Seed Priming with Iron Oxide Nanoparticles Raises Biomass Production and Agronomic Profile of Water-Stressed Flax Plants

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Abstract: The current study is a field experiment set out to comprehend significance of the iron oxide (IO) nanoparticles for use as seed priming agents and their subsequent impact in alleviating water stress and improving agronomic profile of flax plants. The experimental layout consisted of a split-plot factorial design with one main plot divided into two subplots corresponding to drought and well-irrigated environment. Each of the subplots was divided into five rows of the flax plants raised from iron oxide primed seeds. The seed priming concentrations were 0, 25, 50, 75, and 100 ppm. Seed priming increased stem diameter, stem length, height, fresh weights, and dry weights of plant. The yield attributes, such as number of fruit branches, capsules, seeds per capsule, total fresh and dry stem's fiber production, were also predominantly improved. The levels of malondialdehyde and hydrogen peroxide were found to decline by 66% and 71%, respectively, upon seed priming, and an enhancement in activity of antioxidant enzymes superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) was also observed by 28%, 56%, and 39%, respectively, documenting the potential of iron oxide particles in mitigating the water stress.

Keywords: nanopriming; crop protection; water stress; flax plants; flax yield

1. Introduction

Plants have been facing many biotic and abiotic stresses in different times of their life spans. Out of these abiotic stresses, the most prevalent is drought, which is the most critical threat to the food crops and has been a potential yield-limiting agent worldwide. Climate change is going to induce severe droughts, leading to global food security in peril [1]. Water deficiency results in stomata closure due to production of stress hormones [2], resulting in a mitigated inflow of carbon dioxide into the leaves of the plant and thus overall stimulating production of the “reactive oxygen species” (ROS) [3]. Limited water availability leads to deterioration in plastid structure and photosynthetic machinery of the plants [4]. The balance between the formation of ROS and antioxidant enzymes is lost under drought...
and there is an accumulation of ROS, resulting in induction of osmotic stress in plants [5]. Oxidative stress leads to porosity in the membrane structure due to lipid peroxidation, leading to disturbance in cellular transport processes [6].

Flax plant (*Linum usitatissimum* L.), also known as linseed crop, is among the oldest crops cultivated for its oil yield, fiber, and food [7]. The seed oil obtained from flax seeds is a potential means of obtaining “omega-3 fatty acid”, commonly called “linolenic acid” [8]. Flax seed is added in animal fodder as a supplement to enhance the health and reproductive fitness of the animal [8]. In Pakistan, flax is cultivated in an area of around 3164 hectares but, due to more climate-change-mediated pest and stress encounters, lack of farmer education, and high yielding seed varities, the per hectare yield of flax is 722.3 kg, which is quite low [9].

In the modern era, nanotechnology has the potential to limit declines in yield caused due to climate change and has the potential to promote agricultural industry. The inception of nanotechnology is very promising, where nanofertilizers (NFs), nano-agro-medicines (NAMs), nanobiosensors (NBS), nanoclays (NCs), and nanoseed priming (NSP) techniques are prevalently used [10]. The nanotechnology has been an area of exploitation over the past decade, as nano-based formulations are proving to be catalysts to enhance agricultural yields and productivity, to improve the water use efficiency, and limit stress-induced damages on the overall physiology of food crops [11].

Nanopriming or seed preconditioning with eco-friendly and commercially useful nanoparticles is a technology being exploited to provide seed protection, improving plant growth, crop protection, and increasing agricultural yield against environmental stressors such as drought [12]. Seed nanopriming is also proving useful in reducing the fertilizer use and use of pesticides. Researchers are highlighting that seed priming with nanoparticles is useful in expression of genes involved in stress resistance [13–15]. New evidence is suggesting that NSP controls the “epigenetic expression” of certain genes relevant for crop protection and yield enhancement [16]. The use of NSP technology is a novel field in the agriculture sector for maximum yield exploitation, and the research in the field is proving successful and encouraging to the researchers across the world [17].

Iron (Fe) is known as an essential micronutrient for plant metabolism and life sustenance, as well as for better agronomic yield. Iron deficiency is commonly reported in food crops, resulting in yield losses and poor agronomic profile. A large proportion of iron content is entangled along with soil particles [18] and is thus not readily available to the plants [19]. The iron is usually present in agricultural soils in its insoluble ionic form (Fe$^{3+}$). However, aerobic soils with high pH generally lack the soluble form of iron (Fe$^{2+}$) [20].

Iron oxide (IO) nanoparticles (NPs) are of two types, viz, maghemite (with chemical formula of $\gamma$-Fe$_2$O$_3$) and magnetite (with formula of Fe$_3$O$_4$) with particles having diameter in the range of 1 to 100 nm. Due to their super-magnetic properties and eco-friendly nature, the iron oxide NPs are proving to be catalysts in enhancing food security and stress mitigation [21]. Maswada et al. [14] reported the encouraging results on the utilization of iron oxide (IO) NPs as nano seed priming agents and showed that application of iron oxide NPs results in increasing water contents of leaves and increases biomass production of the sorghum plant. Kumar et al. [22] reported the encouraging results on the use of iron oxide NPs on *Capsicum annum* L. Similarly, work reported by Rizwan et al. [15] regarding application of iron oxide NPs on wheat is encouraging in terms of wheat biofortification and induction of tolerance against oxidative, as well as heavy, metals stress.

