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International Journal of Plant Biology & Research

Research Article

Exploring the Potential of Nano-Priming and Magneto-Priming in Enhancing Drought Tolerance Potential of Wheat Seeds

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Submitted: 12 November 2023
Accepted: 04 December 2023
Published: 06 December 2023
ISSN: 2333-6668

Keywords
- Chl a Fluorescence
- Drought
- Seed Germination
- Nanoparticles
- Photosynthesis
- Pulse Magnetic Field

Abstract

The current study was aimed to explore the effects of two priming treatments, Nano-Priming (NP) and Magneto-Priming (MP) on improving the drought tolerance potential in wheat. Study revealed increase in germination efficiency in both the priming treatments compared to control Unprimed (UP) seeds. NP seeds exhibited a significant improvement in parameters such as germination percentage, seedling length, and seedling vigor when compared to both MP and UP seeds. The impact of Drought Stress (DS) on photosynthetic efficiency measured using Chl a fluorescence parameters, revealed a drastic reduction in the number of active Reaction Centers (RC) per chlorophyll molecule, performance of water splitting complex at donor side of PSII (Fv/Fo) and plant Performance Index (PI) in UP and MP plants wheat plants. However, NP+DS plants seemed to have improved the efficiency of primary photochemistry of PSII by showing significantly lesser reduction in these parameters under DS, ultimately resulting in better growth. Furthermore, the investigation of the oxidative status of plants revealed a reduction in the activity of antioxidant enzymes in NP + DS plants, potentially linked to reduced levels of reactive oxygen species production when compared to UP + DS plants. These findings suggest NP as an effective yet simple way for augmenting the drought tolerance potential in wheat.

INTRODUCTION

Priming is a physiological technique of seed hydration and drying to enhance the pre-germinative metabolic process for rapid germination, seedling growth and overall yield [1]. Priming methods are very useful for agricultural practices in improving seedling establishment, especially when environmental conditions are not optimum [2]. The primed seeds show faster and uniform seed germination due to activation of several enzymes, metabolic activities, biochemical process of cell repair and protein synthesis. Moreover, plants raised from primed seeds showed sturdy and quick cellular defense response against abiotic stresses due to improvement of antioxidant defense system as compared to unprimed seeds [1]. There are many techniques of seed priming which are broadly divided into conventional methods (hydro-priming, osmo-priming, nutrient priming, chemical priming, bio-priming, and priming with plant growth regulators) and advanced methods (nano-priming and magneto-priming [3].

Considering present situation the world population is expected to increase to 9-10 billion by 2050, implying that food production will need to rise by 25-70% compared to current levels [4]. Agriculture is currently facing catastrophic losses of agriculturally productivity of important food crops due the effects of global climate change [5]. Among the various abiotic inputs which effects heavily on crop growth and yield, water availability is a big factor and has led to reduced crop production and economic losses [6]. Drought conditions generate stress in plants, impacting their metabolic as well as biochemical and molecular properties [7]. Prominent effect of water stress is seen on plant photosynthesis which is one of the important phenomenon for plant growth [8]. Drought stress results in substantial damage to photosynthetic pigments and deterioration of thylakoid membranes [9] which can result in stunted growth and poor yield. Traditional agricultural practices for drought tolerance in plants rely on continuous application of pesticides and fertilizers, resulting in environmental contamination [10]. Therefore, new technologies need to be deployed in agriculture to ensure sustainability and increase productivity. The use of seed priming in agriculture can improve the quality of seeds and increase resistance against stress conditions. Seed priming is hence widely recommended to farmers to enhance the crop productivity under stressed field conditions. Wheat is one of the major cereals of world with annual production of 718 million tons, feeding one fifth of world population [11]. To fulfill demands of rapidly growing population of world, wheat production needs to be doubled by 2050. Drought often causes serious problems in wheat production worldwide causing yield loss by shortening the life cycle and duration of grain filling [12].

The aim of this study was to evaluate the effects of two different pre-sowing techniques (nano-priming and magneto-priming) for their influence on (HI-1634) seed germination and photosynthetic performance of drought sensitive wheat variety when subjected to drought stress. Nanotechnology has the potential to contribute to a new technology to enhance crop productivity. Nanoparticles, can act as stimulants, improving seed metabolism, seedling vigor, and plant growth by acting in cellular signaling pathways [13]. These effects depend on nanoparticle physical-chemical properties, such as size, zeta potential, and concentration, which are the factors that determine the biological responses of nanoparticles on plant [14]. Nano-Priming (NP) has been applied to seeds in order to provide protection for seeds during storage, improve germination, germination synchronization and plant growth. New studies have also reported NP to activate different genes, related to plant stress resistance during the germination. Thus seed nano-priming is a new area of research, showing promising results to develop stress tolerant crop varieties, while minimizing fertilizer input.

