Nitrogen Use Efficiency Regulates Drought Stress in Pearl Millet Genotypes: Morpho-Physiological Evaluation

Shiv Shankar Shukla and Sanjib Kumar Panda *

Department of Biochemistry, Central University of Rajasthan, Bandar Sindri, Ajmer 305817, India
* Correspondence: sanjib.panda@curaj.ac.in

Abstract: In this paper, the author discusses the effects of drought stress on pearl millet genotypes during the vegetative stage of development and the plant’s mechanisms for survivability under stress with various nitrogen treatment regimes. A total of six treatment conditions were imposed on plants, i.e., N0 (no Nitrogen-0 mM), N0+PEG-10%, LN (low Nitrogen-2.5 mM), LN+PEG-10%, HN (high Nitrogen-7.0 mM), and HN+PEG-10%. Five days of moderate drought stress caused significant morphophysiological changes, as evidenced by a reduction in fresh and dry biomass, as well as relative water content (RWC), relative electrolyte leakage, and chlorophyll fluorescence. A significant reduction in plant biomass and RWC when compared to the controls was seen. Reactive oxygen species (ROS) and lipid peroxidation (MDA) levels increased in the shoot in response to drought stress along with a loss of membrane integrity. A nitrogen treatment regime regulated the drought stress. In relation to N0-treated batches, proline accumulation increased in various N-treated batches. Results revealed that GHB-538 is less tolerant to drought stress in different N treatment conditions, while RHB-234 and 223 showed better tolerance to drought under nitrogen treatments. The significance of this study is to uncover the regulatory role of nitrogen over the drought stress in pearl millet.

Keywords: nitrogen use efficiency (NUE); pearl millet; drought stress

1. Introduction

Pearl millet (Pennisetum glaucum (L.) R Br.) is a nutritive staple food grain that is grown all over the world, notably in some of the torrid rainfed cultivation regions of Africa and some parts of Asia. It is notably accessible for the impoverished rural population. Pearl millet is the fourth most significant grain farmed in the hot semi-arid parts of the world [1,2] and is planted on 33 M hectares globally [3].

Different studies have described that this crop can flourish on degraded land and in extreme climate conditions, which offers strong sustainability features. It is compatible with the various crop systems of both irrigated and dryland farming. The nutritional composition of pearl millet is protein (13.6%), fats (7.8%), starch (63.2% Table S1), and fibres (2.8%). In comparison to wheat, it has more calories, less easily accessible starch, more fibers (1.2 g/100 g, the majority of which is indissoluble), and higher α-amylase activity (8–15 times). Additionally, it is gluten-free and poor in glycemic index [3].

