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Rhizobium leguminosarum Mediated Enhancement of Antioxidant Defense in Drought-Stressed Canola: Implication for Agriculture and Pharmaceutical Research

Sara Fatima Alvi[1], Ume Aiman[2], Tuba Saleem[3*], Muhammad Saad Ali[4], Faiza Mumtaz[5], Talha Ahmad[6*], Naeem Arshad[7], Ayesha Hussain[8], Muhammad Zakriya Khan[8,] Muhammad Dawood[9,] Muhammad Azhar[2]

[1] Henan Medical University, Henan Province, China (Email: saraalvi005@gmail.co)
[2,11] Institute of Microbiology, Faculty of Life Sciences, Government College University Faisalabad, Pakistan. (Email: umeaimanaliedu@gmail.com) , Email: azhargujjar7273@gmail.com.
[3] Government College University Faisalabad, Sub-Campus Sahiwal (Email: ch.hasnat707@gmail.com; ORCID: 0009-0006-6641-905X)
[4] Department of pharmacognosy, The Islamia university of Bahawalpur (Email: Saadalirph@gmail.com)
[5] Department of Pharmacy, The Superior University, Lahore (Email: faiza.mumtaz@superior.edu.pk)
[6] School of Science and Engineering, CAHID (Center of Anatomy and Human Identification), University of Dundee, Scotland, United Kingdom (Email: thkllkn@gmail.com; ORCID:- 0009-0003-3215-1102
[7] Department of Biochemistry, Government College University Faisalabad, Pakistan
[8,9] Department of Botany, Government College University Faisalabad, Pakistan (Email: ayeshahussain0207@gmail.com), (Gmail : muhammadzakriyakhan667@gmail.com)
[10] Sharif Medical City Hospital, Lahore. Email: dawoodix99@gmail.com ORCID:- 0009-0008-3698-8301
Corresponding author's email: Tuba Saleem (Email: ch.hasnat707@gmail.com; ORCID: 0009-0006-6641-905X) and Talha Ahmad (Email: thkllkn@gmail.com; ORCID:- 0009-0003-3215-1102

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Abstract

One of the most critical environmental factors that negatively impact the growth, yield and nutrient status of canola is drought stress caused by nutrient deficiency and oxidative damage. In the present study, the effect of Rhizobium leguminosarum on antioxidant defense mechanism and nutrient status of drought stressed canola plants was highlighted. The activity of important antioxidant enzymes like Superoxide Dismutase (SOD), Catalase (CAT) and Peroxidase (POD) was also found to be significantly improved by R. leguminosarum inoculation which effectively scavenged the Reactive Oxygen Species (ROS) produced in water-deficit condition. Stress tolerance of plants was enhanced by reducing the amount of lipid peroxidation and plant damage caused by the increased antioxidant protection. Furthermore, the bacterial inoculation helped to ensure the uptake and retention of different nutrients (N, P, K, Ca and Mg), and thus improved the physiological performance and metabolic stability under drought stresses. Overall, these two benefits of combined antioxidant and nutrient uptake resulted in improved growth, biomass production and water stress tolerance in plants. Based on the results, use of R. leguminosarum as bioinoculant could be a sustainable strategy to enhance the productivity of canola crops under drought stress. Furthermore, the greater level of anti-oxidants and nutritional characteristics has the potential to enhance the pharmaceutical and nutraceutical applications of canola products in the health industry.

Keywords: drought, antioxidant, canola, agriculture, nutrients, sustainability

Introduction

The drought stress is one of the most important abiotic stresses limiting the productivity of global agriculture and expected to further intensify under climate change and increasing water scarcity (Seleiman et al., 2021). Water stress has a negative impact on plant growth, development and yield by interfering with the physiological and biochemical and metabolic processes. Drought stress in crop plants leads to a decrease in photosynthetic activity, decreases nutrient uptake, disrupts cellular homeostasis and causes accumulation of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide and hydroxyl radicals in excessive amounts (Anjum et al., 2011). These toxic molecules start to cause oxidative damage to proteins, lipids, nucleic acids and cellular membranes which result in a decreased growth and productivity in the plant.