Literature surfing has shown that iron oxide NPs are finding a large number of applications as nanosensors, nano coating materials, in food packaging, as profertilizer, in precision farming, and as nanofertilizer in improving the crop performance. Studies have shown that iron oxide NPs application leads to broader leaf area, increased fresh and dry weights, and increased number of leaves, leading to increased photosynthesis. The iron oxide NPs are involved in the mobilization of endospermic starch contents, increasing the seed germination and overall plant performance. Due to this property, the iron oxide NPs are available commercially as profertilizer in the form of pyrite nanoformulation. Overall,
the strategy of applying the iron oxide NPs as preconditioner is eco-friendly, since the iron oxide NPs are not being released in the soil and are adhered to seed coats, allowing the slow release of the active material from the surface of seed coats without contaminating the soil, unlike the conventional fertilizers which are added to soils. Additionally, the iron oxide NPs, if used as seed priming agent, are required in lower volumetric quantities and hence are cost-effective compared to conventional fertilizers which are applied at higher dose leading to high cost [23–26].

The research on application of iron oxide NPs as seed priming agents is quite new and requires exploration; therefore, the key objectives of the present study were (i) to explore the potential and significance of iron oxide NPs in stress mitigation and (ii) subsequently yield improvement of flax plants under field conditions.

2. Materials and Methods
2.1. Experimental Conditions

The present study is a field experiment which was conducted on a farmland area at Government Graduate College Sarai Alamgir (32.8849090, 73.7571688) District Gujrat, Punjab, Pakistan, as per the advice of the research mentor. In Pakistan, the flax or linseed crop is characterized as “Rabi crop”, which means it is cultivated in mid-October and harvested in April of the following year. The crop was planted on 15 October 2020. Before cultivation, the farmland was ploughed twice for better aeration and land preparation. A basal dose of diammonium phosphate (DAP) was applied at the rate of 100 kg per hectare. The soil characteristics are listed in Table 1, following Chapman and Pratt [27]. The sowing temperature of the crop was around 20°C. The seeds of flax variety LC2023 (1998) were obtained from National Agriculture Research Centre (NARC) Islamabad. The seeds before priming were given treatment with fungicidal thiram at the rate of 2 g per kg.

Table 1. Physiochemical features of experimental trial soil samples.

| Soil Character       | Recorded Value |
|---------------------|----------------|
| Soil Type           | Clayey         |
| EC                  | 1.99 dsM⁻¹     |
| pH                  | 5.46           |
| Organic Matter      | 1.99%          |
| Sand                | 28.01%         |
| Silt                | 23%            |
| Clay                | 47%            |

The research design followed was a split-plot factorial layout involving a main plot which was further split into two subplots corresponding to well-irrigated and drought environment. Each of the subplots was further carved up into five rows of flax plants raised from seeds receiving priming treatments of 0 ppm, 25 ppm, 50 ppm, 75 ppm, and 100 ppm. A distance of 25 cm was maintained among the rows and a distance of 10 cm was spaced between the plants. The cultivation was carried out using drilling method, and rows were drilled at depth of 5 cm and then the seeds were planted carefully. About 20 plants were raised per row in both plots. The number of plants was reduced to 10 after thinning treatment performed after 14 days of seed germination. Three replicates from each row were taken for data collection [28].

2.2. Drought Treatment

The drought stress was imposed using a deficit irrigation technique following Capra et al. [29]. Usually, the flax plants require four irrigations. Four irrigations were given to well-irrigated subplots (i.e., at the time of sowing, 25 days after germination, at the time of flowering, and at the time of fruiting) whereas drought-stressed plots received only two irrigations (i.e., at the time of seed plantation and at the start of flowering).
2.3. Characterization of Iron Oxide Nanoparticles and Treatment Application

Maghemite nanoparticles (n-Fe$_2$O$_3$) were of analytical standard and these were bought from chemical supplier Alpha Genomics, located at Plot 4-C, Main PWD Road, Islamabad, Punjab Pakistan. The physiochemical character analysis of NPs was performed using UV–visible spectroscopy and transmission electron microscopy. The ultraviolet–visible analysis was carried out by employing a spectrophotometer, model ELICO SL-159, with a range of wave length 350–470 nm. Transmission electron microscopy of different selected samples was conducted using FEI Tecnai 12 apparatus supported by a low-dose digital camera from Gatan, and this part of the experiment was conducted at the Institute of Space Technology (IST), Islamabad.

