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

A. Younas*, A. Riaz*, S. Fiaz†, S. Hussain† and S. Huang‡

*Lahore College for Women University, Department of Botany, Lahore, Pakistan
†College of Life Sciences, Yan’an University, Yan’an 716000, Shaanxi, China
‡The University of Haripur, Department of Plant Breeding and Genetics, Khyber Pakhtunkhwa, Pakistan

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 (CaCl₂), reduct priming hydrogen peroxide (H₂O₂) 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 Zhongzaoa 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) (272, 35.45, 371), Final Germination Percentage (FGP) (98.2, 99.1, 99.4%), Seedling Dry Weight Biomass (DWB) (0.01, 0.137, 0.14g), Total Chlorophyll Content (0.502, 13.74, 15.21), antioxidant enzymes Superoxide Dismutase (SOD) (3145, 2559, 3345 µg FWh⁻¹), Catalase (CAT) (300, 366, 3243 µg FWh⁻¹) and decreased Malondialdehyde (MDA) (6.5, 12.2, 6.5 µmol g⁻¹ FW) for Zhongzaoa 39 and KSK 133 rice varieties under low (LT+NTW), optimal temperature (OT+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.
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) (Youñas et al., 2020)

The variations in climatic conditions and upsurge in the human population are continuously posing threat to crop production and management practices (Akrım, 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 CaCl₂ and better sprouting likewise Rehman et al. (2011) testified improved plantlet growth and crop harvest. The suitable amount of Hydrogen peroxide (H₂O₂) was favorable for breaking seed dormancy and improved growth (Lariguet et al., 2013). H₂O₂ 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 (Pakistan 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 (H₂O₂: 50µM Hydrogen peroxide), and Hormonal priming (SA: 150 mg/ LSalicylic acid) methods were utilized. Priming reagents were pre-optimized 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
Table 1. List of rice germplasm used in study at China National Rice Research Institute, 2017/18.

| 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 |

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 1121aroma +LT +NP), PK 1121 aroma, low temperature and Nano treated water priming (PK 1121aroma +LT+NTW), PK 1121 aroma, low temperature and Calcium chloride priming (PK 1121aroma +LT+CaCl₂), PK 1121 aroma, low temperature and H₂O₂ priming (PK 1121aroma +LT+H₂O₂), PK 1121 aroma, 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 1121 aroma, 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 H2O2 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 1121aroma +HT +NP), PK 1121 aroma, High temperature and Nano treated water priming (PK 1121aroma +HT+NTW), PK 1121 aroma, High temperature and Calcium chloride priming (PK 1121aroma +HT+CaCl2), PK 1121 aroma, High temperature and H2O2 priming (PK 1121aroma+ HT+H2O2), PK 1121 aroma, High temperature and SA priming (PK 1121aroma+ 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 seeds)/(Days of final count); GE %=(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 × A 663 – 2.69 × A645;Cb = 22.9 × A665 – 4.68 × A663

Content of chlorophyll = (Ca +Cb)×Va / m leaf

2.6. Antioxidant enzymes activities

2.6.1. Catalase (CAT) µg FW⁻¹ h⁻¹ and Superoxide Dismutase (SOD) µg FW⁻¹ h⁻¹

For CAT activity, the reaction mixture containing 50 mmol sodium phosphate bufer (pH 7.0), 20 mmol H2O2, 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 H2O2 min⁻¹. The reaction mixture of SOD contained 25 mmol sodium phosphate bufer (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, CaCl₂, H₂O₂, 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, H₂O₂ and CaCl₂ showed improved germination but less than nano-priming. 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 OT+ 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 H₂O₂/µg-1 FW, min and SOD 3345 µg-1 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
Table 2. Effect of low temperature (17°C), optimal temperature (27°C) and high temperature (37°C) on GEP Germination energy percentage (%), SG Speed of germination, FGP final Germination percentage (%) of five rice varieties under five priming treatments (Control, NTW, CaCl$_2$, H$_2$O$_2$, SA).

