Impact of Qiangdi 863 Nanosynergids Treated Water on Rice Physiology, Grain Production, and Grain Quality

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Original Article

Keywords: Nano synergid, Catalase, Superoxide Dismutase, Salicylic acid, Malondialdehyde, Jasmonates

Posted Date: December 2nd, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-117667/v1>

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Abstract

Background

Global warming, climate change, environmental stress and population increase have threatened food security and increased food production demand. Increasing Rice productions could be a better solution to solve or at least decrease the severity of the global food crisis. The modern rice cultivation techniques have been insufficient for sustainable rice production. So, Qiangdi 863 nano synergid is hypothesized to enhance the early growth of rice, rice physiology, grain yield, and grain quality.

Results

The present study was carried out to determine the role of Qiangdi 863 nano synergid on the growth and yield of one Chinese rice variety Zhongzao 39 and four Pakistani rice varieties KSK 133, KS 282, Super basmati, and PK 1121 aromatic. Field experiments were conducted during the years 2017, 2018, and 2019. Applying nano-treated water enhanced the germination rate along with radicle and plumule lengths and improved the photosynthesis rate, SPAD value in rice leaves for all five varieties. The results showed that nano-treated water improved the anti-oxidant Catalase activity (CAT), Superoxide Dismutase (SOD) Peroxide (POD) production, declined Malondialdehyde (MDA content), enhanced endogenous Salicylic acid (SA), Jasmonates (JA) Brassinosteroids (BR) hormonal concentration and yield parameters of rice in 2017, 18 and 19 years of data.

Conclusion

These findings indicate that nano synergids can be effectively used for rice cultivation in different environmental conditions (China and Pakistan). This is the first reported application of Qiangdi 863 nano synergid in Pakistani rice varieties with effective outcomes highlighting significant results in KSK 133.

Background

A rapid increase in population by 2050, demands increased crop production using advanced technologies (Kromdijk et al. 2016). In the past 70 years, 2250 rice varieties were introduced but still, there is the necessity to improve rice cultivation for the flourishing world population (Ahloowalia et al. 2004). FAO forecast for worldwide rice production in 2020 stands at 508.7 million tonnes, up to 1.6% from 2019. In 2021 global trade in rice is tentatively pegged at 47.6 million tonnes, 2.8 million tonnes from 2020 (OECD. 2019). In 2019, china's rice production was estimated at 209.6 million tonnes. Rice is an economically important viable cereal cash crop of Pakistan. In 2019, Pakistan's predictable rice production was 11.5 million tonnes (OECD. 2019). During the 2017-18 area under rice, cultivation was 2,899 thousand hectares and the production of rice was 7,442 thousand tonnes. (Chandio and Yuansheng 2018). Due to diversified domestic needs with limited resources (water and land) for a sustainable crop, adoption of the latest agricultural technologies may ensure the improved crop production per unit area (Asfaw et al. 2012).

For sustainable agriculture, nanotechnology has been observed as an advanced technology to cope with the globally increasing population (Ditta et al. 2015). The main aspect of nanotechnology is to endorse the biological metabolism of plants. Nano-treated germinated seed can stimulate crop growth and lead towards the enhanced yield and improved quality (Tuteja and Gill 2012). Though nanotechnology in agriculture is at the initial stage, it is hypothesized that it will cause revolutionary change in crop yield and quality improvement.

The effect of nanoparticles has been explored previously on various plants. Mostly carbon nanomaterials in the form of single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) were used. The effect of these nanoparticles was investigated on tobacco (Khodakovskaya et al. 2013) and different Poaceae members like barley and corn (Lahiani et al. 2013). Some Scientists worked on the effect of MWCNTs on rice seed germination, vigor growth of seedlings, and observed pronounced rate and speed of germination. Earlier studies successfully demonstrated the positive impacts of nanomaterials for disease diagnosis and treatment in lab experiments (Nair et al. 2014). Previously, progressive applications of nano-materials on plants were observed to enhance the impact of nanotechnology in agriculture. But widespread use of nanoparticles addresses the potential threat like nanotoxicology may able to create an adverse effect at their plant cell entry (Dietz and Herth 2011). So, nano-materials that can emit electromagnetic waves or specified energies could bring in support of the growth for the safe and sustainable nanotechnology industry.

Nanosynergid's can generate specific far infrared waves. These nano synergids can build the resonance between far infrared waves and water molecules and at that point, high energy is generated (Huang et al. 2015). The alteration of a water molecule can be analyzed by the Ultraviolet absorption spectrum (UV) and nuclear magnetic resonance (NMR) (Liu and Liao 2008). Nano synergid (Qiangdi 863) activated water molecules that carried high energy and entered into plant cell stimulated the metabolism of the plant cell (Liu et al. 2007a). In China agriculturists used Qiangdi nano-863 biological assistant growth apparatus (disc) to check the breakdown of a macro water molecule into

micro-molecule clusters. The apparatus was dipped in water to absorb and emit electromagnetic waves in specific energies (Pudake et al. 2019). They carried a mass of kinetic energy which was 30% greater than regular water. Nano-863 has been an agricultural high-tech invention and most widely used in China (Liu et al. 2007a).

Because of acclaimed reports, the qualitative nanotechnology applications on rice were noticed. Agriculturally no reporting about nano-material treated water has been done on *Oryza sativa*. Taking into consideration, the present study was designed to examine the effects of Qiangdi nano-863 nano synergids on *O. sativa*. For the proposed study five varieties; one from China and four from Pakistan were selected.

Methods

Plant material and growth conditions

In the experiment cultivated rice (*Oryza sativa* L.) varieties Zhongzao 39 (Chinese Indica variety), PK aromatic 1121, KSK 133, KS 282, and Super Basmati (Pakistani Indica varieties) were utilized as germplasm in China and Pakistan. The experiment was conducted in split block design with 3 replicates having a plot size 3 m X 2m. During crop season of 2017 experiment was done at the State key laboratory of Rice Biology, China National Rice Research Institute (31°4'49" N, 119°56'11" E), and Zhejiang Province, China. The same experiment was repeated in Pakistan at Rice Research Institute, Kala Shah Kaku (31°45'N 74°14'E / 31.750°N 74.233°E) during rice crop season 2018-and 2019.

Nano synergid treated water (NTW) and rice seed soaking

For different treatments, nano-synergid treated water was prepared with a variation of the duration of soaking of the disc in water. Qiangdi nano-863 biological assistant growth apparatus (disc) was placed in a plastic bucket with 20 L water for 24 hours (one day), 48 hours (two days), and 72 hours (three days) to produce nano-treated water. Rice seed was pre-soaked in tap water for 24 hours, and then soaked in nano-treated water for 24 h and were germinated for 36 hours. In three years (2017, 2018, and 2019), each variety containing 100 rice seedlings were sowed for all the treatments in three replicate

Water and fertilizer management

Fertilizer application after the transplanting stage was the same as that of common rice production. Homologous nano-treated water was used to irrigate the rice seedlings originated from nano-treated seeds. The recommended doses of fertilizer NPK were 53 kg N, 16 kg P, and 33 kg K ha⁻¹ for rice.

