Original Article

Physiological and biochemical changes induced by Qiangdi nano-863 biological assistant growth apparatus during rice seed priming under temperature stress

Alterações fisiológicas e bioquímicas induzidas pelo aparelho de crescimento assistente biológico Qiangdi nano-863 durante o priming de sementes de arroz sob estresse de temperatura

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Abstract

A huge amount of rice cultivation and consumption occur in Asia particularly in Pakistan and China. However, multiple abiotic stresses especially high and low-temperature proved to be a substantial threat for rice production ultimately risks for food security. To overcome various types of abiotic stress; seed priming is among the effective approaches to improve the rice seed germination and growth vigor. Therefore, the present study was planned to evaluate physiological and biochemical modifications in Chinese and Pakistani rice varieties by Qiangdi 863 biological assistant growth apparatus nano treated water (NTW), Osmopriming Calcium chloride (CaCl.,), redox priming hydrogen peroxide (H_2O_2) and hormonal priming by Salicylic acid (SA) under temperature stress conditions. The experiment was performed with completely randomize design conditions. Five rice varieties, nomenclature as Zhongzoa 39, (Chinese rice variety) KSK 133, KS 282, Super basmati and PK 1121 aromatic (Pakistani rice variety) were sown under low temperature (LT) (17°C), optimal temperature (OT) 27°C and high temperature (HT) 37°C conditions. The present study indicated that nanopriming were the most effective treatments increased Germination Energy Percentage (GEP) (96.1, 100, 100%), Speed of Germination (SG) (27.2, 35.45, 37.1), Final Germination Percentage (FGP) (98.2, 99.1, 99.4%), Seedling Dry Weight Biomass (DWB) (0.1, 0.137, 0.14g), Total Chlorophyll Content (0.502, 13.74, 15.21), antioxidant enzymes Superoxide Dismutase (SOD)(3145, 2559, 3345 µg-1FWh-1), Catalase (CAT) (300, 366, 3243 µg⁻¹FWh⁻¹) and decreased Malondialdehyde (MDA) (6.5, 12.2, 6.5 µmol g⁻¹ FW) for Zhongzao 39 and KSK 133 rice varieties under low (LT+NTW), optimal temperature (OP+NTW) and high temperature (HT+NTW) stress., Therefore, nano-priming is recommended to cope with the high and low-temperature stress conditions along with improved productivity of rice.

Keywords: antioxidative enzymes, high temperature, low temperature, seed priming.

Resumo

Cultivo e consumo de arroz ocorrem em grandes quantidades na Ásia, particularmente no Paquistão e na China. No entanto, vários estresses abióticos, especialmente de alta e baixa temperatura, provaram ser uma ameaça considerável para a produção de arroz, em última análise, riscos para a segurança alimentar. Para superar vários tipos de estresse abiótico, o priming de sementes está entre as abordagens eficazes que melhoram a germinação e o vigor de crescimento das sementes de arroz. Portanto, o presente estudo foi planejado para avaliar as modificações fisiológicas e bioquímicas em variedades de arroz chinês e paquistanês por Qiangdi 863, aparelho assistente biológico de crescimento com água nanotratada (NTW), Osmopriming cloreto de cálcio (CaC₁₂), peróxido de hidrogênio redox (H_2O_2) e priming hormonal por ácido salicílico (SA) em condições de estresse de temperatura. O experimento foi realizado em condições de delineamento inteiramente ao acaso. Cinco variedades de arroz, nomenclaturas como Zhongzoa 39 (variedade de arroz chinês), KSK 133, KS 282, Super basmati e PK 1121 aromático (variedade de arroz do Paquistão) foram semeadas sob baixa temperatura (LT) (17 °C), temperatura ótima (OT) 27 °C e condições de alta temperatura (HT) 37 °C. O presente estudo indicou que nanocondicionamento foi o tratamento mais eficaz: aumento da porcentagem de germinação (GEP) (96,1%, 100%, 100%), velocidade de germinação (SG) (27,2, 35,45, 37,1), porcentagem de germinação final (FCP) (98,2%, 99,1%, 99,4%), biomassa de peso seco de mudas (DWB) (0,1 g, 0,137 g, 0,14 g), conteúdo total de clorofila (0,502, 13,74, 15,21), enzimas antioxidantes superóxido

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dismutase (SOD) (3145, 2559, 3345 µg-1FWh- 1), catalase (CAT) (300, 366, 3243 µg-1FWh-1) e malondialdeído diminuído (MDA) (6,5, 12,2, 6,5 µmol g-1 FW) para as variedades de arroz Zhongzao 39 e KSK 133 sob baixo (LT + NTW), temperatura ótima (OP + NTW) e estresse de alta temperatura (HT + NTW). Portanto, o nanopriming é recomendado para lidar com as condições de estresse de alta e baixa temperatura, juntamente com a produtividade aprimorada do arroz.

Palavras-chave: enzimas antioxidantes; temperatura alta, temperatura baixa, priming de sementes.

1. Introduction

Rice (Oryza sativa L.) has been nurtured for an immense portion of the realm's populace specifically in Western Asia, the Mid East, and the Caribbean region (FAO, 2005). A huge amount of rice cultivation and consumption occur in Asia specifically in China and Pakistan (Datta and Adhya, 2014). It is cultivated in more than 100 countries which can deliver more than 483 million tons of milled rice every year (Dass et al., 2017). In the future, the demand for rice probably rise due to increase in consumption up to 496 million tons (mt) in 2020, and 555 mt by 2035 (FAO, 2005). Asia is cultivating rice on a vast scale due to excessive field area, and that's why its demand and supply are going smoothly and steadily until now. Expected trade of rice all over the world record in 2022 will be (000 tons) USA 3320 tons, Thailand 11,000 (000 tons), Pakistan 4300 (000 tons), China 1800 (000 tons) (Younas et al., 2020)

The variations in climatic conditions and upsurge in the human population are continuously posing threat to crop production and management practices (Akram, 2007). Climate change now has become the world's most critical issue so, it is necessary to improve the resistance in the agricultural crop against various abiotic stresses (Wang et al., 2016; Scremin-Dias et al., 2011). Climatic change is generally categorized by the present global warming scenario (Fischer et al., 2005; Jerônimo et al., 2011). Therefore, it has become evident that temperature has become the major abiotic stress in the rice cultivation system (Bashir et al. 2007). Low temperature and high temperature are critically impacting rice production and causing more than half of the global yield losses annually (Ribant et al., 2002).

