



## Effect of exogenous plant growth regulators and rhizobial inoculants on physiology traits associated with drought tolerance in blackgram

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### ARTICLE INFO

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### ABSTRACT

Field experiments were conducted at Agricultural College and Research Station, Eachankottai, Thanjavur and Oilseeds Research Station, Tindivanam during Rabi, 2022 & Rabi, 2023 to assess the suitable foliar application of hormones for drought tolerance in blackgram. The experiment was laid out in completely randomized block design and replicated thrice. The treatments are T1-Absolute Control (Without drought), T2-Absolute drought, T3-Rhizobial strain (MB 1), T4-KCL Spray 1% +T3, T5-100 µmol/L melatonin+T3, T6-200 µmol/L melatonin+T3, T7-300 µmol/L melatonin+T3, T8-0.1 mM Putrescine +T3, T9-0.2 mM Putrescine+T3, T10-0.3 mM Putrescine+T3. The growth, physiological and yield parameters were recorded and analysed statistically.

The results revealed that different foliar spray levels had significant influence on pod yield of blackgram. With regard to growth, physiological and yield parameters T6-200 µmol/L melatonin+T3 performed better compared to other treatments. Drought stress reduced the yield to about 52.26% in absolute stress plants compared to the absolute control (Table 6). The combined effect of soil application of rhizobial strain and foliar application of 200 µM melatonin increased the number of pods (25.65) per plant and grain yield (818.70) per plant under drought stress when compared to control plants.

### Introduction

Pulses are the major sources of proteins in India and complement the staple cereals in the diets with proteins, essential amino acids, vitamins and minerals. Black gram (*Vigna mungo* L.) is one of the major rainy season pulse crops grown throughout India. Blackgram is cultivated in about **4.5–5.0 million hectares** across India with a production of nearly **2.8–3.0 million tonnes** and an average productivity of **600–800 kg/ha**. (Department of Agriculture, Cooperation & Farmers Welfare [DAC&FW], 2022; Kannan *et al.*, 2023).

In Tamil Nadu, it occupies around **3.5–4.0 lakh hectares**, producing **2.5–2.8 lakh tonnes**, with a relatively higher productivity of **600–700 kg/ha** (Government of Tamil Nadu, 2022; Balusamy *et al.*, 2023), indicating its importance as a major pulse crop in the state. Enhancing the productivity of blackgram requires the adoption of proper agronomic practices.

Blackgram is highly sensitive to moisture stress, and drought is one of the major constraints limiting its productivity under rainfed and rice-fallow conditions. Drought stress affects germination, root development, photosynthesis, and

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reproductive processes, often leading to heavy flower drop and poor pod filling. However, certain genotypes of blackgram exhibit drought tolerance through adaptive mechanisms such as deeper root systems, osmotic adjustment, efficient stomatal regulation, and activation of antioxidant defense pathways. Improving drought tolerance through the use of resistant varieties, seed priming, balanced nutrition, and moisture conservation practices is crucial to stabilize yields in vulnerable regions. (Kumar et al., 2021; Tavu et al., 2025) Plant growth regulators (PGRs) play a significant role in enhancing drought tolerance in blackgram by modulating physiological and biochemical responses under water-deficit conditions. **Melatonin** and **putrescine** have emerged as important bio-regulators in enhancing drought tolerance in blackgram. Melatonin, a potent antioxidant, mitigates drought-induced oxidative stress by scavenging reactive oxygen species (ROS) and enhancing the activity of antioxidant enzymes such as superoxide dismutase, catalase, and peroxidase. It also improves water-use efficiency, maintains chlorophyll content, and supports root growth under water-deficit conditions. Putrescine, a polyamine, contributes to osmotic adjustment, stabilizes cellular membranes, and regulates ion balance during drought stress. Both melatonin and putrescine help in maintaining photosynthetic efficiency, delaying senescence, and ensuring better flowering and pod set, thereby improving yield under moisture-limited conditions. Exogenous application of these compounds, either individually or in combination, has been shown to significantly enhance drought resilience in blackgram. (El-Beltagi et al., 2023; Moustafa-Farag et al., 2020).

