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Mitigating Drought by Exogenous Potassium-mediated Improvements in Water Relation, Antioxidant Defense, Morpho-physiological and Biochemical Attributes of Black Gram [*Vigna mungo* (L.) Hepper]

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ABSTRACT

Background: Globally, drought stress (DS) incidence in early development and grain-filling stages of crops like black gram has posed a serious constraint to the growth and yield of legume crops. To ensure the food and nutritional security of the rising human population, requisites developing eco-friendly, pro-farmers and cost-effective DS mitigation strategies for imparting yield sustainability to black gram.

Methods: In this trial, treatments included control group entailing control (normal watering), water spray (WA, positive control), control+50 mg L^{-1} K_2SO_4, control +100 mg L^{-1} K_2SO_4, control +150 mg L^{-1} K_2SO_4, while drought treatment included drought stress (plant exposed to 15-20% by suspending water supply), water spray (WA, positive control), drought +50 mg L^{-1} K_2SO_4, drought +100 mg L^{-1} K_2SO_4 and drought +150 mg L^{-1} K_2SO_4.

Result: The results exhibited that the DS seriously declined plant growth, relative water content and water potential by 24.2% and 39.3%, respectively, inducing higher levels of malondialdehyde (MDA) content and hydrogen peroxide (H_2O_2) concentration that reduced cell membrane stability, stomatal conductance and photosynthetic rate, than the control. However, the foliar applied K significantly improved plant growth, plant water status, gas exchange and photosynthetic performance, chlorophyll content and antioxidant enzyme activity. Exogenous application of K further reduced lipid peroxidation, cell membrane injury and hydrogen peroxide by 12.7, 17.6 and 8.70%, respectively.

Key words: Abiotic stresses, Antioxidant defense, Cellular injury, Climate change, *Vigna mungo*, Water suspension.

INTRODUCTION

Recently, drought stress (DS) has emerged as one of the most critical limitations that reduce plant growth and crop yield causing food insecurity and undernourishment in a rapidly increasing human population. It can reduce production efficiency and crop yield by 20% in warm, dry and arid regions. Furthermore, a persistent DS declines the efficiency by 45% of agricultural lands which has impacted over 38% of the world's population livelihood (Zhang *et al.,* 2023). The underlying reason is DS inhibits vegetative growth parameters, including root and shoot development, resulting in a decrease in plant growth. The DS-induced oxidative stress may impair the synthesis of photosynthetic pigment and reduce the relative water content (RWC), leading to an increase in oxygen radical production (Waraich *et al.,* 2020). The DS tends to impair germination, cell division, root proliferation, leaf growth, stem elongation, photosynthetic efficiency, nutrient mobilization, cell turgidity and gas exchange attributes, resulting in lower crop yield. Moreover, it adversely affected assimilate translocation which impaired flowering and pod formation, resulting in pollen grain sterility (Ul-Allah *et al.,* 2020), therefore, eco-friendly, DS management strategies are critically needed for sustainable and smart crop production, otherwise, zero hunger and poverty alleviation related Sustainable Development Goals (SDGs) may not be achieved.

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After nitrogen, potassium (K) is the second-most important macro-nutrient and the most prevalent mineral-nutrient in the kingdom *Plantae* (Waraich *et al.,* 2020). It plays an important role in protein synthesis, which increases resistance to abiotic stresses. Additionally, it may not be the main component of different plant parts and structures, but exogenous and endogenous K may play a significant role in regulating the physicochemical processes involved in better plant morphological growth, yield and quality and mitigating stress and other mineral deficiency in plants in cotton (Zahoor *et al.,* 2017). The K application assisted in upregulating the plant gas exchange and water relation leading to improved crop performance under DS conditions in canola (Waraich *et al.,* 2020). Moreover, Ihsan *et al*. (2013) opined that K foliage supplementation remained effective in mitigating moisture-induced stresses in mung bean as revealed by growth, photosynthesis, gas exchange capacity and Zn analysis of shoot. Furthermore, Martineau *et al*. (2017) have also reported a significant role of K in maize leaf carbon exportation under DS conditions.

