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Elucidating morphogenic and physiological traits of rice with nitrogen substitution through nano-nitrogen under salt stress conditions

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Abstract

Background Sustainable crop production along with best nutrient use efficiency is the key indicator of smart agriculture. Foliar application of plant nutrients can complement soil fertilization with improved nutrient uptake, translocation and utilization. Recent developments in slow releasing, nano-fertilizers in agriculture, begins a new era for sustainable use and management of natural resources. This study aims to explore the effectiveness of nano-nitrogen usage on plant growth, yield attributes and sustaining rice production while optimizing fertilizer N application through conventional (prilled urea) and nano-N source under salt stress conditions.

Results The strategic substitutions of traditional urea by nano-nitrogen was distributed from partial to complete with 33, 50, 66 and 100% applications. Further, the strategic substitutions were compared in saline (ECe ~ 6.0 dSm^{-1}) and sodic stress (pH ~ 9.1) conditions along with normal soils to dissect the beneficial response of nano-N in two rice varieties (CSR 30 and PB 1121). Salt stress affected the plant performance by decreasing leaf relative water content upto 10%, total chlorophyll content by 1.3–1.5%, leaf area upto 29.9%, gas exchange attributes by 10–39%, with concomitant yield reductions upto ~ 4%. Collateral improvement in leaf greenness (SPAD index) crop growth rate and net assimilation rate was observed with foliar application of Nano-N. 0.2–1.64% enhancement in growth traits, 0.93–1.85% in physiological traits, and comparable yield gains with 100% recommended dose of prilled were comparative with nano-substitutions. Salt tolerant rice variety, CSR-30 performed better than PB 1121 with better expression of morphological, physiological and yield traits under stress conditions and nitrogen substitutions.

Conclusions Overall, our experimental findings revealed agricultural use of nano-N in improving the plant physiological efficiency and optimizing rice yields with partial N substitution through nano fertilizers under salt stress

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conditions. These studies are further open for futuristic aspects of long term effects of nano-fertilizers on soil nutrient depletion in correlation to yield enhancement in salt affected soils.

Keywords Nano-nitrogen, Physiological traits, Basmati rice, CSR-30, Salinity, PB1121

Background

Creating a sustainable food future (physical supply, economic access and supply stability) forever galloping population, agricultural production needs to be increased by ~70% linking productivity gains with inevitable cropland expansion, protection of natural ecosystems and enhanced climate resilience and GHG mitigation [1–3]. Sustained rice production in South Asia is vital to global food and nutritional security, as 90% of world's rice production is confined to Asia pacific region. In India, rice-wheat system (RWS) occupies nearly 9.2 million hectares (mha) area, with more than 85% is practiced in Indo-Gangetic Plains of Uttar Pradesh, Punjab, Haryana, Bihar, and West Bengal states [4]. During the post-green revolution period, indispensable use of fertilizers especially nitrogen (N) and realistic genetic improvements allowed significant productivity gains and self-sufficiency in agricultural production. For a better crop stand, plant nutrition, crop quality and food production, the share of fertilizer inputs alone is estimated to be 50–55% at global scale [5]. However, farmer's perception of applying higher N inputs in expectations of better yield realization has negatively outpaced concomitant improvement in yield gains with lower factor productivity. Previous studies have shown that imbalanced and excessive N application beyond crop needs (30–40% utilization efficiency in most agricultural situations) tends to N losses through NO_3^- leaching, NH_3 volatilization and N_2O emission and associated environment pollution [5–7]. In India, the combined consumption of fertilizer N in RWS amounts to 4.15 million tonnes (MT) of total nitrogen usage in agriculture [8].

Mineral nutrients are determinant factors for plant growth and development and are currently recognized as potential signal molecules, specifically under abiotic stress conditions [9]. Limited research investigations are available pointing out that beyond plant nutrition, macro- and micro-elements can act as signaling molecules in plant responses under adverse environmental conditions [10]. Being a vital component of plant metabolic processes, chlorophyll, proteins, metabolites, and nucleic acids as well as for the enzyme activities, N contributes towards yield formation and grain quality [11]. Reactive nitrogen species (RNS), just like reactive oxygen species (ROS) in plants have been reported to be involved in signaling functions along with generation of adequate responses to overcome the plant stress under challenging conditions [12]. Nitrogen is absorbed by plants mainly in the form of nitrate (NO_3^-) and ammonium (NH_4^+) and both these forms are in short supply in agricultural and natural ecosystems [13]. The competition between NO_3^-/NH_4^+ and other ions across the plasmalemma, affects plant tolerance to different types of stress factors including salinity, nutrient deficiency or elemental toxicity where sensitive plants may require more fertilizer N compared with plants encompassing better resilience [14]. Physiological and proteomic analyses revealed that increased N uptake and assimilation imparted plant tolerance to alkali stress, but the underlying mechanism remains unclear [15]. Additionally, nitrogen has also been used for mitigating salinity consequences for its osmoregularity effects depending on the type and amount of nitrogen source supplied, its pattern of mineralization over time and the salinity levels of the soil [16] e.g. using NH_4^+ as sole source of N improves salt stress tolerance of plants [17]. Therefore, exploring the interaction mechanism between plant N nutrition and salt stresses may be helpful to improve plant growth under abiotic stresses further reducing its negative impact on the ecological sustenance.

Systematic initiatives to restore natural resources are required to make intensive agriculture more sustainable while simultaneously guarding ecosystem disruptions with minimal environmental footprints. In view of this, a wide range of nano-fertilizers are being developed for smart nutrient delivery to the plants [18]. Use of these slow release smart nano-fertilizers (small size, high surface area-volume ratio, controlled delivery, easy absorption and high reactivity) is an eco-friendly and novel approach to substitute bulky chemical fertilizers with increased use efficiency, lower production cost while mitigating the environmental footprints [19, 20]. Although there have been several studies on beneficial effects of nano-formulations of Fe, Zn and Cu [20] yet relatively limited information is available pertaining to agricultural use of nano-N fertilizers on nutrient supply, plant growth and yield realization and that too in salt affected soils. With this hypothesis, the present investigation aimed at (i) evaluating the crop performance under variable N application through conventional (prilled urea) and nano-N source under salt stress conditions, and (ii) identifying the specific morpho-physiological traits related to efficient N use and sustaining rice production while optimizing the fertilizer N requirements through nano-N. The information generated herein will help in enabling efficient N supply, better resource conservation and sustainable rice yields under stress conditions while minimizing undesirable consequences of environmental pollution in salt affected agro-ecosystems in India and similar ecologies.