Magneto Priming (MP) is another seed priming treatment that has been reported to increase the rate of germination and seedling vigor of many crops. There are several reports on the metabolic changes occurring during germination in response to MP under stressed and non-stressed environments [15]. Studies have also reported the positive effect of MP on increasing seed germination, seedling vigor by increase in membrane permeability, facilitating the process of water absorption by seeds [16]. MP may affect water and nutrient absorption, as well as improving plant growth. Beyond improving the germination rates, MP-exposed seeds induce positive effects such as increased cell proliferation capacity, which possibly induces plants rapid growth [17]. Several researchers have reported the beneficial effect of both static (SMF) and Pulsed Magnetic Fields (PMF) in different plant species [18]. However there are no reports on the effect of pulsed magnetic field in reclamation of drought sensitive wheat seeds. To our knowledge this is the first attempt to compare and evaluate the effect of nano-priming and magneto priming on physiological and biochemical changes in drought sensitive wheat variety HI-1634. The present study was therefore conducted to assess the

A. Response of NP and MP in improving seed germination performance of drought sensitive wheat seeds.

B. To evaluate role of NP and MP priming in alleviating the effect of DS on plant photosynthetic efficiency by studying the Chl a fluorescence parameters.

C. To investigate the effect of DS on biochemical balance and biomass accumulation in NP and PMF primed wheat plants.

D. To suggest the best priming technique between MP and NP for improving wheat seed performance.

MATERIALS AND METHODS

Plant Material

Triticum aestivum L; (H1-1634), a drought sensitive variety, were obtained from Indian Agricultural Research Institute (IARI), Regional station Indore.

Seed Priming Techniques

Nano-priming: of wheat seeds were done following [19]. Briefly, surface sterilized wheat seeds were soaked in of 10 mg/L ZnO nano suspensions at 25^0C for 18h, with constant gentle agitation. The primed seeds were then given repeated washes with distilled water and dried back to original moisture content. Unprimed seeds (untreated seeds) were considered as control.

Magneto-priming: seed priming with Pulse Magnetic Field (MP) was done following [20]. Briefly, wheat seeds were exposed to pulse magnetic field treatment 150mT for duration 1 hour with on time of 4 minutes and off time of 3 minutes, in a cylindrical-shaped sample holder of 42 cubic cm capacities, made of a non-magnetic thin transparent plastic sheet. The required strength between the two poles was obtained by regulating the current in the coils of the electromagnet keeping the temperature constant at 25 ± 5°C. Unprimed seeds (untreated seeds) were considered as control.

Study of Seed Germination Parameters

Germination parameters were studied following standard germination assay test [21]. Germinating grains were counted based on 2 mm radical emergence on the 2^{nd} day (48h) of germination. Germination percentage and germination rate was calculated according to [22,23]. All further germination data were taken after 5 days of germination [24].

Seedling fresh and dry weight: For seedling fresh weight 10 seedlings were selected randomly per plate followed by overnight drying in oven at 60^0C for 72h for their dry weight was measured [25].

Seed vigour index: Dry weights of 10 seedlings were measured from each replicate after 5 days. Seedling vigour was calculated using following standard protocols [26].

Study of physiological parameters

Ten UP, NP and MP primed seeds each were uniformly sown at depth of 2cm maintaining equal distance between seed to seed, in black polythene pots (20cm diameter and 25cm height) filled with black garden soil and sieved sand (in the ratio 3:1) kept under natural conditions.

Drought stress (DS): Drought Stress (DS) was given 20 days after germination. Drought conditions were ensured by measuring relative soil water content which was maintained

20% of control (UP+W) measured as described previously by [27]. Experimental setup consisted of fully irrigated unprimed (UP+W), hydroprimed (HP+W), nanoprimed (NP+W) and drought stressed unprimed (UP+DS), hydroprimed (HP+DS) and nanoprimed (NP+DS) wheat plants. Where, unprimed fully irrigated wheat plants (UP+W) were considered as control.

Pigment and growth analysis: The content of photosynthetic pigments extracted in 80% acetone (Chl a and b, carotenoids in mg/g fresh leaves) was determined spectrophotometrically (Thermo scientific) following standard protocol [28].

Chl a fluorescence measurements: The chlorophyll a (Chl a) fluorescence induction kinetics was measured at room temperature using a Plant Efficiency Analyzer (PEA), (Hansatech, England). The energy fluxes were calculated by using Biolyzer HP3 software (the chlorophyll fluorescence analyzing program gifted by Bioenergetics Laboratory, University of Geneva, Switzerland).

Antioxidant Enzymes Assay

Activity of antioxidant enzyme, Superoxide Dismutase (SOD) [EC1.15.1.1] was spectrophotometrically assayed following sodium phosphate buffer extraction method at 560nm, as described previously by [29]. The activity was expressed as Units/mg protein wherein one unit of SOD was defined as the amount of enzyme required to cause 50% inhibition in the rate of NBT photo reduction. Activity of antioxidant enzyme Peroxidase (POD) [EC1.11.1.7] was assayed by the method previously described by [30]. For POD the initial and final absorbance was recorded at 470nm for 2min.The activity was calculated as the change in absorbance/min/mg protein. Catalase (CAT) activity measured as the rate of disappearance of H_2O_2 at 240 nm. Protein was estimated by the method of [31] using BSA as the standard.