For ensuring the food and nutritional security of a rapidly increasing population, especially in developing countries of South Asia, legumes hold a strategic position (Iqbal *et al.,* 2019a,b). The leguminous crops contribute over 33% of the global human protein requirement (Iqbal, 2018; Iqbal *et al.,* 2018). Among leguminous crops, black gram [*Vigna mungo* (L.) Hepper] belongs to the Fabaceae family (Sadiq *et al.,* 2023) and is cultivated in various agroecological zones of rain-fed areas of South Asia. It is one of the most important grain legumes in the world due to its high nutritive profile, including vitamins (A, B and C), protein (24.2%), fat (1.42%) and carbohydrate (59.6%) (Hossain *et al.,* 2024). However, DS seriously reduced black gram yield and research gaps exist about the optimized doses of K for mitigating DS effects in black gram.

Therefore, to bridge the research gaps, it has become necessary to explore the exogenous application of numeral nutrition and its importance in boosting plant drought tolerance. The prime objective of this investigation was to explore whether and how the exogenous application of K induces drought tolerance by altering a series of morpho-physiological attributes in black gram. We further intended to optimize the dose of K application for boosting plant defense DS in field conditions.

MATERIALS AND METHODS

Experimental treatments and execution

Drought tolerant black gram genotype (cv. Arooj-11) seed was acquired from the Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan. The trial was conducted in the Department of Agronomy, University of Agriculture Faisalabad, Pakistan. Sowing was done in plastic pots (28 cm diameter 25 cm length) with a drainage hole at the bottom, filled with 6 kg of silica sand [after calculating its field capacity (FC)] on 15 March 2020 in a wire-house. The gravimetric method was used to determine the field capacity (FC) of sand, while 25 ml water was added to 100 g of sand to attain the saturation point. To determine the FC, the saturated sand was oven-dried (90°C) until it had obtained a constant weight. Black gram seedlings were grown in clay pots and water supplementation was gradually suspended as plants were exposed to DS by declining field capacity (FC) from 100% to 15%. Mild drought started at 30% field capacity; however considerable drought effects started at the FC of 15-20%. The treatments included a (i) control group entailing control (normal watering), water spray (WA, positive control), control+50 mg L^{-1} K_2SO_4, control +100 mg L^{-1} K_2SO_4, control +150 mg L^{-1} K_2SO_4, while (ii) drought treatments group included drought stress (plant exposed to 15-20% by suspending water supply), water spray (WA, positive control), drought +50 mg L^{-1} K_2SO_4, drought +100 mg L^{-1} K_2SO_4 and drought +150 mg L^{-1} K_2SO_4. The potassium (K_2SO_4) was sprayed during the reproductive stage (45 DAS). The experiment was set up using a completely randomized design (CRD). Three individual replications were considered for recording the response variables.

Determination of photosynthetic pigments, photosynthesis and transpiration-related parameters

Five plants were randomly selected from each treatment for data recording at 64 DAS during the flowering stage. The chlorophyll content was determined using the method outlined by Arnon (1949) and Davies (1976). The gaseous exchange parameters, photosynthetic rate (*Pn*), inter-cellular CO_2 concentration (*Ci*), stomatal conductance (*gs*) and transpiration rate (*Tr*) were recorded by using an infrared gas analyzer (CI-340 portable, Hoddesdon, England), with the water vapor pressure in the chamber kept between 6.0 and 8.9 m bar, the molar flow of air per unit leaf area kept at 403.3 mmol m^2 s^{-1}, the ambient temperature of 22.4°C-27.9°C, ambient CO_2 concentration (352 mol mol^{-1}), leaf temperature of 28.4-32.4 °C and atmospheric pressure 99.9 KPa.

Estimation of water status and oxidative stress indicators

The plant water relations were recorded at 63 DAS during the flowering stage as described by Scholander *et al.* (1964). Leaf water potential (ψs) and leaf osmotic potential (ψw) were measured with a water potential apparatus and an osmometer, respectively. The pressure potential (ψp) was determined as suggested by Hopkins (1999):

$$\psi p = \psi w - \psi s$$

Following the method outlined by Ahmad *et al.* (2021), the relative water content was determined.