Materials and methods

Experimental setup and treatment details

This pot experiment was carried out for two consecutive years (*kharif* 2021 and 2022) in Randomized Complete Block Design (RCBD) with three replications at the Research Farm of ICAR-Central Soil Salinity Research Institute, Karnal (29°43’ N latitude,76°58’ E longitude). The experimental site represents monsoonal sub-tropical climatic conditions with hot summers (May-June) and cool winters (Dec-Jan) with mean annual precipitation of 750 mm; of which three-fourth is received during July-September months. The effectiveness of N applied via a conventional source [prilled urea; hereafter referred as PU] and N substitution through nano-N [liquid formulation, 4% N (w/v) with 20–50 nm particles; IFFCO product hereafter referred as nN] was tested across two rice varieties [CSR 30 Basmati (CSR 30), Pusa Basmati 1121 (PB 1121)] and three stress (normal, saline and sodic) environments. To understand the plant mechanism and changes in morpho-physiological and yield-related traits in response to N nutrition under salt stress conditions, variable stress environments were created by taking different soil types in 20 kg capacity porcelain pots, filled with 16 kg normal soil ($EC_e \sim 0.62\ dSm^{-1}$ & pH ~ 8.2), sodic ($EC_e \sim 0.91\ dSm^{-1}$ and pH ~ 9.1) and saline ($EC_e \sim 6.0\ dSm^{-1}$ and pH ~ 8.3] soils. The crop water requirement was maintained by applying good quality irrigation water ($EC_{iw} \sim 0.6 \pm 0.05\ dSm^{-1}$) under normal and sodic stress environments while salinity stress was maintained by applying irrigation water of $EC_{iw} \sim 6\ dSm^{-1}$. The pot house was covered with high density transparent polythene sheet to prevent rain water, and also to maintain the desired levels of salt stress.

Four treatments using different nitrogen (N) substitution through nano-N viz., 33% N substitution [1/3 N as PU + 1/3 N as PU + 1/3 N as nN; hereafter, referred as N_{33}], 50% substitution [1/3 N as PU + 1/3 N (½ each as PU and nN) + 1/3 N as nN; N_{50}], 66% substitution [1/3 N as PU + 1/3 N as nN + 1/3 N as nN; N_{66})], 100% substitution [complete N as nN; N_{100}] were evaluated using fixed plot technique measuring 10.75 m × 6 m plot size. In addition, two treatments; absolute control (no Nitrogen-fertilizer; N_0) and recommended dose of N (N_{RD}) were also kept in replicated form for comparison purpose. Treatment-wise fertilizer N, each using a different fertilizer scheduling, was done in 3 equal splits (basal, 25 and 45 days after sowing) using 60 kg N ha^{-1} in CSR 30 and 90 kg N ha^{-1} in PB 1121 in normal soil with additional 25% N in saline and sodic soils [21]. To reduce the experimental error, uniform application of P and K was done in all the treatment pots. Before the conduct of experiment, the initial status of nitrogen (N), phosphorus (P) and potassium (K) in soil was observed as low in N (152–182 kg ha^{-1} $KMnO_4$-N), medium in organic carbon (0.55–0.71%) & available P (20.5–22.7 kg ha^{-1}Olsen’s-P), and high in available K (245–270 kg ha^{-1} NH_4OAc-K). Rice seedlings (30–35 days old) were transplanted during first fortnight of July each year, and the crop was harvested during first fortnight of November depending on the physiological maturity.

Morpho-physiological observations and estimations

Different morpho-physiological and yield-related traits were recorded at reproductive stage, and accordingly variety-oriented growth indices in relation to plant N nutrition under variable salt stress were estimated. Plant height was recorded in randomly selected five plants per plot with measuring scale. The total number of tillers and effective tillers were recorded from five tagged plants. The leaf greenness was measured using the SPAD meter (SAPD-502 Plus, KONICA MINOLTA, INC, Japan). Leaf area index (LAI) was measured using CI-110 Plant Canopy Imager (CID). Leaf area (cm^2) was measured using Portable Laser leaf area meter (Model CI-202, Spectra Agritech). Total chlorophyll content (TCC, mg/gFW) was estimated using acetone method of Arnon [22]. Fresh leaves were initially collected from the plant, cut into small disks, and subsequently incubated at 65˚C until the leaf disks were completely colorless. Absorbance of the samples was measured at 645 nm and 663 nm using 80% acetone as a blank on a UV spectrophotometer (SPECORD 210 PLUS).

$$Total\ chlorophyll\ content\ (mg/gFW) = 20.2\,(A_{645}) - 8.02\,(A_{663}) \times \left[\frac{V}{1000 \times W}\right]$$

Where V – volume made (ml); W – weight of tissue(g).

The relative water content (% RWC) was estimated as per the procedure outlined by Barrs and Weatherly [23]. The representative leaf samples were collected and immediately weighed to record the fresh weight (FW). These leaves were then immersed in distilled water for 4 h in closed petri dishes for estimating the turgid weight (TW). The leaf tissue was then incubated at 65 °C for 72 h, or until a constant dry weight (DW) was attained. The relative water content (%) was calculated using the formula as:

$$A = \pi\, r^2 Relative\ water\ content\ (\%) = \left[\frac{FW - DW}{TW - DW}\right] \times 100$$

Where FW -fresh weight (g); DW-dry weight (g); TW-turgid weight (g).

The fully developed flag leaves were used to measure the photosynthetic rate (Pn, umol m^{-2} s^{-1}), stomatal conductance (gS, mol m^{-2} s^{-1}) and transpiration rate (E, mmol m^{-2} s^{-1}) using Portable Photosynthesis System (LI-6800, LICOR Inc., Lincoln, NE, USA). Cuvette conditions were maintained at a photosynthetic photon flux density (PPFD) of 1000 µmol $m^{-2}s^{-1}$, relative humidity > 60%, ambient CO_2 concentration of 400 ppm and leaf temperature of 25 °C [24]. Instantaneous Water use efficiency (WUEi) was calculated as the ratio of photosynthetic and transpiration rate (Pn/E).

Relative growth rate (RGR) represents the increase in total plant dry weight over a specific time interval, relative to the initial weight. It is expressed as the dry matter increment per unit biomass per unit time, or the grams of dry weight increase per milligrams of initial dry weight, and is expressed as mg $g^{-1}day^{-1}$.

$$\textit{Relative growth rate}\ \left(\mathrm{mg\,g^{-1}day^{-1}}\right) = \frac{log_e W_2 - log_e W_1}{t_2 - t_1}$$

Where, W_1 and W_2 is the whole plant dry weight at t_1 and $t_2$time interval (in days), respectively.

The crop growth rate (CGR) explains the dry matter accumulated per unit area per unit time (g m^{-2} day^{-1}).

$$\textit{Crop growth rate}\ \left(\mathrm{g\,m^{-2}day^{-1}}\right) = \frac{(W_2 - W_1)}{\rho\,(t_2 - t_1)}$$

Where, W_1 and W_2 are whole plant dry weight at time t_1 and t_2;

ρ is the ground area on which W_1 and W_2 are recorded.