In the modern agricultural system, different approaches are employed to lessen the high and low-temperature effects in crops. Among these approaches, seed priming is an operative, beneficial, cost-effective for improved fast, and even germination (Paparella et al., 2015; Jeller et al., 2003). This technique helps to activate the standard metabolic process during the primary stage of sprouting before the root emergence (Hussain et al., 2015). It can also reduce the longevity duration of germination (Hill et al., 2007). The rice sprouting and other developmental stages might be enriched by seed priming (Farooq et al., 2007a). Qiangdi nano-863 biological assistant growth apparatus is an agricultural high tech invention and most widely used in China (Jun-rong et al., 2016). Farooq et al. (2007b) demonstrated Osmo-hardening with CaCl, and better sprouting likewise Rehman et al. (2011) testified improved plantlet growth and crop harvest. The suitable amount of Hydrogen peroxide (H_2O_2) was favorable for breaking seed dormancy and improved growth (Lariguet et al.,

2013). H_2O_2 was involved in the improvement of signaling pathways of hormones (SA, GA, and ABA) El Maarouf-Bouteau et al. (2015). Salicylic acid (SA) was a renounced hormone promoting plant defensive system to biotic and abiotic stress (Yan and Dong, 2014).

Rice is the main food crop and cultivated mostly in colder, tropical, arid, semi-arid, and temperate regions of the world where low and high temperatures are barriers to good rice crop establishment. But few studies have been conducted for temperature based comparable execution of seed priming. The main purpose of current experimentation was to assess the response of various seed priming techniques on the germination of seed under low and high-temperature stress. Moreover, this study also focused to investigate biochemical and physiological characteristics in primed rice seedlings.

2. Materials and Methods

2.1. Plant material and growing conditions

Five rice varieties Zhongzao 39 (Chinese Indica variety), KSK 133, KS 282, Super Basmati, and PK aromatic 1121 (Pakistani Indica varieties) seed were used as experimental germplasm (Table 1). For removal of contamination in the trail of seed priming, germplasm was superficially treated with 3% NaClO solution (domestic bleach diluted 1:1 with distilled water) for 30 minutes and rinsed with sanitized water. The early sprouting of rice seed was > 95-98% in this study. This trial was carried out at the State key laboratory of Rice Biology, China National Rice Research Institute, Hangzhou in 2017.

2.2. Experimental design

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 72 hours to produce nano-treated water. Rice seed was pre-soaked in tap water for 24 hours, and then soaked in nano-treated water for germination.

In the present study Osmopriming (CaCl₂: 150 mg/L Calcium chloride), Redox priming (H_2O_2 : 50µM Hydrogen peroxide), and Hormonal priming (SA: 150 mg/L Salicylic acid) methods were utilized. Priming reagents were preoptimized for their significant level established on seed germination and initial seedlings proliferation execution. Seed was primed at 26°C for 24hours through continual moderate agitation (100rpm). The division of seed mass to primed solutions dimensions (w/v) was 1:5. The seed was dripping in the preparing solutions for 12 hrs and 24 hrs

Rice varieties	Parentage/Pedigree	Year of Release	Breeding Center	Province/Country
Zhongzao 39	Jiayu 253/Zhongzu no.3	2012	CNRRI, Hangzhou	Zhejiang, China
KSK 133	KS 282× 4321	2006	KSK, Lahore	Punjab, Pakistan
KS 282	Basmati 370×IR95	1982	KSK, Lahore	Punjab, Pakistan
Super Basmati	Basmati×10486	1996	KSK, Lahore	Punjab, Pakistan
PK 1121 aromatic	Selection from farmer's fields for extra-long grain and high elongation ratio	2013	KSK, Lahore	Punjab, Pakistan

Table 1. List of rice germplasm used in study at China National Rice Research Institute, 2017/18.

interval. After 24 hrs seed was rinsed by purified water. The seed dried by filter paper and moved to the air-drying oven at 25°C for 48 hrs. to minimize the moistness.

2.3. Temperature treatments

All the five varieties were sown under three different temperatures; low temperature (17 °C), optimum temperature (27°C) and high temperature (37°C).

2.3.1. Low-temperature treatments

Low-temperature treatments included; Variety 1 (Zhongzao 39), Control + low temperature and No priming (Zhongzao 39+LT +NP), Zhongzao 39, low temperature and Nano treated water priming (Zhongzao 39+LT+NTW), low temperature and Calcium chloride priming (Zhongzao 39+LT+CaCl₂), Zhongzao 39, low temperature and H₂O₂ priming (Zhongzao 39+LT+H₂O₂), Zhongzao 39, low temperature and SA priming (Zhongzao 39+LT+SA).

Variety 2 (KSK 133) Control+ low temperature and No priming (KSK 133+LT +NP), KSK 133, low temperature and Nano treated water priming (KSK 133+LT+NTW), low temperature and Calcium chloride priming (KSK 133+LT+CaCl₂), KSK 133, low temperature and H₂O₂ priming (KSK 133+ LT+H₂O₂), KSK 133, low temperature and SA priming (KSK 133+ LT+SA).

Variety 3 (KS 282) Control + low temperature and No priming (KS 282+LT +NP), KS 282, low temperature and Nano treated water priming (KS 282+LT+NTW), KS 282, low temperature and Calcium chloride) priming (KS 282+LT+CaCl₂), KS 282, low temperature and H₂O₂ priming (KS 282+LT+H₂O₂), KS 282, low temperature and SA priming (KS 282+LT+SA).

Variety 4 (Super Basmati) Control + low temperature and No priming (Super bas +LT +NP), Super bas, low temperature and Nano treated water priming (Super bas +LT+NTW), Super bas, low temperature and Calcium chloride priming (Super bas +LT+CaCl₂), Super bas, low temperature and H₂O₂ priming (Super bas + LT+H₂O₂), Super bas, low temperature and SA priming (Super bas + LT+SA)

Variety 5 (PK 1121 Aromatic) Control + low temperature and No priming (PK 1121 aroma +LT +NP), PK 1121 aroma, low temperature and Nano treated water priming (PK 1121 aroma +LT+NTW), PK 1121 aroma, low temperature and Calcium chloride priming (PK 1121 aroma +LT+CaCl₂), PK 1121 aroma, low temperature and H₂O₂ priming (PK 1121aroma+ LT+H₂O₂), PK 1121aroma, low temperature and SA priming (PK 1121aroma+ LT+SA).

2.3.2. Optimum-temperature treatments

Optimum-temperature treatments included; Variety 1 (Zhongzao 39), Control + Optimum temperature and No priming (Zhongzao 39+OT +NP), Zhongzao 39, Optimum temperature and Nano treated water priming (Zhongzao 39+OT+NTW), Optimum temperature and Calcium chloride priming (Zhongzao 39+OT+CaCl₂), Zhongzao 39, Optimum temperature and H₂O₂ priming (Zhongzao 39+OT+H₂O₂), Zhongzao 39, Optimum temperature and SA priming (Zhongzao 39+OT+SA).

