Efficacy of Plant Growth Promoting Rhizobacteria to Improve the Biochemical Properties and Development of Wheat Seedlings under Drought Stress Control

Abstract

The most significant cereal crop is wheat, which ranks third among the world's most demanding staple crops after rice and maize. Temperate climates with moderate rainfall are ideal for growing it. Under the current changing climate, abiotic stress conditions, particularly drought conditions, have a significant impact on wheat crop productivity. Plants under drought stress have less water accessible to them, which slows down germination, growth, and yield. In this study, plant growth promoting rhizobacteria (PGPR) is used to boost the drought tolerance capability of the wheat plant. PGPR isolate KD-3B strain is used to investigate the plant growth characteristic such as, mineral solubilization (zinc, phosphate, and potassium), ammonia production, indole acetic acid (IAA), and the enzyme 1-carboxylic acid (ACC). Moreover, the plant showed drought tolerance at polyethylene glycol (PEG 6000) concentrations of 0%, 5%, 10%, 15%, 20%, and 25% (w/v). Wheat seedlings were exposed to both normal and drought conditions when they interacted with the KD-3B bacterial strain, which possesses many features that promote plant development. In comparison to growth in a non-PEG condition, this strain is tested for relative growth up to 41.05% in a 25% PEG concentration. When inoculum of KD-3B bacterial strain used under normal and drought conditions, the relationship between microbes and plants was examine in wheat seedlings grown under hydroponic condition. Under drought stress condition significant changes were observed in the morphological and biochemical conditions of seedlings. This research concluded that, in drought stress condition, PGPR KD-3B strain inoculated seedlings showed increased chlorophyll content, TSS content, malondialdehyde concentration and decreased proline, H_2O_2 content, SOD and POD activities, as compared to the negative control seedlings. The PGPR stain KD-3B improved their biochemical and physiological parameters and plant microbe interaction decreased the deleterious effect of drought on wheat seedlings.

Keywords: Wheat; PGPR; drought tolerance; morphological; biochemical; plant-microbial interaction.

Introduction

Our world, “The Earth” is critically facing three biggest uncontrolled problems: population explosion, food sustainability and climate change. The busting population strongly affects food production as well as food consumption, and world agriculturists and researchers are seriously concerned about food supply and food sustainability. Among all the edible food communities, wheat (*Triticum spp.)* is the most popular, cultivable (followed by rice and maize) and consumable crop in the world. It has played an important role in alleviating food grain production in the country over the last few years. The wheat crop is important for food security and economic development in many regions of the world.

Climate change is the biggest concern in today's world; it is not only affects humans and animals but also severely disturbs crop development and production scenarios. Drought is one of the foremost abiotic stresses factors that badly affect the wheat growth, production and yield. Drought stress can be basically defined as a scarcity of water that affects morphological, physiological, biochemical and molecular changes (Sallam et al., 2021). To cope with drought stress, the wheat plant performed various physiological and biochemical mechanisms, such as osmotic adjustment, antioxidant defense, and hormonal regulation (Cochard et al., 2002). But these mechanisms are often insufficient to overcome the negative impacts of water deficits, especially under prolonged and severe drought conditions. Therefore, there is a need to explore substitute strategies to enhance the drought tolerance of wheat plants. Drought stress is an edaphic process that affects plant growth and forcefully reduces agricultural production and efficiency in several parts of the world (Comas et al., 2013).

One of the promising approaches is to use plant growth promoting rhizobacteria (PGPR), which are beneficial bacteria that can colonize the roots of plants and improve their growth and development by various mechanisms. PGPR can modulate the plant responses to drought stress by inducing or enhancing the expression of genes involved in osmotic adjustment, antioxidant defense, and hormonal regulation, as well as by altering the root morphology and architecture to facilitate water uptake and transport. PGPR can also mitigate the oxidative damage caused by drought stress by scavenging reactive oxygen species and increasing the antioxidant enzymes and metabolites in plants.

According to recent reports, PGPRs modify gene responses in plants to help them tolerate abiotic stressors (Srivastava et al., 2008). Drought-tolerant native PGPR strains might be more effective in promoting environmental recovery. According to Kaushal and Wani (2016), PGPRs have the extraordinary ability to modify the physiological response to water deprivation, improving plant endurance under drought stress.