Net Assimilation Rate (NAR) is defined as the dry matter increment per unit leaf area or per unit leaf dry weight over a given time period. It serves as a measure of the average photosynthetic efficiency of the leaves in a crop community. NAR is expressed as the milligrams of dry weight increase per unit dry weight or area per unit time (mg $g^{-1}day^{-1}$).

$$\mathrm{Net\ assimilation\ rate}\ \left(\mathrm{mg\,g^{-1}\,day^{-1}}\right) = \frac{(W_2 - W_1)}{(t_2 - t_1)} \times \frac{(log_e L_2 - log_e L_1)}{(L_2 - L_1)}$$

Where,

W_1 and W_2 is the whole plant dry weight at time t_1 and t_2;

L_1 and $L_2$is the leaf weight or leaf area at time t_1 and t_2;

$t_1$and t_2 are time interval in days.

The crop was harvested manually at the time of physiological maturity. After harvesting, the plant produce was bundled, dried in the sun for 5–7 days and their weight was recorded. For each treatment, the data on biological yield and grain yield was expressed as g/plant. Before analysis, observations under each variable were tested for normality (Q-Q plot of residuals) through Shapiro-Wilk (W) test. Violated variables were transformed through appropriate transformation method. The data was statistically analyzed by General Linear Model (GLM) procedure for Randomly Complete Block Design (RCBD) using SAS software of Indian NARS Statistical Computing Portal (ICAR-IASRI, New Delhi) (www.stat.iasri.res.in/sscnarsportal). Pair-wise treatment comparisons were performed using the least significant difference at 5% level of significance. PCA based Correlation matrix of pooled data was generated using ggally package v. 2.1.2 in R v. 4.4.0.

Results

The experiment was conducted for two years during Kharif- 2020 and 2021 to evaluate the effect of nano-N on rice performance under abiotic stress. The data evaluated across different traits has non-significant effect over two years; hence, the data was pooled over and analyzed in factorial completely randomized design (RCBD).

Morpho-physiological traits

Soil nutrition and climatic conditions significantly affects the plant morphology and plant height is an essential quantitative trait for predictive grain production and biomass. We observed that plant height was significantly affected by the prevailing stress conditions of salinity or sodicity, (Table 1) with a decrease of 8–10% although CSR 30 had higher plant height (98.14 cm) than PB-1121 (91.39 cm). Although, different nitrogen substitutions did not alter the plant height very significantly as treatments N_{33} (33% N replacement through nano-N) and N_{50} (50% N replacement through nano-N) depicted non-significant differences in comparison to N_1 (recommended dose of urea). But higher N substitution of urea with nano-N (N_{66}&N_{100}) significantly reduced the plant height.

Relative water content (RWC) represents appropriate measure of plant hydration status since it represents physiological consequence of cellular water deficit. On average, RWC was recorded higher in PB 1121 (68.4%) than CSR 30 (63.05%) with mean values of 68.63% in control, 67.01% in sodic stress and 61.54% in salinity showing more moisture loss under salinity (10.33%) in comparison to sodic stress (2.36%). With N-substitutions significant differences in RWC were observed where, relative water content at N_{33} (71.04%) was statistically at par with recommended dose of urea, N_{RD} (70.39%). Further, increase in N substitution resulted in reduction of RWC in range of 0.94%, 2.13% and 14.95% respectively in comparison to RDN, N_{RD} (Table 1).

Leaf chlorophyll is a vital photosynthetic pigment that largely determines plant greenness related to photosynthetic capability and ultimately plant development. Total chlorophyll content varied significantly within variety

Table 1 Effect of different N-substitutions on morpho-physiological traits in rice under salt stress conditions

Treatments/Traits	Plant height cm	RWC %	TCC mg/g	SPAD	Pn umol m^{-2} s^{-1}	gS mol m^{-2} s^{-1}	E mmol m^{-2} s^{-1}	WUEi
				Variety (V)				
CSR 30	98.14^{a}	63.05^{b}	1.57^{a}	33.68^{a}	15.7^{a}	0.744^{a}	10.08^{a}	1.65^{b}
PB 1121	91.39^{b}	68.4^{a}	1.51^{b}	29.6^{b}	14.29^{b}	0.697^{b}	8.51^{b}	1.72^{a}
LSD (*P* ≤ 0.05)	2.96 (0.0)	1.98	0.037	0.343	0.82	0.027	0.75	0.092
				Stress conditions (S)				
Control condition	101.9^{a}	68.63^{a}	1.78^{a}	34.0^{a}	17.7^{a}	0.917^{a}	10.46^{a}	1.79^{a}
Sodic stress, pH ~ 9.1	91.99^{b}	67.01^{ab}	1.52^{b}	31.53^{b}	14.43^{b}	0.684^{b}	9.37^{b}	1.63^{c}
Saline stress, ECe ~ 6.0 dSm^{-1}	90.4^{c}	61.54^{c}	1.32^{c}	29.38^{c}	12.86^{c}	0.561^{c}	8.05^{c}	1.69^{b}
LSD (*P* ≤ 0.05)	1.15	0.64	0.018	0.421	0.194	0.022	0.185	0.038
				Nitrogen substitutions (N)				
N_0 (Without N)	78.02^{d}	54.4^{d}	1.30^{d}	25.01^{e}	10.75^{d}	0.545^{c}	4.71^{c}	2.28^{a}
N_{RD} RDN through PU	100.72^{ab}	70.39^{a}	1.63^{ab}	35.99^{a}	16.38^{ab}	0.777^{a}	10.98^{a}	1.49^{c}
N_{33} [1/3 PU + 1/3 PU + 1/3 nN]	102.21^{a}	71.04^{a}	1.65^{a}	33.96^{b}	16.6^{a}	0.788^{a}	11.07^{a}	1.50^{c}
N_{50} [1/3 PU + (½ PU + ½ nN) + 1/3 nN]	100.47^{ab}	69.73^{ab}	1.62^{ab}	33.7^{c}	16.31^{ab}	0.789^{a}	11.03^{a}	1.49^{c}
N_{66} [1/3 PU + 1/3 nN + 1/3 nN] –	99.5^{b}	68.89^{b}	1.6^{b}	33.34^{c}	16.17^{b}	0.777^{a}	10.92^{a}	1.49^{c}
N_{100} [1/3 nN + 1/3 nN + 1/3 nN]	87.64^{c}	59.87^{c}	1.44^{c}	27.83^{d}	13.78^{c}	0.645^{b}	7.06^{b}	1.96^{b}
LSD (*P* ≤ 0.05)	1.63	0.91	0.025	0.595	0.275	0.031	0.261	0.053
LSD (*P* ≤ 0.05) S × N	2.14	1.20	0.037	NS	0.296	0.036	0.308	NS
LSD (*P* ≤ 0.05) V× N	1.75	0.98	0.03	0.841	0.242	NS	0.251	0.065
LSD (*P* ≤ 0.05) V× S	1.24	0.69	0.021	0.595	0.171	NS	0.178	0.046
LSD (*P* ≤ 0.05) V× S × N	3.03	1.70	0.052	NS	0.419	NS	0.435	0.113