It has also been demonstrated that microbe-based plant biotechnology is an alternative strategy for effectively improving plant drought resistance (Naseem et al., 2018). The rhizosphere is anatural ecosystem that hosts various kinds of microbes, including bacteria, action bacteria, fungi, algae, and protozoa (Mendes et al., 2013; Rout and South worth, 2013). Plants and the rhizosphere microbiome interacts through several distinct mechanisms. Plants fuel the soil food web through their below ground carbon (C) inputs. In turn, the activities of the rhizosphere microbiome release nutrients for plant growth and determine the balance between C respiration and stabilization in the soil. In addition, the microbiome interacts directly with plantsin the rhizosphere by feeding on (or infecting) roots, forming symbiotic relationships, or promoting plant growth through phytohormone production or the reduction of plant a. The weather conditions of the experimental site. (2022-23)

stress signaling (de Vries et al., 2020). However, despite an increasing understanding of plant-microbiome interaction mechanisms, our understanding of these mechanisms under drought is quite limited over the past few years, the use of exogenous PGRs and microbe-based plant biotechnology to enhance crop drought resistance has become pioneering research because of their better field operability and environmental friendliness.

## Materials and methods

### Conditions of experiment and treatments

The experiments were performed in two locations during 2022-23 and 2023-24 in the Agricultural College and Research Station, Eachangkottai, Thanjavur and Oilseeds Research Station, Tindivanam to study the effect of melatonin and putrescine as foliar application with seed treatment of drought tolerant Rhizobial strain (MB 1) on morphological, physiological and yield characters of blackgram VBN 8. The experiment was arranged in a completely randomized design with three replications. The seeds of blackgram (Vigna mungo)VBN 8 were obtained from National Pulses Research Centre, Vamban and sown during rabi season in the both the locations. Blackgram seeds were treated with drought tolerant Rhizobial strain (MB 1) and kept at room temperature for 1 h. The recommended amounts of nitrogen, phosphorus, and potassium (NPK) fertilizers were added as basal. The weather conditions have been presented in Table 1. The drought stressed plants were exogenously sprayed with melatonin and putrescine during after 30 days from sowing. The experiment includes 10 treatments as follow.

T <sub>1</sub>	:	Absolute Control (Without drought)
T <sub>2</sub>	:	Absolute drought
T <sub>3</sub>	:	Rhizobial strain (MB 1)
T <sub>4</sub>	:	KCL Spray 1% +T <sub>3</sub>
T <sub>5</sub>	:	T <sub>3</sub> +100 µmol/L melatonin
T <sub>6</sub>	:	T <sub>3</sub> +200 µmol/L melatonin
T <sub>7</sub>	:	T <sub>3</sub> +300 µmol/L melatonin
T <sub>8</sub>	:	T <sub>3</sub> +0.1 mM Putrescine
T <sub>9</sub>	:	T <sub>3</sub> +0.2 mM Putrescine
T <sub>10</sub>	:	T <sub>3</sub> +0.3 mM Putrescine

Month	Avg Tmax (°C)	Avg Tmin (°C)	Rainfall (mm)	Relative Humidity (%)	Wind Speed (km/h)
November 2022	32	23	150	75-80	14
December 2022	31	22	40	75-80	15

January 2023	30	20	15	70-75	14
February 2023	32	21	8	65	13
March 2023	34	23	20	60	12
April 2023	36	24	15	58	12
May 2023	37	26	25	60	15

b The weather conditions of the experimental site. (2023-24)

Month	Total Rainfall (mm)	Avg Tmax (°C)	Avg Tmin (°C)	Relative Humidity (%)	Wind Speed (km/h)
November 2023	331.00	30.0	23.0	80	12.5
December 2023	368.20	28.5	21.5	85	12.0
January 2024	27.60	29.0	22.0	80	11.5
February 2024	0.00	30.0	23.0	75	11.0
March 2024	0.30	32.0	24.0	70	10.5
April 2024	0.00	34.0	26.0	65	10.0
May 2024	15.80	35.0	27.0	60	9.5

## Morphological characters

After 30 days from sowing, morphological characters, such as branches number and nodules number per plant were determined.

## Physiological characters

### Determination of chlorophyll content

#### Proline determination ( $\mu\text{g g}^{-1}$ FW)

Free proline was estimated following the method of Bates et al. (1973). Fresh leaf tissue (0.5 g) was homogenized in 3% sulfosalicylic acid, reacted with acid-ninhydrin, heated at 100 °C for 1 h, extracted with toluene, and absorbance measured at 520 nm. Proline content was calculated using a standard curve and expressed on a fresh weight basis.

#### Electrolyte leakage determination (EL%)

Leaf discs were rinsed, incubated in deionized water, and initial conductivity (C1) was measured. Samples were then boiled to release total electrolytes, and final conductivity (C2) was recorded. Electrolyte leakage (%) was calculated as  $(C1/C2) \times 100$ .