| Rice Varieties | Treatment | 17°C | 27°C | 37°C | 17°C | 27°C | 37°C | 17°C | 27°C | 37°C |
|----------------|-----------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
|                | GEP (%)   |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| 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±1.6* | 98.4±2.8* | 97.3±1.2* | 27.2±1.7* | 35.4±2.1* | 37.1±7.7* | 96.6±2.1* | 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$_2$O$_2$ | 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 | 86.6±7.3 | 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±0.0* | 35.2±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±10 | 96.2±3.2 | 92.7±5.7 |
|                | H$_2$O$_2$ | 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 | 80.5±5.9 | 25.9±10.0 | 27.7±14.0 | 30.0±11 | 87.0±10 | 40.0±15 | 83.3±10 |
| 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±100 | 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 | 97.8±5.7 | 93.3±2.8 |
|                | H$_2$O$_2$ | 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±16 | 93.0±7.6 | 68.3±100 | 91.3±5.7 |
| Super Basmati  | 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 |
|                | Nano      | 95.0±3.2* | 98.3±2.8* | 98.3±0.7* | 30.0±1.0* | 38.1±0.7* | 35.2±1.3* | 98.1±2.3* | 98.2±1.2* | 98.8±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 | 86.6±2.3 |
|                | H$_2$O$_2$ | 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 | 98.7±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±100 | 36.7±7.6 |
| aromatic       | Nano      | 33±1* | 68.3±3.2* | 69.3±6.5* | 6.5±1.4* | 25.5±2.5* | 26.5±1.1* | 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 | 28.3±20.0 | 73.6±2.8 | 63.7±5.7 |
|                | H$_2$O$_2$ | 21.6±7.0 | 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±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 | 36.6±100 | 60.7±10.0 |

*Values were mean standard error ± (n=3) with different treatments Mean values are mentioned. P-Value < 0.05.
Nano-priming of rice seed under abiotic stress

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).

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

```
| Treatments                           | Germination | Chlorophyll |
|--------------------------------------|-------------|-------------|
|                                      | DF F-Value  | P-Value     |
| Variety                              |             |             |
|                                      | 4 6715      | ***         |
| Temperature                          |             |             |
|                                      | 2 117       | ***         |
| Priming                              |             |             |
|                                      | 4 2052      | ***         |
| Variety*temperature                  |             |             |
|                                      | 8 358       | ***         |
| Variety*priming agent                |             |             |
|                                      | 12 273      | ***         |
| Temperature*priming agent            |             |             |
|                                      | 6 456       | ***         |
| Variety*temperature*priming agent    |             |             |
|                                      | 24 227      | ***         |

| Treatments                           | DW          | CAT         |
|--------------------------------------|-------------|-------------|
|                                      | DF F-Value  | P-Value     |
| Variety                              |             |             |
|                                      | 4 6.28      | ***         |
| Temperature                          |             |             |
|                                      | 1 28.8      | ***         |
| Priming                              |             |             |
|                                      | 4 1.508     | Non-sig     |
| Variety*temperature                  |             |             |
|                                      | 4 10.46     | ***         |
| Variety*priming agent                |             |             |
|                                      | 12 0.758    | Non-sig     |
| Temperature*priming agent            |             |             |
|                                      | 3 0.603     | Non-sig     |
| Variety*temperature*priming agent    |             |             |
|                                      | 12 2.537    | ***         |

| Treatments                           | SOD         | MDA         |
|--------------------------------------|-------------|-------------|
|                                      | DF F-Value  | P-Value     |
| Variety                              |             |             |
|                                      | 4 2.605     | **          |
| Temperature                          |             |             |
|                                      | 2 7.021     | Non-sig     |
| Priming                              |             |             |
|                                      | 4 5.57      | **          |
| Variety*temperature                  |             |             |
|                                      | 8 1.86      | Non-sig     |
| Variety*priming agent                |             |             |
|                                      | 12 1.46     | Non-sig     |
| Temperature*priming agent            |             |             |
|                                      | 6 2.11      | **          |
| Variety*temperature*priming agent    |             |             |
|                                      | 24 1.31     | **          |
```

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

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 AgNO\(_3\) nanoparticles improved germination, chlorophyll, growth and yield of watermelon (Citrus lanatus). It is
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+CaCl$_2$ exhibited enhanced seed germination but less than nano-priming. According to Ruan et al. (2002a, b) that osmopriming with CaCl$_2$ showed a better germination rate and reduced the germination time. CaCl$_2$ 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.