Means followed by at least one letter common are not statistically significant ($p<0.05$) using LSD test; RWC–relative water content; TCC–total chlorophyll content; Pn–photosynthetic rate; gS–stomatal conductance; E–transpiration rate; WUEi–instantaneous water use efficiency

with higher content in CSR 30 and decreasing gradually with salt stress. Although, PU and nN when used in different combinations had statistically similar values for chlorophyll content (1.6–1.65 mg g^{-1} FW), but with application of nano-N replacing full nitrogen, N_{100}, significant reduction in chlorophyll content to the extent of11.66–12.73% was noticed. Correspondingly, SPAD reading (measure of greenness in leaves) also showed significant variation among varieties, stress condition and different N-substitutions (Table 1). SPAD values reduced significantly (14%) in saline soils albiet to a lesser extent in, CSR 30 than PB 1121. Although the interactive effect of these factors was non-significant but leaf greenness was relatively more with application of recommended dose of urea (N_{RD}) irrespective ofN substitution through nN.

Gas exchange attributes

Gas exchange traits including photosynthetic rate (Pn), stomatal conductance (gS), transpiration rate (E) and instantaneous water use efficiency (WUEi) showed a significant variability among tested varieties, stress conditions and N-substitutions (Table 1). On average, CSR 30 variety recorded higher Pn (9.9%), gS (6.7%) and E (18.4%) than PB 1121, whereas reverse was the trend for WUEi being 4.2% higher in PB1121. A reduction to the tune of 18.5–21.3% in Pn, 25.4–38.8% in gS by and 10.42–23.04% in E was recorded as a result of N substitution through nN in sodic and saline stresses, respectively, with greater reductions in saline conditions. Photosynthetic rate (Pn) was highest (16.6 µmol m^{-2} s^{-1}) in N_{33} followed by N_{RD}(RDN) and N_{50} (50% N replacement through nano-N), and lowest in N_{66} and N_{100}. No significant differences were observed between stomatal conductance and transpiration rate irrespective of treatment combinations. Notably higher WUEi was obtained under N_0 and N_{100} treatments whereas other treatments showed statistical at par WUEi values although the interactive effect of all the factors was significant except for soil × N-substitutions.

Physiological growth analysis

Plant growth analysis is a quantitative description of environmental impacts on different traits used to calculate net photosynthetic production. Among these, leaf area (LA), leaf area index (LAI), relative growth rate (RGR), crop growth rate (CGR), and net assimilation rate (NAR) are particularly essential in measuring crop growth and may be an indication of prospective productivity. Leaf area affects the plant development under stress and with N-substitutions because it represents light interception capacity of plants. Notably, it was observed that leaf area/plant was highest in N_{33} (786.6 cm^2), followed by N_{RD} (783.7 cm^2), N_{50} (777.5 cm^2) and N_{66} (770.6 cm^2), which reduced by 14.06% with N_{100} (Table 2). In general, the significant reduction in Leaf area was recorded with

Table 2 Effect of different nitrogen substitutions on growth traits in rice under salt stress conditions

Treatments/ Traits	**LA cm^{2}**	**LAI m^{2}m^{-2}**	**RGR mg g^{-1}day^{-1}**	**CGR g m^{-2}day^{-1}**	**NAR mg g^{-1}day^{-1}**
	Variety (V)				
CSR 30	763.5a	3.29	90.8^{b}	15.94^{a}	0.227^{a}
PB 1121	714.0b	3.26	97.3^{a}	12.63^{b}	0.221^{b}
LSD ($P \leq 0.05$)	4.74	NS	0.6	0.094	0.005
	Stress conditions (S)				
Control condition	875.8^{a}	3.89^{a}	101.7^{a}	17.21^{a}	0.233^{a}
Sodic stress, pH ~ 9.1	726.5^{b}	3.39^{b}	92.1^{b}	13.33^{b}	0.225^{b}
Saline stress, ECe ~ 6.0 dSm^{-1}	613.9^{c}	2.85^{c}	88.4^{c}	12.31^{c}	0.214^{c}
LSD ($P \leq 0.05$)	5.81	0.046	0.7	0.115	0.002
	Nitrogen substitutions (N)				
N_0 (Without N)	638.0^{e}	2.67^{d}	78.9^{d}	8.85^{d}	0.201^{f}
N_{RD} RDN through PU	783.7^{ab}	3.65^{ab}	99.3^{a}	16.09^{a}	0.236^{b}
N_{33} [1/3 PU + 1/3 PU + 1/3 nN]	786.6^{a}	3.71^{a}	99.5^{a}	16.17^{a}	0.239^{a}
N_{50} [1/3 PU + (½ PU + ½ nN) + 1/3 nN]	777.5^{bc}	3.65^{ab}	99.0^{ab}	15.9^{b}	0.232^{c}
N_{66} [1/3 PU + 1/3 nN + 1/3 nN]	770.6^{c}	3.61^{b}	98.0^{b}	15.75^{b}	0.227^{d}
N_{100} [1/3 nN + 1/3 nN + 1/3 nN]	676.0^{d}	2.98^{c}	89.5^{c}	12.95^{c}	0.208^{e}
LSD ($P \leq 0.05$)	8.21	0.064	1.0	0.163	0.003
LSD ($P \leq 0.05$) S × N	14.22	0.112	1.80	0.283	0.005
LSD ($P \leq 0.05$) V× N	11.61	NS	1.50	0.231	NS
LSD ($P \leq 0.05$) V× S	8.21	0.064	1.0	0.163	0.003
LSD ($P \leq 0.05$) V× S × N	20.12	0.158	2.50	0.4	NS

Means followed by at least one letter common are not statistically significant ($p<0.05$) using LSD test; LA–leaf area; LAI–leaf area index; RGR–relative growth rate; CGR–crop growth rate; NAR–net assimilation rate

salt stress; albiet to a greater extent under saline stress (29.9%) than sodic (17.05%) stress. Further, CSR 30 had higher leaf area/plant of 763.5 cm^2 than PB 1121 (714.0 cm^2).

Leaf area index (LAI) is an assimilatory system of any crop and plays an important role in deciding the plant growth and yield. Although non- significant differences were seen between the two varieties, but with salt accumulation LAI decreased by 12.85% under sodicity and 26.74% under salinity stress. Under different N scenario, LAI was maximum with N_{33} followed by N_{RD} and N_{50} (Table 2). Interaction effects of variety, soil and N-management was also found significant for LAI. Data on RGR depicted that CSR 30 had lesser (6.7%) RGR in comparison to PB 1121. RGR decreased by 9.44% under sodicity and 13.08% under salinity stress. Among different N scenario, N_{RD} and N_{33} had similar effect while further reduction in RGR was observed with increasing N substitution with nN in the sequence of $N_{50} < N_{66} < N_{100}$, (Table 2). Crop growth rate (CGR) is another important radiation interception dependant growth variable that largely depends on the quantity of radiation used and received by the crop. Similar results were also noted for CGR between two varieties, stress type and different N substitutions. Net assimilation rate measures net gain of assimilate per unit of leaf area and time. On average, NAR was higher in CSR 30 (0.227 g m^{-2} day^{-1}) than PB 1121 (0.221 g m^{-2} day^{-1}). Compared to normal soils, a decrease of 3.43% and 8.16% was observed with sodicity and salinity respectively (Table 2). Statistically higher NAR was noticed with N_{33} (0.239 g m^{-2} day^{-1}) than N_{RD}, while other N-combinations revealed a gradual reduction in NAR.