#### Determination of Malondialdehyde ( $\text{nmol g}^{-1}$ fresh weight)

Malondialdehyde (MDA) content, an indicator of lipid peroxidation, was estimated using the thiobarbituric acid

(TBA) assay. Fresh leaf tissue (0.5 g) was homogenized in 0.1% trichloroacetic acid (TCA) and centrifuged. The supernatant was mixed with 0.5% TBA in 20% TCA, heated at 95 °C for 30 min, cooled, and absorbance measured at 532 and 600 nm. MDA concentration was calculated using an extinction coefficient of  $155 \text{ mM}^{-1} \text{ cm}^{-1}$  and expressed as  $\text{nmol g}^{-1}$  fresh weight. (Heath & Packer, 1968).

#### Determination of Superoxide dismutase ( $\text{mg}^{-1}$ protein)

Superoxide dismutase activity was determined by measuring the inhibition of nitroblue tetrazolium (NBT) photoreduction. Fresh leaf tissue (0.5 g) was homogenized in 50 mM phosphate buffer (pH 7.8) and centrifuged to obtain the enzyme extract. The reaction mixture containing enzyme extract, NBT, riboflavin, and methionine was illuminated, and the absorbance was measured at 560 nm. One unit of SOD activity was defined as the amount of enzyme required to inhibit 50% of NBT photoreduction and expressed as  $\text{mg}^{-1}$  protein (Beauchamp & Fridovich, 1971).

#### Enzymes activity

Catalase (CAT) activity was assayed by measuring the decomposition of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) at 240 nm. Fresh leaf tissue (0.5 g) was homogenized in phosphate buffer (50 mM, pH 7.0), centrifuged, and the supernatant used as enzyme extract. The decrease in absorbance at 240 nm was recorded for 1–3 min, and activity expressed as  $\mu\text{mol H}_2\text{O}_2$  decomposed  $\text{min}^{-1} \text{ mg}^{-1}$  protein (Aebi, 1984).

Peroxidase (POD) activity was determined by the oxidation

of guaiacol in the presence of H<sub>2</sub>O<sub>2</sub> at 470 nm. Leaf tissue (0.5 g) was homogenized in phosphate buffer (50 mM, pH 7.0), centrifuged, and the supernatant used as enzyme extract. The increase in absorbance at 470 nm was monitored, and activity expressed as change in absorbance min<sup>-1</sup> mg<sup>-1</sup> protein (Chance & Maehly, 1955).

### Yield and yield parameters

At maturity, five representative plants from each treatment plot were randomly selected to record yield and yield parameters. Number of pods per plant and pod length (cm) were measured. Pods were harvested, sun-dried, and seed yield per plant (g) was recorded. For plot-level yield, pods from each plot were threshed, and grain yield per hectare (kg ha<sup>-1</sup>) was calculated after adjusting for moisture content.

### Statistical analysis

Statistical analysis was done using one-way Anova (ANOVA) procedures [66], using the MSTAT-C statistical software package, and the means between treatments were compared by Duncan [67] when the difference was significant (p ≤ 0.05).

## Results

**Table 1: Effects of exogenous melatonin and putrescine application on the plant height, number of branches and chlorophyll content of blackgram (Rabi 2022-2023 & Rabi 2023 -2024)**

Treatments	Plant height (cm)			Number of branches			Chlorophyll content (SPAD)		
	Rabi 2022-2023	Rabi 2023-2024	Pooled mean	Rabi 2022-2023	Rabi 2023-2024	Pooled mean	Rabi 2022-2023	Rabi 2023-2024	Pooled mean
T <sub>1</sub> - Absolute Control (Without drought)	36.44	35.93	36.19	7.90	6.43	7.17	40.4	39.4	39.90
T <sub>2</sub> -Absolute drought	29.85	28.60	29.23	5.26	6.33	5.79	31.6	32.4	32.00
T <sub>3</sub> -Rhizobial strain (MB 1)	36.64	35.83	36.24	7.10	5.67	6.39	35.4	34.7	35.05
T <sub>4</sub> - KCL Spray 1% +T <sub>3</sub>	36.72	35.10	35.91	7.26	6.10	6.68	35.8	35.1	35.45
T <sub>5</sub> - 100 µmol/L melatonin+T <sub>3</sub>	36.91	35.03	35.97	7.45	6.00	6.73	36.7	37.9	37.30
T <sub>6</sub> - 200 µmol/L melatonin+T <sub>3</sub>	<b>38.47</b>	<b>36.50</b>	<b>37.49</b>	<b>7.86</b>	<b>6.48</b>	<b>7.17</b>	<b>41.1</b>	<b>39.5</b>	<b>40.30</b>
T <sub>7</sub> - 300 µmol/L melatonin+T <sub>3</sub>	38.11	35.03	36.57	<b>7.49</b>	<b>6.28</b>	<b>6.89</b>	36.2	37.4	36.80
T <sub>8</sub> - 0.1 mM Putrescine +T <sub>3</sub>	36.00	34.53	35.27	6.24	6.17	6.21	35.9	35.3	35.60