Drought stress is one the most severe abiotic stress affecting plant production globally. The majority of grain crops, which provide the majority of the food consumed worldwide, are drought vulnerable. Water scarcity is a problem that will likely worsen during the coming decades [4]. To sustain production during droughts, it is crucial to understand the processes behind drought resistance in grain crops and to produce drought-tolerant cultivars. Drought avoidance, drought resistance, drought escape, and drought restoration are all mechanisms used by plants to combat drought stress. Plants respond to dehydration at the molecular and cellular levels by activating both regulatory and functional genes [5]. When pearl millet was subjected to drought treatment, the many DEGs in pearl millet...
roots increased as the stress period lengthened. This could be attributed to an increase in the level of drought stress on plants. To increase its response to drought stress, the plant must produce specific metabolites through the differential expression of additional genes [6]. Numerous research studies have considered the effects of drought stress on leaf pigments [7,8]. Drought stress can reduce chlorophyll concentration and impede its synthesis [9]. Nitrogen plays a cardinal role in plant growth and metabolism. Nitrogen (N) is the primary fundamental nutrient required by plants, and inorganic nutrition serves as one of the most critical components impacting crop output. Higher recommended fertilizer rates can boost yields even though pearl millet has historically been recognized for being nutrient-efficient and maintained with modest fertilizer rates. Plant responses to nitrogen treatments and soil water indicate that N applications may have a favourable impact on soil water content and plant development [10]. When pearl millet was subjected to full irrigation and high nitrogen treatment, maximum profit of fodder production was obtained. However, in a water deficit condition, low nitrogen treatment was suitable for high productivity [11]. Pearl millet genotypic variations in nitrogen use efficiency (NUE) at comparable N absorption levels are significant determinants of fertilizer N consumption. Efficiency in nitrogen usage is a key characteristic of intensive crop production. The higher grain and fodder production conditions had higher nitrogen uptake, suggesting that of dry matter production—rather than seed and straw nitrogen percentage. Higher dry biomass production is principally responsible for the higher nitrogen uptake. To increase agricultural output, it is crucial to improve plant NUE together with management strategies that support greater root dispersion and increased N fertilizer utilisation [12]. It was discovered that NUE was much greater in the drought-resistant genotype than in the susceptible genotype in other plants, such as sugarcane. It generated a hypothesis that increasing NUE would enhance sugarcane’s capacity to withstand drought. Previous research discovered a strong correlation between NUE features and sugarcane production and yield [13]. The global nitrogen use efficiency (NUE) of contemporary field crops agriculture practices has hovered at only about 30% for decades [14]. C4 plants minimize photorespiration and require much less RUBISCO, the primary protein responsible for approximately 50% of the nitrogen content in leaves; they usually use nitrogen more efficiently than C3 plants [14]. Numerous descriptions of plants’ NUE have been made throughout the years. The NUE definitions in this context connect the shoot biomass produced to the nitrogen absorbed or the nitrogen accessible to the plants [13]. No concrete information on the drought stress regulation in contrasting pearl millet varieties by NUE is available. To bridge the gap, the current work was planned with objectives to uncover the regulatory mechanism of N-treatment over drought stress at the morpho–physiological level. It is expected to provide in a drought sensitive and drought tolerant variety of pearl millet, an alleviation of drought stress with improved NUE.

2. Materials and Methods

2.1. Experimental Details

A two-phase screening was conducted in pearl millet (Pennisetum glaucum L.). In the first phase, a total of fifteen (15) varieties of pearl millet were screened on the basis of morphological parameters of drought stress. After the first phase of screening, the author found five varieties have more tolerant and sensitive characteristics (Supplementary Table S1).

In the second phase, the five (5) varieties (RHB-234, RHB-223, HHB-67, GHB-538, and GHB-719) were finally screened on basis of drought and NUE traits. All experiments were conducted at the department of Biochemistry, Central University of Rajasthan, Ajmer, India (26.6231° N, 75.0250° E). Pearl millet seeds were sterilized with 0.1% HgCl₂ for 5 min and then washed with distilled water 3–4 times for 5 min. After washing, the seeds were imbibed for 3–5 days in a Petri plate on Whatman No. 1 filter paper at 28 °C to germinate. After germination, seedlings were transfered to the hydroponic culture. Both the first and second step of screening was conducted in the hydroponic system. Nutrient media was modified Hoagland solution (pH-6.8) [15]. The climatic conditions were...
25/22 °C in day/night, 60–70% relative humidity, and the photoperiod was 14/10 h light/dark, respectively. Nutrient media were changed on every 4th day. After 5 days of seedlings growth, 3 different nitrogen treatments were applied—N0 (0 mM-KNO$_3$ and CaNO$_3$·4H$_2$O), LN (2.5 mM-KNO$_3$ and CaNO$_3$·4H$_2$O), and HN (7.0 mM-KNO$_3$ and CaNO$_3$·4H$_2$O). After 11 days of different N treatment, plants were subjected to drought stress (PEG-6000-10%) for 5 days.

2.2. Morphometric Parameters

The following morphological parameters were assessed on the final day of the drought stress treatment: root and shoot (length, fresh weight, and dry weight). At the time of harvest, fresh weights were measured. While measuring dry weight, plants were kept in an oven at 80 °C for 24 h (up to a constant weight).