Yield and yield attributes

Yield attributing characters are the resultant of dry matter accumulation by plants translocation which has direct correlation with the grain yield. A significant impact of different N substitutions strategies and stress conditions (saline or sodic) was observed on yield variables, such as effective tillers, panicle length, grains per panicle, and 1000 grain weight (Table 3). On average, CSR 30 produced more effective tillers (4.5%), grains per panicle (2.3%) and more specifically healthier grains (30.5%) than PB 1121. With sodicity and salinity stresses, significant reduction to the extent of 13.56 and 19.45% reduction in total tillers and 14.24 and 18.18% in effective tillers, 10.1 and 19.8% in panicle length, 18.8 and 29.3% in grains per panicle and 1.3 and 4.9% in 1000-grain weight was recorded, respectively was observed, respectively (Table 3). Statistically significant differences were found for average panicle length ranging from 8.55 to 11.13 cm under different N-scenario, whereas rice plants receiving no-nitrogen (N_0) had panicle length of 7.53 cm (Table 3).

N substitution through N_{33} acclaimed highest number of effective tillers, grains per panicle (53.67) and1000 seed weight (23.4 g) though remained at par with N_{RD}. Among different N-scenario, highest test weight (23.41 g) was observed with N_{33} (33% replacement of nitrogen through nano-N) followed by N_{RD} (22.6 g) and N_{50} (22.1 g), which were statistical at par (Table 3). A downfall of 21 and 16% in grain yield of CSR 30 and PB 1121 was observed when rice plants were exposed to sodicity stress while the corresponding reductions elevated to 30 and 27%, respectively under saline stress. N substitution through nN showed marginal yield gains with N_{33}across different soil types i.e., 2.04 & 1.79% under normal soil, 1.45% & 2.23% under sodic stress, 6.43% & 1.4% under saline stress in CSR 30 and Pusa 1121, respectively. The interactive effect of different N substitutions along with variety and stress

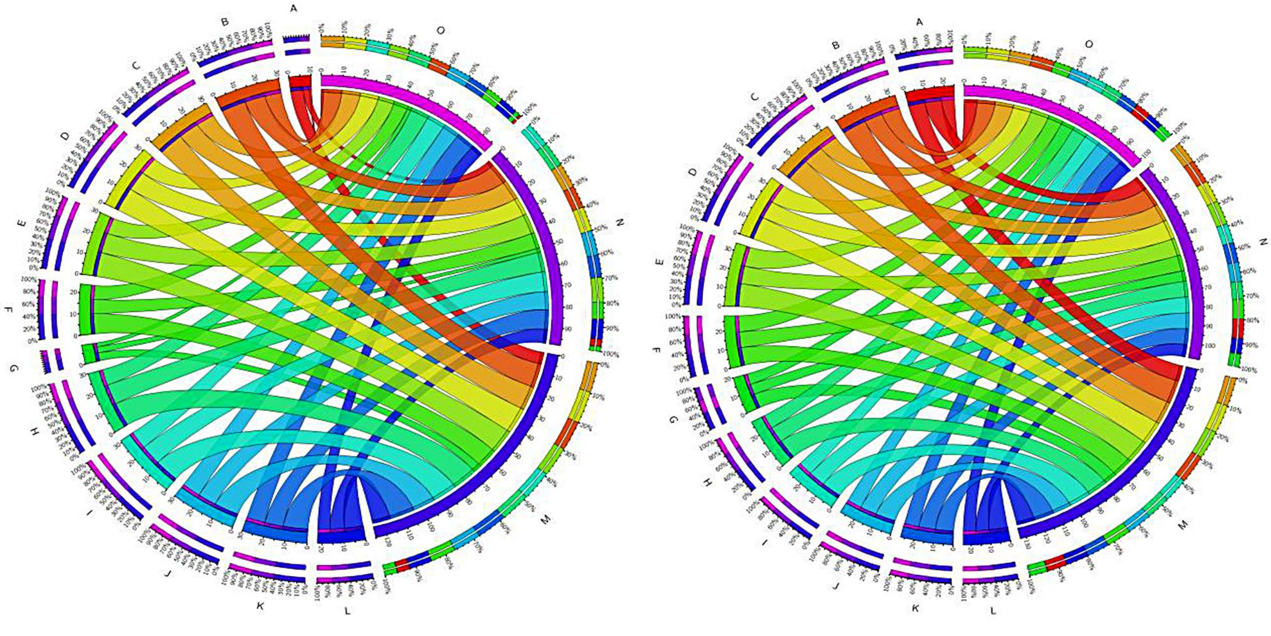
Table 3 Yield-related traits of rice in response to variable N nutrition, crop variety and salt stress conditions

Treatments/Traits	**Total tillers per hill**	**Effective tillers per hill**	**Panicle length (cm)**	**Grains per panicle**	**1000-grain weight(g)**
	Variety (V)				
CSR 30	6.68^{a}	6.02^{a}	9.87^{a}	43.53	24.19^{a}
PB 1121	6.31^{b}	5.76^{b}	9.50^{b}	42.56	18.54^{b}
LSD ($P \leq 0.05$)	0.13	0.11	0.23	1.56	0.59
	Stress conditions (S)				
No stress	7.30^{a}	6.6^{a}	10.76^{a}	51.25^{a}	21.82^{a}
Sodic stress, pH ~ 9.1	6.31^{b}	5.66^{b}	9.67^{b}	41.64^{b}	21.54^{b}
Saline stress, ECe ~ 6.0 dSm^{-1}	5.88^{c}	5.40^{c}	8.63^{c}	36.25^{c}	20.74^{c}
LSD ($P \leq 0.05$)	0.16	0.14	0.29	1.20	0.72
	Nitrogen substitutions (N)				
N$_{0}$ (Without N)	4.40^{d}	3.79^{c}	7.53^{e}	27.56^{f}	17.38^{d}
N$_{RD}$ RDN through PU	7.21^{ab}	6.55^{a}	10.86^{a}	51.78^{b}	22.79^{ab}
N$_{33}$ [1/3 PU + 1/3 PU + 1/3 nN]	7.31^{a}	6.58^{a}	11.13^{a}	53.67^{a}	23.41^{a}
N$_{50}$ [1/3 PU + (½ PU + ½ nN) + 1/3 nN]	7.15^{ab}	6.47^{a}	10.30^{b}	47.50^{c}	22.60^{ab}
N$_{66}$ [1/3 PU + 1/3 nN + 1/3 nN]	7.06^{b}	6.45^{a}	9.78^{c}	42.94^{d}	22.10^{ab}
N$_{100}$ [1/3 nN + 1/3 nN + 1/3 nN]	5.91^{c}	5.47^{b}	8.55^{d}	34.83^{e}	19.78^{c}
LSD ($P \leq 0.05$)	0.224	0.19	0.405	1.701	1.02
LSD ($P \leq 0.05$) S × N	NS	NS	NS	NS	1.76
LSD ($P \leq 0.05$) V× N	0.317	0.269	NS	2.406	1.44
LSD ($P \leq 0.05$) V× S	0.224	0.19	0.405	1.701	1.02
LSD ($P \leq 0.05$) V× S × N	NS	0.466	NS	NS	2.49