Canola (Brassica napus L.) is one of the most important world-wide oilseed crops, and a major source of animal feed, edible oil and raw material for industries. It is an important crop due to its nutritional and high-quality fatty acid content (Dutta et al., 2025). Canola is also especially vulnerable to drought during the critical growth periods, causing decreased biomass, seed yield, oil quality and nutrient levels. So, enhancing drought tolerance in canola is a key research focus to ensure food security and agricultural sustainability (Akhatar et al., 2025).

Well developed and advanced defense system towards drought induced oxidative stress in the plants. These are enzymatic antioxidants (such as superoxide dismutase [SOD], catalase [CAT] and peroxidase [POD]) that are needed to neutralize ROS and preserve cell integrity (Fujita & Hasanuzzaman, 2022). Such defense mechanisms are crucial to avoid stress-induced damage, but are not always effective under high or long drought stress (Fathi et al., 2025). Therefore, other strategies will have to be pursued to improve water stress tolerance and productivity in plants.

Recently, rhizobacteria referred to as plant growth promoting rhizobacteria (PGPR) have attracted renewed interest for the promotion of plant growth and stress tolerance in recent years, which are an alternative, environment-friendly and sustainable approach (Al-Turki et al., 2023). The beneficial microorganisms colonize the rhizosphere and bring their positive influence to the plant's performance in various ways, including through biological nitrogen fixation, production of plant hormones, nutrient solubilization, stimulation of root growth and induction of systemic stress tolerance (Vocciante et al., 2022). Among the PGPR, Rhizobium leguminosarum is a well established bacterium that is known for its ability to form symbiotic relationship with plants and enhance the nutrition and activities of plants under stress (Goyal & Habtewold, 2023).

The available data showed that R. leguminosarum was able to improve the antioxidant defense system, osmotic balance and nutrient uptake, resulting in improved drought tolerance in both leguminous and non-leguminous crops (Fahde et al., 2023). The upregulation of antioxidant enzyme activity can decrease the amount of ROS accumulation and reduce oxidative stress, while the increased acquisition of essential nutrients like nitrogen, phosphorus, potassium, calcium and magnesium can help to maintain plant metabolism and growth during stress (Y. Zhang et al., 2019). The positive impacts result in increased plant survival, biomass gain and productivity under water stress. Beyond its nutritional use as an agricultural crop, canola has a significant nutraceutical and pharmaceutical potential, because of its bioactive compound contents with antioxidant properties. Enhancing canola and its product's antioxidant and nutritional value through the use of microbial inoculants could serve as an additional benefit of canola. Thus it could be useful for improved sustainable agriculture and preparation of functional food and pharmaceuticals given the fact that it can boost plants antioxidant activity and nutrient under water stress.

This study aimed to compare the activity of antioxidant enzymes, nutrient absorption and growth parameters of canola under drought stress with and without the use of Rhizobium leguminosarum. Another aspect of the study is to explore the potential use of this beneficial bacterium as a sustainable bioinoculant for enhancing crop quality and resilience in water limited situations with potential agricultural and pharmaceutical applications.

Materials and Methods

Experimental Site and Plant Material

The experiment was carried out in the greenhouse with Brassica napus L, canola and Rhizobium leguminosarum (R. trifolii, R. meliloti and R. lupinicola) to investigate the effects of R. leguminosarum on growth, antioxidant defense and nutrient uptake under drought stress conditions. All certified seed and healthy seed for all study years were from an approved seed supplier of canola. The greenhouse environment was kept suitable to achieve uniform plant growth by regulating the environmental factors such as the temperature, humidity and photoperiod.