**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$_2$O$_2$, SA). Vertical bars above mean indicate standard errors of three replicates.
**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, CaCl$_2$, H$_2$O$_2$, SA).

| Rice varieties | Treatment | Chlorophyll a | Chlorophyll b | Total chlorophyll |
|----------------|-----------|---------------|---------------|------------------|
|                | 17°C | 27°C | 37°C | 17°C | 27°C | 37°C | 17°C | 27°C | 37°C |
| Zhongzao 39    |       |       |       |       |       |       |       |       |       |
| Control        | 0.02  | 1.62  | 8.04  | 0.04  | 0.52  | 2.34  | 0.06  | 2.14  | 10.38 |
| 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$_2$O$_2$     | 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 Basmati  |       |       |       |       |       |       |       |       |       |
| Control        | 0.03  | 0.68  | 2.26  | 0.05  | 0.23  | 0.62  | 0.08  | 0.91  | 2.89  |
| 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$_2$O$_2$     | 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  |

*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+H$_2$O$_2$ improved germination and dry biomass but less than NTW+HT treatment conditions. H$_2$O$_2$ at appropriate concentration helpful for seed dormancy broken and germination improvement but the accumulation of H$_2$O$_2$ 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$_2$O$_2$ (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...
Younas, A. et al. 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 Osmo-priming 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...
Nano-priming of rice seed under abiotic stress

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 $\alpha$-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 $\alpha$-amylase activity (Mahakham et al., 2017). The initiation of biosynthesis of $\alpha$-amylase is dependent on the activity of Gibberellic acid (GA$_3$). It showed there is direct proportional relation between GA$_3$ activity and $\alpha$-amylase synthesis (Figure 4).

Moreover aquaporin’s promotes GA$_3$ production and suppress abscisic acid synthesis. A signaling crosstalk pathway existing between nano waves, $\alpha$-amylase and 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 salicylate cooperated with other hormonal pathways which caused an increase in resistance of seedling osmotic stress (Ding and Wang, 2003).

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, CaCl$_2$, H$_2$O$_2$, SA).

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 $\alpha$-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 $\alpha$-amylase activity (Mahakham et al., 2017). The initiation of biosynthesis of $\alpha$-amylase is dependent on the activity of Gibberellic acid (GA$_3$). It showed there is direct proportional relation between GA$_3$ activity and $\alpha$-amylase synthesis (Figure 4).

Moreover aquaporin’s promotes GA$_3$ production and suppress abscisic acid synthesis. A signaling crosstalk pathway existing between nano waves, $\alpha$-amylase and
Brazilian Journal of Biology, 2023, vol. 83, e245206

Younas, A. et al.

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. CaCl$_2$, H$_2$O$_2$ 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.

**Acknowledgements**

The authors are thankful to Prof. Shiwen Haung from the State key laboratory of Rice Biology, Hangzhou China for providing lab and necessary facilities.

**Funding**

The publication of the present work is supported by the Natural Science Basic Research Program of Shaanxi Province (grant no. 2018JQ5218) and the National Natural Science Foundation of China (51809224), Top Young Talents of Shaanxi Special Support Program.

**References**

ACHARYA, P., JAYAPRAKASHA, G.K., CROSBY, K.M., JIFON, J.L. and PATIL, B.S., 2020. Nanoparticle-Mediated Seed Priming Improves Germination, Growth, Yield, and Quality of Watermelons...
Nano-priming of rice seed under abiotic stress

FISHER, G., SHAH, M., TUBIELLO, F.N. and VAN VELHUZEN, H., 2005. Socio-economic and climate change impacts on agriculture: an integrated assessment, 1990–2080. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, vol. 360, no. 1463, pp. 2067-2083. http://dx.doi.org/10.1098/rstb.2005.1744. PMid:16433094.

FOOD AND AGRICULTURE ORGANIZATION – FAO, 2005. Food and Agriculture Organization of the United Nations. Rome: FAO.

GILL, S.S. and TUTEJA, N., 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry, vol. 48, no. 12, pp. 909-930. http://dx.doi.org/10.1016/j.plaphy.2010.08.016. PMid:20870416.

HILL, H.J., CUNNINGHAM, J.D., BRADFORD, K.J. and TAYLOR, A.G., 2007. Primed lettuce seeds exhibit increased sensitivity to moisture content during controlled deterioration. Horticultural Science (Prague), vol. 42, pp. 1436-1439.