Variety 2 (KSK 133) Control+ Optimum temperature and No priming (KSK 133+OT +NP), KSK 133, Optimum temperature and Nano treated water priming (KSK 133+OT+NTW), Optimum temperature and Calcium chloride priming (KSK 133+OT+CaCl2), KSK 133, Optimum temperature and H2O2 priming (KSK 133+ OT+H2O2), KSK 133, Optimum temperature and SA priming (KSK 133+ OT+SA).

Variety 3 (KS 282) Control + Optimum temperature and No priming (KS 282+OT +NP), KS 282, Optimum temperature and Nano treated water priming (KS 282+OT+NTW), KS 282, Optimum temperature and Calcium chloride) priming (KS 282+OT+CaCl₂), KS 282, Optimum temperature and H₂O₂ priming (KS 282+OT+H₂O₂), KS 282, Optimum temperature and SA priming (KS 282+OT+SA).

Variety 4 (Super Basmati) Control + Optimum temperature and No priming (Super bas +OT +NP), Super bas, Optimum temperature and Nano treated water priming (Super bas +OT+NTW), Super bas, Optimum temperature and Calcium chloride priming (Super bas +OT+CaCl₂), Super bas, Optimum temperature and H₂O₂ priming (Super bas + OT+H2O2), Super bas, Optimum temperature and SA priming (Super bas + OT+SA)

Variety 5 (PK 1121 Aromatic) Control + Optimum temperature and No priming (PK 1121aroma +OT +NP), PK 1121 aroma, Optimum temperature and Nano treated water priming (PK 1121aroma +OT+NTW), PK 1121 aroma, Optimum temperature and Calcium chloride priming (PK 1121aroma +OT+CaCl₂), PK 1121 aroma, Optimum temperature and H₂O₂ priming (PK 1121aroma+OT+H₂O₂), PK 1121aroma, Optimum temperature and SA priming (PK 1121aroma+OT+SA).

2.3.3. High-temperature treatments

High-temperature treatments included; Variety 1 (Zhongzao 39), Control + High temperature and No priming (Zhongzao 39+HT +NP), Zhongzao 39, High temperature and Nano treated water priming (Zhongzao 39+HT+NTW), High temperature and Calcium chloride priming (Zhongzao 39+HT+CaCl₂), Zhongzao 39, High temperature and H_2O_2 priming (Zhongzao 39+HT+H₂O₂), Zhongzao 39, Optimum temperature and SA priming (Zhongzao 39+HT+SA).

Variety 2 (KSK 133) Control+ Optimum temperature and No priming (KSK 133+OT +NP), KSK 133, Optimum temperature and Nano treated water priming (KSK 133+OT+NTW), Optimum temperature and Calcium chloride priming (KSK 133+OT+CaCl2), KSK 133, Optimum temperature and H2O2 priming (KSK 133+ OT+H2O2), KSK 133, Optimum temperature and SA priming (KSK 133+ OT+SA).

Variety 3 (KS 282) Control + High temperature and No priming (KS 282+HT +NP), KS 282, High temperature and Nano treated water priming (KS 282+HT+NTW), KS 282, High temperature and Calcium chloride) priming (KS 282+HT+CaCl₂), KS 282, High temperature and H₂O₂ priming (KS 282+ HT+H2O2), KS 282, High temperature and SA priming (KS 282+ HT+SA).

Variety 4 (Super Basmati) Control + High temperature and No priming (Super bas +HT +NP), Super bas, High temperature and Nano treated water priming (Super bas +HT+NTW), Super bas, High temperature and Calcium chloride priming (Super bas +HT+CaCl₂), Super bas, High temperature and H₂O₂ priming (Super bas + HT+H₂O₂), Super bas, High temperature and SA priming (Super bas + HT+SA)

Variety 5 (PK 1121 Aromatic) Control + High temperature and No priming (PK 1121 aroma +HT +NP), PK 1121 aroma, High temperature and Nano treated water priming (PK 1121 aroma +HT+NTW), PK 1121 aroma, High temperature and Calcium chloride priming (PK 1121 aroma +HT+CaCl₂), PK 1121 aroma, High temperature and H₂O₂ priming (PK 1121 aroma + HT+H₂O₂), PK 1121 aroma, High temperature and SA priming (PK 1121 aroma + HT+SA).

The abiotic stresses of low temperature and high temperature were applied in growth chambers via controlling the daylight and dark temperatures at 17°C and 37°C. Optimal temperature (27°C) was maintained in a separate growth chamber for priming technique. All three growth chambers were provided with 12hrs light period and 60% humidity at 17°C, 27°C, and 37°C.

In all treatments, 30 seeds were placed into each plate. Vigorous seed from each treatment (with three replications) was consistently sprouted in glass plates per/by two layers of filter paper covered through the lid. After adding 10ml of water to each treatment, Petri plates were placed on the metal shelves in growth chambers. All treatments were arranged in a completely randomized design (CRD) for recording the physiological and biochemical attributes in replicates. The seed were considered to be germinated when the radical was just emerged (1-2cm). The germination test was ended when there was no germination till 8 days of sowing. Seedlings were transferred to hydroponic media for observing the physiological and biochemical attributes at the leaf stage (30 days).

2.4. Germination/seedling development and dry biomass

Germination was recorded daily by following Association for official seed analysists (AOSA, 1990) until it turned into constant. The speed of germination (SG), germination energy percentage (GE %), and final germination percent (FGP %) were estimated by using the following formulae. Dry biomass was weighed by using 10 random rice sprouts. Root and shoot dry weights were documented after oven drying at 70°C for one day. The formulae are SG= (Number of germinated seed)/ (Days of first count) +.... + (Number of germinated seed at DAS) / (Total number of seed) × 100; FGP = ((Number of final germinated seed) / (Total number of seed) × 100

2.5. Chlorophyll content/mg g⁻¹ Fw

0.25g of seedling was used for the extraction of Chlorophyll content. The seedling sample was soaked in a 25ml mixture of acetone and alcohol ratio v: v = 1:1 for one day in the dark at room temperature. 663, 645, and 470 nm using UV-2600, Shimadzu, Japan absorbance were used for measurement of chlorophyll a, chlorophyll b and carotenoids contents according to Marschall and Proctor (2004). The equations are $Ca = 12.7 \times A \ 663 - 2.69 \times A645$; $Cb = 22.9 \times A645 - 4.68 \times A663$