Cell membrane injury (CMI) measurements were made by recording the electrical conductivity of leachates from black gram leaves that had been soaked in distilled water at 100°C for an overnight period (Deshmukh *et al.,* 1991). Equal-sized pieces of leaves (100 mg) were placed in tubes of distilled water (10 ml) in two sets. The electric conductivities C1 and C2 of the two sets were measured after one set was left overnight at room temperature and the other set was placed in a boiling water bath for 15 minutes. The CMI was calculated as follows:

$$\text{Cell membrane stability} = 1- \frac{C1}{C2} \times 100$$

$$\text{Cell membrane injury \%} = 100\text{-CMS}$$

The malondialdehyde (MDA) content was estimated by following Heath and Packer (1968).

Measurement of metabolite accumulation and antioxidant enzyme activity

The metabolite content was determined by following the procedure outlined by Lowry *et al.* (1951) that estimated total soluble protein. A spectrophotometer was used to measure the absorbance at 620 nm and total phenolic content was calculated (Chaovanalikit and Wrolstad, 2004). The phenolic content of an acetone extract was assessed using the Folin Ciocalteu method and an absorbance measurement at 700 nm.

Plant sample extraction was made using the protocol suggested by Chauhan *et al.* (2022) and catalase (CAT) activity was measured (Kabir *et al.,* 2023). Enzyme extracts (100 µL) were combined with 100 L of freshly made hydrogen peroxide (5.9 mM; 35% pure) to begin the procedure. A microplate reader (ELX800, Bio-Tek Instruments, Inc., Winooski, VT, USA) monitored the rate of absorbance fall for three minutes and recorded the H_2O_2 disappearance rate at 240 nm and superoxide dismutase (SOD) activity was assessed (Ju *et al.,* 2021). Each test tube contained KH_2PO_4 (500 µL) buffer (5 pH, 50 mM), 22 µM methionine (200 µL), 0.1 µM Triton X (200 µL), 20 µM NBT (100 µL) and 0.6 µM riboflavin (100 µL) as a substrate. In the end, 800 µL of distilled water was mixed with 100 µL of enzyme extract. At 560 nm, the absorbance of a microplate reader (ELX800, Bio-Tek Instruments, Inc., Winooski, VT, USA) was measured.

Statistical analysis

The recorded data were arranged and subjected to analysis of variance (ANOVA) with the help of Statistix (version 10.0, USA) statistical package. Thereafter, the least significant difference (LSD) test was employed at the probability level of 5% to determine the significance among treatment means (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Plant growth attributes

The results revealed that K application had a significant ($P \leq 0.05$) effect on root length, shoot length, leaf area, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight, respectively, by 12.9%, 0.85%, 11.8%, 18.9%, 9.06% and 16.9% in comparison to the control treatment. However, the maximum improvement in growth attributes of black gram was noted for K_{100} (100 mg L^{-1} potassium) followed by K_{150} (150 mg L^{-1} potassium) and K_{50} (50 mg L^{-1}

Table 1: Impacts of potassium application on black gram's growth and yield attributes under drought stress conditions.