Preparation of Bacterial Inoculum

A pure culture of Rhizobium leguminosarum was isolated from a microbial culture collection, and grown in Yeast Extract Mannitol Broth (YEMB). The bacterial suspension was cultured in a bench top culture to achieve an appropriate cell concentration of 108cfu/mL. Canola seed was surface sterilized with sodium hypochlorite solution and then washed a few times with sterile distilled water prior to planting in pots. Next, seeds were inoculated with the bacteria and then covered with the bacteria to ensure good rhizosphere colonization.

Experimental Design and Drought Treatment

An experiment was laid out as a completely randomized design (CRD) in 4 treatments and 3 replications. The treatments included well-watered control, drought stressed plants, plants inoculated under normal irrigation and plants inoculated under drought stress. Drought stress was applied at the vegetative growth stage by keeping the soil water at about 50% of the field capacity while the control plants were kept at 100% field capacity. The soil moisture was measured periodically during the experiment.

Measurement of Growth Parameters

At the end of the experiment the various growth parameters were recorded and the plant performance was measured. The height of the plant was recorded with the help of a measuring scale and the length of root was recorded after carefully removing the plant from the soil and washing the root with distilled water (Romero-Aranda et al., 2001). Fresh biomass was obtained at harvest and dry biomass was obtained after drying the plant samples to constant dry weight at 70°C.

Determination of Antioxidant Enzyme Activities

Fresh leaf samples collected from all treatments and kept at low temperature for analysis. Enzyme extracts were made with phosphate buffer and antioxidant enzyme activity was spectrophotometrically measured. Superoxide Dismutase (SOD) activity was measured as the inhibition of the photo-reduction of Nitro Blue Tetrazolium (NBT). The rate of hydrogen peroxide decomposition was used to determine catalase (CAT) activity and the rate of guaiacol oxidation was used to determine peroxidase (POD) activity. Enzymes activities were expressed as per g FW (Guo et al., 2012).

Assessment of Lipid Peroxidation

Lipid membrane oxidation was estimated by the amount of malondialdehyde (MDA) which is an indicator of oxidative damage to membrane lipid. Tissues of leaves were homogenized and subjected to reaction with thiobarbituric acid reagent. Absorbance of the reaction mixture was determined spectrophotometrically and the amount of MDA concentration was determined with the appropriate extinction coefficient (S.-Z. Zhang et al., 2008).

Nutrient Analysis

Plant samples were oven dried, ground to fine powder and analyzed for nutrients. The nitrogen content was measured by the Kjeldahl method and the concentration of phosphorus was determined by spectrophotometric measurement after acid digestion. The concentrations of potassium, calcium and magnesium were determined by AA spectrophotometry. Concentrations of nutrients were reported per dry weight.

Statistical Analysis

Data obtained were analysed with Analysis of Variance (ANOVA) using statistical software. Means of treatments were compared by using Tukey's Honest Significant Difference (HSD) at significance level ($P \leq 0.05$). All data presented as mean ± SEM.

Results

Effect of Rhizobium leguminosarum on Antioxidant Enzyme Activities

Drought stress had a major impact on the antioxidant defense system of the Canola plants. The activities of Superoxide dismutase (SOD), Catalase (CAT) and Peroxidase (POD) were found to be higher in the drought stressed plants as compared to the well watered plants in the control. But when inoculated with Rhizobium leguminosarum, these enzyme activities are further increased, thus providing a more robust antioxidant defense system to cope with drought-induced oxidative stress. The enzyme activities under drought stressed with inoculated plants of R. leguminosarum were maximum (Table 1).

Table 1. Effect of Rhizobium leguminosarum **on antioxidant enzyme activities in canola under drought stress**

Treatment	SOD (U mg^{-1} protein)	CAT (U mg^{-1} protein)	POD (U mg^{-1} protein)
Control	45.3 ± 1.2	28.6 ± 0.9	18.4 ± 0.7
Drought Stress	58.7 ± 1.5	36.8 ± 1.1	25.2 ± 0.9
R. leguminosarum	63.5 ± 1.7	41.3 ± 1.2	29.7 ± 1.0
Drought + R. leguminosarum	78.4 ± 2.1	52.9 ± 1.5	39.8 ± 1.3

Values are means ± SE (n = 3).