2.3. Physio–Biochemical Parameters

2.3.1. Relative Water Content

Pearl millet shoots were harvested after the experiment and weighed right away (fresh weight, or FW). The tissues were completely turgid after being rehydrated in distilled water for 4 h. They were then surface-dried, reweighed (TW), and finally dried in an oven for 24 h at 80 °C., then reweighed again (DW). The formula below was used to determine the shoot’s relative water content 

\[
\text{RWC} \% = \left[ \frac{(FW - DW)}{(TW - DW)} \right] \times 100
\]

2.3.2. Chlorophyll Fluorescence

The maximal photochemical quantum yield of photosystem II (Fv/Fm) of pearl millet leaves were measured using junior PAM (Heinz, Walz, Germany) following a 30 min acclimation period in the dark at room temperature in the laboratory [18,19]. Three elements comprise measurements performed with a PAM fluorometer: measuring light, actinic light, and saturation pulses. Photosynthetic activity is stimulated by actinic light, and saturation pulses are used to calculate the maximal fluorescence production. The modulated measurement light, the third component, generates hardly negligible photosynthesis by itself. Two different types of cellular antennas and reaction centres, known as Photosystems I and II, are used by plants during photosynthesis. Only Photosystem II of the two photosystems provides the standards required for PAM fluorometry, enabling a fluorometric measurement of photosynthesis. Initially, we imposed a dark condition for 30 min, then gave a dim measuring light beam (125 µmol photon m$^{-2}$·s$^{-1}$) until its fluorescence became stable. After that, we measured the minimal fluorescence yield (Fo,Fm). Then, light conditions were imposed on the plant leaf for 5 min before measuring the Fo’ and Fm’ by activating the actinic light (285 µmol photon m$^{-2}$·s$^{-1}$) and saturation pulse (1500 µmol photon m$^{-2}$·s$^{-1}$). To determine photosynthesis using fluorometry, the primary measurement is photochemical yield in the light.

The maximum quantum efficiency (yield) of PSII (Fv/Fm) in dark-adapted leaves was calculated as (Fm – Fo)/Fm.

\[
\text{Fo’} = \text{Min. fluorescence level after a saturation pulse.}
\]

\[
\text{Fm’} = \text{Max. fluorescence level during saturating light pulse.}
\]

2.3.3. Relative Electrolyte Leakage

By quantifying the ions that were pouring from the leaflets into Milli-Q water, electrolyte leakage was assessed. After being submerged in 20 mL of Milli-Q water for 4 h at room temperature, plant materials’ conductivity (C1) was measured using an EC measuring device. After that, it was kept for 20 min in a water bath at 100 °C. Its conductivity was also observed after it had cooled to ambient temperature (C2) [20–22].

\[
\text{Relative Electrolyte leakage} = [1 - (\text{C1}/\text{C2})] \times 100
\]
2.3.4. Lipid Peroxidation

The Heath and Packer et al. (1968) method was used to determine the MDA level in leaf samples. Leaf tissue (0.2 g) was homogenized in 0.1% (w/v) tri-chloroacetic acid (0.5 mL) on ice, and the supernatant was carefully collected after centrifuging at 12,000× g for 15 min at 4 °C.

The supernatant (0.5 mL) was next consolidated with 0.5% TBA-TCA (1.5 mL), which was then incubated for 25 min at 95 °C in a water bath. The reaction was stopped by keeping it on ice for 10 min. After a further 15 min centrifugation at 15000× g of the solutions, the UV-visible absorbance at 532 nm was measured and normalized with non-specific absorbance at 600 nm. As a consequence of the difference in absorbance at 532 and 600 nm and an extinction coefficient of 155 mM⁻¹ cm⁻¹, the amount of lipid peroxidation was expressed as µmol of MDA content [23,24].