Treatment	Potassium application (K)	Shoot length (cm)	Root length (cm)	Leaf area (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	No. of pods plant^{-1}	1000-seed weight (g)	Yield/ plant (g)
Control	NA	14.3c	15.8c	4.06d	1.60d	0.056c	0.59g	0.018f	18.4abc	36.1b	3.99d
	WA	14.4c	15.9c	4.08d	1.62c	0.057c	0.61g	0.018ef	18.7abc	36.6b	4.13d
	K_{50}	15.1b	17.03b	4.64c	1.75b	0.062b	0.66f	0.020e	19.5abc	41.2a	4.84c
	K_{100}	16.4a	17.9a	5.35a	1.79a	0.065a	0.69de	0.023bc	21.4a	42.9a	5.70a
	K_{150}	15.3b	17.5a	4.91b	1.76b	0.064ab	0.68ef	0.022cd	20.1ab	42.3a	5.11b
Drought stress	NA	10.7e	12.7g	3.48f	1.14i	0.031f	0.71cd	0.021d	15.1cd	28.5d	2.58f
	WA	10.7e	12.8fg	3.53f	1.16h	0.032f	0.72c	0.022cd	15.5bcd	28.8d	2.68f
	K_{50}	12.05d	13.2ef	3.76e	1.25g	0.037e	0.77b	0.024b	11.5d	32.08c	3.21e
	K_{100}	12.6d	14.2d	4.03d	1.32e	0.041d	0.81a	0.027a	18.1abc	35.0a	3.92d
	K_{150}	12.1d	13.3e	3.88de	1.29f	0.038e	0.79a	0.026a	17.1abc	32.9c	3.38e
LSD		0.58	0.40	0.22	0.02	2.32	0.02	1.39	4.66	1.57	0.24

NA=No application, WA=Water spray, K_{50}, K_{100} and K_{150} represent potassium application at the rate of 50, 100 and 150 mg L^{-1}, respectively. Numerical values sharing typical letters within the same column do not differ significantly ($P \leq 0.05$) according to the LSD test.

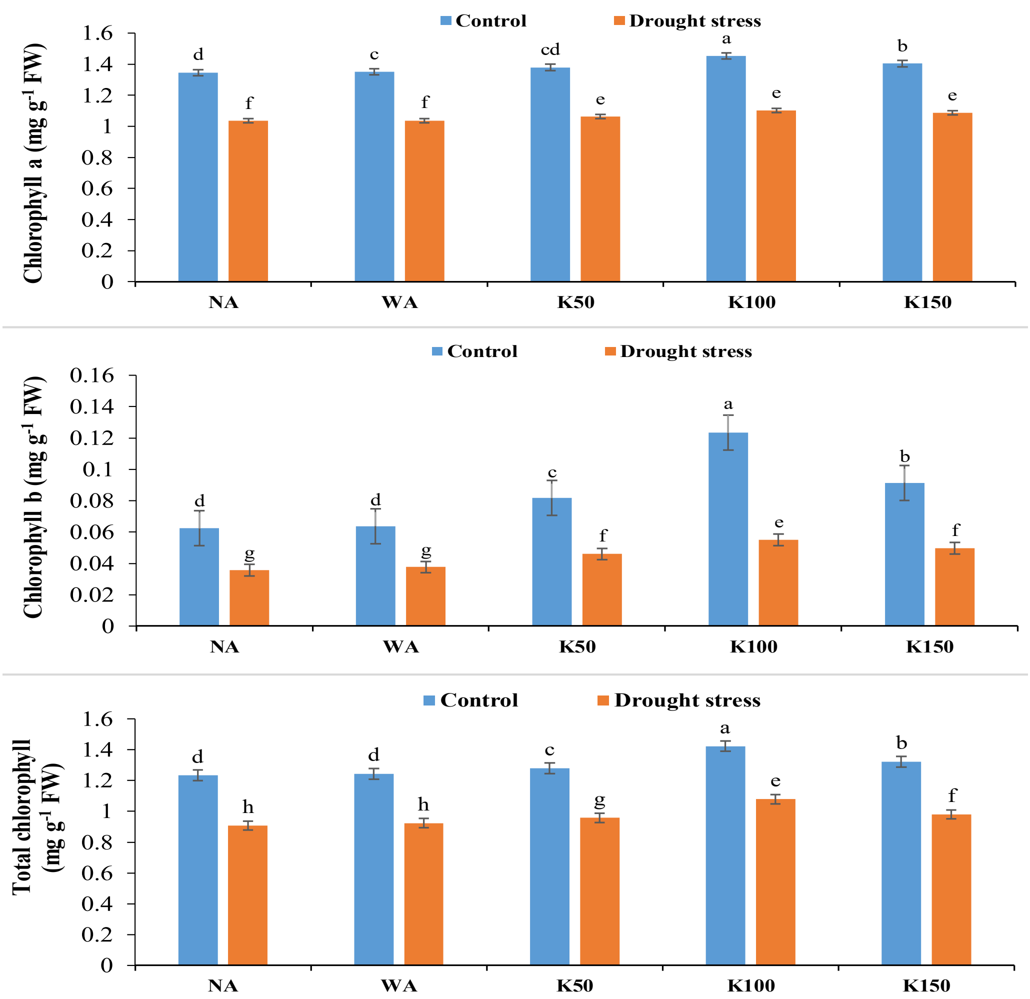
potassium) (Table 1). Root length and leaf area in black gram were significantly affected by the interactions between DS and K applications. Black gram yield components were significantly ($P \leq 0.05$) affected by the DS and K application in different concentrations. Compared to control (no stress) plants, the number of pods per plant, 1000-seed weight and yield decreased by 21.2%, 21.06% and 37.80% under DS, to counteract the negative effects of DS, K was applied; this increased the number of pods per plant, 1000-seed weight and yield when compared to the control (no potassium applied). Exogenous potassium application increased pods per plant, 1000-seed weight and yield under DS conditions by 5.14, 14.6 and 27.6%, respectively, in comparison to control (no potassium applied). However, when compared to control (no K applied), the maximum improvement in seed yield and associated characters was observed for K_{100} which was followed by K_{150} and K_{50} (Table 1). For yield and associated traits in black gram, the interaction between DS and K application was not significant.