Germination, Seedling Growth, and dry biomass

Germination of seed was documented daily according to AOSA (association official seed analysis) till it became persistent. After compiling data, speed of germination (SG), final germination percent (FGP %) and germination energy percentage (GE %) was calculated using the following formulae. Ten random seedlings/ treatment were selected for measuring the dry biomass. Shoot and root dry weights (10 seedlings) were recorded after oven drying at 70 °C for 24 hours in a drying oven (Islam et al. 2012).

$$SG = \frac{\text{Number of germinated seeds}}{\text{Days of first count}} + \dots + \frac{\text{Number of germinated seeds}}{\text{Days of final count}}$$

$$GE(\%) = \frac{\text{Number of germinated seeds at 4DAS}}{\text{Total number of seeds}} \times 100$$

$$FGP = \frac{\text{Number final germinated seeds}}{\text{total number of seed tested}} \times 100$$

Chlorophyll content/mg g⁻¹ Fw

Chlorophyll was extracted from 0.2 g of fresh leaves soaking in 25 ml of acetone and alcohol (1:1) for 24 h in the dark at room temperature. The absorbance of the extract was measured at 663, 645, and 470 nm by using a UV-VIS spectrophotometer (UV-2600, Shimadzu, Japan) to estimate chlorophyll a, chlorophyll b, carotenoids contents and total chlorophyll content (Marschall and Proctor 2004).

$$C_a = 12.7 \times A_{663} - 2.69 \times A_{645}$$

$$C_b = 22.9 \times A_{645} - 4.68 \times A_{663}$$

$$C_t = (\mu\text{g}\cdot\text{cm}^{-2}) = [(1000 \times A_{470}) - (1.9 \times C_a) - (63.14 \times C_b)]/214$$

$$\text{Chlorophyll Content} = (C_a + C_b) \times V_a / m_{\text{leaf}}$$

Physiological parameter

I. SPAD Value

Chlorophyll content was characterized as SPAD values of the rice seedling in this trial (Esfahani et al., 2008). SPAD values were measured by selecting rice flag leaves, the second and third leaves from the top with 10 d intervals by using chlorophyll meter (SPAD-502 plus).

II. Antioxidant enzymes activities

Catalase (CAT) $\mu\text{g}^{-1}\text{FW h}^{-1}$

For CAT activity, the reaction mixture containing 50 mmol sodium phosphate buffer (pH 7.0), 20 mmol H_2O_2 , and 0.04 ml of extracted rice sample. This absorbance was measured at 240 nm for 300 seconds (Yuan et al. 2011). The calculation for CAT was performed according to the molar coefficient of H_2O_2 ($36 \text{ mM}^{-1} \cdot \text{cm}^{-1}$) and expressed as $\text{nmol H}_2\text{O}_2 \cdot \text{mg}^{-1} \text{ Pro} \cdot \text{min}^{-1}$.

Superoxide dismutase (SOD) $\mu\text{g}^{-1}\text{FW h}^{-1}$

The activity of SOD was determined by the method described by Zheng et al. (2016). This activity is measured through inhibited photo-reduction of nitro-blue tetrazolium (NBT). The reaction mixture of SOD contained 25 mmol sodium phosphate buffer (pH 7.8), 13 mmol methionine, 2 μmol riboflavin, 10 μmol EDTA- Na_2 , 75 μmol NBT, and 0.1 ml leaf extract. The total quantity of reaction mixture was 3 ml. The test tube containing reaction solutions was irrigated with light (fluorescent lamps $300 \mu\text{mol m}^{-2} \text{ s}^{-1}$) for 20 min and the activity was measured at 560 nm wavelength.

Peroxidase (POD) $\text{U g}^{-1} \text{FW}$

The POD activity was based on the determination of guaiacol oxidation at 470 nm by H_2O_2 and was expressed as $\text{U g}^{-1} \text{FW}$. The change in absorbance at 470 nm was recorded for every 20 s by spectrophotometer. One unit of POD activity is the amount of enzyme that will cause the decomposition of 1 μg substrate at 470 nm (HITACHI U-3900) for 1 min in 1 g fresh sample at 37 °C Zheng et al. (2016).

Malondialdehyde (MDA)

The MDA content level was determined by the method of enzyme extracted solution (2 ml) was added in 1 ml 20% (v/v) trichloroacetic acid and 0.5 ml (v/v) thiobarbituric acid (Chun and WANG 2003). The mixture was heated in a pre-heated water bath at 95°C for 20 min and cooled at room temperature. The solution was centrifuged at 10,000 rpm $\times$ g for 10 min after cooling. The lipid peroxidation absorbance was measured at 450, 532 and 600 nm by spectrophotometer (UV-VS Spectrophotometer-2600 Shimadzu), its content was expressed as $\mu\text{mol g}^{-1} \text{FW}$.

Quantifications of Plant Growth Hormones

Jasmonates (JA) commonly present in plants and act as a plant growth regulator (Ahmad et al. 2016) Brassinosteroids (BR) plays an important part in monitoring the broad spectrum of developmental processes and plant growth. (Sharma et al. 2015) Salicylic acid (SA) is a major endogenous signal in plant disease resistance, flowering, and thermogenesis. (Yang et al. 2004) Jasmonates (JA), Salicylic acid (SA), and Brassinosteroids (BR) were quantified by MULTISKAN MS (instrument).

Parameters of Yield Determination /Quantitative data of rice

Parameters for yield measurement Plant height, Biomass, number of panicle, number of seed per panicles, filled grains per panicle, unfilled grains per panicles, and thousand-grain weight were tested.

Statistical Analysis

All the data recorded from the five rice varieties (Zhongzao 39, KSK 133, KS 282, Super basmati and PS 2) was subjected to statistical analysis as the mean $\pm$ standard error (SE) of three replicates. Statistical analyses of the data were performed using standard analyses of variance (two-way ANOVA). Analyses were performed by using the software SPSS v. 17 (Zheng et al. 2016) The mean-variance of the data was examined using the least significant difference (LSD) test at the 0.05 probability level.

Results And Discussion

Effect of nano-treated water on seedling emergence characteristics

Seed germination is a fast activity in the plant life cycle and mainly tests offer numerous benefits like ease, sensitivity, low cost and suitability for unstable chemicals or samples (Wang et al. 2005). In this study, germination was observed for 24 hours (one day), 48 hours (two days) and 72 hours (three days) nano treated water and in control. Speed of emergence (SE), percentage emergence (PE) and seed emergence energy percentage (SEEP) were considered to evaluate the impact of nano treated water on the emergence of rice seed. According to the results 24 hours and 48 hours of nano water treatment were proved non-effective nano synergid treatment for rice germination, so did not proceed for further analysis. However, 72 hours of nano water treatment showed significant results than control. Speed of emergence (SE) and was improved for KSK 133, super basmati, and PK 1121 Aromatic than KS 282 and Zhongzao 39. Highest SEEP was observed in KSK 133 (40% in 2017; 55% in 2018; 45% in 2019) and lowest in Zhongzao 39 (5% in 2017; 5% in 2018; 10% in 2019) Fig. 1. Effect of nanometer pottery trays (NPTs), high energy nanomaterials showed better rice seed germination (Jun-rong et al. 2016). In the growing seasons of 2017, 2018 and 19 years after NTW application most prominent seedling emergence were recorded in KSK 133 and lowest in Zhongzao 39.

Effect of nano-treated water on growth characteristics at the early seedling stage

Qiangdi 863 nano synergid is manufactured from composite nano far infrared technology which covers beneficial/captivate vibration frequency (λ) and releases far infrared waves. Nano treated water (NTW) exhibited significant growth in radicle and plumule lengths in all rice varieties. The effects of four-nanometer pottery trays (NPTs) treatment on biological properties of rice were alike to their influence on seed germination and seedling early growth (Jun-rong et al. 2016). Overall, three years (2017, 2018 and 2019) of NTW data revealed improved radicle growth. Highest radicle length was observed in KSK 133 (75% in 2017; 63% in 2018; 83% in 2019) and lowest in Zhongzao 39(10% 2017; 30% in 2018; 21% in 2019). Similarly improved plumule length was noticed in KSK 133 (73%, 72%, 79% in 2017; in 2018 and 2019 respectively) and the lowest plumule length was noted in Zhongzao 39(36%, 28%; 39% in 2017, 2018 and 2019 respectively) (Table 2). According to the present experimental observation, a nano synergid is a good tool for the enhancement of germination and growth.