2.3.5. Proline Content

Proline content was determined according to Bates et al. (1973). A 3% sulfosalicylic acid solution was used to homogenise the tissues, and the homogenate was then centrifuged at 9000× g for 10 min. The reaction mixture, which was heated at 100 °C for one hour, contained 2 mL of supernatant, 2 mL of glacial acetic acid, and 2 mL of acid ninhydrin. Then, the reaction mix was cooled on ice to stop the reaction. The reaction mixture was separated with 4 mL of toluene once the reaction on ice came to an end, and the absorbance was measured at 520 nm [25–27].

2.3.6. Hydrogen Peroxide

There were only minor alterations made to the hydrogen peroxide quantification assay (H₂O₂). After being homogenized in 0.1% (w/v) tri-chloroacetic acid (5 mL) on ice, leaf tissue (200 mg) was centrifuged at 12,000× g, 15 min at 4 °C. The reaction mixture contained one millilitre of potassium iodide (1M), 0.5 mL of potassium phosphate buffer (10 mM, pH 7.0), and 0.5 mL of the sample extract. The samples were incubated for 20 min. at room temperature with no light. For standard curve 1 mL of KI (1M), 0.5 mL of phosphate buffer (10 mM), and 0.5 mL of 0.1% TCA, were used as a reaction mix. It was then seeded with known concentrations (50–250 µmol) of H₂O₂. At 390 nm, absorbance was measured, and results were calculated in micromoles by comparing to the standard curve [28,29].

2.4. Statistical Analysis

All the data presented were the mean of three replicates with standard error of mean (± SE). All the datasets were statistically analysed by one way ANOVA using the Tukey–Kramer multiple comparison tests to evaluate the significant difference among the treatments. Tukey’s test compares the mean of each treatment. Significance was determined at p < 0.01 for all means, either using Microsoft Excel 2007 (Microsoft Inc. Redmond, Washington, DC, USA) or GraphPad prism 9.3.1.

3. Results

3.1. Morpho–Physiological Attributes

Pearl millet was affected by drought stress, as evidenced by changes in the total chlorophyll fluorescence as well as morphological characteristics, such as plant growth, dry and fresh biomass, and relative water content (Figure 1 and Supplementary Figure S1). These characteristics demonstrated a very significant drought-related impact when compared to the corresponding controls. The application of nitrogen enhanced the vegetative growth and greenness of plants compared with the NO condition (Figure 1). Biomass accumulation was observed in all five varieties with different nitrogen treatments. A nitrogen treatment increased the fresh weight and shoot length and also enhanced the root dispersion in RHB-223, RHB-234, and HHB-67 varieties. The highest increment of shoot length (124% and 151%) and shoot fresh biomass (642% and 958%) was observed in RHB-234 among all varieties in LN and HN condition, respectively. In the GHB-538
variety, increased fresh biomass accumulation (154%) and shoot length (80%) was found with LN treatment, while it showed the least growth and survivability among all other varieties with N0 treatment batches. RWC was remarkably influenced by both drought and nitrogen treatments.

Figure 1. Vegetative plant of pearl millet; control (C) and stress (S) plant in different N treatments (without N (N0), low N (LN), high N (HN)) at day 5 of PEG-6000 (10%) treatment. GHB-719 (A); RHB-223 (B); HHB-67 (C); RHB-234 (D); GHB-538 (E).

Under drought stress conditions, the N0 treatment batch of GHB-538 showed the maximum drop (4.5%) in RWC value, whereas RHB-234 showed the least reduction (0.2%) in RWC value. GHB-538 exhibited the maximum decline in RWC with LN (13%) and HN (11%) treatment batches, while RHB-223, HHB-67, and GHB-719 showed modest reduction (2–5%), with the least (2%) in RHB-234 (Figure 2).
Different N treatments significantly increase the dry weight of the pearl millet plant (Figure 2c). In the N0 treatment batch, the lowest dry biomass was observed in GHB-538 and also in the N0+P batch among all five varieties, while RHB-223 showed a higher value in the N0 batch. Dry biomass accumulation was clearly observed in the LN and HN treatment batches. Soot dry biomass was significantly increased in both LN (355%) and HN (566%) treatment in RHB-234, while RHB-223 showed a significant increase (200%) in HN treatment. These results may be due to varietal differences and different NUE at different N treatments. The lowest dry biomass was obtained in GHB-538 at all N treatments and their respective stress condition.