For seed priming treatment, different concentrations of iron oxide NPs were prepared, which included 0 ppm as control treatment, 25 ppm, 50 ppm, 75 ppm, and 100 ppm (Table 2). Initially, a little volumetric quantity of iron oxide NPs was placed in deionized water. The mixture was ultrasonicated for 30 min to make uniform dispersions, and subsequently the desired concentrations of iron oxide NPs were raised. The control seeds were soaked in deionized water while the rest of the seeds were dipped in their respective concentration range for 24 h under dark, provided with continuous aeration treatment during priming [30].

Table 2. Priming treatments layout.

| Treatment  | Concentration mg/L or ppm |
|------------|---------------------------|
| Control    | One liter distilled water (D.W.) |
| 25 ppm     | 25 mg of nanoparticles/L of D.W. |
| 50 ppm     | 50 mg of nanoparticles/L of D.W. |
| 75 ppm     | 75 mg of nanoparticles/L of D.W. |
| 100 ppm    | 100 mg of nanoparticles/L of D.W. |

2.4. Analysis of Biomass Production (ABP)

The biomass production or yield was measured for fresh and dry plants parts separately using electric measuring balance. To calculate biomass production, fresh weight (FW) and dry weight (DW) of selected parts of plants were measured for each trial and repeated in triplicate fashion. For DW measurement, the fresh parts of plants were oven-dried at 70 °C temperature for 48 h duration. The difference of FW and DW culminated into “biomass”.

The plant height and technical stem length of stem were measured using a common meter scale bearing units of centimeters. The samples of each experimental trials were labeled properly, and length or height was measured by scale and recorded on a notepad. The stem diameter was also noted on an Excel sheet for analysis of variance studies.

2.5. Measurement of Total Chlorophyll Contents (MTCC)

The chlorophyll quantity of each plant sample was measured by applying the protocol of Arnon [31] and repeated in a triplicate manner. The sample leaves with age of 25 days were collected, devoid of any disease symptoms. The samples of fresh leaves (FLs) weighing ca. 0.25 g were collected, crushed/extracted in 80% acetone on ice, and then the solution was kept overnight at room temperature. The solution of extraction was agitated slowly and then centrifuged at a speed of 10,000× g for a time of five min. The supernatant obtained was kept in dark overnight at 20 °C and then used for measuring reading at 663, 645, and 480 nm by a spectrophotometer (model version: Hitachi-U2001, Tokyo, Japan).

2.6. Extraction of Anti-Oxidant Enzymes

To analyze conc. or contents of different antioxidant enzymes, healthy and same-age aged leaf samples were collected and extracted in triplicates. The leaf tissue macerates were prepared by crushing fresh leaf weighing about 0.5 g by grinding in mortar and pestle.
using 5 mL of 50 mM chilling phosphate buffer solution. The macerate solution was filtered and centrifuged at 15,000 rpm for 20 min at 4 °C. Functioning of SOD, POD, and CAT was analyzed in units/mg protein from the prepared enzyme extract.

2.6.1. Estimation of Superoxide Dismutase (SOD) Activity

For recording activities of SOD, a method proposed by Giannopolitis and Ries [32] was used. SOD functioning depends upon photochemical reduction of nitroblue tetrazolium (NBT) present in the reaction mixture at 560 nm. The reaction mixture contained 50 µM NBT, 50 µL enzyme extract, 1.3 µM riboflavin, 50 mM phosphate buffer, 75 nM MEDTA, and 13 mM methionine. For photochemical oxidation purposes, the light source of 30 W was used for 15 min and reaction was performed in a fluorescent chamber. The photochemical reduction of NBT produces formazone which can be measured at 560 nm by using a UV—visible spectrophotometer.

2.6.2. Determination of Peroxidase (POD) Activity

POD undertakings were assayed using the protocol of Chnace and Mehely [33] with small modifications. The concentration of POD correlates with guaiacol oxidation. The reaction mixture was supplied with 100 µL enzyme extract, 50 mM phosphate buffer, 20 mM guaiacol, and 40 mM H₂O₂. POD activity was measured by measuring the variation absorbance which was recorded after 20 s, at wavelength of 470 nm.

2.6.3. Determination of Catalase (CAT) Activity

The CAT functioning was recorded as disappearance of H₂O₂ present in the reaction mixture. The reaction mixture comprised 50 mM phosphate buffer (pH 7.8), 0.1 mL enzyme extract, and 59 mM H₂O₂. The decrease in absorbance was recorded as hydrogen peroxide disappeared from the reaction mixture.

2.7. Malondialdehyde Contents

Malondialdehyde contents MDA values (umol/g FW) were recorded in the mesophyll tissues produced upon oxidation of membrane lipids due to stress following the method of Cakmak and Horst [34]. A 10% solution of TCA was prepared in distilled water. In the 10 mL TCA solution, one gram of leaf tissue was ground. The supernatant from the sample homogenized was added to 0.5 mL of thio barbituric acid. The mixture was heated at 95 °C for 50 min and was cooled in chilled water. After centrifugation (10,000 × g) for 10 min, the absorbance of the colored part was read at 600 and 532 nm. The content of MDA was calculated using the formula:

\[
\text{MDA (nmol)} = \Delta (A_{532 \text{ nm}} - A_{600 \text{ nm}})/1.56 \times 105
\]

Absorption coefficient for the calculation of MDA is 156 mmol⁻¹ cm⁻¹.