T <sub>9</sub> - 0.2 mM Putrescine+T <sub>3</sub>	<b>37.25</b>	<b>36.40</b>	<b>36.83</b>	6.85	6.25	6.55	<b>38.4</b>	<b>37.9</b>	<b>38.15</b>
T <sub>10</sub> - 0.3 mM Putrescine+T <sub>3</sub>	36.55	36.80	36.68	7.11	5.98	6.55	34.1	38.6	36.35
<b>SEd</b>	<b>0.395</b>	<b>0.233</b>		<b>0.042</b>	<b>0.019</b>		<b>0.135</b>	<b>0.125</b>	
<b>CD (5%)</b>	<b>1.47</b>	<b>0.63</b>		<b>0.089</b>	<b>0.039</b>		<b>0.284</b>	<b>0.262</b>	

Fig:1: Effects of exogenous melatonin and putrescine application on the plant height, number of branches and chlorophyll content of blackgram

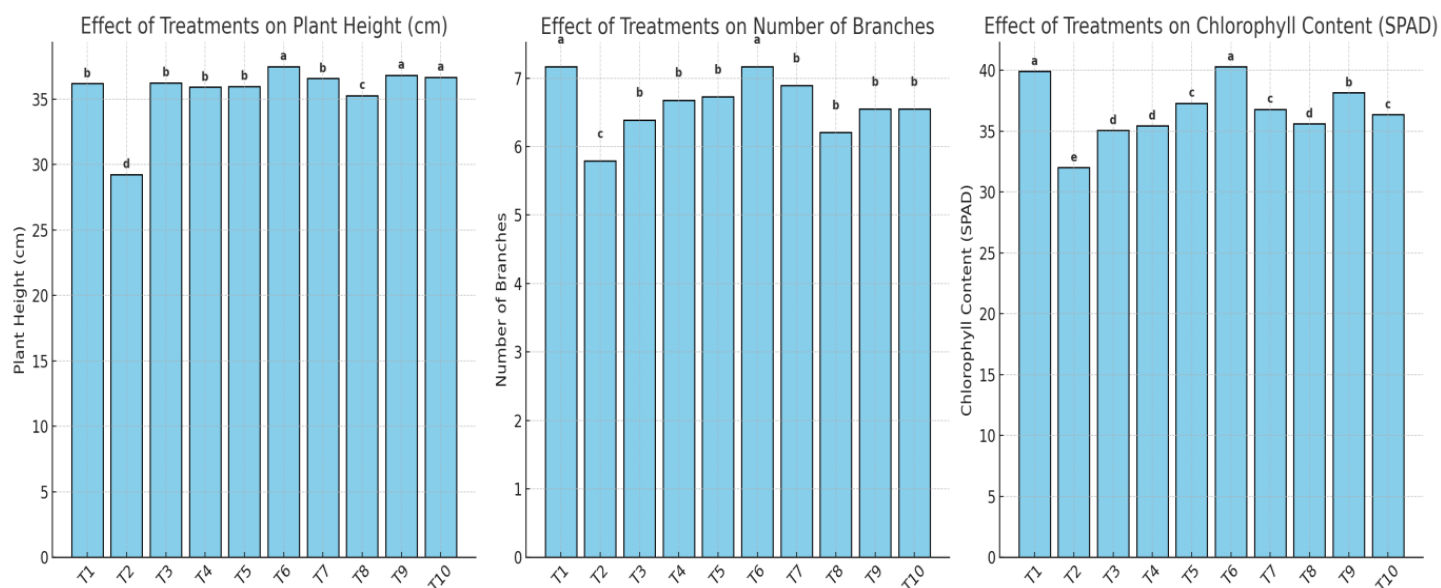


Table 2: Effects of exogenous melatonin and putrescine application on the root length and Number of nodules of blackgram(Rabi 2022-2023 & Rabi 2023 -2024)