Junior PAM is utilized for investigations, such as Quenching evaluation and light curve study, as well as for the assessment of the rather sluggish photosynthetic process. Without much or any external influence, PAM fluorometry monitors the level of photosynthetic activity within a leaf. Several fundamental and regulatory systems that regulate the photosynthetic process may be measured by focusing a beam of illumination on a leaf and monitoring the re-emitted light by the leaf. Understanding PAM fluorometry requires knowledge about term "physiological condition". That is typically connected to recent environmental experiences, especially those that induce stress and how they appear physically. Drought can modify the plant’s biology even after the stress has subsided, altering light use and photosynthesis rates through altering the photosynthetic system. The plant detects these modifications. The C3 cycle activity cannot sustain electron transport activity, as seen, for instance, by PS II and the cytochrome b6f, which release more hydrogen ion in the lumen area than ATP-synthase can utilize. Then, lumen becomes quite acidic, which hinders the flow of electrons to PS I and activates a process that causes the Photosystem II antenna to emit more heat.

Figure 2. (a–d) Morpho–physiological parameters of pearl millet’s shoot treated with different N treatments (N0, LN, HN) and drought stress (P (PEG-6000-10%)). Bars are means ± SE (n = 3).

(a) Fv/Fm (b) RWC (%) (c) Shoot dry biomass (g) (d) REL (%)
Fv/Fm is defined as the maximum photosynthetic quantum yield. Drought stress has a significant impact on the photosynthetic apparatus. The majority of Photosystem-II reaction centres are in the open state and capable of converting excitation energy into photochemical reactions with maximum efficiency, which results in maximal photochemical quenching, in the darkness. Plants that adapt to low light levels spend more on Photosystem-II reaction centres having smaller antennas and higher Calvin-cycle capacities. A higher percentage of open photosystem leads to maximum photochemical yields. While saturating pulse is essential for 0%, an open photosystem results into zero photochemical yields. In this study, we determined the maximum and minimum photochemical yields in term of minimum and maximum fluorescence, respectively.

Fluorescence signal $\propto \frac{1}{\text{photochemical yield}}$

In the N0 treated batch, relative decreases of the Fv/Fm value under stress conditions were in the order, GHB-538 < GHB-719 < RHB-234 < HHB-67 < RHB-223. In the LN batch, all varieties performed well under stress conditions, while in the HN batch, only RHB-234, and 223 performed very well under stress conditions. HHB-67 did not show a better Fv/Fm value in the HN condition in comparison to other varieties. In HHB-67, GHB-719 varieties, the lowest Fv/Fm value was found in the HN+P treatment. RHB-223 showed the lowest value in N0+P treatment, while it performed better in N0 condition (Figure 2a).

Relative electrolyte leakage acts as an indicator of membrane stability during stress. RHB-234 showed a minimum REL value compared to all other varieties in all N treatments and their respective stress condition, while a higher value of REL was obtained in GHB-538, and RHB-223 in the N0 condition, HHB-67, RHB-223, and GHB-538 in the LN condition, and RHB-223 in the HN condition. In N0+P treatment, GHB-538 showed the highest value, and in the LN+P condition HHB—67 showed the highest value, while in the HN+P condition, GHB-719 showed the highest value of REL. In the N0 batch, the maximum increase in REL was observed in RHB-234 (46%) and GHB-538 (22%) compared to stress, while RHB-223 and HHB-67 showed a minimum (2–5%). In the LN batch, RHB-234 and RHB-223 expressed 17% and 6% reduction, respectively, in the context of the N0 batch (Figure 2d).