2.8. Hydrogen Peroxide Values

The hydrogen peroxide contents were noted following the method of Velikova et al. [35]. For this purpose, the test mixture was prepared by grinding the 0.1 g leaf tissues in 5 mL volume of 0.1% TCA. The grinding was performed in an ice bath. The grinding was centrifuged at 12,000 rpm for 5 min. A test sample (0.5 mL) was added to the same volumetric quantity of phosphate buffer in a test tube. A total of 1 mL of 1 M KI was added to the test tube. After shaking, the H₂O₂ values were noted at 390 nm using a spectrophotometer.

2.9. Agronomic Profile

In mid-April 2021, flax crop raised through the primed seeds with iron oxide NPs was harvested. The plants were air-dried for evaluation of yield attributes. The yield profile comprising total dry yield (g), 1000 seeds weight (g), number of capsules/plant, seed yield/capsule, number of fruiting branches/plant, and total fresh yield was evaluated and compared to control under both water-deficit and well-irrigated conditions.
2.10. Statistical Analysis

The data were subjected to statistical tools XLSTAT (Addinsoft 1995–2014, Paris, France) and Co-STAT version 6.3 (developed by Cohort Software Berkley, CA, USA) for ANOVA studies, LSD tests, and principal component analysis and Spearman correlation test among the variables.

3. Results

3.1. Characterization

In the case of the chemical synthesis method, iron oxide nanoparticles are known to exhibit a characteristic surface plasmon band that can be measured by UV–Vis spectroscopy. Figure 1A shows the plasmon band of iron oxide nanoparticles (IONPs) suspensions, showing a typical absorbance peak for nanoparticles centered at 298 nm.

Successful imaging of nanoparticles using TEM depends on the contrast of the sample relative to the background. Samples for TEM were prepared for imaging by drying iron oxide nanoparticles on a copper grid that was coated with a thin layer of carbon (Table 3; Figure 1B).

Table 3. Characterization of iron oxide NPs.

| Character           | Value         |
|---------------------|---------------|
| Purity (%)          | 98.7          |
| Surface Area        | 179 m²/g      |
| Density             | 5.2 kg/L      |

3.2. Biomass Production

The present study illustrates the findings related to total increase in biomass of flax plants raised through iron oxide nanoparticles (iron oxide NPs) primed seeds. The soil moisture contents of the drought subplot were reduced, and the water deficiency symptoms were quite obvious on the flax plants which received zero dose of seed prime treatment. The data presented in Figure 2A,B show the plant biomass, which predicts that fresh weight (FW) and dry weight (DW) is drastically reduced upon exposure to a water-shortage environment. The seed preconditioning with iron oxide NPs enhanced fresh weights and dry weights compared to their corresponding control levels. The maximum increase in plant fresh weight was observed with priming concentration of 75 ppm and 100 ppm, whereas the maximum increase of 41% in dry weights was observed with 75 ppm priming.
concentration in drought-stressed flax plants. The overall morphology of the flax plants produced after seed priming with IONPs also improved, and the symptoms of leaves discoloration and rolling which appear due to water shortage were also found to disappear. The seeds preconditioning with iron oxide NPs has been found to be ameliorative in flax plants under drought stress due to observation in increased biomass.

**Figure 2.** Growth and total chlorophyll values of flax plant samples; where (A) represents fresh weight (FW); (B) shows dry weight (DW); (C) shows plant height (PG); (D) depicts total chlorophyll contents (TCC); (E) shows technical stem length (TSL); and (F) shows stem diameter (SD). Impacts of different conc. (doses) of IONPs in different water scarcity and well-irrigated trials (measured in mean ± SE; replica (n) = 3).
Plant height is an indicator of morphological and physiological health of the plants. In the current study, the data presented in Figure 2C depict that water scarcity causes reduction in plant height by 20% in flax plants. Increase in the height of the plants has a strong correlation of 0.9359 and 0.9506 with the increased fresh weight and dry weights (Table 4). The seed priming with iron oxide NPs significantly affects the plant height and improves it under both water-shortage and well-irrigated environments, as shown in Figure 2C. All the concentrations of iron oxide NPs have been found to be ameliorative and increased the plant height; however, the priming concentration of 100 ppm has been optimum to increase the plant height under both drought and well-irrigated environments.