Content of chlorophyll =
$$(Ca + Cb) \times Va / m \ leaf$$
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2.6. Antioxidant enzymes activities

2.6.1. Catalase (CAT) $\mu g^{-1}FW$ h^{-1} and Superoxide Dismutase (SOD) μg^{-1} FW h^{-1}

For CAT activity, the reaction mixture containing 50 mmol sodium phosphate buffer (pH 7.0), 20 mmol H_2O_2 , and 0.04ml of extracted rice sample. This absorbance was measured at 240 nm for 300 seconds. One unit of CAT was defined as the amount of enzyme required to oxidize 1µmol H_2O_2 min⁻¹. The reaction mixture of SOD contained 25 mmol sodium phosphate buffer (pH 7.8), 13 mmol methionine, 2 µmol riboflavin, 10 µmol EDTA-Na₂, 75 µmol NBT, and 0.1ml leaf extract. The total quantity of reaction mixture was 3 ml. The test tube containing reaction solutions was irrigated with light (fluorescent lamps 300 µmol m⁻² s⁻¹) for 20 mins and the activity was measured at 560 nm wavelength. Determination of catalase and Superoxide Dismutase enzymes was done (Zheng et al., 2016).

2.6.2. Malondialdehyde (MDA) µmol g-1 FW

Malondialdehyde was done by the method of Chun and Ren (2003). 2 ml Seedling extract was added in 0.5 ml (v/v) thiobarbituric acid, 1ml 20% (v/v) trichloroacetic acid. The mixture was heated in a pre-heated water bath at 95°C for 20 mins, cooled at room temperature, and centrifuged at 10,000 rpm × g for 10mins. 450, 532 and 600 nm absorbance was used for measurement of lipid peroxidation by UV-VS Spectrophotometer-2600 Shimadzu. Calculation of malondialdehyde done by an extinction coefficient of 155 mM⁻¹ cm⁻¹ and expressed in terms of µmol g⁻¹ FW.

2.8. Statistical analysis

The data of five rice varieties (KSK 133, Zhongzao 39, Super basmati, KS 282, and PK 1121 aromatic) was recorded and subjected to statistical analysis. The analysis was performed by standard analyses of variance (Three-way ANOVA) using the software SPSS v. 20 Zheng et al. (2016). The comparison of mean values was done by using the least significant difference (LSD) test at the 0.05 probability level (P < 0.05).

3. Results

3.1. Priming enhanced seed germination at Low temperature (17°C), optimal conditions (27°C) and high temperature (37°C)

Data on germination was collected on daily basis. The seed primed with NTW, $CaCl_2$, H_2O_2 , and SA showed variations in germination rate under low, optimum, and high-temperature conditions. But the most significant results were showed in nano-priming. The germination energy percentage, speed of germination and final germination percentage significantly increased in NTW primed seed at all temperatures.

At LT+NTW, OT+NTW and HT+NTW the percentage germination of five rice varieties Zhongzao 39, KSK 133, KS 282 and Super basmati and PK1121 aromatic (98.4%, 99.4%, 98.78%, 98.8% and 78% respectively) was recorded, at high temperature (Table 2). At all temperatures, H_2O_2 and Cacl₂ showed improved germination but less than nanopriming. However, LT+SA, OT+SA and HT+SA showed least significant improvement in germination. Three-way ANOVA for collected data indicated that the interaction between all five varieties, three temperatures, and four priming agents was significant (Table 3).

3.2. Seed priming elevated dry weight at low temperature (17°C), optimal (27°C) and high temperature (37°C) conditions

Under the influence of LT+NTW treatment, dry weight of Zhongzao 39 and KSK 133 (0.11 and 0.12g respectively) exhibited the highest and Aromatic PK 1121 lowest (0.01g) biomass. All varieties showed improved biomass production with LT+H₂O₂ and LT+CaCl₂ treatment but lesser than NTW treatment (Figure 1A). However, KSK 133+OT+NTW and KS 282+OT+NTW depicted an increase in dry weight (0.14 and 0.13g respectively) at treatment conditions (Figure 1B). As compared to control, Zhongzao 39+HT+NTW and KS 282+HT+NTW were exhibited significantly higher biomass (Figure 1C). These two treatments KSK 133+HT+H₂O₂ and KS 282+HT+H₂O₂ were statistically similar (P < 0.05) with each other in dry biomass evaluation (Figure 1C). Three-way ANOVA for biomass data indicated that dependent factor dry biomass with five rice varieties, three temperatures, and four priming agents displayed significant results (Table 3).

3.3. Seed priming improved the accumulation of chlorophyll content at low (17°C), optimal (27°C), and high temperature (37°C) conditions

All priming agents considerably increased the chlorophyll content in all five varieties under low, optimal, and high-temperature conditions (Table 4). The KSK 133+LT+NTW treatment led to enhanced chlorophyll contents (up to 0.201 mg/g FW). Whereas, chlorophyll contents of Zhongzao 39, KS 282 and PK 1121 aromatic were moderate (0.132, and 0.094 mg/g FW), and Super Basmati (0.068 mg/g FW) showed the least improvement at LT+NTW (Table 4). At OT+NTW all five rice varieties showed improved chlorophyll content than OT+ NP. Zhongzao 39+OT+NTW and KSK 133+OT+NTW showed 13.74 and 23.86 mg/g FW, respectively had elevated chlorophyll content than other rice varieties (Table 3).

Compared with NP+HT, chlorophyll contents promoted in KSK 133+HT+NTW (22.01 mg/g FW), KS 282+HT+NTW (17.77 mg/g FW) however, the least improvement was observed in super basmati+HT+CaCl₂ (3.587 mg/g FW) (Table 4). Three-way ANOVA for chlorophyll content data for all treatment indicated that all five varieties of three temperatures and four priming agents had significant interaction.

3.4. Seed priming enhanced the oxidative enzyme catalase (CAT) and Superoxide dismutase (SOD) activity at low-temperature 17°C optimal (27°C) and high temperature (37°C) conditions

Data regarding oxidative enzyme CAT and SOD increased at LT, OT, and HT rice seedlings with priming agents. CAT and SOD enzymes of five rice varieties revealed significant improvement at LT+NTW (Figure 2A, 2D). All five varieties exhibited the highest catalase activity at LT+NTW treatment and least CAT improved at LT+SA treatment (Figure 2A). Compared with OT+NP, Zhongzao 39 and KSK 133 showed exhibited maximum improvement in CAT and SOD activities at OT + CaCl₂. At optimal temperature PK 1121 aromatic +OT + NTW, KS 282+OT +NTW, and KSK 133+OT + NTW treatments expressed a higher quantity of CAT and SOD (Figure 2B, 2E).