Measurement of Total Biomass

Fresh and dry weights of over the ground plant parts (shoots and leaves) were measured after 30 days of plant growth. For dry weight estimation individual plants were oven-dried at 80°C for 24h [22].

Statistical and Data Analysis

Statistical analysis were done using Origin Pro8 and Graphpad Prism 5.01 Software (GraphPad Software Inc.) Results were analyzed using one-way ANOVA followed by the Dunnets comparison test. Significance was determined at $P < 0.001$ (*, $P < 0.05$, **, $P < 0.01$,$^{***}< 0.001$) and results expressed as mean values SD. Each experiment was done thrice with three replicates.

RESULTS

Germination Assay Parameters

The results of the germination analysis on wheat seeds subjected to different priming treatments are presented in (Figure 1). As evident from figure, Unprimed Seeds (UP) displayed a Germination Percentage (GP) of 65%. However, a substantial improvement in germination performance was observed in Nano Primed (NP) seeds with a 100% GP and moderately in Magneto Primed (MP) seeds with a 90% GP. Furthermore, the Germination Rate (GR) was also positively affected by priming. NP seeds exhibited a 25% increase in GR, while MP seeds showed a 20% increase, as compared to UP seeds. Upon assessing the seedling growth parameters, significant enhancements were observed in both Root Length (RL) and Shoot Length (SL) of NP-primed seeds in comparison to UP seeds. NP seeds displayed an impressive 94% increase in RL and a 69% increase in SL after 5 days of germination. On the other hand, MP seeds exhibited a comparatively moderate increase of 35% in RL and 65% in SL. In terms of seed vigor, the seed vigor index (SVI and SVII) demonstrated significant improvements in response to both priming treatments. Notably, NP seedlings exhibited the most prominent increase in vigor indices, further underscoring the effectiveness of nanopriming. Collectively, these results highlight the pronounced positive effects of nanopriming and outperformed both MP and UP seeds on various germination parameters including germination percentage, germination rate, seedling length, and seedling vigor.

Effect of Drought Stress on Plant Physiological Parameters

Effect of drought stress on pigment content: The impact of drought stress on chlorophyll pigment content in wheat plants subjected to different priming treatments is presented in (Table 1). Among the irrigated plants, the highest levels of chlorophyll a (Chl a), chlorophyll b (Chl b), and total chlorophyll content were observed in irrigated nanoprimed (NP+W) plants, followed by magneto primed (MP+W) plants, in comparison to unprimed (UP+W) wheat plants. Upon exposure to drought stress, there was a noticeable increase in chlorophyll content across all drought-stressed plant groups. Notably, the increase in chlorophyll content was particularly remarkable in NP+DS plants, surpassing the levels seen in MP+DS and UP+DS wheat plants. Furthermore, this enhancement was accompanied by a subsequent elevation in carotenoid content in NP+DS plants. However, it's worth noting that there were no significant changes observed in carotenoid content in UP+DS wheat plants under drought stress conditions. In summation, the results indicate that while all drought-stressed plants exhibited increased chlorophyll content, the most notable and significant improvements were observed in NP+DS wheat plants, followed by a subsequent rise in carotenoid content, highlighting their potential contribution to plant photo protection strategies.

Effect of drought stress on Chl a fluorescence parameters: The Chl a fluorescence transient curves for fully irrigated (UP, NP, MP) and drought-stressed (UP, NP, MP) primed wheat plants are depicted in (Figure 2). For irrigated wheat plants, there were no substantial changes observed in the values of fluorescence parameters. However, when subjected to Drought Stress (DS), a significant increase in Fo was evident in UP+DS wheat plants, accompanied by a notable reduction in other parameters.

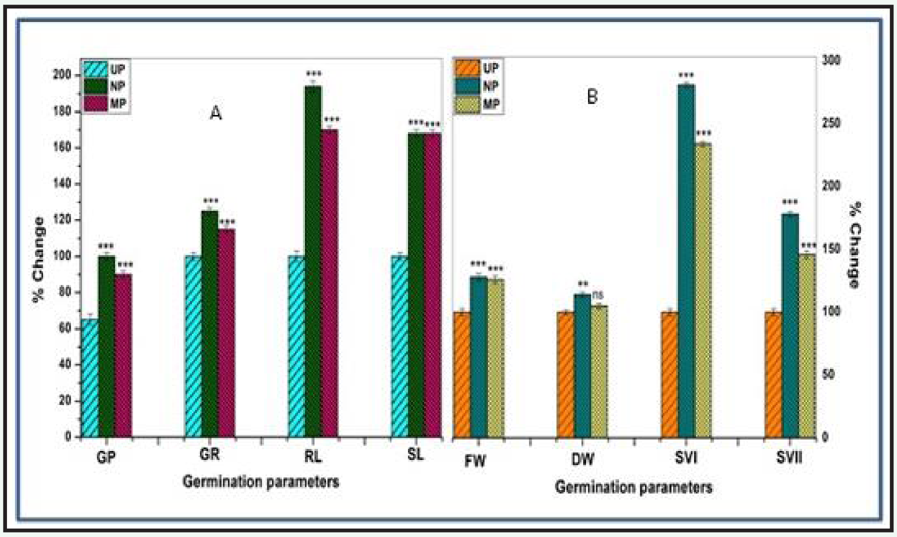

Figure 1 Impact of different priming treatments on
A. Percent Germination (GP), Germination Rate (GR), Root Length (RL), Shoot Length (SL).
B. Seedling Fresh (FW) and Dry Weight (DW), Seed Vigour Index-I (SVI), Seed Vigour Index- II (SVII) of wheat seeds.