Table 4. Physiochemical parameters of flax seeds measured after seed priming using iron oxide NPs and analyzed through ANOVA (with mean square and p values).

| Variation Source | df | T.CHLO | TSL | SD | NFBP | H$_2$O$_2$ | MDA |
|------------------|----|--------|-----|----|------|-----------|-----|
| Water Stress (WS) | 1  | 12.857b,*** | 132.720 *** | 855.895 ns | 9.633 *** | 5.482 *** | 41.418 *** |
| Priming Treatment (PT) | 4  | 1.207 *** | 16.463 *** | 889.664 ns | 2.666 *** | 112.908 *** | 1.782 *** |
| WS X PT | 4  | 0.096 *** | 0.452 ns | 890.287 ns | 0.133 ns | 1.513 *** | 0.765 *** |
| Error | 20 | 0.012 | 0.248 | 0.898 | 0.233 | 0.083 | 0.015 |

A brief analysis regarding total chlorophyll contents is also part of the study, since the overall wellbeing of the plant is highly correlated with photosynthetic pigments, particularly the chlorophyll contents. Drought stress severely affects the total chlorophyll values of water-stressed flax plants and decreases the total chlorophyll contents by 32%, as shown in Figure 2D. Total chlorophyll values significantly correlate with plant biomass and yield characteristics, as shown in Tables 4 and 5. A significant correlation of total chlorophyll contents’ correlation was found with plant fresh weights (FW), plant dry weight (DW), and plant height (PH) of 0.9513, 0.9735, and 0.9686, respectively, which means that increase in chlorophyll contents leads to increased biomass production. Under the current experiment, the maximum increase in total chlorophyll contents was found with
priming concentration of 75 ppm and 100 ppm under both subplots, corresponding to drought and good irrigation.

Table 5. Spearman correlation matrix for studied variables and biomass and yield attributes of flax plants raised from iron oxide NPs primed seeds.

| Variables | PFW * | PDW | PH | T.Chlo | TSL | SD | NFBP | NCP | NSC | TFY | DSY | 1000 SW |
|-----------|-------|-----|----|--------|-----|----|------|------|-----|-----|-----|--------|
| PFW       | 1     | 1   |    |        |     |    |      |      |     |     |     |        |
| PDW       | 0.962 * | 1   |    |        |     |    |      |      |     |     |     |        |
| PH        | 0.935 * | 0.950 * | 1   |        |     |    |      |      |     |     |     |        |
| T.Chlo    | 0.951 * | 0.973 * | 0.968 * | 1   |    |    |      |      |     |     |     |        |
| TSL       | 0.965 * | 0.975 * | 0.979 * | 0.987 * | 1 |    |      |      |     |     |     |        |
| SD        | 0.875 * | 0.871 * | 0.861 * | 0.854 * | 0.863 * | 1 |    |      |      |     |     |        |
| NFBP      | 0.817 * | 0.871 * | 0.774 * | 0.821 * | 0.828 * | 0.743 * | 1 |      |      |     |     |        |
| NCP       | 0.964 * | 0.976 * | 0.982 * | 0.980 * | 0.992 * | 0.863 * | 0.823 * | 1 |      |     |     |        |
| NSC       | 0.866 * | 0.867 * | 0.811 * | 0.829 * | 0.839 * | 0.775 * | 0.785 * | 0.846 * | 1 |    |     |        |
| TFY       | 0.932 * | 0.944 * | 0.978 * | 0.970 * | 0.972 * | 0.838 * | 0.828 * | 0.972 * | 0.804 * | 1 |     |        |
| DSY       | 0.880 * | 0.935 * | 0.913 * | 0.911 * | 0.904 * | 0.801 * | 0.859 * | 0.909 * | 0.796 * | 0.928 * | 1 |        |
| 1000 SW   | 0.953 * | 0.978 * | 0.972 * | 0.979 * | 0.997 * | 0.853 * | 0.817 * | 0.984 * | 0.822 * | 0.964 * | 0.918 * | 1 |
| MDA       | −0.962 * | −0.966 * | −0.977 * | −0.983 * | −0.993 * | −0.858 * | −0.812 * | −0.992 * | −0.838 * | −0.963 * | −0.892 * | −0.976 * |
| H₂O₂      | −0.965 * | −0.981 * | −0.974 * | −0.981 * | −0.988 * | −0.863 * | −0.839 * | −0.988 * | −0.846 * | −0.963 * | −0.924 * | −0.983 * |
| SOD       | −0.533 * | −0.527 * | −0.474 * | −0.522 * | −0.550 * | −0.397 * | −0.212 * | −0.527 * | −0.430 * | −0.394 * | −0.338 * | −0.531 * |
| POD       | −0.350 * | −0.314 * | −0.471 * | −0.516 * | −0.530 * | −0.409 * | −0.206 * | −0.527 * | −0.430 * | −0.394 * | −0.300 * | −0.514 * |
| CAT       | −0.544 * | −0.521 * | −0.460 * | −0.515 * | −0.515 * | −0.408 * | −0.219 * | −0.524 * | −0.445 * | −0.383 * | −0.319 * | −0.515 * |

a PFW: plant fresh weight; T.CHLO: total chlorophyll; TSL: technical stem length; SD: stem diameter; NFBP: number of fruit branches per plant; H₂O₂: hydrogen peroxide; MDA: malondialdehyde; NCP: number of capsule per plant; NSC: number of seeds per capsule; TFL: total fresh yield; DSY: dry stem yield; POD: peroxidase, CAT: catalase, 1000 seeds wt.; PH: plant height; PDW: plant dry weight; SOD: superoxide dismutase. Values with * are different from 0 with a significance level alpha = 0.05.