It was important to note, in Zhongzao 39, KSK 133, KS 282, and PK 1121 aromatic CAT and SOD activities were significantly improved with HT+NTW treatment. The most pronounced enhancement was observed in KSK 133 (CAT $3243 \text{ H}_2\text{O}_2/\mu\text{g}^{-1}\text{FW}$. min and SOD $3345 \,\mu\text{g}^{-1}\text{FW}$ h-1), with HT+NTW treatment (Figure 2C, 2F). Three-way ANOVA indicated that, dependent factor antioxidant enzymes showed a significant interaction between all five varieties, three different temperatures, and four priming agents (Table 3).

3.5. MDA content declined by seed priming at low temperature (17°C), optimal (27°C) and high temperature (37°C) conditions

Lipid peroxidation causes a decrease in the growth and destruction of the plant. Therefore, the current study was deal with lipid peroxidation in the form of MDA content. It was observed that MDA contents were amplified in NP+LT. As compared to control LT+NTW minimum MDA content was recorded in all rice varieties (Figure 3A). In NP + OT displayed higher lipid peroxidation than NTW treatment. In OT+NTW treatments shown lower MDA contents in all rice varieties. MDA content decreased as the chlorophyll content and oxidative enzyme production

perature (17°C), optimal temperature (27°C) and high temperature (37°C) on GEP Germination energy percentage (%), SG Speed of germination, FGP final Germination percentage	nder five priming treatments (Control, NTW, CaCl ₂ , H ₂ O ₂ , SA).
), optim.	five priming tre

Rice	Two and the set		GEP (%)			SG (seeds/day)			FGP (%)	
Varieties		17°C	27°C	37°C	17°C	27∘C	37∘C	17°C	27°C	37∘C
Zhongzao 39	Control	65.0±1.8	66.0±5.7	85.0±5.9	19.7±7.4	30.2±4.4	19.0±14	75.0±7	60.0±3	90.0±5.1
	Nano	$96.8 \pm 1.6^{*}$	98.4±2.8*	97.3±1.2*	27.2±1.7*	35.4±2.1*	$37.1\pm7.7^{*}$	$96.6\pm2.1^{*}$	97.5±2.1*	98.5±1.5*
	$CaCl_2$	94.6±5.7	94.0±7.5	83.3±5.7	23.6±1.9	34.1±2.5	24.6±8.7	92.0±5.7	96.2±2.8	94.1±2.8
	H_2O_2	95.1±2.8	93.0± 8.6	87.3±10.0	26.8±2.0	32.4±1.9	29.1±6.4	95.0±2.8	94.6±5.7	96.3±5.7
	SA	93.3±7.6	87.0±11.0	86.0±1.5	22.9±7.0	31.9±2.9	22.3±3.2	86.67±2.3	93.3±11	91.0±8.6
KSK 133	Control	71.7±28.0	86.7±2.8	68.3±28.0	5.7±5.6	23.2±9.2	23.1±11.0	75.0±15.0	87.3±9.0	67.0 ±3.0
	Nano	96.1±5.1*	100.0±0.0	$100.0\pm0.0^{*}$	35.0±1.4*	36.1±0.3*	38.6±1.8*	98.2±2.8*	99.1±2.1*	99.4±2.8*
	$CaCl_2$	91.7±14.4	99.0±1.3*	84.2±1.1	31.2±6.1	36.1±0.7	31.2±1.0	94.4±1.0	96.2±3.2	92.7±5.7
	H_2O_2	93.3±7.6	98.3±2.8	87.0±2.8	33.4±2.9	34.4 ±0.4	33.4±1.0	96.6±5.7	94.6±5.7	96.8±6.1
	SA	80.0±10	78.3±37.0	80.0±5.9	25.9±10.0	27.7±14.0	30.04 ± 1.1	87.0±10.0	40.0±15.0	83.34±10.0
KS 282	Control	43.3±27.0	93.3±11.0	53.3±40.0	16.4±3.2	20.8 ± 4.0	19.0±14.0	86.7±23.0	90.0±10.0	88.0±11.5
	Nano	94.0±1.4*	98.3±2.8*	99.0±0.5*	33.0±0.8*	38.5±0.21*	37.2±1.0*	98.5±2.3*	98.0±1.3*	98.8±1.5*
	$CaCl_2$	85.0±10.0	97.2±3.0	96.3±2.8	25.3±1.0	35.8±0.8	34.7±2.3	96.0±5.7	98.7±5.7	93.3±2.8
	H_2O_2	91.7±2.8	96.6±5.7	98.7±5.7	31.6±1	33.2 ±1.7	26.4±2.8	98.0±2.8	96.3±2.8	95.0±8.6
	SA	83.3±11.0	70.0±27.0	93.0±4.3	17.8±1.7	23.5±9.2	31.6±1.6	93.0±7.6	68.3±10.0	91.3±5.7
Super	Control	50.0±32.0	73.3±10.0	80.0±17.3	16.6± 3.0	14.9±3.2	10.4 ± 0.7	90.0±8.6	66.7±2.8	61.7±10.0
Basmati	Nano	$95.0\pm3.2^{*}$	98.3±2.8*	98.3±0.7*	$30.0\pm1.0^{*}$	38.1±0.7*	35.2±1.3*	98.1±2.3*	98.2±1.2*	98.88±1.5*
	$CaCl_2$	73.3±10.0	94.3±3.1	93.7±7.6	20.9±1.2	36.0±1.1	26.6±2.8	96.0±5.7	98.3±2.8	86.67±2.3
	H_2O_2	93.3±5.7	92.6±5.7	96.7±5.7	27.4±3.4	31.4±4.9	30.5±1.9	98.0±2.8	96.7±5.7	98.3±2.8
	SA	65.0±10.0	85.0±10.0	91.0±5.0	18.5±9.3	18.0 ± 4.0	23.1±2.9	93.3±5.0	93.3±11.0	83.7±5.7
PK 1121	Control	1.7 ± 2.8	56.7±14.0	30.0±5.0	1.3 ± 0.7	17.6±3.1	9.0±0.8	10.0 ± 5.0	61.7±10.0	36.7±7.6
aromatic	Nano	33±1*	68.3±3.2*	69.3±6.5*	6.5±1.4*	$25.5\pm2.5*$	$26.5\pm1.1^{*}$	42.2±2.3*	77.0±1.3*	78.0±3.2*
	$CaCl_2$	13±4	65.4±5.7	50.0±8.6	4.9±1.9	22.2±4	16.9±1.2	28.34±20.0	73.6±2.8	63.7±5.7
	H_2O_2	21.67±1.0	63.0±5.0	52.3±7.6	6.0 ± 4.1	21.1±3.0	20.3±1.5	38.4±2.6	66.3±14.0	71.3±7.6
	SA	10±5	31.6±15.0	35.0±5.0	3.53±0.9	9.5 ±5.7	10.7 ± 3.0	26.7±7.6	36.67±10.0	60.7±10.0

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Table 3. Three way ANOVA, analysis of dependent variables (germination, chlorophyll content, dry weight, anti-oxidant enzyme CAT, SOD and MDA).