Table 1

Emergence data of rice varieties SEEP% seed emergence energy percentage, SE speed of emergence and FEP% final emergence percentage

Year	Rice cultivars	Treatment	SEEP (%)			SE			FEP (%)		
			24 hours	48 hours	72 hours	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
			(1 day)	(2 days)	(3 days)	(1 day)	(2 days)	(3 days)	(1 day)	(2 days)	(3 days)
2017	Zhongzao 39	Con	32.5 ± 1.4	33.1 ± 1.5	35 ± 1.3	7.1 ± 2.0	8.5 ± 1.6	9.2 ± 2.5	35 ± 3.5	37 ± 3.2	40 ± 4.1
		NTW	32.6 ± 1.5	33.1 ± 2.1	40 ± 0.8	7.2 ± 2.3	8.52 ± 2.1	11.7 ± 1.4	35 ± 2.1	37 ± 2.7	65 ± 2.3
	KSK 133	Con	36.5 ± 2.3	37.5 ± 1.3	40 ± 2.5	10.7 ± 3.1	11.3 ± 2.4	14.6 ± 3.4	50 ± 2.4	52 ± 1.5	70 ± 1.0
		NTW	36.6 ± 2.1	37.4 ± 1.0	80 ± 0.5	10.6 ± 3.4	11.4 ± 2.1	25.3 ± 0.8	50 ± 1.4	52 ± 1.46	100 ± 0.2
	KS 282	Con	38.2 ± 1.1	39.1 ± 2.3	40 ± 0.8	8.7 ± 2.5	9.3 ± 1.4	12.9 ± 1.9	51 ± 1.5	53 ± 2.1	60 ± 2.3
		NTW	38.2 ± 1.4	39.3 ± 2.1	45 ± 1.1	8.71 ± 2.3	9.3 ± 1.6	14.9 ± 0.9	51 ± 1.6	53 ± 1.1	70 ± 0.6
	Super basmati	Con	37.1 ± 1.2	37.5 ± 2.0	40 ± 2.1	11.3 ± 1.1	12.1 ± 2.5	14.5 ± 2.1	53 ± 1.4	54 ± 1.5	60 ± 1.7
		NTW	37.3 ± 1.5	37.8 ± 1.4	65 ± 0.7	11.4 ± 0.7	12 ± 0.5	19.9 ± 0.8	53 ± 1.1	54 ± 2.1	90 ± 0.4
	PK 1121 Aromatic	Con	36.2 ± 2.2	37.4 ± 1.1	40 ± 1.0	10.2 ± 1.1	11.4 ± 2.1	14.5 ± 3.2	50 ± 2.5	51 ± 3.1	55 ± 3.2
		NTW	36.3 ± 1.6	37.5 ± 1.5	60 ± 1.5	10.4 ± 1.5	11.5 ± 2.3	16.2 ± 2.4	50 ± 2.6	51 ± 3.5	80 ± 0.6
2018	Zhongzao 39	Con	32.5 ± 1.3	33.4 ± 1.1	35 ± 3.3	7.3 ± 3.5	8.1 ± 2.8	9.2 ± 4.1	35 ± 2.1	36 ± 1.8	40 ± 2.2
		NTW	32.7 ± 1.6	33.5 ± 1.5	40 ± 0.7	7.4 ± 3.2	8.2 ± 2.7	13.7 ± 3.3	35 ± 2.0	36 ± 1.7	55 ± 1.6
	KSK 133	Con	36.1 ± 2.2	37.2 ± 1.4	40 ± 1.6	10.1 ± 3.0	11.0 ± 2.5	14.7 ± 2.5	51 ± 1.3	53 ± 1.5	80 ± 0.7
		NTW	36.2 ± 2.1	37.3 ± 1.5	95 ± 0.4	10.2 ± 2.8	11.1 ± 2.3	35.6 ± 1.2	51 ± 1.4	53 ± 1.6	100 ± 0.2
	KS 282	Con	38.3 ± 2.2	39.1 ± 1.4	55 ± 3.2	8.5 ± 2.2	9.1 ± 1.5	20.9 ± 2.1	50 ± 1.2	52 ± 1.5	70 ± 1.1
		NTW	38.4 ± 2.1	39.3 ± 1.5	70 ± 2.2	8.6 ± 2.3	9.2 ± 1.7	21.4 ± 1.3	50 ± 1.2	52 ± 1.6	80 ± 1.1
	Super basmati	Con	37.3 ± 1.4	37.8 ± 1.1	40 ± 1.4	10.1 ± 0.8	11.2 ± 1.4	14.0 ± 1.1	51 ± 2.0	52 ± 1.3	60 ± 2.1
		NTW	37.5 ± 1.3	37.9 ± 1.0	80 ± 0.7	10.3 ± 0.7	11.3 ± 1.1	16.5 ± 1.2	51 ± 2.3	52 ± 1.2	85 ± 1.1
	PK 1121 Aromatic	Con	36.2 ± 1.5	37.1 ± 1.2	40 ± 3.2	10.1 ± 3.2	11.3 ± 2.5	14.6 ± 2.2	50 ± 1.1	51 ± 1.4	75 ± 2.3
		NTW	36.3 ± 1.4	37.3 ± 1.3	65 ± 1.0	10.3 ± 3.1	11.5 ± 2.7	19.3 ± 1.0	50 ± 1.6	51 ± 1.0	95 ± 0.7
2019	Zhongzao 39	Con	32.5 ± 2.5	33.1 ± 3.4	35 ± 4.8	7.1 ± 4.5	8.0 ± 4.6	9.4 ± 5.6	35 ± 3.1	36 ± 3.6	40 ± 4.6

Key: Con: Control. NTW: Nano treated water

Values were standard mean and standard error ± (n = 3) with control and nano synergid treatment SEEP% seed emergence energy percentage, SE speed of emergence and FEP% final emergence percentage

Year	Rice cultivars	Treatment	SEEP (%)			SE			FEP (%)		
		NTW	32.6 ± 2.6	33.4 ± 3.1	45 ± 3.6	7.2 ± 4.2	8.1 ± 4.5	12 ± 2.2	35 ± 3.3	36 ± 3.5	67 ± 3.2
	KSK 133	Con	36.0 ± 2.2	37.1 ± 2.5	45 ± 1.3	11.4 ± 3.5	12.1 ± 3.3	15 ± 4.7	51 ± 1.4	53 ± 2.2	75 ± 1.3
		NTW	36.1 ± 2.1	37.2 ± 2.3	90 ± 0.4	11.5 ± 3.2	12.3 ± 3.1	32.3 ± 0.3	51 ± 1.6	53 ± 2.1	100 ± 0.2
	KS 282	Con	38.1 ± 1.5	39.3 ± 1.2	42 ± 1.2	10.4 ± 1.1	11.1 ± 2.2	14 ± 2.3	50 ± 2.4	51 ± 2.1	65 ± 3.5
		NTW	38.3 ± 1.0	39.4 ± 1.2	49 ± 1.6	10.5 ± 1.0	11.3 ± 2.0	18 ± 1.1	50 ± 2.0	51 ± 2.3	70 ± 2.3
	Super basmati	Con	37.3 ± 1.3	38 ± 2.0	43 ± 2.2	11.2 ± 3.2	12.0 ± 2.7	15 ± 4.3	51 ± 1.1	52 ± 2.3	60 ± 4.2
		NTW	37.5 ± 1.1	38 ± 2.1	67 ± 1.3	11.3 ± 3.1	12.1 ± 2.8	20 ± 1.6	51 ± 1.2	52 ± 2.2	90 ± 0.6
	PK 1121 Aromatic	Con	36.1 ± 2.4	37.3 ± 3.1	45 ± 3.2	10.3 ± 1.8	11.4 ± 1.8	15 ± 2.8	50 ± 1.7	51 ± 1.5	65 ± 1.8
		NTW	36.3 ± 2.7	37.5 ± 3.5	65 ± 2.3	10.5 ± 2.0	11.6 ± 2.0	16.4 ± 1.4	50 ± 1.8	51 ± 1.7	80 ± 0.8
Key: Con: Control. NTW: Nano treated water											
Values were standard mean and standard error ± (n = 3) with control and nano synergid treatment SEEP% seed emergence energy percentage, SE speed of emergence and FEP% final emergence percentage											