3.2. Biochemical Attributes

3.2.1. Proline Accumulation

Proline is the major osmolyte that accumulates in pearl millet during drought stress. In the N0 treatment batch, increased proline accumulation was found in RHB-223 (****), and GHB-538 (****) in stress conditions. In all varieties, proline concentrations were elevated significantly (****) in LN and HN conditions concerning stress. The highest proline accumulation was observed in HHB-67 in the HN+P condition. N-treated batches (LN and HN) also showed increased proline in comparison to N0 in all varieties except RHB-234 and HHB-67 (Figure 3).

3.2.2. Lipid Peroxidation

The MDA content was calculated for the lipid peroxidation level in shoot tissue, and it was observed that MDA content significantly increases in N0+P treatment in GHB538 (****), RHB-223 (***), and RHB-234 (****). A striking increase in MDA content was observed in GHB-538 (****) and RHB-223 (****) in HN+P and LN+P treatment, respectively. An elevated level of MDA was found in GHB-719 under both the HN+P and LN+P condition (Figure 4).
Figure 3. Proline content in the leaf of pearl millet treated with different N and drought stress (PEG-6000-10%). Bars are means ± standard error (n = 3). In the above bar (****, ***, **) represents differences among treatments (Tukey’s test, $p \leq 0.0001$), while ns represents no significant difference.

Figure 4. MDA content in the leaf of pearl millet treated with different N and drought stress (PEG-6000-10%). Bars are means ± standard error (n = 3). In the above bar (****, ***, **, *) represents differences among treatments (Tukey’s test, $p \leq 0.0001$), while ns represents no significant difference.
3.2.3. Hydrogen Peroxide

ROS activity was recorded as H$_2$O$_2$ content in shoot tissue. A significantly elevated level of H$_2$O$_2$ was observed in all varieties except HHB-67 (ns) in the N0 treatment batch with the respective stress condition (Figure 5). A significant decrement was also observed in the N-treated batch of all varieties concerning the N0 batch. Among all varieties, an elevated level of H$_2$O$_2$ was recorded in GHB-538, while in the rest of the varieties, average concentrations were observed. In HN, the treatment batch significantly increased the level of H$_2$O$_2$ found in RHB-234 and GHB-719 (***)

![Figure 5](image-url) Hydrogen peroxide content in the shoot of pearl millet treated with different N and drought stress (PEG-6000-10%). Bars are means ± standard error (n = 3). In the above bar (***, **, *) represents differences among treatments (Tukey’s test, p ≤ 0.0001), while ns represents no significant difference.

4. Discussion

Drought is the major abiotic stress in an arid and semi-arid region and has a significant negative impact on plant growth and survivability. Nitrogen is an important ingredient of plant nutrients that has a positive impact on growth and development as evidenced in this experiment. Additionally, H$_2$O$_2$ enhances proline accumulation, which improves plant growth and photosynthetic efficiency [30]. In RHB-234, moderate H$_2$O$_2$ content produced under N treatment and higher proline accumulation in LN+P treatment meant this moderate enhancement in the H$_2$O$_2$ level in stress condition results in stress tolerant capability of pearl millet via accumulation of proline. Moreover, RHB-223 also represented the elevation in H$_2$O$_2$ and proline content in LN+P treatment. While GHB-538 revealed a higher H$_2$O$_2$ content, it did not show high proline accumulation in different N treatments.

In this study, a crop eco-physiological paradigm was developed to describe how nitrogen and drought stress interact to affect the development and survivability of pearl
millet. According to Hakeem et al. [12] N is the most restrictive nutrient for grain yield on degraded semi-arid soils. To achieve optimal growth, increased NUE, and decreased N losses, monitoring plant N is required to make sure an appropriate supply is available for plant requirements. By adjusting N management in rain-fed agriculture cropping systems under water restrictions, it may be possible to reduce N losses and raise NUE. Nitrogen absorption, N translocation, its assimilation, and mobilization are only a few of the processes and elements that have an impact on NUE. Despite the possibility of needing crop-specific management measures, NUE remains a crucial goal for crop improvement.