Means followed by same letter not statistically significant ($p < 0.05$) using LSD test

levels on grain and biological yield of rice (supplementary Table 1), are plotted as Circos plot (Fig. 1) (www.circos.ca). In the outer ring, columns A-F and G-L represent the nitrogen substitutions as N$_{0}$, N$_{RD}$, N$_{33}$, N$_{50}$, N$_{66}$, N$_{100}$ for variety Pusa 1121 and variety, CSR-30, respectively. In the inner ring, the gradual transition of ribbons colors from orange red to green, yellow and blue refers to the exact variations in the yield where the width of each colored ribbon is proportional to the average grain yield or biological yield respectively. Different ribbons under three shades of purple in the inner ring (under columns M, N and O) depict respective effect of the nitrogen doses in normal (M), sodic (N) and saline (O) stress treatments in two varieties with 12 ribbons (2 varieties x 6 N doses) each. Under the outer ring M, the inner purple ring with scale of 0-120 with 12 ribbons (different shades of red, orange, yellow, green & blue) depicts grain yield under no-stress environment corresponding to different N substitutions in two varieties longing towards G-L for variety CSR-30 and A-F for variety Pusa1121. For example, the intensity of red ribbons in column A (N$_{0}$) shows the reducing effect on yield from control (5.64) decreasing to 3.28 & 1.09 in sodic and saline stress respectively which can be clearly seen as narrow red ribbons joining with 0–10 scale of inner rings under M & N. Similarly, in column B (N$_{RD}$), the wider, medium and narrow orange rings correspond to grain yield in normal (11.75), sodic (9.87) and saline (8.57) conditions joining their respective ends with M (normal), N (sodic), O (saline). Again, in column C (N$_{33}$), three ribbon widths clearly differentiate the grain yield in three environments corresponding to M, N & O exactly proportional to yield reduction from 11.96 (no stress) to 9.58 (sodic) to 8.52 (saline) conditions. Similarly, width of three yellow ribbons (column D), four shades of green (12 ribbons in columns E, F, G, H) and four shades of blue (12 ribbons in columns I, J, K L) correspond to respective change in grain yield and biological yield under growing environments of normal (M), sodic (N) and saline (O) stresses respectively.

Further, the pearson correlation analysis of pooled data showed a significant positive correlation of grain yield with chlorophyll content, RWC, photosynthesis, growth rate (RGR, CGR), total tillers and biological yield (Fig. 2). Chlorophyll content (0.878), RWC (0.869), NAR (0.732) and RGR (0.865) are highly and significantly associated with grain yield under salinity stress as compared to normal conditions indicating that yield is an interplay and final expression of these traits under salt stress. Higher number of tillers under salinity (0.855) and sodicity (0.837) stress were responsible for higher photosynthetic rate, thereby, depicting a higher correlation between photosynthesis and rice yield under salinity (0.612) and sodicity stress (0.761).



Grain Yield

Biological Yield

Fig. 1 Circos plot depicting the interactive effect of different N substitutions on grain and biological yield of rice under salt stress conditions. Columns A-F and G-L represents N substitutions as N_0, N_{RD}, N_{33}, N_{50}, N_{66}, N_{100} for variety Pusa 1121 and variety, CSR-30 under normal (M), sodic (N) and saline (O) stress treatments, respectively