Table 2
Effect of nano-material on growth characteristics at the early seedling stage

Rice variety	Treatment	Year 2017		Year 2018		Year 2019	
		Length of the radicle (cm)	Length of Pulmule (cm)	Length of radicle (cm)	Length of Pulmule (cm)	Length of radicle (cm)	Length of Pulmule (cm)
Zhongzao 39	Con	1.2 ± 0.75	0.24 ± 0.12	0.7 ± 0.25	1.41 ± 0.36	0.44 ± 0.35	0.27 ± 0.12
	NTW	1.3 ± 0.53*	0.6 ± 0.21*	1.0 ± 0.27*	1.69 ± 0.5a*	0.65 ± 0.21*	0.66 ± 0.21*
KSK 133	Con	0.5 ± 0.35	0.46 ± 0.12	1.2 ± 0.5	0.52 ± 0.16	1.7 ± 0.26	0.37 ± 0.19
	NTW	1.25 ± 0.72*	1.19 ± 0.25*	1.7 ± 0.33*	1.24 ± 0.27*	2.35 ± 0.52*	0.58 ± 0.27*
KS 282	Con	0.93 ± 0.28	0.21 ± 0.095	1.17 ± 0.6	0.96 ± 0.24	1.09 ± 0.70	0.49 ± 0.19
	NTW	1.34 ± 0.4*	0.65 ± 0.23*	1.8 ± 0.51*	1.3 ± 0.32*	1.6 ± 0.47*	1.02 ± 0.25*
Super bas	Con	2.09 ± 0.49	0.52 ± 0.34	1.41 ± 0.48	0.64 ± 0.3	1.91 ± 0.37	1.25 ± 0.34
	NTW	2.95 ± 0.32*	1.24 ± 0.15*	1.97 ± 0.39*	1.1 ± 0.3	2.46 ± 0.30*	1.3 ± 0.15*
PK1121 Aromatic	Con	0.36 ± 0.35	1.40 ± 0.35	0.40 ± 0.17	0.25 ± 0.12	0.8 ± 0.26	1.39 ± 0.32
	NTW	0.5 ± 0.23*	1.44 ± 0.34*	0.73 ± 0.22*	0.62 ± 0.21*	1.31 ± 0.35*	1.45 ± 0.33*
Note: Mean and Standard error ± SE from triplicate samples; Asterisk represents significant values and P < 0.05.							

Table 3
Effect of nanotechnology on rice seedlings before transplanting

Rice variety	Treatment	Whole plant length (cm)			Length of root (cm)			Length of shoot (cm)			Length of leaves (cm)		
		2017	2018	2019	2017	2018	2019	2017	2018	2019	2017	2018	2019
Zhongzoa 39	Con	430 ± 1.26	462 ± 0.13	452 ± 0.12	75 ± 1.85	44 ± 0.22	58 ± 0.23	83 ± 0.15	81 ± 0.15	78 ± 0.143	80.96 ± 0.003	78.5 ± 0.07	75.8 ± 0.04
	NTW	506 ± 1.72	521 ± 0.09	515 ± 0.14	86.67 ± 3.2	71.5 ± 0.15	74.3 ± 0.14	93.5 ± 0.36	93 ± 0.29	95.5 ± 0.44	126.3 ± 0.002	123 ± 0.25	120 ± 0.21
KS 282	Con	466.7 ± 1.37	465 ± 1.00	450 ± 1.02	67.7 ± 0.87	92 ± 0.15	78 ± 0.91	96 ± 0.14	91.5 ± 0.22	90 ± 0.13	96.26 ± 0.07	91 ± 0.17	95 ± 0.13
	NTW	555 ± 0.75	553 ± 0.12	543 ± 0.52	100 ± 1.68	101.5 ± 0.21	98.34 ± 1.13	121.5 ± 0.19	124 ± 0.37	123 ± 0.43	121.6 ± 0.16	164 ± 0.17	157 ± 0.20
KSK 133	Con	468.3 ± 2.7	466 ± 0.13	459 ± 0.11	96.33 ± 1.5	97.5 ± 0.07	90.22 ± 1.43	99.5 ± 0.21	109.5 ± 0.46	100 ± 0.42	108.7 ± 0.15	119.5 ± 0.13	111 ± 0.16
	NTW	576.7 ± 1.1	574.5 ± 0.15	579.2 ± 1.2	113.3 ± 1.26	106 ± 0.14	110 ± 1.30	134 ± 0.24	137.5 ± 0.06	139 ± 0.11	172.28 ± 0.11	185 ± 0.08	176 ± 0.12
Super basmati	Con	500 ± 2.08	496 ± 0.25	490 ± 0.14	80 ± 1.71	87 ± 0.1	82 ± 1.41	100 ± 0.07	105.5 ± 0.06	98.3 ± 0.06	87 ± 0.07	80.5 ± 0.13	84 ± 0.1
	NTW	626.7 ± 0.5	636 ± 0.6	630 ± 0.53	126 ± 1.88	121.5 ± 0.06	129 ± 0.91	161 ± 0.11	155 ± 0.16	159 ± 0.10	132 ± 0.05	138 ± 0.15	135 ± 0.12
PK 1121 Aromatic	Con	521.6 ± 0.4	594.5 ± 0.32	558.2 ± 0.33	58.3 ± 0.99	54 ± 0.22	56.5 ± 0.73	97.5 ± 0.21	98.5 ± 0.21	90 ± 0.11	87.9 ± 0.003	86.5 ± 0.07	80 ± 0.06
	NTW	610 ± 1.6	615.5 ± 1.26	620 ± 1.3	70 ± 2.84	65.5 ± 0.10	75 ± 2.15	124 ± 0.12	125 ± 0.12	126 ± 0.13	97.9 ± 0.003	93.5 ± 0.21	94 ± 0.05
*Mean and standard error from triplicate samples and P < 0.05													

A dry weight of early seedlings

Dry weight was significantly increased in all experimental varieties due to nano water treatment. Overall in three growing seasons highest seedling dry weight was observed KSK 133. The improvement was 89%, 53% 2017, 52% in 2017, 2018 and 2019 respectively. The lowest dry weight enhancement was in Zhongzoa 39 (31%, 31% and 21% in 2017, 2018 and 2019 respectively). Total seedling dry matter production is considered very important to interpret the yield of the rice crop (Fig. 3A-C). In previous studies, carbon-based nanomaterials showed improvement in morphology due to activate cell growth. They hypothesized that nanomaterials showed a sound effect on plants with different morphologies (Lahiani et al. 2016).