Effect on Lipid Peroxidation

Malondialdehyde (MDA) concentration was significantly higher in the drought-stressed plants and indicated more oxidative stress-induced damage to membranes. Under drought stress, the inoculation with R. leguminosarum resulted in a significant reduction in the MDA level, which is a good indicator of resistance to oxidative stress (Table 2).

Table 2. Effect of Rhizobium leguminosarum **on lipid peroxidation in canola**

Treatment	MDA (nmol g^{-1} FW)
Control	8.6 ± 0.4

Drought Stress	18.5 ± 0.8
R. leguminosarum	7.3 ± 0.3
Drought + R. leguminosarum	11.2 ± 0.5

Values are means ± SE (n = 3).

Effect on Nutrient Uptake

Under drought stress the concentration of the essential nutrients nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) was significantly lowered. Concentrations of nutrients were higher in inoculated plants than in the uninoculated plants under both conditions—normal and drought—were recorded. The most stressed plants (inoculated and stressed by drought) had the greatest nutrient concentrations (Table 3)".

Table 3. Effect of Rhizobium leguminosarum **on nutrient uptake in canola**

Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
Control	2.85 ± 0.08	0.42 ± 0.02	2.10 ± 0.07	1.25 ± 0.04	0.48 ± 0.02
Drought Stress	2.15 ± 0.06	0.30 ± 0.01	1.62 ± 0.05	0.95 ± 0.03	0.35 ± 0.01
R. leguminosarum	3.18 ± 0.09	0.50 ± 0.02	2.45 ± 0.08	1.42 ± 0.05	0.57 ± 0.02
Drought + R. leguminosarum	2.89 ± 0.08	0.44 ± 0.02	2.18 ± 0.07	1.29 ± 0.04	0.49 ± 0.02

Values are means ± SE (n = 3).

Effect on Growth and Biomass Production

In general, drought stress significantly reduced plant height, root length, fresh weight and dry weight of canola. However, inoculation with R. leguminosarum always gave a substantial boost to growth under both normal and water-deficit conditions. The biomass accumulation of non-inoculated drought-stressed plants was significantly lower than that of drought-stressed inoculated plants (Table 4).

Table 4. Effect of Rhizobium leguminosarum **on growth attributes of canola**

Treatment	Plant Height (cm)	Root Length (cm)	Fresh Weight (g plant^{-1})	Dry Weight (g plant^{-1})
Control	42.6 ± 1.3	18.5 ± 0.7	18.9 ± 0.8	4.85 ± 0.15
Drought Stress	30.8 ± 1.1	14.2 ± 0.5	11.6 ± 0.5	2.96 ± 0.11
R. leguminosarum	48.7 ± 1.5	22.4 ± 0.8	22.7 ± 0.9	5.93 ± 0.18
Drought + R. leguminosarum	39.5 ± 1.2	19.8 ± 0.7	17.3 ± 0.7	4.38 ± 0.14

Values are means ± SE (n = 3).

Discussion

Oxidative stress, diminished cellular metabolism and nutrient uptake are all known areas of drought stress that are detrimental to plant growth and productivity (Anjum et al., 2011). The present study indicated that under drought stress, growth parameters, nutrient accumulation were decreased and oxidative damage increased in canola plants as observed from the increased lipid peroxidation. Inoculation with Rhizobium leguminosarum however, alleviated the drought stress from the plants, including the presence of higher antioxidant enzyme activities, uptake of nutrients and general growth of the plants. Based on the research work carried out, it is concluded that