Photosynthetic pigments

According to the results, DS significantly ($P \leq 0.05$) declined chlorophyll *a*, *b* and total chlorophyll in black gram by 23.1, 46.9 and 25.4%, respectively to control treatment (Fig 1). The K_{100} significantly improved the chl a, b and total chlorophyll compared to NA (no application of K with normal watering) and WA (only water spray). It was revealed that when K_{100} was applied under DS, chlorophyll *a*, *b* and total chlorophyll were increased by 3.93%, 34.1% and 6.77%, respectively than control (no K applied) (Fig 1). Overall, the maximum enhancement in photosynthetic pigments was noted for K_{100}, that was followed by K_{150} which in turn was followed by K_{50} (Fig 1). The DS and K application interaction was also significant for chlorophyll content in black gram.

Photosynthesis-associated parameters

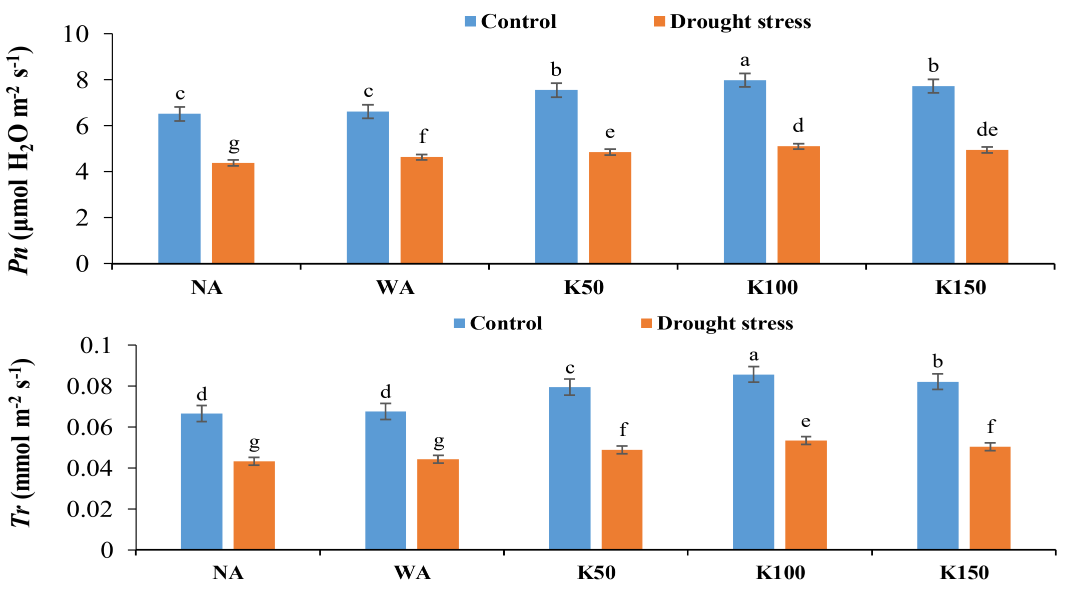
The result exhibited that DS significantly influenced the photosynthesis-associated traits (photosynthetic rate, stomatal conductance, transpiration rate and intercellular