Soil-Plant Analyses Development (SPAD) Value:

The relation between leaf Nitrogen level and chlorophyll in plant leaves can be calculated in terms of SPAD reading (Swain and Sandip 2010). All five varieties showed variation in SPAD values after the application of nano-treated water (Figs. 3D–F). Data showed that SPAD values in Zhongzoa 39 increased by 2%; 1.67% and 4.3% in 2017, 2018, and 2019 respectively. The SPAD values in KSK 133 rice, flag leaves were improved by 17.01% in 2017, 15% in 2018 and 18% in 2019 (for observation flag leaves were used). The results suggested that nano-treated water had a significant effect on deferring the senescence of rice seedling. The effect of nano-TiO₂ has experimented on photosynthetic rate, showed improved photochemical reaction activity like absorbance of light, the transformation of light energy to electron energy, photophosphorylation efficacy and oxygen progression (Hong et al. 2005).

Effect of nanotechnology on rice seedlings (root and shoot lengths) before transplanting

Nanotechnologies or nano-materials are a double-edged weapon because they have both beneficial and negative consequences as well (Service 2004). Therefore, crop treating with nano-material, the specific nano synergids, or nanotechnologies with suitable energies should be selected to reduce negative effects. Nano synergid cannot penetrate plant cells; nano synergid had specific energies which only took part in the breakage of water molecules. So, these energies were destroyed after the formation of activated water and reduced the chances of nanotoxicity effects on plant cells (He 2005). There was no recent data based on nano synergid so the present study considers some nanomaterial examples. Recent data collection showed whole plant lengths, root, shoot length was significantly increased due to nano synergid treated water in all rice varieties, respectively (Fig. 2: Table 4). Earlier studies revealed that nano fertilizers may have a synergistic effect for improved nutrient uptake by plant cells, resulted in optimal growth (Morteza et al. 2013).

Table 4
Effect of nano-treated water on chlorophyll content chl a, chl b, Carotenoid and total chlorophyll content.

Rice variety	Treatment	Chlorophyll a mg g ⁻¹ Fw			Chlorophyll b mg g ⁻¹ Fw			Carotenoids mg g ⁻¹ Fw			Total chlorophyll content mg g ⁻¹ Fw		
		2017	2018	2019	2017	2018	2019	2017	2018	2019	2017	2018	2019
Zhongzoa 39	Control	10.72 ± 1.40	10.4 ± 1.26	10.00 ± 1.13	2.03 ± 0.630	1.94 ± 0.55	2 ± 0.18	826.5 ± 13.1	791 ± 12.5	787 ± 13.4	839 ± 13.2	803 ± 12.00	815 ± 12.2
	NTW	11.06 ± 0.1*6	12.3 ± 1.08*	12 ± 0.14*	2.75 ± 0.12*1	2.5 ± 0.72*	2.63 ± 0.13*	884.9 ± 11.1*	994 ± 11.2*	968 ± 11.0*	898 ± 1.10*	1009 ± 1.10*	956 ± 1.21*
KS 282	Control	10.09 ± 1.38	10.1 ± 2.6	10.4 ± 2.10	2.05 ± 0.66	1.9 ± 1.14	1.87 ± 1.16	804.6 ± 14.2	794 ± 25.1	800 ± 15.3	817 ± 14.3	782 ± 25	785 ± 16.0
	NTW	14.15 ± 2.70*	12.9 ± 3.09*	13.7 ± 2.64*	2.78 ± 1.220*	2.48 ± 1.42*	2.81 ± 1.15*	1104.5 ± 24*	912 ± 24.6*	1012 ± 17*	921 ± 24.0*	996.4 ± 24.0*	983 ± 20.1*
KSK 133	Control	6.32 ± 2.930	6.81 ± 2.83	6.52 ± 2.13	1.62 ± 1.310	1.92 ± 0.01	1.72 ± 1.13	508.3 ± 28.7	775.5 ± 20.2	573 ± 20.13	516 ± 28.9	587.5 ± 0.20	598 ± 0.25
	NTW	11.30 ± 0.48*	14.7 ± 0.16*	12.11 ± 0.15*	2.14 ± 0.210*	2.89 ± 0.02*	2.62 ± 0.19*	971.1 ± 4.6*	1179.6 ± 0.6*	1080 ± 5.13*	885 ± 4.64*	897 ± 0.60*	900 ± 1.23*
Aromatic 1121	Control	3.717 ± 0.58	5.75 ± 0.1	4.89 ± 0.13	1.698 ± 0.24	1.71 ± 0.10	1.64 ± 0.23	983 ± 5.31	995 ± 1.8	950.2 ± 1.13	287 ± 5	207 ± 1.8	265 ± 3.13
	NTW	13.14 ± 0.22*	13.7 ± 0.11*	12.78 ± 0.13*	2.65 ± 0.190*	2.7 ± 0.10*	2.69 ± 0.13*	1051.3 ± 3.8*	1100.9 ± 1.8*	1095 ± 1.4*	1067 ± 3.8*	1017 ± 1.76*	1023 ± 2.40*
Super basmati	Control	13.63 ± 0.09	13.9 ± 0.04	12.8 ± 0.07	2.61 ± 0.060	2.78 ± 0.09	2.54 ± 0.03	1056.2 ± 0.3	1097.8 ± 1.9	1084 ± 1.5	1072 ± 0.31	1014 ± 0.43	1035 ± 0.25
	NTW	18.98 ± 0.30*	17.3 ± 1.41*	16.81 ± 1.5*	3.76 ± 0.260*	3.42 ± 0.75*	3.54 ± 0.54*	1412.2 ± 7.3*	1376 ± 13.0*	1400 ± 6.1*	1435 ± 7.3*	1397 ± 7.50*	1400 ± 5.05*
*Mean and standard error (± S.E) from triplicate samples (n = 3) * showed significance at P < 0.05													

Oxidative enzymes: Superoxide Dismutase Activity (SOD); Catalase (CAT); Peroxidase (POD)

Oxidative enzymes play a key factor in abiotic and biotic stress like pathogen cell death in plants (Grant and Loake 2000). Activation of defensive genes activated by H₂O₂ which performs as a secondary messenger (Pellinen et al. 2002). The nano-treated water was showed effective results in biochemical components like enzymes (antioxidant and oxidant enzymes), reactive oxygen species (ROS), protein, starch, and amino acid. Among the enzymatic antioxidants, SOD is a main superoxide scavenger due to its enzymatic activity (Sharma et al. 2012). Compared to the SOD activity of control, three years (2017, 2018 and 2019) depicted that the SOD activity of Zhongzoa increased by 26%,

14%, and 27%. KSK 133 increased by 86% in 2017 and 2018, 60% in 2019 respectively (Figs. 4G–1). The other 3 rice varieties KS 282, Super basmati and PK 1121 aromatic were better than control.