rhizobacteria can be used as a beneficial source which promotes water-deficit resistance of plants through their production of beneficial compounds.
Under drought stress, the antioxidant defense mechanism of canola plants was increased by inoculation with R. leguminosarum as shown by higher activities of antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) in inoculated plants compared to the uninoculated ones. Excessive accumulation of the reactive oxygen species (ROS) due to drought can result in significant damage to proteins, lipids and nucleic acids. The more active SOD becomes, the more it will convert the superoxide radical into hydrogen peroxide which then will be converted to water and oxygen by CAT and POD (Branch, 2009). Under this condition, higher activities of these enzyme activities in the inoculated plants were observed which indicated more active ROS scavenging system and less oxidative stress experienced by the plants, thus maintaining the integrity of cell. Similar findings were reported in other plant growth promoting rhizobacteria studies where the growth of different crops increased and the antioxidant enzyme activity was enhanced, and stress tolerance increased (Duan et al., 2022).
This decrease in malondialdehyde (MDA) also suggests that R. leguminosarum is also shielding some of the plants against oxidative damage when they were drought-stressed. MDA is a known stress indicator to membranes and is a commonly used parameter of lipid peroxidation. The lower levels of MDA found in inoculated plants suggest a lower level of cellular structure damage, and a more stable structure (Garg & Kaur, 2013). Increased antioxidant defense system may be responsible for this decrease, as it was able to effectively quench the lipid oxidation that occurred with ROS. Under stress, such as adverse environmental stress, plants can only survive if maintaining membrane integrity to continue plant's metabolic processes.
A second factor that may impact plant responses during drought is the amount of nutrients that have been absorbed by the plants. Nutrient mobility and uptake may be affected in water deficient conditions and result in nutrient imbalance and growth losses (Ahanger et al., 2016). Drought significantly reduced the concentration of nitrogen, phosphorus, potassium, calcium and magnesium in canola plants in this experiment. Inoculation with R. leguminosarum, however, boosted the uptake of these important nutrients under normal and drought conditions. The enhanced nutrient status may be linked to the activities of bacteria that fix nitrogen, mobilize P and stimulate root growth, all of which lead to increased nutrient uptake and availability. Improved nutritional status optimises photosynthesis, as well as enzyme production and osmosis regulation and energy metabolism, which all improve drought tolerances.
The plant growth parameters also improved when treated with R. leguminosarum confirming its beneficial effect in reducing the drought stress. The plants inoculated were higher in plant height, root length, fresh biomass as well as dry biomass than non-inoculated plants under drought condition. The improved root system structure may have aided its ability to extend its roots to reach the available water and nutrients in the surrounding soil, which assisted with water scarcity (Ahmad et al., 2021). Additionally the rhizobacteria produced some phytohormones, which could have contributed to the overall growth and development, cell division and elongation of the roots. Under this drought stress, inoculated plants exhibited these physiological advantages and had the ability to grow well and produce more.
The enhancement of antioxidant activities and nutritional composition observed also has significant implications other than agricultural productivity. Canola is a good source of oilseed and is used as a nutritive and industrial source (Shen et al., 2023). This will be beneficial in enhancing bioactive compounds synthesized by the plants and improving nutritional and medicinal values as well as product values of canola parts. Antioxidants have also been reported to play an important role in protection of human cells against the oxidative damage and are very widely used for formulation of nutraceuticals, functional foods and drugs. Therefore, the use of

R. leguminosarum can contribute to the sustainability in the production of crops, and improve the economic value of canola production by developing nutritionally beneficial products.

Conclusion

In summary, this study shows that Rhizobium leguminosarum possess various complementary mechanisms for increasing the drought resistance of canola such as antioxidant defense mechanisms, reduced oxidative damage, improved nutrient uptake, and improved plant growth. The results presented here are in favor of using R. leguminosarum as an eco-friendly bioinoculant to help improve the drought resistant ability of crops in arid localities. Future research under field conditions and on the molecular level would provide a better understanding of the action of the rhizobial stress tolerance mechanism and would lead to the development of new microbial technologies for improving sustainable agriculture and for the production of pharmaceuticals.

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