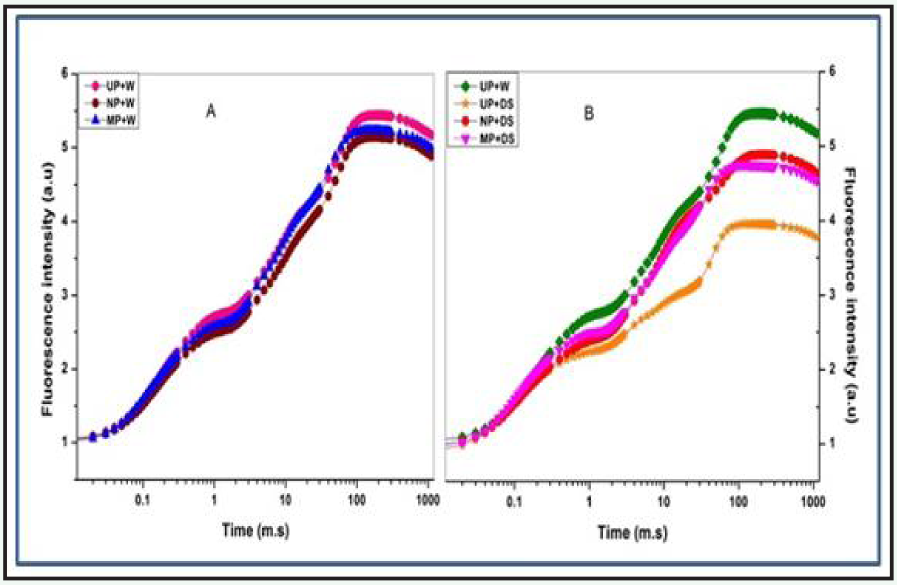

Figure 2 Chl a transient curves for
A. Irrigated Un Primed (UP), Hydroprimed (HP), Nanoprimed (NP) and Mageto Primed (MP).
B. Drought stressed Un Primed (UP), Hydroprimed (HP), Nanoprimed (NP) and Mageto Primed (MP) wheat plants. Transients have been plotted on logarithmic time scale.

Particularly, the maximum fluorescence (Fm) exhibited a drastic decrease in UP+DS plants, followed by a significant decrease in the efficiency of water splitting complex on the donor side of Photosystem II (Fv/Fo). This reduction in Fv/Fo was correlated with a significant decrease in the number of active reaction centers per chlorophyll molecule (RC/Abs) in UP+DS plants. In contrast, the reduction in these parameters was less significant in NP+DS and MP+DS plants compared to fully irrigated untreated wheat (UP+W). While Fm and Fv/Fo showed less significant change in NP+DS plants, the number of active reaction centers was notably reduced in MP+DS plants compared to UP+W plants. Further, remarkably lesser decrease in the overall photochemical efficiency of the plants for primary photochemistry, reflected by PI, was observed in NP+DS plants compared to UP+W wheat

Table 1: Effect of drought stress on Chlorophyll a (Chl a); Chlorophyll b (Chl b); total chlorophyll content (total Chl) and Carotenoid (Car) content in irrigated and drought stressed Un Primed (UP), Hydro Primed (HP), Nano Primed (NP) and Mageto Primed (MP) wheat plants.

Treatments	Chl a (mg/g)	Chl b (mg/g)	Total Chl content (mg/g)	$Car_{(a+b)}$(mg/g)
UP+W	0.42 ± 0.03	0.26 ± 0.03	0.68 ± 0.01	0.21 ± 0.04
NP+W	0.94 ± 0.02***	1.00 ± 0.1***	1.94 ± 0.1***	0.35 ± 0.03***
MP+W	0.92 ± 0.03***	0.78 ± 0.02***	1.71 ± 0.2***	0.35 ± 0.02***
UP+DS	0.61 ± 0.04***	1.09 ± 0.2***	1.70 ± 0.2***	0.19 ± 0.03ns
NP+DS	0.96 ± 0.05***	1.19 ± 0.2***	2.15 ± 0.3***	0.40 ± 0.02***
MP+DS	0.86 ± 0.05***	0.79 ± 0.01***	1.66 ± 0.06***	0.35 ± 0.01***

plants. This enhancement indicated a minimized energy loss through heat dissipation represented by (Fo/Fm). On the contrary, Fo/Fm showed a significant increase accompanied by a substantial decrease in Performance Index (PI) in MP+DS wheat plants, compared to UP+W plants. In summation, the fluorescence parameter analysis reveals distinct responses to drought stress. While NP+DS plants exhibited improved photochemical efficiency and reduced energy loss, MP+DS plants displayed an increase in energy dissipation and decreased performance index. These findings suggest a higher tolerance of NP+DS plants to drought stress compared to both UP+DS and MP+DS plants.