Treatments	Root length (cm)			No. of Nodules		
	Rabi 2022-2023	Rabi 2023 -2024	Pooled mean	Rabi 2022-2023	Rabi 2023 -2024	Pooled mean
T1 - Absolute Control (Without drought)	13.48	14.15	13.82	16.89	17.73	17.31
T2 -Absolute drought	9.11	9.57	9.34	11.80	12.39	12.10
T3 -Rhizobial strain (MB 1)	13.45	14.12	13.79	34.55	36.27	35.41
T4 - KCL Spray 1% +T3	13.51	14.19	13.85	24.90	26.14	25.52
T5 - 100 µmol/L melatonin+T3	15.14	15.90	15.52	27.87	29.26	28.57
T6- 200 µmol/L melatonin+T3	16.46	17.28	16.87	28.81	30.25	29.53
T7- 300 µmol/L melatonin+T3	15.50	16.28	15.89	26.88	28.22	27.55
T <sub>8</sub> - 0.1 mM Putrescine +T <sub>3</sub>	<b>16.01</b>	16.81	16.41	28.11	29.52	28.82
T <sub>9</sub> - 0.2 mM Putrescine+T <sub>3</sub>	<b>14.94</b>	<b>15.68</b>	<b>15.31</b>	<b>31.22</b>	<b>32.78</b>	<b>32.00</b>
T <sub>10</sub> - 0.3 mM Putrescine+T <sub>3</sub>	<b>14.83</b>	<b>15.57</b>	<b>15.20</b>	<b>28.93</b>	<b>30.38</b>	<b>29.66</b>

<b>SEd</b>	<b>0.131</b>	<b>0.173</b>	<b>0.042</b>	<b>0.019</b>
<b>CD 5%</b>	<b>0.275</b>	<b>0.363</b>	<b>0.089</b>	<b>0.039</b>

### Physiological parameters

Drought stress significantly affected the biochemical and physiological responses of the crop. The absolute drought treatment (T2) increased proline accumulation (81.80  $\mu\text{mol g}^{-1}$  FW) relative to the control (T1, 66.55  $\mu\text{mol g}^{-1}$  FW), indicating osmotic adjustment under water deficit. Correspondingly, membrane stability index (MSI) declined sharply under drought (T2: 27.10%; T1: 82.35%), reflecting oxidative damage. Malondialdehyde (MDA) levels, an indicator of lipid peroxidation, were highest under drought (T2: 0.41  $\mu\text{mol g}^{-1}$  FW), while superoxide dismutase (SOD) activity increased (T2: 11.09  $\text{mg}^{-1}$  protein), showing activation of antioxidative defense mechanisms.

Rhizobial inoculation (T3) mitigated drought effects by enhancing proline content (100.48  $\mu\text{mol g}^{-1}$  FW), improving MSI (72.29%), reducing MDA (0.33  $\mu\text{mol}\cdot\text{g}^{-1}$  FW), and increasing SOD activity (12.58  $\text{mg}^{-1}$  protein). Supplementation with KCl (T4) provided minor additional improvements. Exogenous melatonin, especially at 200  $\mu\text{mol/L}$  + T3 (T6),

maximized stress tolerance: proline (208.35  $\mu\text{mol g}^{-1}$  FW), MSI (77.06%), lowest MDA (0.18  $\mu\text{mol g}^{-1}$  FW), highest SOD (14.89  $\text{mg}^{-1}$  protein), and elevated peroxidase (POD) (1.91 Units  $\text{g}^{-1}$  FW  $\text{min}^{-1}$ ) and catalase (CAT) activities (0.95 Units  $\text{g}^{-1}$  FW  $\text{min}^{-1}$ ).

The results indicate that drought induces oxidative stress, evidenced by elevated MDA and enhanced SOD, POD, and CAT activities. Rhizobial inoculation enhances osmotic adjustment, membrane stability, and antioxidant defenses (Egamberdieva et al., 2019). Melatonin further augments these effects by stabilizing membranes, scavenging reactive oxygen species (ROS), and stimulating antioxidant enzymes (Zhang et al., 2023). Putrescine also contributes to stress tolerance through similar mechanisms (Shi et al., 2021).

Overall, the combination of rhizobial inoculation with 200  $\mu\text{mol/L}$  melatonin was the most effective in enhancing osmolyte accumulation, protecting membranes, reducing oxidative damage, and strengthening enzymatic antioxidant defense, providing a promising strategy for improving crop resilience under drought conditions.

**Table 3: Effect of melatonin and Putrescine concentrations on proline content and membrane Stability Index in blackgram(Rabi 2022-2023 & Rabi 2023 -2024)**

Treatments	Proline ( $\mu\text{mol g}^{-1}$ FW)			Membrane stability Index (%)		
	Rabi 2022-2023	Rabi 2023 -2024	Pooled mean	Rabi 2022-2023	Rabi 2023 -2024	Pooled mean
T1 - Absolute Control (Without drought)	64.60	68.50	66.55	80.74	83.96	82.35
T2 -Absolute drought	79.40	84.20	81.80	26.57	27.63	27.10
T3 -Rhizobial strain (MB 1)	97.55	103.40	100.48	70.87	73.70	72.29
T4 - KCL Spray 1% +T3	116.50	123.50	120.00	72.43	75.33	73.88
T5 - 100 $\mu\text{mol/L}$ melatonin+T3	199.95	211.90	205.93	73.97	76.92	75.45
T6- 200 $\mu\text{mol/L}$ melatonin+T3	202.30	214.40	208.35	75.55	78.57	77.06
T7- 300 $\mu\text{mol/L}$ melatonin+T3	172.15	182.50	177.33	74.99	77.98	76.49
T8- 0.1 mM Putrescine +T3	169.00	179.10	174.05	74.06	77.02	75.54
T9- 0.2 mM Putrescine+T3	174.75	185.20	179.98	74.53	77.51	76.02
T10- 0.3 mM Putrescine+T3	166.15	176.10	171.13	73.72	76.67	75.20
SEd	0.131	0.173		0.042	0.019	
CD 5%	0.275	0.363		0.089	0.039	