Plant Growth-Promoting Rhizobacteria (PGPR) stimulates the stress tolerance mechanism at the time an abiotic stress condition forms. Barnawal et al. (2017) reported that the PGPR strain *Bacillus subtilis* (LDR2) can improve tolerance to wheat under drought.

When drought stress conditions are present, these PGPR strains improve photosynthetic efficiency. Furthermore, in situations of salt and drought stress, these PGPR strains raise the amount of indole-3-acetic acid (IAA) in wheat. Few studies have been done to date that show how PGPRs help wheat plants that are stressed by drought (Gontia-Mishra et al., 2016; Sood et al., 2020; Gontia-Mishra et al., 2017; Kour et al., 2020). From the rice rhizosphere, we have revealed a large number of PGPRs in our laboratory. These bacteria exhibited several traits that aid in plant growth, such as the solubilization of potassium, phosphate, and zinc, the activity of ACC deaminase, the formation of volatile compounds, and indole-3-acetic acid (IAA). This research concentrated on microbial interaction by plants against drought stress conditions by screening the microbial performance and wheat morpho-physiological and biochemical characteristics.

Material and methods

Analysis of PEG tolerance of PGPR isolate and inoculum preparation

Pre-isolated PGPR strain KD-3B from the rhizosphere of rice plants was used; it was isolated from Kundam block in Jabalpur district of Madhya Pradesh. This strain produced NH_3, indole acetic acid (IAA), and the enzyme 1-carboxylic acid (ACC), among other plant growth-promoting (PGP) characteristics. Mineral solubilization (zinc, phosphate, and potassium) was also demonstrated. To evaluate the growth of the isolate under drought, an overnight-grown culture was inoculated in Luria Bertani broth (LB) medium accompanied by several doses of polyethylene glycol 6000 (PEG) (0, 5%, 10%, 15%, 20%, and 25% (w/v)). At 600 nm, the optical density of the bacterial cultures was determined by using a UV-vis spectrophotometer (V550, Jasco, Japan). Relative growth at different concentrations of PEG was calculated compared to growth in control (LB media without PEG).

The method described by Gontia-Mishra et al. (2016) was used to prepare the inoculum for the plant-microbe interaction experiment. Overnight-grown culture of isolate KD-3B was centrifuged at 5000 rpm for 5 min and mixed in half the concentration of MS medium (Murashige and Skoog, 1962), maintaining an optical density of 0.6 at 600 nm (10^8 colony-forming units).

PEG Tolerance Screening in Wheat

The wheat variety GW 322 seeds were obtained from the Breeder Seed Production Unit, JNKVV, Jabalpur. After selecting the healthy seeds, they were sterilized for 10 minutes with a 3% sodium hypochlorite solution (v/v), and then they were given three washes with sterile distilled water. Hydroponically grown wheat seeds were tested for seedling susceptibility to PEG by growing them in half-concentration MS media with different PEG concentrations

(Nil, 5%, 10%, 15%, and 20% mM). 20% PEG concentration was selected for the final experiment because plants had a modest inhibitory effect on seedlings, which could be overwhelmed by the use of PGPR, which is drought resistant.

PGPR Infusion and Treatment for Drought Stress

This experiment was carried out using methodology suggested by Gontia Mishra et al. (2016a). The wheat seedlings were treated with a 20% PEG concentration treatment or half MS medium before being placed in plastic cups and netted for hydroponic development. The uninoculated seedlings with 20% PEG concern served as the negative control, while the seedlings without bacterial isolate and 20% PEG concentration treatment served as the positive control. Two types of treatments were planned: seedlings that were inoculated with the PGPR strain KD-3B without receiving a 20% PEG concentration treatment, and seedlings that received the same strains but additionally received a 20% PEG treatment. Total four different sets were prepared, first is positive control (seedlings without bacteria and 20% PEG concentration), second is (seedlings +20% PEG concentration) negative control, third is (seedlings + strain KD-3B) and last is (seedlings + strain KD-3B +20% PEG concentration). These experiments were conducted in triplicate. After the 10-days of treatment, leaf and root samples were collected for various studies.