Catalase enzyme activity (CAT) was one of the ROS-scavenging enzymes of plants. Three-year experiments exposed an increase in CAT activity with nano-treated water in all rice varieties (Fig. 4J-L). CAT content in 2017 was improved by 37%, 35% in 2018 and 37% in 2019 for Zhongzao 39, 55% in 2017, 74% in 2018 and 52% in 2019 for KSK 133. In Super basmati, KS 282 and PK 1211 aromatic showed improved CAT activity respectively as compared to non-treated water. Lu et al. (2002) stated that the increased antioxidant enzymes peroxidase (POD), superoxide dismutase (SOD), catalase (CAT) activities of soya bean germinated seed with nano-SiO₂ and nano-TiO₂ could significantly promote the seedling growth (Lu et al. 2002). POD is an important element to overcome the cascade of uncontrolled oxidation and protect the plant from oxidative damage (Fahad et al. 2016). The lowest enhanced value of POD was observed in Zhongzao 15%, 11.8% and 16.9% in 2017, 2018 and 2019 respectively. The highest increased of POD was in KSK 133 by 52% in 2017, 50% in 2018, 56% in 2019. In the present study, CAT and POD were significantly improved with nano-synergid. (Fig. 5: L-N)

The present study showed that nano-synergid treated water exposure had an effective impression on SOD, CAT, POD antioxidant enzymes. SOD can exchange negatively charged oxygen molecule⁻O₂ into H₂O₂ and positively charged oxygen and CAT and POD can transform the H₂O₂ into water and positively charged oxygen molecule (Scavenging of H₂O₂)(Anjum et al. 2015). Therefore, anti-oxidant enzymes can maintain the ROS and reduce the toxicity of ROS and protect the rice cells from damage. Increased CAT activity under nano-treated water might be the most important cause out detoxify the reactive oxygen activity (ROS) and decreased MDA contents (Fig. 7).

Malondialdehyde (MDA)

MDA content is an important tool for describing the amount of lipid peroxidation; higher concentrations affect the plant or indicate cell membrane damage. The increased amount of MDA content is produced when polyunsaturated fatty acids in the membrane undergo oxidation by the accumulation of free oxygen radicals. Increased lipid peroxidation is the main indicator of oxidative damage in plants (Bor et al. 2003). Present experimentation displayed higher MDA content in control treatments in all five varieties. The previous studies exhibited, decreased MDA content mediated by calcium phosphate nanoparticles (NP) in both root and shoot as compared to control. The MDA contents of root and shoot reduced with calcium phosphate NP may be due to variation of ROS in plants (Upadhyaya et al. 2017). The MDA content decreased in all nano-synergid treated varieties in ascending order of Zhongzao 39 > KS 282 > PK 1121 aromatic > Super basmati > KSK133 (Fig. 50-Q).

Chlorophyll Content (Chl a, b, Carotenoids and total chlorophyll)

In the present study, we speculated that chlorophyll a, b, and total chlorophyll content enhanced by nano-treated water. It can be closely related to photochemical reaction activity. The effect of nano-TiO₂ has experimented on photosynthetic rate, showed improved photochemical reaction activity like absorbance of light, the transformation of light energy to electron energy, photophosphorylation efficacy and oxygen progression (Hong et al. 2005). Total chlorophyll content (CHL a, b, carotenoids) decreased with control and increased in NTW observed in different rice varieties (Table 5). Though nano-materials and nanotechnologies make a positive effect on the plant seed germination and growth, should address some serious challenges like nanomaterial reaction lower the photosynthetic activity, phytotoxicity (Nair et al. 2010).

Table 5
Quantifications of Plant Hormones, Jasmonates (JA), Salicylic acid (SA) and Brassinosteroids (BR) by MULTISKAN MS.

Quantifications of Plant Hormones, Jasmonates (JA), Salicylic acid (SA) and Brassinosteroids (BR) by HPLC/MS/MS.										
Year	Rice variety	Salicylic acid (SA)		Jasmonates (JA)			Brassinosteroids (BR)			
		Curve	R ²	LOQ (pmol/L)	Curve	R ²	LOQ (pmol/L)	Curve	R ²	LOQ (pmol/L)
2017	Zhongzao 39 Con	11.087 + 693.21X	0.9987	429.77	-3.06 + 544.4X	0.9986	354.90	-6.2575 + 73.973X	0.9984	41.95
	Zhongzao 39 NTW			454.03*			384.84*			45.27 *
	KS 282 Con			1275.48			1084.41			231.08
	KS 282 NTW			1968.5*			1963.33*			598.6*
	KSK 133 Con			3755.58			2700.60			381.52
	KSK 133 NTW			6186.86 *			5175.65*			618.20*
	Aromatic 1121 Con			1618.62			1225.96			167.33
	Aromatic1121 NTW			2703.49 *			2224.95*			287.54*
	Super bas Con			848.50			777.13			100.55
	Super bas NTW			1452.25*			1288.56 *			142.18*
2018	Zhongzao 39 Con	9.169 + 587.35X	0.9975	437.56	-2.0482 + 453.5X	0.9981	367.01	-4.1457 + 65.652X	0.9994	36.78
	Zhongzao 39 NTW			467.11*			393.21*			40.32*
	KS 282 Con			1135.25			979.02			146.54.
	KS 282 NTW			4156.50 *			4321.02*			623.11*
	KSK 133 Con			1024.54			910.76			145.64
	KSK 133 NTW			5823.22 *			4231.01*			546.83*
	Aromatic 1121 Con			1645.77			1576.05			187.53
	Aromatic1121 NTW			2154.37			2034.65			265.33
	Super bas Con			783.66			814.25			121.76
	Super bas NTW			1375.43*			1145.53*			156.23*
2019	Zhongzao 39 Con	13.167 + 700.37X	0.9995	421.12	-5.3705 + 678.9X	0.9985	345.54	-8.4879 + 80.706X	0.9986	33.76
	Zhongzao 39 NTW			460.43*			381.4*			42.55 *
	KS 282 Con			2075.48			1898.41			278.12
	KS 282 NTW			6018.1*0			4134.55*			522.1*
	KSK 133 Con			1985.34			2032.30			301.2
	KSK 133 NTW			5922.12*			5014.21*			582.10*
Mean values form triplicate samples (n = 3) * showed P < 0.05 significant values										

Year	Rice variety	Salicylic acid (SA)	Jasmonates (JA)	Brassinosteroids (BR)
	Aromatic 1121 Con	1921.54	1343.21	172.54
	Aromatic1121 NTW	2521.54 *	1954.55*	260.36*
	Super bas Con	679.53	792.51	139.81
	Super bas NTW	1145.23 *	1176.21*	167.32*
Mean values form triplicate samples (n = 3) * showed P < 0.05 significant values				

Quantifications of Plant Hormones, Jasmonates (JA), Salicylic acid (SA) and Brassinosteroids (BR) by instrument MULTISKAN MS.

Brassinosteroids (BRs) are polyhydroxylated steroidal hormones or growth regulators, which are associated with different physiological functions e.g. seed germination, cell elongation, cell divisions, root development, also respond to various biotic and abiotic stress (De Vleeschauwer et al. 2012). BR signaling genes improved rice architecture and increased grain yield (Bajguz 2011). In a present piece of work, KSK 133 showed the highest amount of BR, lowest in Zhongzao 39 (Table 5). In previous studies claimed that BR activated the specific transcription factors which can stimulate BR- targeted genes. BR regulated the antioxidant enzyme activities, SPAD value (photosynthetic capacity), chlorophyll contents to improve plant growth (Anwar et al. 2018). In conformity with earlier reports, it was observed that BR promotes growth and act as plant immunity against Blast fungal disease (*Magnaporthe grisea*) in rice (Yang et al. 2013).

Jasmonic acid (JA) are originated from lipid, known as α -linolenic acid, which plays important role in rice defense system from microbial infection (bacterial and fungal)(Yang et al. 2013). The lipid-derived compounds help in plant biotic and biotic stress response or protection. (Schaller and Stintzi 2009). In a recent study, JA and nano synergid showed a stimulatory effect on rice immunity. Earlier studies stated that JA is involved in a range of processes from development to light responses (Wasternack and Kombrink 2010). Jasmonates cannot work individually but work in a complex signaling network and collective plant hormone signaling pathways (Ahmad et al. 2016). The present study showed an increased level of endogenous JA in rice (Table 5)

Salicylic acid (SA) is an important phenolic compound present in plants at various levels e.g. rice contains high basal SA levels (5000–30,000 ng g⁻¹ fresh weight). SA is produced from benzoic acid. SA is present in leaves as the free acid. In rice maintain a high level of SA because of free SA in leaves and shoot and lesser in roots (Hu et al. 2017). SA helps in control the redox reactions, protects from oxidative stress and biotic and abiotic stress as well (Hu et al. 2017). The present study increased antioxidant, photosynthetic activity, and increased level of endogenous SA in rice leaves (Table 5).