**Table 4: Effect of melatonin and putrescine concentrations on malondialdehyde (MDA) activity and super oxide dismutase activity in blackgram (Rabi 2022-2023 & Rabi 2023 -2024)**

Treatments	Malondialdehyde (MDA) activity ( $\mu\text{mol}\cdot\text{g}^{-1}$ FW)			Superoxide dismutase ( $\text{mg}^{-1}$ protein)		
	Rabi 2022-2023	Rabi 2023 -2024	Pooled mean	Rabi 2022-2023	Rabi 2023 -2024	Pooled mean

T1 - Absolute Control (Without drought)	0.17	0.17	0.17	10.18	10.59	10.39
T2 -Absolute drought	0.41	0.42	0.41	10.87	11.30	11.09
T3 -Rhizobial strain (MB 1)	0.32	0.33	0.33	12.33	12.82	12.58
T4 - KCL Spray 1% +T3	0.29	0.30	0.30	12.30	12.79	12.55
T5 - 100 µmol/L melatonin+T3	0.21	0.22	0.22	12.62	13.12	12.87
T6- 200 µmol/L melatonin+T3	0.18	0.18	0.18	14.60	15.18	14.89
T7- 300 µmol/L melatonin+T3	0.23	0.24	0.24	12.68	13.18	12.93
T8- 0.1 mM Putrescine +T3	0.24	0.25	0.25	12.79	13.30	13.05
T9- 0.2 mM Putrescine+T3	0.19	0.19	0.19	13.78	14.33	14.06
T10- 0.3 mM Putrescine+T3	0.27	0.28	0.28	12.96	13.48	13.22
SEd	0.17	0.17	0.17	10.18	10.59	10.39
CD 5%	0.275	0.363		0.089	0.039	

Table 5: The effects of different concentrations of exogenous melatonin and putrescine on the activities of antioxidant enzymes peroxidase and catalase (Rabi 2022-2023 & Rabi 2023 -2024)

Treatments	Peroxidase activity (Units g-1 fr.wt min-1)			Catalase activity (Units g-1 fr.wt min-1)		
	Rabi 2022-2023	Rabi 2023 -2024	Pooled mean	Rabi 2022-2023	Rabi 2023 -2024	Pooled mean
T1 - Absolute Control (Without drought)	1.19	1.25	1.22	0.36	0.38	0.37
T2 -Absolute drought	1.56	1.63	1.60	0.48	0.51	0.50
T3 -Rhizobial strain (MB 1)	1.59	1.67	1.63	0.66	0.69	0.68
T4 - KCL Spray 1% +T3	1.64	1.72	1.68	0.70	0.73	0.72
T5 - 100 µmol/L melatonin+T3	1.67	1.76	1.72	0.87	0.91	0.89
T6- 200 µmol/L melatonin+T3	1.86	1.95	1.91	0.93	0.97	0.95
T7- 300 µmol/L melatonin+T3	1.76	1.85	1.81	0.90	0.94	0.92
T8- 0.1 mM Putrescine +T3	1.78	1.86	1.82	0.86	0.90	0.88
T9- 0.2 mM Putrescine+T3	1.79	1.88	1.84	0.91	0.95	0.93
T10- 0.3 mM Putrescine+T3	1.74	1.83	1.79	0.88	0.92	0.90
SEd	0.17	0.17	0.17	10.18	10.59	10.39
CD 5%	0.275	0.363		0.089	0.039	