Effect of drought stress on pattern of energy flow in PSII: The energy flow pattern within Photosystem II (PSII) was assessed using an energy pipeline leaf model for wheat plants subjected to different priming treatments, including fully irrigated (UP, NP, MP) and drought-stressed (UP, NP, MP) conditions, as depicted in (Figure 3). The width of the arrows in the model reflects the extent of energy flow, indicating photons captured and utilized for efficient primary photochemistry or electron transport within the system. The model also provides insights into the proportion of active and inactive PSII reaction centers per cross-section (RC/CSm), depicted by open and closed circles, respectively. Among irrigated plants, parameters like ABS/CSm (efficiency of light absorption), ETo/CSm (efficiency of electron trapping), and TRo/CSm (efficiency of electron transport) showed no significant changes in NP+W and MP+W wheat plants compared to UP+W plants. However, upon subjecting the plants to drought stress, UP+DS plants, showed a notable reduction in RC/CSm, followed by reductions in ABS/CSm, ETo/CSm, and TRo/CSm per cross-section. Similarly, in MP+DS plants, a significant reduction in RC/CSm was observed, leading to subsequent decreases in ABS/CSm, TRo/CSm, and ETo/CSm efficiencies per cross-section. Conversely, NP+DS plants exhibited an intriguing response, with the number of active RC/CSm being comparable to that in non-stressed UP+W plants. Collectively, these findings highlight the variations in energy flow patterns in response to drought stress and priming treatments. While UP+DS and MP+DS plants exhibited substantial reductions in energy flow efficiencies, NP+DS plants demonstrated a unique resilience, maintaining similar active PSII reaction center proportions as non-stressed plants. This suggests that Nano Priming (NP) could potentially confer a protective mechanism against the adverse effects of drought stress on PSII functionality (Table 2).

Effect of Drought Stress on Antioxidant Defense System

At the cellular level, the imposition of drought stress triggers an elevation in the generation of Reactive Oxygen Species (ROS) such as O_2^-, OH^-, and H_2O_2 radicals. These ROS, in turn, exert harmful effects on plant cellular components including cell membranes, proteins, chlorophyll, and nucleic acids. In light of this phenomenon, the equilibrium between ROS production and their elimination was investigated by assessing the activity of antioxidant enzymes, specifically Peroxidase (POD) and Superoxide Dismutase (SOD), in both fully irrigated and drought-stressed wheat plants, as presented in (Table 3). Upon exposure to drought stress, a significant increase in the activity of both Peroxidase (POD) and Superoxide Dismutase (SOD) enzymes was observed in UP+DS and MP+DS plants. Interestingly, NP+DS plants exhibited a distinct response, displaying a notably lesser increase in the activity of both POD and SOD enzymes when compared to UP+DS and MP+DS plants.

Effect of Drought Stress on Plant Biomass

The influence of water stress condition on the overall biomass accumulation in wheat plants under irrigated and drought-stressed conditions is depicted in (Figure 4). The results highlight variations in Fresh Weight (FW) and Dry Weight (DW) among different priming treatments and stress conditions. Specifically, in irrigated conditions, there was a decrease observed in both FW and DW of magnetoprimed (MP+W) plants, while no significant changes were observed in nanoprimed (NP+W) plants compared to untreated (UP+W) plants. When subjected to drought stress, UP+DS and MP+DS experienced significant reductions in FW and DW. However, this reduction in biomass accumulation was considerably less pronounced in NP+DS plants when compared to the other priming treatments. The results emphasize the differential responses of wheat plants to various priming treatments and drought stress conditions. Among the treatments subjected to drought stress, NP+DS wheat plants exhibited the most favorable growth attributes, showcasing a relatively milder decrease in biomass accumulation compared to UP+DS and MP+DS plants.