Morphological study

After 10 days of experiment, various plant growth parameters, including shoot and root length, dry shoot and root weight (DW), and fresh shoot and root weight (FW), were observed.

Biochemical study

Chlorophyll content was investigated according to the Arnon (1949) method. The proline content in the leaves was determined using the technique suggested by Bates et al. (1973). The H_2O_2 concentration in the leaves was analyzed by the prescribed method of Velikova et al. (2000). The total soluble sugar was estimated using the anthrone reagent technique (Shukla et al., 2012). The Bradford test (Bradford, 1976) was used to determine the protein content increases or decrease during stress condition. According to Hodges et al. (1999), malondialdehyde (MDA) was analyzed; it is the main reactive metabolite utilized to measure the degree of lipid peroxidation in plant tissues. The approach used by Lutts et al. (1996) allowed for the evaluation of the electrolyte loss in the leaf tissues.

Peroxidase (POD) and superoxide dismutase (SOD) activities

Using guaiacol as the substrate, the POD activity was measured by determining the amount of H_2O_2 at 470 nm that caused guaiacol oxidation. This method was described by Rao et al.

(1996). The SOD activity was measured in accordance with Sharma et al. (2010). A unit of SOD activity was defined as the amount of enzyme that stopped 50% of the dye nitro-blue tertazolium (NBT) from degrading. The absorbance was measured at 560 nm.

Statistical evaluation

Data were collected for every experiment of three replications from ten seedlings of wheat; the mean values and standard deviations were calculated. By using the Web Agri Stat Package (WASP), which is available at http://www.ccari.res.in, a single factor ANOVA analysis was accomplished for PEG tolerance (20% concentration) and bacterial treatment. At the 0.05 level, critical difference (C.D.) values were calculated, the statistically significant differences between the means of the bacterial treatment under control and 20% PEG stress. Values were indicated by different alphabets.

Result

PEG's Effect on the growth of KD-3B Strain

The isolate KD-3B exhibited remarkable growth up to 25% of PEG in LB broth, as shown in Fig 1. It was observed that the LB broth's PEG concentration rises, bacterial growth was detected decreases, simultaneously. At a 25% PEG concentration in the LB broth, 41.05% of relative growth was observed.