T		Germinati	on	Chlorophyll			
Treatments	DF	F-Value	P-Value	DF	F-Value	P-Value	
Variety	4	6715	***	4	27.00	***	
Temperature	2	117	***	2	270.00	***	
Priming	4	2052	***	4	5.05	***	
Variety*temperature	8	358	***	8	7.08	***	
Variety*priming agent	12	273	***	12	4.47	***	
Temperature*priming agent	6	456	***	6	5.46	***	
Variety*temperature*priming agent	24	227	***	24	1.53	**	
		DW			CAT		
Treatments	DF	F-Value	P-Value	DF	F-Value	P-Value	
Variety	4	6.28	***	4	0.524	Non-sig	
Temperature	1	28.8	***	2	32.70	***	
Priming	4	1.508	Non-sig	4	3.04	**	
Variety*temperature	4	10.46	***	7	0.40	Non-sig	
Variety*priming agent	12	0.758	Non-sig	12	1.48	Non-sig	
Temperature*priming agent	3	0.603	Non-sig	6	2.86	**	
Variety*temperature*priming agent	12	2.537	***	19	1.73	**	
Transformente		SOD			MDA		
Treatments	DF	F-Value	P-Value	DF	F-Value	P-Value	
Variety	4	2.605	**	4	4.01	***	
Temperature	2	7.021	Non-sig	2	7.70	***	
Priming	4	5.57	**	4	14.70	***	
Variety*temperature	8	1.86	Non-sig	8	10.12	***	
Variety*priming agent	12	1.46	Non-sig	12	4.33	***	
Temperature*priming agent	6	2.11	**	6	3.47	***	
Variety*temperature*priming agent	24	1.31	**	24	3.47	***	

Asterick showed *** highly significant, ** significant. * Relationship between variety and temperature, DF degree of freedom, DW dry weight, SOD Superoxide Dismutase, CAT Catalase, MDA Malondialdehyde

increased (Figure 3B). In two varieties Zhongzao 39 and KSK133 expressed reduced MDA (6.2 and 6.5 nmol g⁻¹ FW) at HT+NTW. In all five varieties significantly lessen MDA content with HT+NTW treatment conditions than control. (Fig, 3C). Three-way ANOVA disclosed that dependent factor MDA content (Malondialdehyde), exhibited significant interaction between varieties, temperatures, and priming agents (Table 3).

4. Discussion

Temperature stress is one of the abiotic stresses limiting growth in cash crop fields. Rice is the major food of more than half of the world's population. So, our main focus on evitable targets to tackle future environmental change on rice production. Therefore, the present study was based on the comparative proficiency of nano, chemical, osmo and hormonal priming at low, optimal, and high temperatures with hydroponic conditions. In current study nano-priming showed most persistent results in all three temperatures. This was the first study scoping all the four types of priming and three different temperatures to take comprehensive results regarding improvement in seed germination, rate of germination, rate of photosynthesis, chlorophyll content.

In recent study found that seed priming treatments led better sprouting and seedling dry weight than control under low-temperature conditions (Table 2, Figure 1A). The previous study has documented seed priming made easy availability of metabolites for germination at low temperature (Hussain et al., 2016). In earlier studies Acharya et al. (2020), demonstrated seed priming with AgNO₃ nanoparticles improved germination, chlorophyll, growth and yield of watermelon (*Citrullus lanatus*). It is

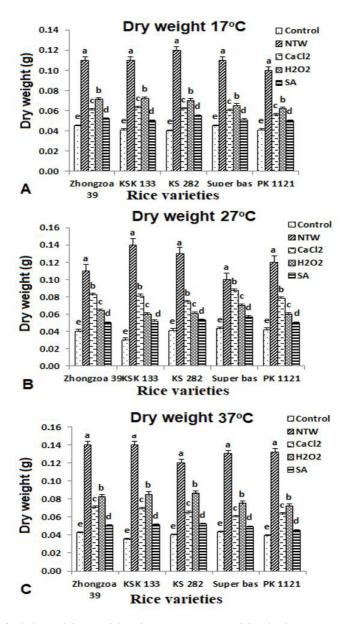


Figure 1. Influence of seed priming on (A) Dry weight at low temperature 17°C, (B) optimal temperature 27°C, (C) high temperature 37°C of five varieties of rice under five priming treatments (Control, NTW, $CaCl_2$, H_2O_2 , SA). Vertical bars above mean indicate standard errors of three replicates.

confirmed from the present study that primed seed had increased germination, chlorophyll content, growth than LT+NP (Table 3). The nano-priming improved the chlorophyll content in *Oryza sativa* at low temperature (Table 4). Seed priming increased the chlorophyll content, rate of photosynthesis, improved biomass and yield was advocated by Mohajeri et al., 2017.

In OT+NTW treatments were significantly enhanced germination of Oryza sativa. (Table 2) was observed. The current study highlighted considerably rate of photosynthesis, chlorophyll content, biomass, and oxidative enzymes (Table 4) (Figure 1B, Figure 2B, E) under

optimum temperature Mohajeri et al. (2017) advocated same observation in the primed seed of beans.

In recent study OT+CaCl₂ exhibited enhanced seed germination but less than nano-priming. According to Ruan et al. (2002a, b) that osmopriming with CaCl₂ showed a better germination rate and reduced the germination time. CaCl₂ is highly soluble in water at ordinary temperatures, but crystallization will occur under certain temperature and concentration conditions (Song et al., 2011). So, present study didn't show worthy results at low and high temperature.