DISCUSSION

The increase in germination parameters including GP, GR and SV in NP treated seeds, could be attributed to the involvement of zinc (Zn) in activating essential enzymes pivotal for initiating key germination-related metabolic activities and protein synthesis [32,33]. The significant enhancement in plumule and radicle development in NP seedlings might stem from Zn-triggered auxin production, resulting in augmented cell elongation and division. Previous research [2] has already underscored the connection between increased seed germination and seedling establishment in NP-treated seeds with augmented seed water uptake facilitated by nanopore formation. This rapid water intake prompts the activation of seed amylase enzymes, consequently accelerating the rate of starch hydrolysis crucial for robust

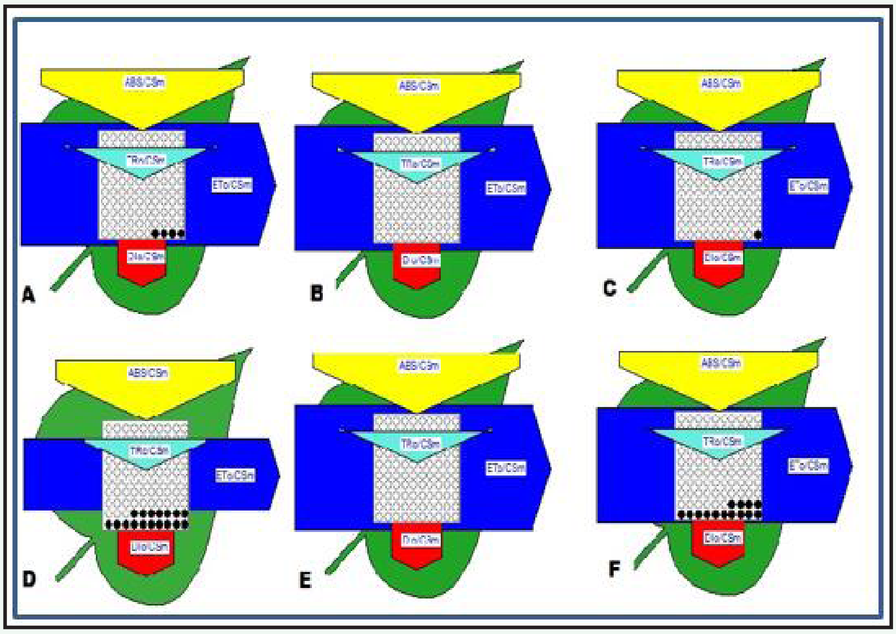

Figure 3 Representative energy pipeline leaf model for
A. Control (UP+W).
B. Nanoprimed irrigated (NP+W).
C. Pulse magnetic field primed irrigated (MP+W).
D. Unprimed drought (UP+DS).
E. Nanoprimed drought (NP+DS).
F. Pulse magnetic field primed drought stressed (MP+DS) wheat plants. The fluxes used represent light absorbance per cross section (ABS/CSm), excitation energy trapping per cross section (TR/CSm), electron transport per cross section (ET/CSm). Empty and full black circles indicate, the percentage of active (QA reducing) and non-active (non QA reducing) reaction centers of PSII respectively.

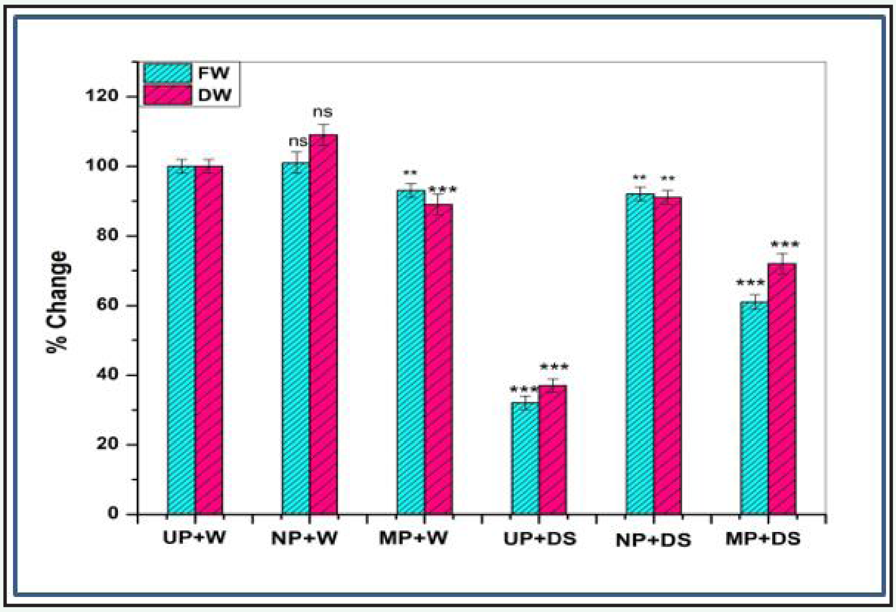

Figure 4 Response of Fresh Weight (FW) and Dry Weight (DW) in irrigated and drought stressed Un Primed (UP), Nano Primed (NP) and pulse magnetic field Primed (MP) wheat plants.

Table 2: Various Chl *a* transient parameters for irrigated and drought stressed Un Primed (UP), Nano Primed (NP) and pulse magnetic field Primed (MP) wheat plants.