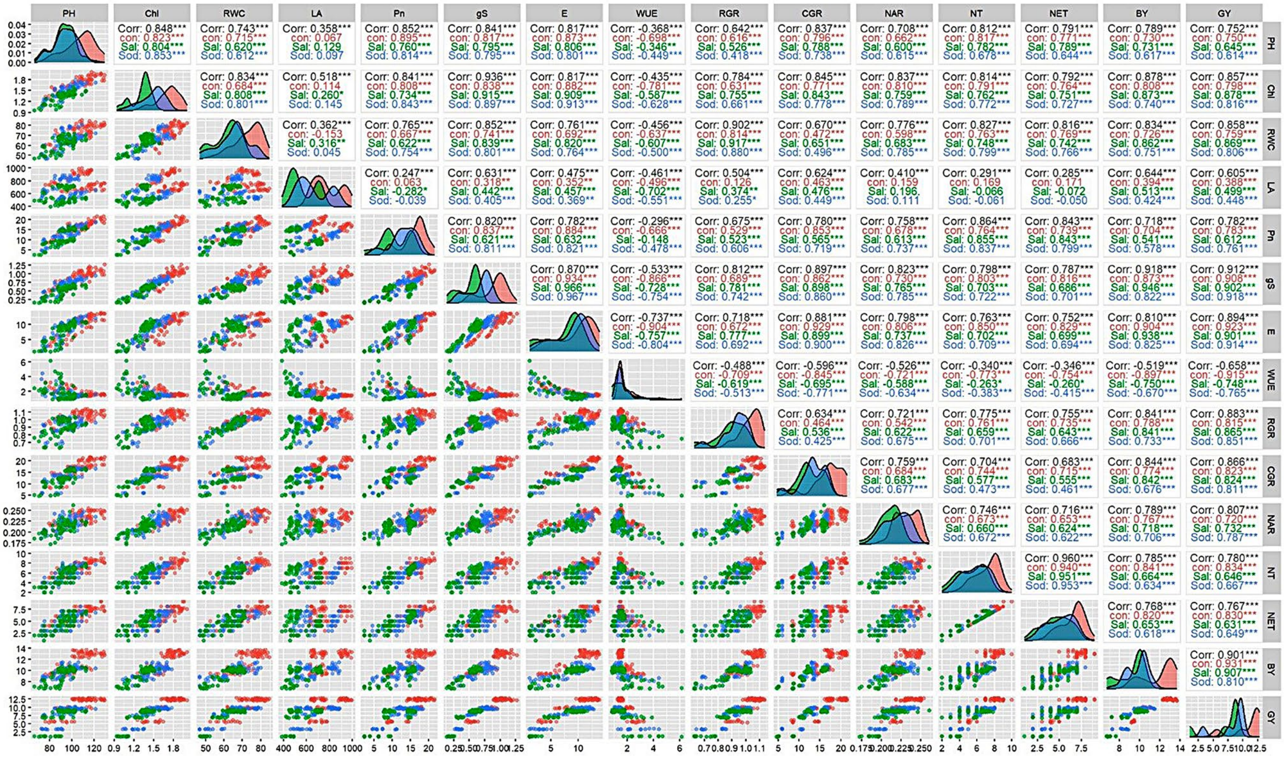


Fig. 2 Pearson correlation matrix between different parameters of crop growth and yield under no-stress (red color), salinity stress (green color) and sodicity stress (blue color).*, **, and *** significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. (PH -plant height; Chl-chlorophyll; LA-leaf area; RWC-relative water content; Pn-photosynthesis; gS-stomatal conductance; E-transpiration; WUE- instantaneous water use efficiency; RGR- relative growth rate; CGR- comparative growth rate; NAR- net assimilation rate grain; NT- number of total tillers; NET-number of effective tillers; BY-biological yield; GY-grain yield

Discussion

Status of plant cell nutrients is a detrimental factor for plants growth and productivity in addition to the mode of delivery of these nutrients. Nutrient application through leaves is also equally effective for its cell requirement because of quick absorption and appropriate translocation in desired concentration [25, 26]. The foliar sprayed nano-nitrogen easily enters the plant cell through stomata and other membrane pores, gets distributed within the plant cell and further assimilated in routine plant cell process via apoplastic and symplastic pathways. These nano-particles are transported from one cell to the other through plasmodesmata. The entry of nanoparticles is due to the negative charge on the plant cell's surface which allows the movement of negatively charged compounds into the cells via their membrane transporters [27]. The uptake, translocation, and aggregation of nanoparticles vary with plant growth stage, environmental conditions and plant species as well. Raliya et al. [28] have reported the exclusive mechanism of uptake and translocation of foliar nano-particles through apoplastic and symplastic movements against the pressure gradient or mass flow of the photosynthetic product inside the plant cell. Further translocation of nanoparticles from leaf to root occurs via the phloem transport mechanism being validated and quantified through ICP-MS showing 28.0% of nano particles in leaf, 58.6% recovered in the stem and 13.4% in the roots. Nitrogen is the main building block of plant protoplasm and most important nutrient being a source of amino acids, proteins, nucleotides and nucleic acids, leaf chlorophyll and enzymes etc. Therefore, efficient N application, its uptake and metabolism by the plants is vital for appropriate crop growth and yield. The present study provides useful insights in exploring the comprehensive understanding of morpho-physiological changes in rice crop in response to altered N nutrition in the form of Nano-N under stress conditions. This might be the first report of Nano-N application on plant growth and development under abiotic stress condition although very few reports are available for the field use of nano-N in normal soil conditions.

Altered morpho-physiological response suppressed important growth and yield-related traits; revealing repressive effects of salinity stress on rice yield. Better crop performance of CSR 30 can be mainly attributed to its adaptive capacity and response mechanism to prevailing salt stress conditions in comparison to PB 1121. Plant height and relative leaf water content increased with N applied as N_{33}however further increase in N substitution with nN revealed negative effect on these traits (Table 1). The nano particles promote plant growth by altering the leaf organization and regulating the development of vascular bundles in leaves [29] leading to more vegetative growth. Similar results obtained by Singh et al. [30] reported taller plants with 75% NPK and nano nutrients (N, P, K, Zn) in wheat under controlled conditions. Similarly, beneficial effects of nano-nutrient (NPK) has been reported by Mehta [31] and with nano-Zn by Munir et al. [32]. In general, the growing plants under stress accumulate other toxic ions and probably, nano-N in the soluble form or its ionic form in the plant cell tends to maintain the ionic homeostasis as well to counter-act the adverse effect of harmful ions, imparting the plant cell favorable cellular mechanisms. Rizwan et al. [33] have also reported the enhanced effect of Zn and Fe nano-fertilizers on morpho-metric traits like plant height, spike length, root-shoot fresh and dry weights and grain weight in wheat. This could be ascribed to elevated IAA concentrations through upregulation of auxin-related genes, which expedites the biosynthesis and transportation of indoleacetic acid (IAA) in wheat tissues, thereby, increasing plant growth after application of chitosan nanoparticles of iron [34]. In our studies also, a significant increase in rate of photosynthesis, stomatal conductance and other gas-exchange attributes of rice plants was observed in both saline-sodic soils with N_{33} (33% urea replacement with nano-N) (Table 1). This may be due to more penetration of nano-N through leaves revealing better gaseous exchange under stress conditions. Application of nano-N in different combinations with conventional source (N_{33}, N_{50} and N_{66}) increased the gas-exchange attributes but lower than the control plants, N_{RD} (receiving 100% N PU). The foliar application of Nano-N, ensured accelerated and prolonged N availability within the plant cell which accelerated the leaf growth by the production of necessary proteins required for cell development, cell division as well as chlorophyll synthesis and photosynthesis. Nitrogen is an essential component of chlorophyll, And there is a widely recognized relationship between light-saturated photosynthetic rate and the N status of the leaves since N gets integrated into structure of proteins and amino acids proteins which are responsible for carrying out the essential photochemical and biosynthetic reactions of photosynthesis. Therefore, the supplemental use of nano fertilizers along with chemical fertilizer leads to greater photosynthates accumulation and translocation to the plant's economic parts, resulting in higher yield, mainly credited to increased source and sink strength through uniform distribution of nano-N through phloem [35]. Crops provided with the exact amounts of nutrients (controlled release through nano-fertilizer) in the right proportions helps in reducing stomatal resistance and increasing stomatal conductivity, providing the plant with adequate carbon dioxide and water to continue photosynthesis and remove nutrients from the soil which ultimately resulted in increased yield [36–38]. Similarly, Di et al. [39] also noted that CuO-NPs exposure notably increased the biomass, root length, and root tip number by 22.0%, 22.7%, and 82.9%, respectively, whereas Cu- NPs and $CuSO_4$ significantly reduced root biomass, net photosynthetic rate (PN), and root length by 31.2% and 44.2%, 24.5% and 32.2%, and 43.4% and 40.6%, respectively in bok choy (*Brassica chinensis* L.) under hydroponic conditions. Similarly, nano fertilizers synergistically improved plant growth through better absorption and utilization of N in rice [38] and maize (*Zea mays* L.) [40].