The data presented in Figure 2E,F clearly show that drought stress depicts a landmark reduction in technical stem length, as well as in stem diameter. Exogenous application of all the treatments significantly enhances the technical stem length and stem diameter; however, the increasing effect is treatment- and concentration-specific. A seed priming concentration with IONPs with conc. of 75 ppm dose proves best among all other treatments in increasing stem diameter under drought environment, whereas a treatment concentration of 100 ppm was found best in increasing the technical stem length of the flax plants. The analysis of variance studies given in Table 5 justify the results, where a significant contribution of priming treatments in raising biomass accumulation is listed.

3.3. Agronomic Profile

The yield parameters of the current study included measuring or counting the number of fruit branches, capsules, seeds per capsule per each plant, total fresh yield, dry stem yield, and 1000 seeds weight of flax plants. A look at the data presented in Figure 3A shows that drought leads to significant decrease in number of fruit branches per plant. The drought stress reduced number of fruit branches to as low as three. The flax plants raised from iron oxide NPs primed seeds significantly increased the number of fruit branches in each of the flax plants. The maximum number of fruit branches was six in well-irrigated plots, from 75 ppm iron oxide NPs treatment. A similar result was seen in corresponding drought treatment as well, where five branches were observed, as compared to three branches, with 0 ppm treatment. A strong correlation of 0.8215 was observed between total chlorophyll values and number of fruit branches per plant. Water shortage leads to decreased number of capsules on flax plants (Figure 3B) and, subsequently, the seed yield per capsule (Figure 3C). The seed priming treatment enhanced both the parameters; however, the increasing effect is treatment specific. A strong correlation of number of fruit branches per plant and number of capsules per plant is recorded and mentioned in Tables 4 and 5. Seed preconditioning with 100 ppm was found best to increase number of capsules per plant and number of seeds per capsule (Figure 3B,C).
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It was explored that water stress or scarce treatment produces decrease in the total fresh yield, dry stem yield, and 1000 seed weights by 15%, 38%, and 19%, respectively (Figure 3D–F). Seed priming treatments significantly enhance these parameters. The maximum increase in total fresh yield was obtained by 100 ppm concentration. Similarly, maximum increase in 1000 seeds weight was observed with 100 ppm concentration. An increase of 61% of dry stem yield was observed with plants raised through 75 ppm iron oxide NPs primed seeds.

Figure 3. Yield attributes of rice plants. (A) Number of fruit branches per plant. (B) Number of capsules per plant. (C) Number of seeds per capsule. (D) Total fresh yield. (E) Dry stem yield. (F) 1000 seeds weight. As influenced by various levels of IONPs under water-scarce and well-irrigated environment (mean ± SE; n = 3).
It was explored that water stress or scarce treatment produces decrease in the total fresh yield, dry stem yield, and 1000 seed weights by 15%, 38%, and 19%, respectively (Figure 3D–F). Seed priming treatments significantly enhance these parameters. The maximum increase in total fresh yield was obtained by 100 ppm concentration. Similarly, maximum increase in 1000 seeds weight was observed with 100 ppm concentration. An increase of 61% of dry stem yield was observed with plants raised through 75 ppm iron oxide NPs primed seeds. A look at Tables 4 and 5 clearly indicates the potential of iron oxide NPs in updating yield profiles of flax plants, where a highly significant correlation is tabulated between various studied attributes. The increase in chlorophyll has strong correlation of 0.9780, 0.9136, and 0.9793 with total fresh yields, dry stem yields, and 1000 seeds weight of flax plants. Similarly, iron-oxide-NPs-mediated increased biomass of the plants significantly correlates with all of the yield attributes (Tables 4 and 5).

3.4. Osmotic Stress Indicators and Antioxidant Analysis

Data presented in Figure 4A,B represent the levels of osmotic stress indicators H$_2$O$_2$ and malondialdehyde (MDA). It is clear that drought induces the accumulation of lipid peroxidation product (MDA) and hydrogen peroxide. The increased level of these products suggests highest levels of oxidative damages, and subsequently these indicators regulate plant yield as well. Seed priming with iron oxide NPs has been found to be ameliorative and decreases the levels of both of these parameters. The 100 ppm concentration decreased MDA values by 66%, and H$_2$O$_2$ values by 71%. The current experimental trials’ activities proved that conc. of enzymes superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) was predominantly increased upon imposition of drought stress (Figure 4C,D,F, respectively). The increase for SOD, POD, and CAT was observed as 88%, 50%, and 149%, respectively. Plants grown from seed with pretreatment of IONPs depicted increased activities of SOD, POD, and CAT enzymes. A similar significant correlation is also presented in Figure 5, depicting principal component analysis (PCA). Overall, it can be inferred that seed preconditioning with iron oxide NPs significantly enhances the yield attributes of flax plants. Priming of seeds with conc. of 75 ppm proved to be optimum and it resulted in enhancement of antioxidant enzymes conc., which were SOD, POD, and CAT, bearing each of 28%, 56%, and 39%, respectively.
Figure 4. Values of osmotic stress indicators and antioxidant enzymes activities in flax plants (A) Malondialdehyde contents. (B) Hydrogen peroxide levels. (C) Superoxide dismutase. (D) Peroxidase. (E) Catalase activities. As influenced by various treatment levels of IONPs under water-scarce and well-irrigated environment (mean ± SE; n = 3).
Figure 4. Values of osmotic stress indicators and antioxidant enzymes activities in flax plants. (A) Malondialdehyde contents. (B) Hydrogen peroxide levels. (C) Superoxide dismutase. (D) Peroxidase. (E) Catalase activities. As influenced by various treatment levels of IONPs under water-scarce and well-irrigated environment (mean ± SE; n = 3).