Treatment	Fo	Fm	RC/Abs	Area	Fv/Fo	Fo/Fm	PI
UP+W	470 ± 37	2557 ± 158	0.74 ± 0.02	37972 ± 1001	4.44 ± 0.4	0.23 ± 0.02	1.90 ± 0.2
NP+W	487 ± 28^{ns}	2504 ± 143^{ns}	0.77 ± 0.01^{ns}	36795 ± 995^{ns}	4.12 ± 0.3^{ns}	0.23 ± 0.01^{ns}	1.96 ± 0.3^{ns}
MP+W	476 ± 25^{ns}	2492 ± 101**	0.72 ± 0.02^{ns}	28458 ± 617***	4.23 ± 0.2^{ns}	0.24 ± 0.03^{ns}	1.83 ± 0.3^{ns}
UP+DS	600 ± 19***	2371 ± 113***	0.54 ± 0.04***	20023 ± 891***	2.95 ± 0.2***	0.41 ± 0.01***	0.88 ± 0.1^{ns}
NP+DS	518 ± 22^{ns}	2531 ± 143^{ns}	0.73 ± 0.03^{ns}	33277 ± 990***	3.88 ± 0.5**	0.25 ± 0.04^{ns}	1.76 ± 0.1***
MP+DS	524 ± 23^{ns}	2488 ± 111**	0.64 ± 0.03***	23564 ± 977***	3.74 ± 0.4**	0.29 ± 0.03***	1.39 ± 0.2^{ns}

Table 3: Activity of Peroxidase (POD) and Superoxide Dismutase (SOD), in irrigated and drought stressed Un Primed (UP), Nano Primed (NP) and pulse magnetic field Primed (MP) wheat plants.

Treatments	POD (Units/mg protein)	SOD (Units/mg protein)
UP+W	0.15 ± 0.02	3.46 ± 0.5
NP+W	0.11 ± 0.01^{ns}	0.57 ± 0.01***
MP+W	0.17 ± 0.03^{ns}	3.00 ± 0.3^{ns}
UP+DS	0.71 ± 0.03***	7.88 ± 0.4***
NP+DS	0.20 ± 0.02^{ns}	5.34 ± 0.2***
MP+DS	0.56 ± 0.02***	6.64 ± 0.2***

seedling growth in NP-treated seeds [34]. Similarly, the increased germination efficiency observed in Magneto Primed (MP) seeds could be attributed to heightened amylase activity, creating an advantageous scenario during germination [35]. These findings are also in accordance with previous research [36] highlighting improved germination performance in wheat, cucumber and maize seeds upon exposure to pulse magnetic field. This elevation allows for greater availability of soluble sugars, essential for generating energy necessary for seedling growth, thus resulting in increased germination rate and seedling length in both NP and MP primed seeds.

Chlorophyll, a key component of chloroplasts, maintains a positive correlation with photosynthetic rate [37]. The highest chlorophyll content, including Chl a, Chl b, and total Chl content, was observed in NP+W followed by MP+W wheat plants compared to UP+W plants. The observed increase in chlorophyll content in NP+W plants might be attributed to enhanced water and nutrient uptake facilitated by the presence of Zn, leading to improved physiological performance and growth compared to UP plants [38]. However, under drought stress, a noteworthy increase in chlorophyll content was observed across all stressed plant groups, with NP+DS plants exhibiting a remarkable increase compared to MP+DS and UP+DS wheat plants. This increase can be attributed to an adaptive response toward drought stress. Plant exposure to moderate stress levels often induces increased synthesis of chlorophyll pigment as a component of the plant's adaptive defense mechanism, in contrast to the catabolic pathway leading to chlorophyll hydrolysis under severe stress conditions [39]. Role of Zn extends beyond chloroplast development; it also aids in the repair of Photosystem II by recycling damaged D1 protein during drought stress [40]. Zn is a crucial component for safeguarding the sulfhydryl group of chlorophyll molecules which might explain the advantageous impact of ZnO NP priming on chlorophyll content, particularly in the face of drought stress [32]. The subsequent increase in carotenoid content observed in NP+DS plants, playing a vital role in plant photo protection mechanisms [41], further validates our findings. Conversely, no significant changes were noted in carotenoid content in MP+DS and UP+DS wheat plants. Hence, the increase in chlorophyll and carotenoid content in NP+DS plants indicates an adaptive response aimed at combating drought stress.

The study of various chlorophyll fluorescence parameters, including Fo, RC/Abs, Fv/Fo, and Fo/Fm, presents a rapid and accurate method for detecting and quantifying a plant's tolerance to drought stress [42]. No remarkable change in values of fluorescence parameters were observed in irrigated wheat plants. However, significant increase in Fo in plants subjected to DS resulted in considerable reduction in other parameters in UP+DS. The reduction magnitude of fluorescence parameters was notably lower in NP+DS and MP+DS plants when compared to fully irrigated UP+W plants. This relatively minor reduction in Fv/Fo in NP+DS plants might be attributed to an enhanced PSII complex efficiency in performing primary photochemistry [19]. Furthermore, the rise in the number of active reaction centers under drought stress signifies more effective transfer of absorbed light energy to these active reaction centers in NP+DS plants. This increase may be attributed to enhanced association between LHC II and the PSII complex might lead to increased electron flow from QA to QB in NP plants [42]. This inference is supported by the relatively smaller reduction in the area under the fluorescence curve, indicating the quinone pool size in the electron transport chain in NP+DS [43], in comparison to UP+W plants. While Fm and Fv/Fo demonstrated no significant change in NP+DS plants, the number of active reaction centers significantly decreased in both MP+DS plants compared to UP+W plants. These observations suggest that DS did not substantially affect the water splitting complex of MP+DS plants but did reduce photosynthetic efficiency by influencing the number of active reaction centers. The increase in overall photochemical efficiency in NP+DS plants, thereby resulted in minimizing energy loss through heat dissipation (Fo/Fm). The corresponding lesser reduction in the Performance Index (PI) further elucidate the ability of NP+DS plants to maintain a higher photosynthetic efficiency under drought stress. On the contrary, the increase in Fo/Fm accompanied by a substantial PI decrease in MP+DS wheat plants compared to UP+W plants suggests a different response. Thus, of the two priming treatments ZnO nano-priming proves to be better in maintaining photosynthetic efficiency of light reaction, absorbance per reaction center and net photosynthesis even under severe drought conditions.