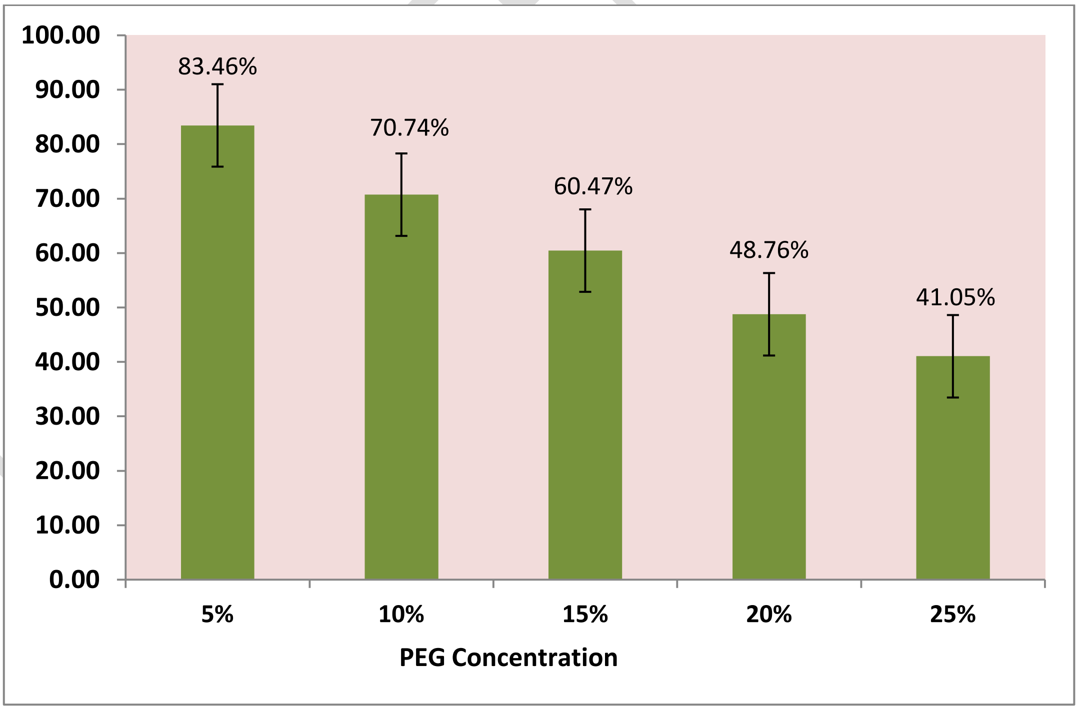

Fig 1. Comparative growth of the isolate KD-3B strain at various PEG concentrations

Impact of the bacterial KD-3B strain on wheat seedlings under normal and drought conditions

It was investigated that when PGPR strain KD-3B shown lessen the negative effects of drought on wheat seedlings grown on a half strength MS medium supplemented with 20% PEG. When PGPR inoculated seedlings were compared to positive control seedlings grown under normal conditions, the shoot and root length, as well as the fresh and dry weight of the shoot and root, were shown significantly higher values. When seedlings of the positive control compared with KD-3B treated seedlings, the drought effect dramatically decreased (Table 1). All growth indicators showed an improvement in KD-3B inoculated seedlings, indicating a decreased impact of drought. The lengths of the shoots and roots under drought and normal conditions noticeably differed from one another, and the inoculation of isolate KD-3B showing increased length of the shoots and roots (Fig. 2).

Effects of PGPR, KD-3B strain on wheat seedlings in biochemical parameters

The result of drought on wheat seedlings in the presence of drought-tolerant PGPR strains was studied by evaluating a range of plant biochemical parameters (**Table 2**). The chlorophyll content using KD-3B, positive control, KD-3B + 20% PEG and negative control were found 0.45±0.08, 0.41±0.03, 0.37±0.01 and 0.34±0.05 mg per gram of fresh weight, respectively. The observed values showing highest in PGPR (KD-3B) and lowest in negative control condition. Similarly The Total Soluble Protein (TSP) content in KD-3B, KD-3B + 20% PEG, positive control and negative control treatment were found 273.6±3.58, 248.4±5.09, 246.5±4.78 and 221.2±3.34 microgram per gram of fresh weight, respectively. The observed values showing highest in PGPR KD-3B and lowest in negative control condition. The amino acid, proline found highest in negative control than positive control, KD-3B + 20% PEG, and lowest in KD-3B treatment drought stress condition and the concentration were found was 41.21±1.88, 32.61±1.99, 23.34±1.46 and 16.36±1.23 microgram per gram of fresh weight, respectively.

The observed values showing highest in negative control and lowest in KD-3B treated condition. The total soluble solids content observed negative control highest, than KD-3B + 20% PEG, KD-3B, and last lowest in positive control treatment were found 3.92±0.15, 2.45±0.21, 2.11±0.19 and 1.74±0.19 microgram per gram of fresh weight, respectively. The observed values were maximum in negative control and minimum in positive control condition under stress condition.

Fig. 2: Impact of drought on wheat seedling morphology under plant microbe interaction. A) Positive control, B) Negative control, C) KD-3B, D) KD-3B with 20% PEG