Endogenous SA displays a vital antioxidant role in defending rice from oxidative stress. So a high amount of SA can directly be related to triggering antioxidant responses, modulate redox balance and scavenge ROS (Grant and Loake 2000). In a recent study increased SA showed in KSK 133 and lowest in Zhongzao 39. In the foliage of *Alternanthera tenella* SA exhibited improved antioxidant activity and increased in betacyanin content, which are compounds with a normal antioxidant action (Lucho et al. 2019)

The cross-talk of plant hormones is the best way to respond to plant stress. SA and JA are resistant factors and BR responsible for above-ground plant growth. So, the present study stated that endogenous hormones play an important in growth, the developmental process, and plant immunity (Fig. 7). The same observation expressed in previous studies like BR and JA pathways have involved a balance between growth and defense, SA controls early defense gene expressions and JA tempts late defense based gene expressions (He et al. 2017).

Yield of rice

The nano synergid application had a significant impact on yield –attributing characters i.e., Plant height (cm), Remaining biomass (R.B), Branch weight without seeds (g), Panicle weight (g), No of Panicle, Total no of seeds per panicle, Filled grain per panicle, unfilled grain per panicle and 1000 grain weight as compared to the control (Table 6). In previous studies, root shoots and grains were improved by ZnO nanoparticles which act as nano fertilizer (Bala et al. 2019) same observations were made in recent studies. The yield parameters were

significantly higher with nano synergid in KSK 133. Earlier studies showed that seed primed by Qiangdi nano-863 can achieve good yield in japonica rice (Liu et al. 2007a).

Table 6
Effect of nano-synergid on agronomic data after ripening of rice plant

Year	Rice variety	Plant height (cm)	Remaining biomass (R.B) (g)	Branch weight without seeds (g)	Panicle weight (g)	No of Panicle	Total no of seeds per panicle	Filled grain per panicle	Unfilled grain per panicle	1000 grain weight (g)
2017	Zhongzao 39 Con	900	27.5	1.8	14.3	10	600	280	320	12.2
	Zhongzao 39 NTW	1210*	32.3*	1.9*	15.43*	12*	644*	300*	344*	14.8*
	KS 282 Con	500	27.8	1.60	7.6	9	851	600	451	17.3
	KS 282 NTW	550*	29*	1.7*	9.5*	13*	1032*	739*	547*	20.3*
	KSK 133 Con	468	20	1.50	13.8	6	900	600	300	17.8
	KSK 133 NTW	877*	47.40*	2.2*	18.1*	15*	1286*	802*	384*	22.92*
	Aromatic1121 Con	883	28.2	1.9	9.1	9	535	287	248	10.2
	Aromatic1121 NTW	1002*	33.3*	2.2*	11.9*	11*	700*	328*	232*	12.5*
	Super bas Con	600	40.07	1.7	15.8	12	878	600	278	17.4
	Super bas NTW	850*	63.04*	2.0*	22.34*	19*	1100*	794*	300*	21.14*
2018	Zhongzao 39 Con	875	47.2	1.6	12.1	7	639	354	285	12.7
	Zhongzao 39 NTW	1245*	57.3*	2.1*	17.5*	15*	657*	370*	287*	13.5*
	KS 282 Con	540	28.7	1.8	6.8	11	975	597	478	17.4
	KS 282 NTW	570*	31*	2.0*	7.9*	15*	1277*	731*	564*	22.8*
	KSK 133 Con	450	20	1.47	4.1	6	920	775	145	17.9
	KSK 133 NTW	880*	58*	2.48*	20.5*	17*	1209*	1100*	109*	22.1*
	Aromatic 1121 Con	870	30	1.7	8	7	500	200	290	11.2
	Aromatic1121 NTW	1115*	34.3*	2.0*	10*	9*	545*	368*	117*	12*
	Super bas Con	588	39	1.6	14.1	10	959	409	250	17.2
	Super bas NTW	900*	66*	2.5*	24.14*	20*	1104*	1051*	760*	21.5*
2019	Zhongzao 39 Con	822	52.2	1.8	13.9	9	585	285	300	11.45
	Zhongzao 39 NTW	1196*	57.6*	2.0*	15.43*	12*	635*	325*	310*	13.63*
	KS 282 Con	525	27.1	1.5	7.0	10	711	611	400	16.72
	KS 282 NTW	562*	30*	1.8*	9.8*	14*	998*	660*	538*	19.34*
	KSK 133 Con	575	22	1.6	14	7	937	600	337	18.3
	KSK 133 NTW	885*	46*	2.4*	18.3*	16*	1275*	802*	373*	22.56*
	Aromatic 1121 Con	850	26	1.7	8.8	8	535	287	248	11.45

*Mean from triplicate samples and Asterisk showed significance at P < 0.05

Year	Rice variety	Plant height (cm)	Remaining biomass (R.B) (g)	Branch weight without seeds (g)	Panicle weight (g)	No of Panicle	Total no of seeds per panicle	Filled grain per panicle	Unfilled grain per panicle	1000 grain weight (g)
	Aromatic1121 NTW	1025*	34*	2.4*	12*	12*	692*	360*	232*	13.1*
	Super bas Con	578	38.29	1.6	15.4	11	878	600	278	17.4
	Super bas NTW	880	64.11*	2.2*	23*	18*	1094*	794*	300*	20.34*
*Mean from triplicate samples and Asterisk showed significance at P < 0.05										

As pointed out previously, nano synergid enhanced grain yield in rice, is already used in agriculture due to their lack of toxicity, biodegradability, and edibility (Lemraski et al. 2017; Sirisena et al. 2013). In KSK 133 maximum plant height was recorded 877 cm in 2017, 880 cm in 2018 and 885 cm in 2019. The remaining biomass was 47.4 g in 2017, 58 g in 2018 and 46 g in 2019. Branch weight without seed was 2.2 g in 2017, 2.48 g in 2018 and 2.4 g in 2019. Branch weight and panicle weight was improved in NTW KSK 133 and lowest in Zhongzoa 39. No of the panicle indicate the yield of grains in the rice plant. KSK 133 exhibited filled grains were per panicle 802 in 2017, 1100 in 2018 and 802 in 2019, unfilled grain per panicle was 384 in 2017, 109 in 2018 and 373 in 2019. In previous studies, nanomaterials showed an increase in root 31–37%, 12–35% and overall leaves area also improved (Liu et al. 2007b). The agronomist recommended that nano-fertilizers significantly influenced the biomass yield and grain yield (Janmohammadi et al. 2016; Morteza et al. 2013)

Multivariate analysis (Principle Component Analysis)

PCA in the recent study used for better practice in the interpretation of complex data, physiological parameters, biochemical parameters, and yield among 5 rice varieties was shown by PC1 for 2017, PC2 for 2018 and PC 3 for 2019 (Fig. 6). In plots of principal components, 1, 2 and 3 of PCA results obtained from bio-chemicals, physiological features and yield of Chinese and Pakistani rice exposed to nano treated water. In the year 2017 principal component, 1 (PC 1) describes 33.45% and principal component 2 describes 24.10%. The cumulative percentage of PC 1 was 57.55%. It was clear that CAT, POD, chlorophyll content and 1000 grain weight grouped with positive loading on the upper side of the biplot, suggesting that these parameters had a positive correlation among themselves. Dry weight, JA, SA and BR were observed in the lower side positive and SPADE, SOD were negatively correlated in the biplot. In the year 2018 PCA results describe PC 1 45.63% and PC 2 was 18.83%. In PC 2 cumulative percentage was 64.46%. Dry weight, SA, JA, BR, MDA and 1000 grain weight were on the right upper side of the biplot, suggesting that these parameters had a positive correlation among themselves. CAT, SOD, POD chlorophyll and SPADE were on the lower side of the biplot. In the year 2019 PCA 3 cumulative was 63.49%. In PC3 first component was 46.75% and the second component was 16.75%. The upper right side of the PC3 had a positive correlation showed in dry weight, chlorophyll, SPADE, CAT, POD, SOD, MDA and 1000 grain weight. All hormones JA, SA and BR were on the lower side of the biplot which were also positively correlated.