Figure 5. (A) Spearman correlation map of the studied variables. (B) PCA correlation circle. T.CHLO: total chlorophyll; TSL: technical stem length; SD: stem diameter; NFBP: number of fruit branches per plant; H$_2$O$_2$: hydrogen peroxide; MDA: malondialdehyde; NCP: number of capsule per plant; NSC: number of seeds per capsule; TFL: total fresh yield; DSY: dry stem yield; POD: peroxidase; CAT: catalase, 1000 seeds weight; PH: plant height; PFW: plant fresh weight; PDW: plant dry weight; SOD: superoxide dismutase.
4. Discussion

The current study involved use of nanopriming of flax seeds with iron oxide NPs and it proved beneficial in increasing biomass production, yield profile, and mitigating drought stress. Seed priming with nanoparticles acts epigenetically in expression of certain genes involved in stress-tolerance mechanism [36]. Moreover, seed nanopriming (NPS) has been reported to enhance food quality and yield [16]. In Figure 4A, a Spearman correlation heat map is presented, showing highly significant correlation among studied variables. The principal component analysis (PCS) is presented in Figure 4B, where it is evident that two key components, which were “F1” and “F2”, contributed enhancement of 75.66% and 12.82%, respectively, showing correlation of variance among the analyzed parameters. In the analysis, it was found that both factorial components depicted cumulative participation of 88.48% in determining the variance. All the studied attributes are strongly correlated with the yield attributes in the flax plants under study.

Growth parameters, such as plant height, fresh weight, dry weight, technical stem length, and stem diameter, of flax plants were monitored in the current experiment. The present study concludes that water shortage leads to decline in all of these parameters, reducing the biomass production. However, the flax plants raised through iron oxide NPs treated seeds showed significant increase in all growth attributes The results are congruent to the priming experiments conducted on the plant Glycine max by Chau et al. [37], who reported nearly coinciding results due to seed priming treatments with nanoparticles of molybdenum and cobalt. The iron NPs have been found to be fruitful in increasing biomass of wheat plants [38]. The analysis proves that there was an increase in biomass which is probably due to the pivotal role of iron oxide NPs, because application of IONPs increases conc. of photosynthetic pigments and increases root proliferation [39]. The use of nanopriming with iron oxide NPs must have supplied iron, which acts as cofactor enzymes involved in general plant metabolism, which are “cytochrome P450s” and “Fe (II)/2-oxoglutarate-dependent oxygenase enzyme” [40]. The increased metabolism is thus a contributing factor in uplifting the biomass of a plant.

The total chlorophyll analysis was performed to comprehend the status of photosynthesis. The current study found that contents of total chlorophyll are reduced drastically upon exposure to water shortage in the flax plants. Seed nanopriming with iron oxide NPs proved significantly beneficial in updating the level of total chlorophyll contents and thereby increasing biomass of the flax plants (Table 5). These outcomes are similar to past research work conducted by Li et al. [41], where application of iron oxide NPs proved beneficial in uplifting total chlorophyll contents in water melon. The biosynthetic pathway of chlorophyll involves the formation of d-aminolevulinic acid and its action as precursor in biosynthesis of chlorophyll molecule. The iron is involved in the formation of d-aminolevulinic acid; thus, it indirectly helps in raising total chlorophyll contents [42].