Rice	Turnet	Chlorophyll a			Chlorophyll b			Total chlorophyll		
varieties	Treatment	17°C	27°C	37°C	17°C	27°C	37°C	17°C	27°C	37ºC
Zhongzao	Control	0.02	1.62	8.04	0.04	0.52	2.34	0.06	2.14	10.38
39	Nano	0.20*	8.81*	11.41*	0.30*	4.93*	3.80*	0.50*	13.74*	15.21*
	CaCl ₂	0.10	8.22	9.25	0.16	2.76	3.50	0.26	10.98	12.75
	$H_{2}O_{2}$	0.15	2.90	10.14	0.27	1.03	3.19	0.42	3.94	13.33
	SA	0.07	0.47	7.06	0.04	0.27	3.92	0.11	0.88	10.97
KSK 133	Control	0.04	1.07	3.25	9.15	0.42	0.99	9.19	1.49	4.24
	Nano	0.13*	8.93*	15.00*	20.10*	3.21*	4.50*	24.01*	23.86*	22.01*
	CaCl ₂	0.08	8.45	8.37	17.93	3.01	2.36	23.1	11.47	10.73
	$H_{2}O_{2}$	0.11	6.53	14.64	15.60	2.41	4.31	23.71	8.94	18.95
	SA	0.06	3.26	7.46	9.76	1.54	2.07	10.81	4.80	9.53
KS 282	Control	0.01	1.62	8.04	0.02	0.22	2.34	0.03	1.83	10.38
	Nano	0.09*	10.32*	13.52*	0.048*	2.94*	4.53*	0.14*	13.65*	17.77*
	CaCl ₂	0.04	8.22	12.24	0.04	1.04	3.24	0.08	9.26	15.48
	H_2O_2	0.08	9.90	13.15	0.04	2.73	4.30	0.13	12.6	17.45
	SA	0.02	0.47	10.05	0.03	0.54	2.87	0.05	1.01	12.92
Super	Control	0.03	0.68	2.26	0.05	0.23	0.62	0.08	0.91	2.89
Basmati	Nano	0.07*	4.42*	9.11*	0.12*	2.54*	2.88*	0.19*	7.00*	11.98*
	CaCl ₂	0.06	4.21	2.44	0.06	2.33	1.15	0.12	6.54	3.59
	H_2O_2	0.06	3.56	8.86	0.07	2.24	2.53	0.13	5.79	11.39
	SA	0.04	1.13	6.46	0.059	0.79	1.80	0.10	1.92	8.27
PK 1121 aromatic	Control	0.01	0.04	0.10	0.01	0.65	1.73	0.02	0.69	1.83
	Nano	0.05*	1.95*	10.64*	0.10*	1.12*	5.01*	0.15*	3.10*	15.65*
	CaCl ₂	0.04	1.44	10.07	0.06	1.25	4.76	0.09	2.69	14.83
	$H_{2}O_{2}$	0.04	1.76	6.33	0.08	1.10	3.87	0.13	2.87	10.20
	SA	0.035	1.39	4.71	0.04	0.05	2.77	0.08	1.45	7.47

Table 4. Effect of low temperature (17 °C) Optimal (27 °C) and high temperature (37 °C) on Chlorophyll a, chlorophyll b and total chlorophyll content of five rice varieties under five priming treatments (Control, NTW, CaCl₂, H_2O_2 , SA).

*Mean values are mentioned. P-Value < 0.05.

Exposure of high temperature in field conditions gave rise to weaken and un-even germination in addition to deprived plantlet formation (Lal et al., 2018). Zheng et al. (2016) verified that seed priming significantly improved the development of seedling and its growth performance under poor water environments. In our results showed that HT+H₂O₂ improved germination and dry biomass but less than NTW+HT treatment conditions. H₂O₂ at appropriate concentration helpful for seed dormancy broken and germination improvement but the accumulation of H₂O₂ simply caused cell injury (Jeevan Kumar et al., 2015). In the current study, eminent dry weight biomass achieved at NTW+HT (Figure 1C). In KSK 133 and KS 282 varieties pronounced increase in chlorophyll content with NTW priming treatments at high temperature (Table 4) was observed. Literature showed that severe high-temperature stress resulted in incomplete seedling emergence. The seed

priming improved the germination rate but less than optimal conditions were concluded by Wahid et al. (2007).

In current study nano-treated water was showed effective results in biochemical components like antioxidant enzymes, reactive oxygen species (ROS), protein, starch, and amino acid. In earlier studies, the effect of priming on antioxidant capacity was found to be correlated with increased transcription (mRNA) levels of enzymatic antioxidants (Christou et al., 2014). In the current study antioxidative enzymes (CAT, SOD) improved in primed seed than NP + LT (Figure 2 A, D). In previous studies, antioxidant enzymes were relatively higher in nano primed seed and more applicable for ROS (Mahakham et al., 2017).

SOD reflects the main role of catalyzing the dismutation of superoxide, whereas CAT pays in scavenging of H_2O_2 (Fahad et al., 2015; Borges et al., 2018). The results revealed that oxidative enzymes CAT and SOD increased at LT with NTW (Figure 2A, D). Seed priming enhanced

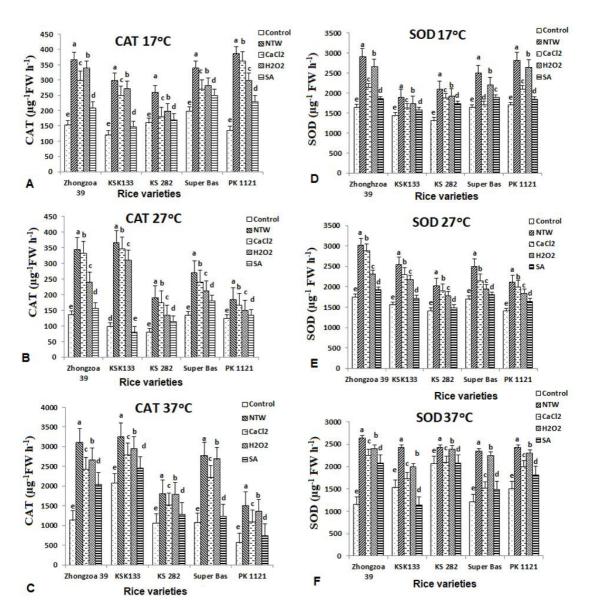


Figure 2. Influence of seed priming on (A) Oxidative enzyme Catalase (CAT) at low temperature 17° C, (B) (CAT) optimal temperature 27° C, (C) (CAT) high temperature 37° C (D) Oxidative enzyme Superoxide dismutase (SOD) at low temperature 17° C, (E) (SOD) optimal temperature 27° C, (F) (SOD) high temperature 37° C. Vertical bars above mean indicate standard errors of three replicates.