NA=No application, WA=Water spray, K_{50}, K_{100} and K_{150} represent potassium application at the rate of 50, 100 and 150 mg L^{-1}, respectively. Numerical values sharing typical letters do not differ significantly ($P \leq 0.05$) according to the LSD test.

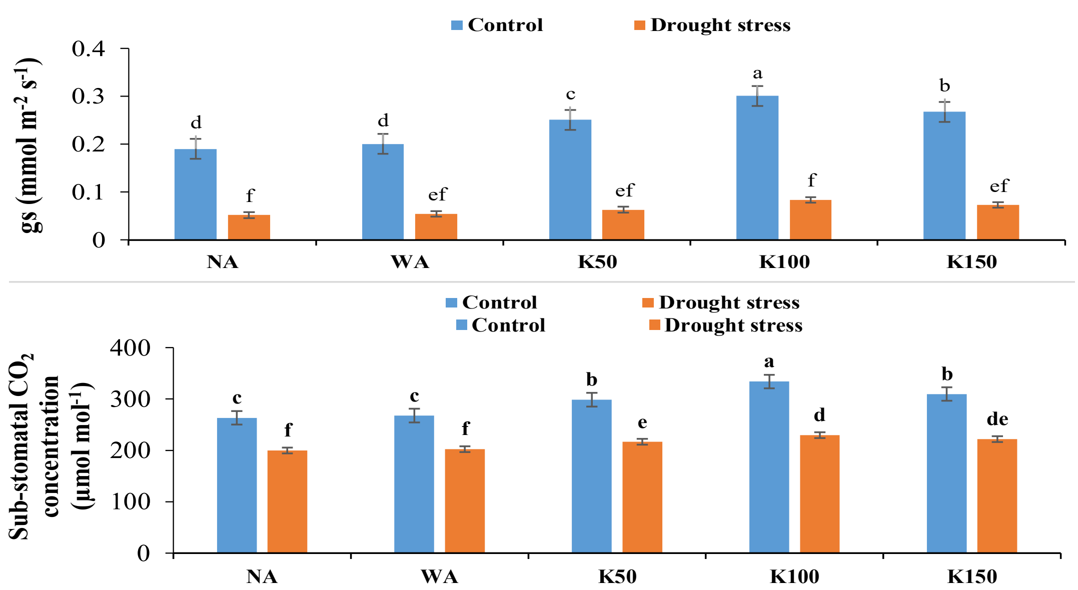
Fig 1: Effect of potassium application on chlorophyll *a*, chlorophyll *b* and total chlorophyll of black gram under drought stress.

CO_2 concentration were decreased by 34.2%, 73.02%, 37.1% and 27.3%, respectively compared to control treatment) of black gram (Fig 2). The K application increased photosynthetic rate (12.03%) and transpiration rate (14.7%). However, due to the improvement of photosynthesis rate (*Pn*) in black gram in response to DS, the transpiration (*Tr*) was enhanced compared to other K treatments. Additionally, K supplementation significantly improved stomatal conductance by 31.4% and sub-stomatal CO_2 concentration by 9.84% (Fig 3). Out of these K treatments (K_{50}, K_{100} and $K1_{50}$), the K_{100} showed better performance for improving photosynthesis system in black gram under DS.