Nitrogen absorption, N translocation, its assimilation, and mobilization are only a few of the processes and elements that have an impact on NUE. Despite the possibility of needing crop-specific management measures, NUE remains a crucial goal for crop improvement. The extension of agricultural production onto degraded areas with limited nutrient availability requires the efficient use of inorganic fertilizer, which may be achieved by improving NUE [12]. Regarding intrinsic reasons of economy, the atmosphere, and yield, it is crucial to understand NUE in pearl millet all over the agro-ecological zones. This is a result of improper application, excessive fertilizer costs, or a lack of fertilizer availability, which poses a significant challenge to farmers who lack access to resources. Additionally, the usage of nitrogen fertilizers is growing quantitatively due to the promotion of better production techniques and cultivars, yet responsive outputs of N are significantly declining over time in all crops.

In order to better understand the link between NUE and drought tolerance capability in pearl millet, this study was carried out to assess growth, biomass output, NUE, and drought tolerability of several pearl millet varieties in drought stress during the early stage of growth. In the initial screening, we noted that RHB-234, 223, and 233 were highly tolerant, while GHB-538, MPMH-17, and GHB-719 were sensitive, and the rest were moderately tolerant to drought. Then, we further screened five genotypes on NUE and drought traits. Among all five varieties, RHB-234, GHB-719, and HBB-67 showed better growth and survival mechanism in LN treatment, and RHB-234 and RHB-223 showed greater biomass in LN conditions. In these varieties, the drought impact was regulated efficiently with HN and LN treatments. Thus, RHB-234 showed the most tolerant mechanism in both LN and HN treatment and also in the respective stress treatments. RHB-234 also performed better in N0 treatment. The GHB-538 variety had minimum shoot length, fresh biomass, and dry biomass in comparison to other varieties as revealed in Figure 1. That means higher NUE variety somehow regulates the drought stress in different N treatments. In the N0 treatment, all varieties showed diminished growth due to a lack of N in nutrients. Many works are in progress on pearl millet to increase its productivity under harsh climate conditions [1,11,13,31,32]. So, uncovering the interactive mechanism of NUE and drought stress in pearl millet will be helpful to increase productivity and survivability.

5. Conclusions

The goal of this screening experiment was to find tolerant and sensitive variety of different N regimes during drought stress. This study has shown that N application physiologically influenced the growth, biomass accumulation, RWC, etc., of all varieties under drought conditions. The overall result of this study reveals that the GHB-538 variety is less tolerant to drought stress with different N applications as it showed lower values in Fv/Fm, RWC, DW, and proline and higher values in REL and H2O2 content, while RHB-234 and RHB-223 were found to be the most tolerant varieties, with elevated photosynthetic efficiency, RWC, etc. The future prospect of this work is to explore the molecular regulatory mechanism of N treatments on drought stress in pearl millet.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agriculture13030680/s1, Figure S1: Vegetative plant of pearl millet; GHB-719 (A); RHB-223 (B); HBB-67 (C); RHB-234 (D); GHB-538 (E). Table S1: Varieties of pearl millet on basis of morphological parameters related to drought stress.

Author Contributions: Conceptualization and design of experiment, S.K.P.; experiments and data analysis, S.S.S.; paper writing, S.S.S. and S.K.P. All authors have read and agreed to the published version of the manuscript.
Funding: We thankfully acknowledge the DBT BUILDER Project, BT/INF/22/SP44383/2021 for financial assistance.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All the data underlying the results are available as the part of this article. All the data summarized in an Excel sheet file is available with the first author.

Acknowledgments: Guidance of my supervisor throughout is gratefully acknowledged.

Conflicts of Interest: The authors declare no conflict of interest.

References


**Disclaimer/Publisher’s Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.