HUSSAIN, S., KHAN, F., HUSSAIN, H.A. and NIE, L., 2016. Physiological and biochemical mechanisms of seed priming-induced chilling tolerance in rice cultivars. Frontiers of Plant Science, vol. 9, pp. 116. http://dx.doi.org/10.3389/fpls.2016.00116.

HUSSAIN, S., PENG, S., FAHAD, S., KHALIQ, A., HUANG, J., CUI, K. and NIE, L., 2015. Rice management interventions to mitigate greenhouse gas emissions: a review. Environmental Science and Pollution Research International, vol. 22, no. 5, pp. 3342-3360. http://dx.doi.org/10.1007/s11356-014-3760-4. PMid:25354441.

JEEVAN KUMAR, S.P., RAJENDRA PRASAD, S., BANERJEE, R. and THAMMINENI, C., 2015. Seed birth to death: dual functions of reactive oxygen species in seed physiology. Annals of Botany, vol. 116, no. 4, pp. 663-668. http://dx.doi.org/10.1093/aob/mcv098. PMid:26271119.

JELLER, H., PEREZ, S.C. and RAIZER, J., 2003. Water uptake, priming, drying and storage effects in Cassia excelsa Schrad seeds. Brazilian Journal of Biology = Revista Brasileira de Biologia, vol. 63, no. 1, pp. 61-68. http://dx.doi.org/10.1590/S1519-69842003000100008. PMid:12914415.

JÉRÓNIMO, G.T., SPECK, G.M., CECHINEL, M.M., GONÇALVES, E.L.T. and MARTINS, M.L., 2011. Seasonal variation on the ectoparasitic communities of Nile tilapia cultured in three regions in southern Brazil. Brazilian Journal of Biology = Revista Brasileira de Biologia, vol. 71, no. 2, pp. 365-373. http://dx.doi.org/10.1590/S1519-69842011000200005. PMid:21755153.

JISHA, K.C. and PUTHUR, J.T., 2016. Seed priming with beta-amino butyric acid improves abiotic stress tolerance in rice seedlings. Rice Science, vol. 23, no. 5, pp. 242-254. http://dx.doi.org/10.1007/jrs.2016.08.002.

JUN-RONG, H., AI-JUAN, W., GUO-RONG, W., LIAN-MENG, L. and SHI-WEN, H., 2016. Quality of irrigated water with nanomter pottery tray treatment and its effects on seed soaking. Rice Science, vol. 23, no. 2, pp. 88-95. http://dx.doi.org/10.1007/jrs.2016.02.003.

LAJ, S.K., KUMAR, S., SHERI, V., MEHTA, S., VARAKUMAR, P., RAM, B., BORPHUKAN, B., JAMES, D., FARTYAL, D. and REDDY, M.K., 2018. Seed priming: an emerging technology to impart abiotic stress tolerance in crop plants. In: A. RAKSHIT and H. SINGH, eds. Advances in seed priming, pp. 41-50. Singapore: Springer. http://dx.doi.org/10.1007/978-981-13-0032-5_3.

LARIGUET, P., RANOCHA, P., DE MEYER, M., BARBIER, O., PENEL, C. and DUNAND, C., 2013. Identification of a hydrogen peroxide signaling pathway in the control of light-dependent germination in Arabidopsis. Planta, vol. 238, no. 2, pp. 381-395. http://dx.doi.org/10.1007/s00425-013-1901-5. PMid:23716184.

MAHAKHAM, W., SARMAH, A.K., MAENSIRI, S. and THEERAKULPISUT, P., 2017. Nanopriming technology for enhancing germination and
starch metabolism of aged rice seeds using phytosynthesized silver nanoparticles. *Scientific Reports*, vol. 7, no. 1, pp. 8263. http://dx.doi.org/10.1038/s41598-017-08669-5. PMid:28811584.

MARSCHALL, M. and PROCTOR, M.C., 2004. Are bryophytes shade plants? Photosynthetic light responses and proportions of chlorophyll a, chlorophyll b and total carotenoids. *Annals of Botany*, vol. 94, no. 4, pp. 593-603. http://dx.doi.org/10.1093/aob/mch178. PMid:15319230.

MOHAIJERI, F., RAMROUDI, M., TAGHVAEI, M. and GALAVI, M., 2017. Effects of seed priming on chlorophyll content and yield components of pinto beans. *International Journal of Biology, Pharmacy and Allied Sciences*, vol. 6, pp. 1069-1085.