Oxidative Stress and Antioxidant Enzyme Activity Measurements

In drought stress conditions, wheat seedlings found a markedly higher concentration of H_2O_2. When wheat seedlings were treated with the isolate KD-3B strain during drought stress conditions, the quantity of H_2O_2 in their leaves was noticeably decreased in KD-3B inoculate treatment, the higher concentration was found in negative control treatment. In the ascending order it was found that H_2O_2 was lowest in KD-3B, than positive control, KD-3B + 20% PEG and highest in negative control. The concentration was 2.21±0.4, 3.46±0.8, 6.08±0.3 and 8.92±0.9 microgram per gram of fresh weight respectively (Table 2). The seedlings under drought stress exhibited extensively higher SOD and POD activity. The comparative analysis study of SOD and POD reported that the maximum concentration was found in negative control treatment and minimum was in positive control. When the PGPR KD-3D inoculated in drought stress wheat seedlings it was observe that the concentration reduced as compared to the negative control. The order of treatment was negative control, KD-3B + 20% PEG, KD-3B and positive control and the concentration of the treatment was 0.367±0.17, 0.221±0.08, 0.178±0.16 and 0.161±0.14 U per milligram fresh weight respectively. In the same manner the POD activity was found in drought stress condition. Highest concentration of POD was observed in negative control, KD-3B + 20% PEG, KD-3B and lowest in positive control, the concentration of the treatment was 8.6±0.11, 4.5±0.12, 3.3±0.06 and 2.3±0.15 microgram per gram of fresh weight per min respectively (Table 2). The amount of malondialdehyde in the roots and shoots was determined during a drought stress condition. The PGPR isolate KD-3B inoculated seedlings' shoots exhibited much lower levels of malondialdehyde than the negative control seedlings'. In comparison to the roots of positive control seedlings and inoculated KD-3B strain seedlings, it was found that there was a significant fall in malondialdehyde concentration compared to those of negative control seedlings. The order of treatment was like that highest in negative control, than KD-3B + 20% PEG, KD-3B and last lowest in positive control and the concentration of the treatment was found 58.73±3.7, 41.82±2.1, 35.21±3.2 and 31.63±2.1 nM per gram in fresh weight respectively.

Similar patterns were observed when EL leakage was estimated in seedlings grown in drought conditions. The EL leakage in the negative control plants was higher than that of the positive control and KD-3B inoculated seedlings under both normal and drought conditions (Table 2). The EL leakage content under negative control, KD-3B + 20% PEG, KD-3B and positive control were found 61.92±3.5, 48.37±2.7, 41.65±3, and 40.69±1.4 in percentage respectively.

Table 1. Effect of isolate KD-3B on wheat seedlings in various growth parameters under control and drought condition

Treatment	**Shoot length (cm)**	**Root length (cm)**	**Shoot fresh weight (mg)**	**Root fresh weight (mg)**	**Shoot dry weight (mg)**	**Root dry weight (mg)**
Positive control	12.10±0.31^{ab}	6.10±0.1^{b}	34.53±0.8^{b}	23.93±0.5^{a}	18.00±0.4^{a}	10.13±0.5^{b}
Negative control	9.73±0.22^{c}	4.33±0.06^{d}	29.73±0.9^{c}	15.93±0.5^{c}	11.93±0.5^{c}	6.00±0.4^{d}
KD-3B	12.63±0.57^{a}	7.67±0.31^{a}	35.87±0.4^{a}	24.53±1.1^{a}	18.73±0.8^{a}	11.80±0.7^{a}
KD-3B+ 20% PEG	11.73±0.07^{b}	5.23±0.42^{c}	33.27±1.0^{b}	19.73±0.7^{b}	15.67±0.6^{b}	8.67±0.3^{c}

Data are given as mean ± standard deviation of three replicates; means followed by same letters are not significant at 5% level

Table 2. Effect of isolate KD-3B on leaf tissue of wheat seedling in various growth biochemical parameters under control and drought stress condition

Treatment	**Proline (µg g^{-1} FW)**	**Total Soluble Sugar (mg g^{-1} FW)**	**Total Soluble Protein (µg g^{-1} FW)**	**MDA equivalent (nmol g^{-1} FW)**	**H_2O_2 (mM g^{-1} FW)**	**Total Chlorophyll (mg g^{-1} FW)**	**SOD (U/mg FW)**	**POD (µM/ g^{-1} FW Min^{-1})**	**Electrolyte leakage (%)**
Positive control	32.61±1.99	1.74±0.19	246.5±4.78	31.63±2.1	3.46±0.8	0.41±0.03	0.161±0.14	2.3±0.15	40.69±1.4
Negative control	41.21±1.88	3.92±0.15	221.2±3.34	58.73±3.7	8.92±0.9	0.34±0.05	0.367±0.17	8.6±0.11	61.92±3.5
KD-3B	16.36±1.23	2.11±0.19	273.6±3.58	35.21±3.2	2.21±0.4	0.45±0.08	0.178±0.16	3.3±0.06	41.65±3.1
KD-3B + 20% PEG	23.34±1.46	2.45±0.21	248.4±5.09	41.82±2.1	6.08±0.3	0.37±0.01	0.221±0.08	4.5±0.12	48.37±2.7