CAT and SOD activities of rice observed in this research is supported by Hussain et al. (2016). The current study highlighted considerably increased rate of photosynthesis, chlorophyll content, biomass, and oxidative enzymes (Table 4) (Figures 1B, 2B, E) under optimum temperature. The oxidative enzymes CAT and SOD improved in redox and Osmopriming but less than nano-priming (Figure 2B, E). Osmo-priming induced increases in antioxidative enzymes (CAT and SOD) activities of rice seedlings have been reported by Wojtyla et al. (2016). The recent investigation confirmed that nano-seed priming under high-temperature conditions effectively enhanced the germination, biomass, chlorophyll content, and antioxidant enzymatic activities (Table 2, 4, Figure 2C, F). In earlier reports recommended that primed seeds showed robust antioxidant system than non-primed seed germination, early seedling growth and enhanced antioxidant enzymes in primed seed can improve seedling growth (Zheng et al., 2016).

MDA is responsible for the synthesis of lipids which possibly decrease carbohydrate content in seed resulted in poor germination. Low temperature enhanced MDA content accumulation in control treatment in all rice varieties (Figure 3A). Previously, an optimistic approach of seed priming in preventing the MDA content and ROS

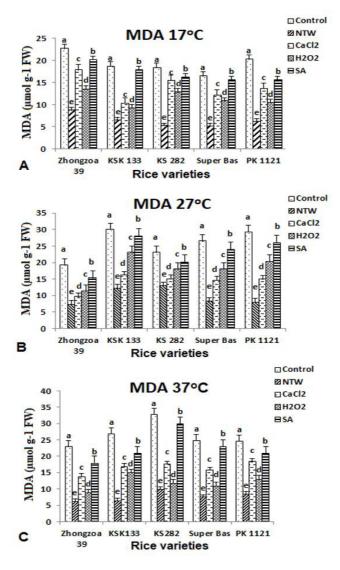


Figure 3. Influence of seed priming on (A) MDA content at low temperature 17°C, (B) Optimal temperature 27°C, (C) High temperature 37°C of five varieties of rice under five priming treatments (Control, NTW, CaCl₂, H₂O₂, SA).

generation was observed for rice (Jisha and Puthur, 2016) seedlings. Similarly, in the current study, all the primed seed significantly lower the MDA under LT. The results from the current study revealed that LT+NTW were most efficient under low temperature than all other seed priming treatments in five rice varieties.

Moreover, a decrease was noticed for MDA contents in primed seed under drought stress (Figure 3C). Consequently, dropping of ROS concentrations and lipid peroxidation may be the main reason in high-temperature tolerance achieved by plantlets of primed seeds (Gill and Tuteja, 2010). Present study results revealed that hormonal priming exhibited lower MDA content in all rice varieties. In previous studies, it was noticed that salicyicate cooperated with other hormonal pathways which caused an increase in resistance of seedling osmotic stress (Ding and Wang, 2003).

5. Mechanism of Action for Nano-treatments

Nano electromagnetic waves entered through seed coat into nanoprimed seeds and initiate molecular events for germination. These waves trigger the α -amylase activity to convert starch into sugar. This converted sugar provides energy for young dormant embryo to initiate growth. Previous studies reported that nanopriming involves rapid starch degradation as indication of α -amylase activity (Mahakham et al., 2017). The initiation of biosynthesis of α -amylase is dependent on the activity of Gibberellic acid (GA₃). It showed there is direct proportional relation between GA₃ activity and α -amylase synthesis (Figure 4). Moreover aquaporin's promotes GA₃ production and suppress abscisic acid synthesis. A signaling crosstalk pathway existing between nano waves, α -amylase and

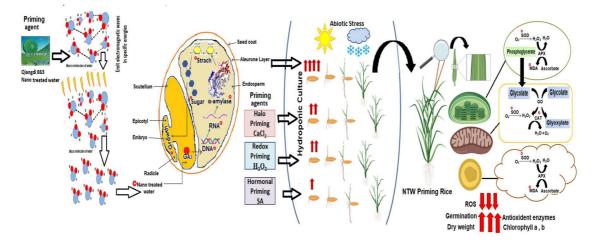


Figure 4. Proposed mechanism of Qiangdi nano-863 treated water induced seed germination physiological and biochemical attributes ofrice. Qiangdi nano-863 emits electromagnetic waves, can declustered water molecules (super-critical water) which increase reaction rates, hydration and solubility of kinetically stable water molecules. Seed priming with nano treated water can enhance seed germination by influx of nano-treated water into seed trigger the expression of genes. Due to activation of genes antioxidant enzyme system (CAT, SOD and MDA) maintain the ROS in optimum range and oxidative systems act as signaling the plant organelles for triggering essential metabolic activity of rice seedling development (germination, dry weight and chlorophyll content.) and growth.

phytohormones in nanoprimed seeds. Previously, reported studies about an association between nanoparticles bond, photochromic had impact on seed germination in a nanoprimed seed (Chandrasekaran et al., 2020). Antioxidant enzymes (e.g. CAT, SOD) act as scavenging system that tightly regulates ROS concentration and enables them to act as cellular messengers. The accumulation of ROS e.g. hydrogen peroxide (H₂O₂), hydroxyl radicals (OH) and superoxide radicals (O_2) , enhanced the dynamics of seed germination in rice seed. Regulatory proteins of ROS, phytohormones complex along with antioxidant scavengers involved in transduction of ROS signaling cascade. Physiological and biochemical reactions studies may improve more understanding about regulatory role of nanopriming in initiation of seed germination under severe environmental stress conditions.

6. Conclusion

Conclusively, exposure of low and high temperature at 17°C and 37°C harshly delayed the growth and seedling propagation. However seed priming treatments neutralized the hostile properties of low and high temperature. Improved growth and healthy sprout development allocated to seed priming linked with better chlorophyll content, improved anti-oxidant enzymatic activities, and decreased MDA content in a primed seed. Priming treatments exhibited trend in the fashion of NTW>H2O2 > CaCl2 > SA at low temperature, NTW>CaCl2>H2O2 > SA at optimal temperature and showed NTW>H2O2>CaCl2> SA at high temperature

It was concluded from all results that nano-priming is most efficient in rate of germination, seedling growth, and chlorophyll content and oxidative enzymes systems in all three temperatures. Nanometer Qiangdi 863 Nano disc has strong light absorbing properties and ceramic material act as carrier. The ceramic material has high absorbing activity which promoting chemical reaction of nano treated water. Qiangdi nano-863 disc emits electromagnetic waves that produced declustered water molecules or activated water molecules of high energy and entered into plant cell stimulated the metabolism. So, temperature had no effect on reaction rate, solubility and kinetic energy of water molecule. But temperature fluctuations affect other priming agents e.g. CaCl₂, H₂O₂ and SA. Nano priming showed most persistent results at low, Optimal and high temperature conditions. Therefore, it recommended as remedial technological to cop temperature stress for rice production. Ultimate it will contribute toward food security.

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