PAPARELLA, S., ARAÚJO, S.S., ROSSI, G., WIJAYASINGHE, M., CARBONERA, D. and BALESTRAZZI, A., 2015. Seed priming: state of the art and new perspectives. *Plant Cell Reports*, vol. 34, no. 8, pp. 1281-1293. http://dx.doi.org/10.1007/s00299-015-1784-y. PMid:25812837.

REHMAN, H., BARS, S.M.A., FAROOQ, M., AHMED, N. and AFZAL, I., 2011. Seed priming with CaCl, improves the stand establishment, yield, and quality attributes in direct-seeded rice (*Oryza sativa*). *International Journal of Agriculture and Biology*, vol. 13, no. 5, pp. 5.

RIBANT, J.M., BANZIGER, M. and HOISINGTON, D., 2002. Genetic dissection and plant improvement under abiotic stress conditions: drought tolerance in maize as an example JIRCAS. *Working Reports*, vol. 85, pp. 92.

RUAN, S., XUE, Q. and TYLKOWSKA, K., 2002a. Effects of seed priming on germination and health of rice (*Oryza sativa L.*) seeds. *Seed Science and Technology*, vol. 30, pp. 451–458.

RUAN, S., XUE, Q. and TYLKOWSKA, K., 2002b. The influence of priming on germination of rice (*Oryza sativa L.*) seeds and seedling emergence and performance in flooded soils. *Seed Science and Technology*, vol. 30, pp. 61–67.

SCREMIN-DIAS, E., LORENZ-LEMKE, A.P. and OLIVEIRA, A.K., 2011. The floristic heterogeneity of the Pantanal and the occurrence of species with different adaptive strategies to water stress. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 71, no. 1, suppl. 1, pp. 275-282. http://dx.doi.org/10.1590/ S1519-69842010000200006. PMid:21537600.

SONG, X., ZHANG, L., ZHOU, J., XU, Y., SUN, Z., LI, P. and YU, J., 2011. Preparation of calcium sulfate whiskers using waste calcium chloride by reactive crystallization. *Crystal Research and Technology*, vol. 46, no. 2, pp. 166-172. http://dx.doi.org/10.1002/crat.201000420.

WAHID, A., PERVEEN, M., GELANI, S. and BASRA, S.M.A., 2007. Pretreatment of seed with H₂O₂ improves salt tolerance of wheat seedlings by alleviation of oxidative damage and expression of stress proteins. *Journal of Plant Physiology*, vol. 164, no. 3, pp. 283-294. http://dx.doi.org/10.1016/j.jplph.2006.01.005. PMid:16545492.

WANG, H., WANG, H., SHAO, H. and TANG, X., 2016. Recent advances in utilizing transcription factors to improve plant abiotic stress tolerance by transgenic technology. *Frontiers in Plant Science*, vol. 7, pp. 67. http://dx.doi.org/10.3389/fpls.2016.00067. PMid:26904044.

WOJTYLA, L., LECHOWSKA, K., KUBALA, S. and GARNCZARSKA, M., 2016. Different modes of hydrogen peroxide action during seed germination. *Frontiers in Plant Science*, vol. 7, pp. 66. http://dx.doi.org/10.3389/fpls.2016.00066. PMid:26870076.

YAN, S. and DONG, X., 2014. Perception of the plant immune signal salicylic acid. *Current Opinion in Plant Biology*, vol. 20, pp. 64-68. http://dx.doi.org/10.1016/j.pbi.2014.04.006. PMid:24840293.

YOUMAS, A., YOUSAF, Z., RIAZ, N., RASHID, M., RAZZAQ, Z., TANVEER, M. and HUANG, S., 2020. Role of nanotechnology for enhanced rice production. In: R. MEENA, eds. *Nutrient dynamics for sustainable crop production* Singapore: Springer, pp. 315–350. http://dx.doi.org/10.1007/978-981-13-8661-2_11.

ZHENG, M., TAO, Y., HUSSAIN, S., JIANG, Q., PENG, S., HUANG, J., CUI, K. and NIE, L., 2016. Seed priming is dry direct-seeded rice: consequences for emergence, seedling growth, and associated metabolic events under drought stress. *Plant Growth Regulation*, vol. 78, no. 2, pp. 167–178. http://dx.doi.org/10.1007/s10725-015-0083-5.