Abiotic stress involves the formation of ROS, which react with membrane lipids and stimulate their degradation [43]. In the current experiment, the levels of hydrogen peroxide and malondialdehyde (MDA) were found to be increased, indicating the level of osmotic stress. Seed priming treatment decreased the contents of both osmotic stress indicators. The current study also found that the activities of antioxidant enzymes are enhanced upon water shortage. It was confirmed that conc. and activities of different antioxidant enzymes was increased in response to oxidative stress, which may be an improvement in plant internal defense mechanisms [6]. Seed priming with iron oxide NPs further increased the activities of antioxidant enzymes SOD, POD, and CAT. These results are in accordance with the experiment of Das et al. [44], where nano-iron pyrite was used in seed priming of rice. Both SOD and POD contain iron in their basic structure, and SOD converts the superoxide radical (A form of ROS) into molecular oxygen and provides the cellular defense against abiotic stress, whereas POD is a scavenger of hydrogen peroxide, converting it into water [45]. The activities of the antioxidant enzymes are declined upon exposure to deficiency of iron [46]. Seed priming with iron oxide NPs may have served as a catalyst in elevating the activities of these enzymes and thereby reducing the endogenous ROS levels, and
ultimately the levels of MDA and H$_2$O$_2$. Contents of H$_2$O$_2$ generally tend to elevate upon exposure to iron-deficient soils, and plants under such circumstances produce ROS, leading to osmotic damage. Iron-oxide-NPs-mediated seed priming causes increased activities of POD; hence, a decrease in H$_2$O$_2$ contents occurs, enabling the plant to survive oxidative damage. Additionally, due to different oxidation states, the Fe$^{+2}$ and Fe$^{+3}$ are promising candidates in ROS scavenging, thereby limiting oxidative damages. Higher reactivity of iron oxide NPs enables them to better adhere with the seed coats and thus potentially act as promising fertilizer. Increased plant biomass due to iron-oxide-NPs-mediated seed priming results in increased synthesis in chlorophyll a, increased light reactions, and promising internal defense due to increased SOD, POD, and CAT activities. Furthermore, the iron conc. occurring in the Earth is usually insoluble in the water and is thus not available to the plants. However, the iron oxide NPs contain 9% of iron which is soluble in water and is thus readily available as a mineral source under the stress conditions [23–25]. Enhanced ability of the flax plants to mitigate drought stress might be due to iron-oxide-NPs-induced availability of transcription factors, methylation of DNA, and modification of chromatin. It was found that a decrease in MDA conc. might be due to more accumulation of osmolytes caused by the priming with iron oxide NPs, enhanced process of activities of SOD, POD, and CAT, and better internal defense responses.

Food security is a major problem associated with increasing world population in the context of climate-change-mediated increased encounters of plants with abiotic and biotic stressors [39]. In the current experiment, yield profile of flax plants was studied. The water-shortage environment led to decreased yield attributes in the current study. However, the seed nanopriming with IONPs enhanced the food production and agronomic attributes in terms of total fresh yield, number of fruit branches, number of capsules, number of seeds per capsule, and 1000 seeds weight. These key results are predominantly congruent with the previous findings of Yasmeen et al. [47], where application of seed priming with copper and iron particles caused significant improvement in spike length, number of grains per spike, and grain endospermic contents in wheat. Furthermore, iron-oxide-NPs-mediated increase in plant photosynthetic ability plays a major role in increasing the yield quality. The seed priming with iron oxide NPs is helpful in alleviating drought-induced yield declines, as iron acts as a metabolism booster by increasing the enzymatic activities, and the expression of several genes in flowering and fruiting is epigenetically modulated by priming treatments [16]. Better agronomic traits show better nitrogen use efficiency caused by priming treatment with iron oxide NPs. Iron increases the bioavailability of nutrients and improves nitrogen use efficiency. Studies have shown that iron oxide NPs penetrate through nanopores of seed coats, becoming the part of embryonic tissues from where they are easily translocated and transported through symplastic pathways. Additionally, iron oxide NPs possess super-paramagnetic properties that buoy the plant metabolism, increasing the biochemical performance. Iron oxide NPs are potential candidates in boosting the performance of photosystems, transcription-regulating machinery, and auxin activity. The role of iron as a cofactor for several enzymes is also evident from the literature. Additionally, enhanced protein synthesis induced by seed priming iron oxide NPs might be a mechanism behind stress mitigation by the flax plants [23–25].

Cost effectiveness of nanofertilizers and nano-based formulations depends upon the approach used for their synthesis. Although the physical mode of synthesis of a nano-based formulation is easy to manage and well controlled, it is not cost-effective, and particle size and shape are not often controlled as desired. The chemical mode of synthesis ensures better control over particle size and dimensions; however, the method requires great skill and thus is not cost-effective. The biological synthesis or green synthesis is highly laborious, yet highly eco-friendly and cost effective. The iron oxide NPs, if used as seed priming agent, are required in lower volumetric quantities and hence are cost-effective compared to conventional fertilizers, which are applied at higher dose, leading to high cost [23]. However the term cost-effectiveness is not always related to crop economics; rather, it may be taken in terms of energy usage. The seed priming reduces the energy of the plant
required to trigger a stress tolerance mechanism; hence, it enables the plant to conserve energy investments, proving cost-effective in a broader spectrum [26].

5. Conclusions

The present study concludes that seed priming acts as a catalyst in improving growth and yield attributes in flax plants. The levels of osmotic stress indicators such as malondialdehyde and hydrogen peroxide were found declined by 66% and 71%, respectively, upon seed priming, and an increase in activity of antioxidant enzymes SOD, POD, and CAT was also observed by 28%, 56%, and 39%, respectively, documenting the potential of iron oxide particles in mitigating water stress. It can be inferred that nanopriming with iron oxide NPs is helpful in alleviating water stress and increasing the yield profile of flax. Nanotechnology surely has a great future in sustainable agriculture and in mitigating climate change, but the field of applying nanomaterials in agriculture still requires a lot of work, and further experimentation is required to explore the benefits of nanoseed priming technology.

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