Likewise, the pattern of energy flow within PSII exhibited a remarkable reduction in RC/CSm followed by a decline in ABS/CSm, ETo/CSm, and TRo/CSm in UP+DS plants (Figure 3). The diminished number of active reaction centers (RC/CSm) in these conditions corresponds to the production of dissipative heat sinks (increased DIo/CSm) from excitation energy, as reflected in the increased Fo/Fm ratio in UP+DS plants as discussed earlier (Table 1). Similarly, MP+DS plants demonstrated a significant reduction in RC/CSm, leading to subsequent decreases in ABS/CSm, TRo/CSm, and ETo/CSm efficiencies per cross-section. In contrast, NP+DS plants exhibited a number of active RC/CSm similar to that of non-stressed UP+W plants. This increase in the number of active RCs signifies improved absorption and trapping by antennae pigments, thereby facilitating more efficient electron transport per cross-section, even under severe drought conditions. This suggests that ZnO nanopriming assists in maintaining the photosynthetic apparatus' efficiency, protecting it from extreme damage under water-stressed conditions. Furthermore, the decrease in SOD and POD activity in NP+W plants indicates a lesser extent of stress compared to other priming treatments. However, upon exposure to drought stress, both SOD and POD activities significantly increased in UP+DS and MP+DS plants, whereas the increase was notably lower in NP+DS plants. This indirect observation suggests that NP+DS plants experience a milder level of stress, associated with a reduced rate of Reactive Oxygen Species (ROS) production in response to drought stress compared to other priming treatments. These results can be validated in terms of overall biomass accumulation. Drought-induced reductions in both Fresh Weight (FW) and Dry Weight (DW) were significant in both UP+DS and MP+DS plants. This decline in biomass could be directly linked to the decrease in the photosynthetic parameters (discussed above). Moreover, increased ROS production in these plants results in chloroplast dysfunction and altered metabolic pathways, collectively contributing to decreased biomass. While overall biomass accumulation decreased in NP+DS plants, the reduction was notably milder compared to the other priming treatments. Consequently, our results demonstrate that among the three treatments subjected to drought stress, NP+DS wheat plants exhibited the most favorable growth attributes. In summary, our findings underscore the differential responses of wheat plants to various priming treatments and drought stress conditions. NP+DS plants exhibited enhanced growth attributes, marked by better germination rates, enhanced chlorophyll content and increased photosynthetic efficiency as compared to MP+DS and UP+DS wheat plants.

CONCLUSION

Both magneto-priming and nano-priming of drought sensitive wheat seeds enhanced early growth measurements individually compared to UP seeds. Various germination parameters including germination percentage, and seedling length as well as seedling vigour were significantly increased in both priming techniques. However, between the two priming methods nano-priming was found to be the best-performing pre-sowing technique in enhancing plant performance under normal as well as water stressed conditions. As evident from results photosynthetic performance and accumulation of overall biomass showed significantly lesser reduction in both primed (MP and NP) plants. However, NP plants showed a better protection of photosynthetic apparatus in comparison to MP plants under water stress conditions. As an effect of DS, NP plants showed lower level of ROS production as compared to MP and UP plants; thereby protecting the plant cells from ROS induced cellular injury. Taken together, results suggest that Zn nano-priming could be considered as a potential plant growth enhancement strategy for the improvement of crop drought tolerance potential in comparison to MP. Also, magneto-priming of seeds is time consuming and requires expensive equipment. On the contrary pretreatment of seeds with nanoparticles without soil application proves to be simpler, cost effective as well as ecofriendly way to enhance seed performance for sustainable agriculture in future.

AUTHORS' CONTRIBUTION

Study was designed by AJ, PR and SM. Experiments were performed and analysed by PR and SM. Manuscript was written by PR, SM and edited by AJ.

ACKNOWLEDGEMENT

AJ thanks Devi Ahilya University for seed money grant (Dev/Seed money 2.0/2020-21/654). PR thanks University Grants Commission (UGC) India for fellowship [F.16-(DEC.2016)/2017(NET)].

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