Data are given as mean ± standard deviation of three replicates; means followed by same letters are not significant at 5% level

Discussion

In drought stress a constrained water supply alters physiological and biochemical processes that impact development and production, reduces crop productivity. Crop productivity can be increased by inoculating plants with PGPR to enable plant growth under such harsh circumstances. The present investigation seeks to better understand the biological processes by which PGPRs facilitate the response of wheat seedlings to drought stress. It was clear that PGPR inoculation enhanced wheat development during drought stress and the plant's subsequent recovery, as evidenced by the improved length, fresh weight and dry weight of roots and shoots (Table 1). Our findings support earlier research on the amelioration of osmotic stress mediated by PGPR (Gontia-Mishra et al., 2016; Tiwari et al., 2016; Gontia-Mishra et al., 2017). Ethylene biosynthesis is increased during drought stresses, which results in reduced root and shoots growth. PGPR strain helped seedlings to tolerate drought stress, as indicated in inoculated plants under drought stress (Table 1).

Proline and TSS are significant biochemical markers of plants' ability to withstand stress. Plant cells use proline production to counteract the negative effects of drought by stabilizing subcellular structures, adjusting the osmotic balance, and scavenging free radicals (Hare et al., 1998). Under drought stress, another way to acclimate to osmotic adjustment is to accumulate soluble carbohydrates as osmolytes. Thus, under drought stress, our study also documents an increase in proline and TSS content in wheat seedlings, which are subsequently restored to normal levels upon recovery. In contrast to non-inoculated plants under drought, PGPR-inoculated seedlings displayed a notable decrease in proline and TSS during drought stress (Table 2). Similar observations have previously been reported in previous research (Grover et al., 2014; Tiwari et al., 2016; Gontia-Mishra et al., 2016; Gontia-Mishra et al., 2017). It is evident that PGPR positive treated plants did not meet significant drought stress, and consequently, less proline and TSS buildup occurred in the presence of PGPR.

Plant oxidative stress is thought to be reflected in the amount of protein content. Under drought stress, the total protein content of non-inoculated plants dropped considerably. It is thought that increased oxidative stress and ROS production occur during drought stress, which may have led to oxidative damage and a decrease in protein content (Gontia-Mishra et al., 2016). It is shown that under drought stress, the protein content of PGPR-inoculated plants was higher than that of non-inoculated controls.

Reactive oxygen species such as O_2, H_2O_2, and OH^-, are produced in response to drought stress and harm membranes and macromolecules (Mittler 2002). In this work, during drought stress, PGPR-inoculated seedlings showed a significant decrease in

H_2O_2concentration compared to control seedlings. It's probable that during drought stress, PGPR-inoculated seedlings produced less H_2O_2 because they were not under as much stress (Gusain et al. 2015). The degree of oxidative membrane damage caused by the stress is indicated by the EL and MDA levels, which are byproducts of lipid peroxidation (Gontia-Mishra et al. 2016). The current work found that under drought stress, PGPR-inoculated seedlings had very reduced EL in shoot tissues, indicating that PGPR shielded plant cell membrane integrity against the damaging impacts of dehydration (Table 2).

Conclusion

This research validated that PGPR isolate KD-3B inoculation enhanced the biochemical and physiological condition of wheat seedlings under drought stress and improved their ability to endure drought stress as compared to uninoculated seedlings. The results of this research suggest a potential role for PGPR inoculation in mitigating the significance of drought stress. In order to decrease the damage to wheat seedlings under drought stress conditions, PGPR plant inoculation may be a practical possibility. However, extra research is required to see whether this strain will be helpful to wheat crops in typical field conditions under drought stress.

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