### Yield and yield parameters

Drought stress significantly reduced reproductive performance in the crop. The absolute drought treatment (T<sub>2</sub>) recorded the lowest number of pods per plant (13.55), grain yield per plant (9.25 g), and grain yield per hectare (545.40 kg/ha), compared to the absolute control (T<sub>1</sub>: 25.16 pods, 19.06 g/plant, 823.53 kg/ha), highlighting the adverse effect of water deficit on reproductive development and yield formation. Rhizobial inoculation (T<sub>3</sub>) mitigated the effects of drought, increasing pods per plant (21.28), grain yield per plant (17.12 g), and grain yield per hectare (740.33 kg/ha). Combined treatments with KCl (T<sub>4</sub>) maintained similar

improvements (21.25 pods, 18.06 g/plant, 746.60 kg/ha). Exogenous application of melatonin, particularly at 200 µmol/L + T<sub>3</sub> (T<sub>6</sub>), resulted in the highest pooled values for pods per plant (25.65), grain yield per plant (19.81 g), and grain yield per hectare (818.70 kg/ha), even approaching or exceeding control levels. Putrescine treatments (T<sub>8</sub>-T<sub>10</sub>) also improved yield parameters (pods: 22.36-22.90, grain/plant: 16.56-18.49 g, yield/ha: 766.50-787.28 kg/ha), although slightly less than T<sub>6</sub>.

These results indicate that drought reduces yield primarily through impairment of reproductive growth and pod formation. Rhizobial inoculation enhances yield by improving nutrient acquisition and plant vigor (Egamberdieva *et al.*, 2019), while melatonin strengthens

reproductive performance under stress, likely through osmotic adjustment, antioxidant activity, and maintenance of physiological functions (Zhang *et al.*, 2023). Putrescine also contributes to mitigating drought effects by regulating growth and stress tolerance (Shi *et al.*, 2021). Overall,

integrating rhizobial inoculation with 200  $\mu\text{mol/L}$  melatonin proved most effective in sustaining pod formation and grain yield under drought stress, offering a viable strategy for improving crop productivity under water-limited conditions.

Table 6: The effects of different concentrations of exogenous melatonin and putrescine on the yield components of blackgram(Rabi 2022-2023 & Rabi 2023 -2024)

Treatment details	No. of pods per plant (Number)			Grain yield per plant (g/plant)			Grain yield (kg/per ha)		
	Rabi 2022-2023	Rabi 2023-2024	Mean	Rabi 2022-2023	Rabi 2023-2024	Mean	Rabi 2022-2023	Rabi 2023-2024	Mean
T1-Absolute Control (Without drought)	31.71	18.60	25.16	18.52	19.60	19.06	824.40	822.65	823.53
T2-Absolute drought	17.70	9.40	13.55	9.05	9.45	9.25	543.30	547.50	545.40
T3-Rhizobial strain (MB 1)	26.05	16.50	21.28	16.59	17.65	17.12	735.20	745.45	740.33
T4-KCL Spray 1% +T3	25.10	17.40	21.25	17.51	18.60	18.06	746.60	746.60	746.60
T5-100 $\mu\text{mol/L}$ melatonin+T3	27.92	16.90	22.41	16.98	18.05	17.52	787.00	791.60	789.30
T6-200 $\mu\text{mol/L}$ melatonin+T3	32.19	19.10	25.65	19.21	20.40	19.81	820.30	817.10	818.70
T7-300 $\mu\text{mol/L}$ melatonin+T3	29.75	18.50	24.13	18.55	19.70	19.13	796.30	798.15	797.23
T8-0.1 mM Putrescine +T3	28.71	16.00	22.36	16.07	17.05	16.56	787.80	786.75	787.28
T9-0.2 mM Putrescine+T3	27.44	18.20	22.82	18.20	19.40	18.80	781.40	779.25	780.33
T10-0.3 mM Putrescine+T3	28.80	17.00	22.90	17.77	19.20	18.49	761.30	771.70	766.50
SEd	2.53	2.16		1.35	1.28		37.9	35.44	
CD 5%	5.35	5.16		2.87	2.59		80.5	79.44	

### Economics of Cultivation

The cost of cultivation varied slightly among treatments, with the absolute control ( $T_1$ ) at ₹20,756 and marginally higher costs in treatments involving growth regulators, particularly putrescine applications ( $T_8$ - $T_{10}$ : ₹23,500–23,800) due to additional inputs.

### Gross Returns and Net Returns

Drought stress ( $T_2$ ) significantly reduced gross return (₹37,800) and net return (₹17,100) compared to the absolute

control ( $T_1$ : ₹58,555 gross; ₹37,799 net), reflecting the adverse impact of water deficit on yield and profitability. Rhizobial inoculation ( $T_3$ ) improved gross and net returns to ₹50,505 and ₹29,695, respectively, demonstrating the economic benefit of microbial inoculants under stress conditions. Supplementation with KCl ( $T_4$ ) and exogenous growth regulators further enhanced profitability. Among these, 200  $\mu\text{mol/L}$  melatonin +  $T_3$  ( $T_6$ ) recorded the highest gross return (₹58,492), net return (₹37,142), and a favorable B:C ratio of 2.74, closely followed by 100  $\mu\text{mol/L}$  melatonin +  $T_3$  ( $T_5$ ) and 300  $\mu\text{mol/L}$  melatonin +  $T_3$  ( $T_7$ ). Putrescine treatments ( $T_8$ - $T_{10}$ ) also improved returns compared to  $T_3$  alone but were

slightly less profitable than melatonin treatments.