Salt stress negatively impacted the crop performance and the photosystem traits like Pn, gS, E and WUEi (Table 3). This may be due to presence of toxic Na^+ ions in the transpirational stream causing photo-inhibition and thus, stomatal opening. Na^+ accumulation within chloroplast causes reduction/denaturation of photosynthetic pigments, impairs photosynthetic efficiency by lowering the electron transport rate (ETR) and quantum yield of photosystem II [41, 42]. Osmotic stress, higher ion accumulation in the photosynthesis apparatus, causes physiological drought at the cellular level, resulting in an indirect reduction in the photosynthetic rate [43–45]. Furthermore, under salt stress due to a lack of fully functional osmoregulation and lower K^+ uptake, the incapability of guard cells leads to stomatal closure, which directly reduces the photosynthetic activity [46, 47]. Different studies have predicted that use of nano materials combat the consequences of salinity by maintaining the adequate water balance and potential [48]. Faizan et al. [49] reported substantial improvement in fluorescence, chloroplast structures, and photosynthesis-related parameters in response to ZnO-NPs in rice and increased chlorophyll in wheat was reported with iron-nanoparticle application at lower concentration [50]. This could be ascribed to improved Pn by application of ZnO-NPs through stabilization of the photosynthetic apparatus and enhancing the biosynthesis of photosynthetic pigments, and neutralizing the ionic toxicity. In our studies also, maximum leaf greenness was observed with N_{RD} which gradually decreased under salt stress (Table 1). With N substitution through nN at N_{33}, N_{50} and N_{66}, significant enrichment of green pigment was noticed in rice leaves but it remained lower than the plant leaves without stress. Nano-formulation provided the required nutrition with long lasting and slow release at target site contributing to the stay green trait under stress. Almost all the plants are capable of osmoregulation through biosynthesis of compatible osmolytes at the cost of 10 times energy demand which gets more with increasing stress type. The exogenous application of nano-N might ameliorate salinity-induced effects to some extent with continuous nitrogen supply, an integral part of proteins, thus, maintaining cell stability. While studying the effect of different combinations of nano-N with PU in mustard, an increased LAI and SPAD have been reported, 50% at par with control [51]. Salinity of 120 mM NaCl significantly affected plant growth attributes, physiological performance, nutrient profiles, antioxidant activity, plant yield, and yield-contributing characteristics of maize plants. Foliar application of ZnO-NPs successfully alleviated these salinity effects on LGR, PGR etc. and significantly improved all studied parameters, except transpiration rate (TR) and intrinsic water use efficiency (WUEi) [52]. Foliar spray of iron source has also shown enhanced SPAD values, chlorophyll content in addition to maintaining the membrane stability in groundnut cultivars under iron deficiency [53] depicting the targeted delivery and beneficial effects of foliar nutrients.

Notably, RGR and CGR in rice declined under both saline and sodic conditions (Table 2). However, significant improvement was observed with by foliar applications of N_{33}, N_{50} & N_{66}Herein the slow release of nano-nitrogen form the plant cell under stress conditions leads to better translocation of nitrogen in maintaining the plant functioning and hence, the better crop growth rate. Additionally, nN provide higher surface/absorption area with greater diffusion rates for various metabolic processes, resulting in increased size and efficiency of source, which in turn might have contributed towards better expression of yield attributes and grain yield depicting improved source-sink translocation [54]. Manikandan and Subramanian [55] have also reported significantly higher grain yield & weight and crude protein with zeolite based N fertilizers in maize due to its slow and controlled release and prolonged availability throughout crop growth period. We have also observed similar beneficial effects of N nutrition (N_{33}) on rice plants imparting similar yield gains comparable with N_{RD} (Table 3; Fig. 1). Recently, Zarinkoob et al. [56] have also reported increased grain yield and harvest index in wheat with Manganese Ferrite Nanoparticles ($MnFe_2O_4$) concomitant with a 14% enhancement in the grain number per spike. The improvement in yield characteristics with strategic application of nano-urea might be brought by effective nutrient and water intake (higher absorption of nutrient and their deep penetration into leaves) and metabolic output. This also provides enough time to plants to perform its nutritional efficiency and growth more effectively than reflected [38, 57]. Al-Juthery et al. [37] also revealed a significantly enhanced wheat plant height, 1000-grain weight, grains, straw and biological yields with foliar application of nano fertilizer. Many reports in literature have shown a 15–20% increased crop yield with foliar spray of nano-N in rice [57], sweet corn [58], maize [59], rice [60] and wheat [61]. Such increased crop yield with nano-fertilizer might be due to high efficiency of nanoparticles in slow release of nitrogen for plant use.

Briefly, our findings depicted that foliar application of nano-nitrogen helps rice plants to maintain the cell homeostasis and water balance with active photosynthetic machinery, thereby, improving relative plant growth rate, crop growth rate and net assimilation rate of the photosynthates and metabolites. Better expression of plant physiological functions contributes to more effective tillers, grains per panicle and improved 1000-grain weight culminating in receipt of better grain yield under salt stress conditions. These are preliminary studies for effects of nano-particle on plant performance in stress conditions, further studies are required for deep down regulation of nano-N signals, plant responses at metabolic, genetic and molecular levels for varietal individual responses. Further, the effective metabolism of nano-fertilizers can be defined in experiments which can be conducted on larger scale for longer durations.

Conclusions

From these studies, we can summarize that the application of nN alleviated the harmful effects of salt stress realizing comparable yield gains at N_{33}similar to that obtained with recommended dose of prilled urea (N_{RD}). Although, these preliminary field insights, suggest that application of nano-N may be a prospective solution in partially substituting the conventional fertilizer to extent possible while improving the agronomic productivity of stressed plants, further studies are required to understand and validate the actual mechanism of uptake, translocation and phytotoxicity of nano-particles on the plant growth. The magnetized levels of nano-particle in grain, leaves, stem and root need to be traced for tissue-translocation and metabolism in plant cell. Although nano-particles have been extensively used in heavy metal toxicity remediation but the nano-fertilizers have not been utilized for mitigation of abiotic stresses, here, our studies will be helpful in providing an initial preview of mechanism of action of nano-nitrogen source which can be elaborated in different crop plants under abiotic stress conditions. As of now, the interactions between N status and abiotic stress in plants is crucial to optimize the use of N fertilizers, while keeping the balance between application and the adverse effects of abiotic stresses. In future, it may be expected that nano fertilizers will upgrade the sustainable farming practices being more efficient fertilizer resource, while lowering reliance on hazardous chemicals and thus, saving our ecosystem over time in addition to bioremediation of abiotic stresses. Similar to other biological issues, many open-ended questions still instigate further investigations about agricultural use of nano-fertilizers.

Supplementary Information

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Supplementary Material 1

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Author contributions

Conceptualization of the experiments was done by AK, PS, AKB, and AM. Plant samplings and data analysis, and writing the original draft were done by NK, SD, MR, KM. ArK and AKB helped in various analyses and PS, AK, AM, reviewed and edited the manuscript. All authors contributed to the writing of the manuscript and reviewed the draft. All authors approved the final manuscript.

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Data availability

All necessary data used to evaluate the conclusions in the manuscript are either included in the article itself or are readily available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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