	Straw yield(kg/ha)	Harvest index (%)
SPAD	1				
Leaf N content (%)	0.897171	1			
Grain yield (kg/ha)	0.905184	0.880839519	1		
Straw yield (kg/ha)	0.901707	0.782869104	0.928893172	1	
Harvest index (%)	-0.07493	-0.20292973	-0.295112667	-0.372824779	1

d

	SPAD	Leaf N content (%)	Grain yield (kg/ha)	Straw yield(kg/ha)	Harvest index (%)
SPAD	1				
Leaf N content (%)	0.894956	1			
Grain yield (kg/ha)	0.941841	0.834201457	1		
Straw yield (kg/ha)	0.844301	0.81310385	0.928893172	1	
Harvest index (%)	-0.30338	-0.270030862	-0.295112667	-0.372824779	1

e

	SPAD	Leaf N content (%)	Grain yield (kg/ha)	Straw yield(kg/ha)	Harvest index (%)
SPAD	1				
Leaf N content (%)	0.902997	1			
Grainyield (kg/ha)	0.925962	0.81507275	1		
Straw yield(kg/ha)	0.937232	0.851480637	0.928893172	1	
Harvest index (%)	-0.20323	-0.335903009	-0.295112667	-0.372824779	1

f

	SPAD	Leaf N content (%)	Grain yield (kg/ha)	Straw yield(kg/ha)	Harvest index (%)
SPAD	1				
Leaf N content (%)	0.680058	1			
Grain yield(kg/ha)	0.863956	0.732663282	1		
Straw yield (kg/ha)	0.9401	0.598833934	0.928893172	1	
Harvest index (%)	-0.42165	0.01034452	-0.295112667	-0.372824779	1

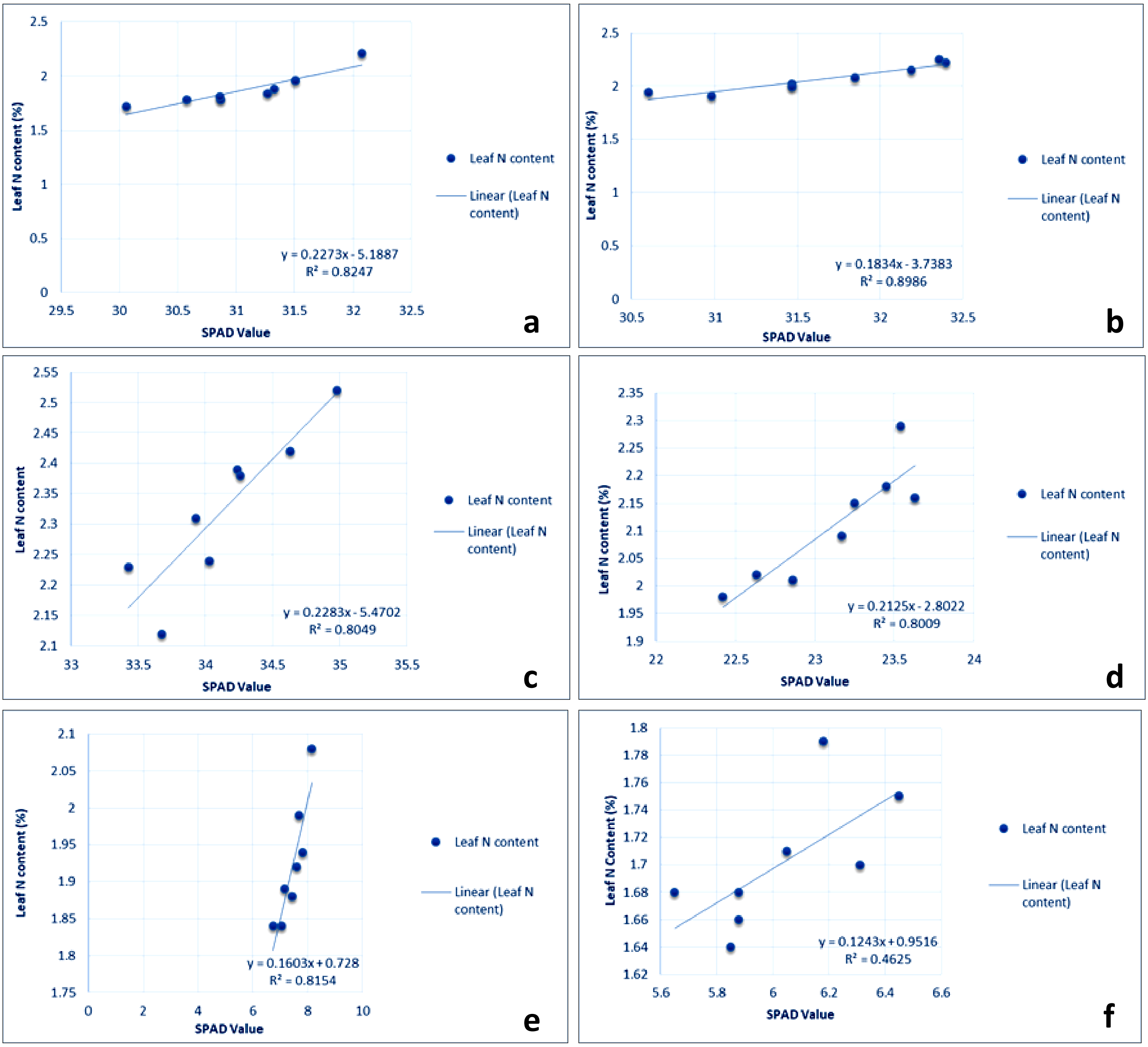

Fig. 2. Relationship between SPAD values and the leaf nitrogen content (%) of rice as affected by different nitrogen and zinc management practices at (**a**) 20 DAS; (**b**) 40 DAS; (**c**) 60 DAS; (**d**) 80 DAS; (**e**) 100 DAS and (**f**) at harvest.

associated with better plant health and higher productivity in terms of grain and straw production throughout the stages of growth of the rice crop. A similar positive correlation between SPAD value, leaf nitrogen content and yields were obtained from some experiments (14, 21).

Conclusion

From the study we can conclude that 125 % higher RDN along with one soil application and 3 sprays of zinc sulphate resulted in higher pooled SPAD value, leaf nitrogen content and higher yield in the rice crop. Additionally, there was a positive correlation among periodic SPAD levels and corresponding leaf nitrogen concentrations. Also, the rice crop yields. Thus the aforesaid treatment combination assures better growth of the rice crop in the agro-climatic zone, i.e., East and South-eastern coastal plain of Odisha.

Acknowledgements

The authors are thankful to Siksha 'O' Anusandhan Deemed to be University for providing the required facilities for carrying out the field research and publication.

Authors' contributions

SR was responsible for carrying out the work, manuscript preparation and communication of the manuscript, SD has reviewed the manuscript and AKM has provided guidance and helped throughout the whole process.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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