### B:C Ratio

The benefit-cost ratio (B:C) was lowest under drought (T2: 1.83) and highest under T6 (2.74), indicating that integrating rhizobial inoculation with 200 µmol/L melatonin provided maximum economic efficiency by enhancing yield while optimizing input costs. Overall, the results suggest that drought reduces profitability, but application of rhizobia in combination with melatonin, especially at 200 µmol/L, effectively improves economic returns, making it a viable strategy for sustainable and profitable crop production under water-limited conditions (Egamberdieva et al., 2019; Zhang et al., 2023).

Table 7: The effects of different concentrations of exogenous melatonin and putrescine on economics of blackgram (AC&RI, Eachangkottai)

Treatment details	Cost of cultivation	Gross return	Net return	B:C ratio
T1-Absolute Control (Without drought)	20756	58555	37799	2.82
T2-Absolute drought	20700	37800	17100	1.83
T3-Rhizobial strain ( MB 1)	20810	50505	29695	2.43
T4-KCL Spray 1% +T3	20940	52808	31868	2.52
T5-100 µmol/L melatonin+T3	21220	54992	33772	2.59
T6-200 µmol/L melatonin+T3	21350	58492	37142	2.74
T7-300 µmol/L melatonin+T3	21350	56056	34706	2.63
T8-0.1 mM Putrescine +T3	23800	55860	32060	2.35
T9-0.2 mM Putrescine+T3	23600	55580	31980	2.36
T10-0.3 mM Putrescine+T3	23500	52332	28832	2.23

### Conclusion

Drought stress severely affected plant growth, physiological and biochemical traits, reproductive performance, and economic returns. The absolute drought treatment reduced

root growth, nodule formation, pod number, grain yield, and profitability, while increasing oxidative stress indicators such as MDA and activating antioxidant enzymes.

Rhizobial inoculation (MB 1) a drought tolerant strain significantly mitigated drought effects by enhancing nutrient uptake, root architecture, osmotic adjustment, and antioxidant defense. Exogenous application of melatonin, particularly at 200 µmol/L combined with rhizobial inoculation, was most effective in improving morphological traits, maintaining membrane stability, reducing lipid peroxidation, enhancing antioxidant enzyme activity, increasing yield attributes, and maximizing economic returns. Putrescine treatments also provided benefits but were slightly less effective than melatonin.

Overall, integrating rhizobial inoculation with 200 µmol/L melatonin is a promising strategy to enhance drought tolerance, sustain crop growth and yield, and improve economic feasibility under water-limited conditions. This approach offers a practical and sustainable solution for mitigating drought stress in crops.

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Month	Avg Tmax (°C)	Avg Tmin (°C)	Mean Temp (°C)	Rainfall (mm)	Relative Humidity (%)	Wind Speed (km/h)
November	32	23	28-29	150 mm	75-80	14
December	31	22	26-27	40 mm	75-80	15
January	30	20	25-26	15 mm	70-75	14
February	32	21	26-27	8 mm	65	13
March	34	23	28.5	20 mm	60	12
April	36	24	30-31	15 mm	58	12
May	37	26	31-32	25 mm	60	15

Month (2023–24)	Monthly total rainfall (mm)
Jun 2023	143.40
Jul 2023	61.40
Aug 2023	239.90
Sep 2023	291.60
Oct 2023	55.30
Nov 2023	331.00

Month (2023–24)	Monthly total rainfall (mm)
Dec 2023	368.20
Jan 2024	37.60
Feb 2024	0.00
Mar 2024	0.30
Apr 2024	0.00
May 2024	15.80
Annual total	1544.50

Month	Total Rainfall (mm)	Avg Max Temp (°C)	Avg Min Temp (°C)	Avg Humidity (%)	Avg Wind Speed (km/h)
<b>November 2023</b>	331.00	30.0	23.0	80	12.5
<b>December 2023</b>	368.20	28.5	21.5	85	12.0
<b>January 2024</b>	37.60	29.0	22.0	80	11.5
<b>February 2024</b>	0.00	30.0	23.0	75	11.0
<b>March 2024</b>	0.30	32.0	24.0	70	10.5
<b>April 2024</b>	0.00	34.0	26.0	65	10.0
<b>May 2024</b>	15.80	35.0	27.0	60	9.5