Conclusion

Nano synergid Qiangdi 863 has a great potential for application in cash crops, but there is still a long way down to reach the field. It is impossible to know all the details about how Nano synergid work in a particular crop but need to start the field experiments solving some problems like mounting the production and avoiding hazardous and toxic materials. The present study deals with nano synergid Qiangdi 863 in a field experiment and they exhibited prolong effective nutrient supply; involve all the steps of the crop cycle; from sowing to transplanting and harvest. The first time reported the potential of Qiangdi 863 nano synergid treated water to improve yield efficiency of Pakistani varieties (KS 133, Super basmati, KS 282 and 1121 aromatic). These efforts led to nano synergid based design and development. Nanotechnology-based agriculture could be more effective than fertilizer counterparts.

Nano synergid emits electromagnetic waves that generated high energy (resonance) between water molecules. The nano synergid treated water enhanced the light absorption at a specific wavelength that changes the water molecules' structure and energy. This alteration of water structure and energy is also called activated water. Activated water absorbed by the seed and it enhanced amylase activity, continuously strikes the cell for germination. The cell energy is activated and their function is stimulated which enhances the metabolism of rice seedling. Nano synergid treated water enters into an oxidative enzyme system like SOD is a main superoxide scavenger exchange negatively charged oxygen molecule O_2^- into H_2O_2 , Catalase (CAT) and Peroxidase (POD) transform H_2O_2 into water and positively charged oxygen molecule. These oxidative enzymes reduce the toxicity of reactive oxygen species (ROS) in kerb cycle, citric acid cycle in cytosol and mitochondria. Malondialdehyde (MDA) is polyunsaturated fatty acids in the membrane that undergo oxidation by an accumulation of free oxygen radicals.

MDA was negatively correlated with the activities of ROS scavenging enzymes. So, oxidative enzymes lower the MDA content in rice. The rice performance is improved and their immune competencies are enhanced. Plant endogenous hormones Jasmonates (JA), Salicylic acid (SA) and Brassinosteroids (BR) also play an important role in growth, development and rice immunity (protect from biotic and abiotic stress). Nano synergid treated water will induce a higher germination rate, improved vegetative growth, increase no of productive panicles and a good harvest can be achieved (Fig. 7).

List Of Abbreviations

Nano treated water (NTW)

single-walled carbon nanotubes (SWCNTs)

Multi-walled carbon nanotubes (MWCNTs)

Nanometer pottery trays (NPTs)

Speed of emergence (SE)

Percentage emergence (PE)

Seed emergence energy percentage (SEEP)

Soil-Plant Analyses Development (SPAD)

Superoxide Dismutase (SOD)

Catalase (CAT)

Peroxidase (POD)

Malondialdehyde (MDA)

Reactive oxygen activity (ROS)

Jasmonates (JA)

Salicylic acid (SA)

Brassinosteroids (BR)

Declarations

Acknowledgements

The present study received financial support from the China National Rice Research Institute of China (CNIRRI) Open Program of State key laboratory of Rice Biology, China National Rice Research Institute, Hangzhou 310006, China Open Fund from State key laboratory of Rice Biology, China National Rice Research Institute, Hangzhou 310006, China.

Funding

This research was sponsored by the China National Rice Research Institute of China (CNIRRI) Open Program of State key laboratory of Rice Biology, China National Rice Research Institute, Hangzhou 310006, China

Authors' contributions

AY, SF and MR designed carried out the experiments, ZY designed the experiments, analyzed and interpreted data. AY, MR, NR and AA drafted the manuscript. SF and SH analyzed and interpreted data, and helped to draft the manuscript. All authors read and approved the final manuscript.

Ethics declarations

Competing interests

The authors declare that they have no competing interests.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The data and materials used and analyzed in the current study can be provided by the corresponding author for scientific, non-profit purposes.

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Figures

Figure 1

Effect of Qiangdi 863 Nano synergid treated water on rice seed germination a Zhongzao 39 control b Zhongzao 39 NTW c KSK 133 control d KSK 133 NTW e KS 282 control f KS 282 NTW g Super basmati control h Super basmati NTW i PK 1121 aromatic control j PK 1121 aromatic NTW

Figure 2

Effect of nano-treated water on rice seedlings A Zhongzao 39 control B Zhongzao 39 NTW C KSK 133 control D KSK 133 NTW E KS 282 control F KS 282 NTW G Super basmati control H Super basmati NTW I PK 1121 Aromatic control J PK 1121 Aromatic NTW.

Figure 3

Influence of nano-treated water on (A) Dry weight in 2017, (B) Dry weight in 2018, (C) Dry weight in 2019 (D) Spade value 2017 (E) Spade value 2018 (F) Spade value 2019. Vertical bars above mean indicate standard errors of three replicates. The mean value of each treatment with different showcase letters represents significant differences by LSD-test ($P < 0.05$).

Figure 4

Effect of nano-treated water on (G) SOD in 2017 (H) SOD in 2018, (I) SOD in 2019, (J) CAT in 2017, (K) CAT in 2018, (L) CAT in 2019. Vertical bars above mean indicate standard errors of three replicates. The mean value of each treatment with different showcase letters represents significant differences by LSD-test ($P < 0.05$).

Figure 5

Effect of nano-treated water on (L) POD in 2017 (M) POD in 2018 and (N) POD in 2019, (O) MDA in 2017 (P) MDA in 2018 and (Q) MDA in 2019. Vertical bars above mean indicate standard errors of three replicates. The mean value of each treatment with different showcase letters represents significant differences by LSD-test ($P < 0.05$).

Figure 6

Principle Component Analysis (PCA) year 2017, 2018 and 2019 with all physiological parameter (Dry weight, Chlorophyll, SPADE) biochemical parameters (antioxidant enzymes CAT, SOD, POD and MDA), Endogenous hormones (SA, JA and BR) Yield parameter (1000 grain weight).

Figure 7

Qiangdi nano-863 nano synergid release electromagnetic waves which break the water macro-molecules into micro-molecules. Micro-molecules of water enter into seed activates the hormone (GA) to amylase and speed up the germination process. Oxidative enzymes activation (SOD, CAT) and riddance of reactive oxidation species (ROS) lower the production of MDA and maintain the redox reactions in different subcellular structures. H₂O₂ is generated in normal metabolism via the different organelle electron transport chain in mitochondria, chloroplasts PS-1 and PS II and cytosol. SA and JA also helped in oxidative response and rice immunity. BR promotes growth and antioxidant activity. Rice plant will have faster germination, establishing root system, enhanced tillers, flowering and full filled grains.