NA=No application, WA=Water spray, K_{50}, K_{100} and K_{150} represent potassium application at the rate of 50, 100 and 150 mg L^{-1}, respectively. Numerical values sharing typical letters do not differ significantly ($P \leq 0.05$) according to the LSD test.

Fig 2: Effect of potassium application on photosynthetic rate and transpiration in black gram under drought stress.



NA=No application, WA=Water spray, K_{50}, K_{100} and K_{150} represent potassium application at the rate of 50, 100 and 150 mg L^{-1}, respectively. Numerical values sharing typical letters do not differ significantly ($P \leq 0.05$) according to the LSD test.

Fig 3: Effect of potassium application on stomatal conductance (gs) and sub-stomatal CO_2 concentration in black gram under drought stress.

Water relations

The K application remained effective in significantly (*P*≤ 0.05) influencing the water relations in black gram under DS conditions. It was noted that the water potential, pressure potential, osmotic potential and leaf-relative water content decreased by 39.3%, 21.5%, 9.64% and 24.2%, respectively, compared to the control treatment (Table 2). On the other hand, the K application increased the water relations, as well as the water potential and pressure potential under DS control. The water potential and leaf relative water content were improved under DS by 9.27% and 9.83%, respectively in comparison to the control treatment. The highest increment was observed for K_{100} when compared to the control-no K application during DS conditions. Nonetheless, in comparison to control (no K-applied) plants, pressure potential increased by 47.03%, 36.1% and 26.4% in response to $K_{150,}$ $K_{100\ and}$ K_{50}, respectively (Table 2). The pressure potential was also significantly influenced by the interaction of DS and K dose application.

Metabolites regulation

Stress-induced metabolites mediate antioxidant defenses under oxidative stress in plants, while these also help to cope with stress in plants. The DS and K application had a significant (*P* ≤0.05) effect on the osmolyte content of black grams (Table 3). Additionally, the DS increased total soluble protein and total phenolics than the control treatment. Exogenous K application, on the other hand, increased total soluble proteins and total phenolics compared to the control (no potassium applied). Under DS, K application increased total soluble proteins and total phenolics by 7.53 and 30.7%, respectively, when compared to no potassium. Furthermore, the maximum increment in osmolyte content was observed for the K_{100} treatment. Nonetheless, K application increased total phenolics content by 4.57%,16.9% and 10.04% by K_{100}, K_{150} and K_{50}, respectively than the control plants receiving no K-application (Table 3). Moreover, the interaction of DS and K application was not significant for osmolyte content in black gram.

Table 2: Impact of potassium application on biochemical attributes and oxidative stress indicators of black gram under drought stress.

Treatment	Potassium application (K)	Water potential (-MPa)	Osmotic potential (-MPa)	Pressure potential (MPa)	Relative water content (%)
Control	NA	-0.76d	-1.34a	0.58e	62.2d
	WA	-0.75d	-1.34a	0.59e	62.4d
	K_{50}	-0.70c	-1.39b	0.68c	65.7c
	K_{100}	-0.65a	-1.43c	0.78a	71.7a
	K_{150}	-0.68b	-1.41b	0.73b	68.3b
Drought stress	NA	-1.06i	-1.46d	0.39h	46.4h
	WA	-1.03h	-1.48d	0.44g	46.7h
	K_{50}	-0.98g	-1.53e	0.55f	49.3g
	K_{100}	-0.91e	-1.57f	0.65d	55.06e
	K_{150}	-0.95f	-1.55f	0.60e	52.7f
LSD		0.021	0.021	0.027	2.30

Table 3: Impact of potassium application on photosynthetic pigments and physiological attributes of black gram under drought stress.

Treatment	Potassium application (K)	Total soluble proteins (mg g^{-1} FW)	Total phenolics content (mg g^{-1} FW)	Catalase (Unit mg^{-1} FW)	Peroxidase (Unit mg^{-1} FW)	Superoxide dismutase (FW g^{-1})
Control	NA	32.1f	11.7f	39.4f	41.2f	27.3g
	WA	32.4f	12.0f	40.0f	42.5f	27.4g
	K_{50}	33.3ef	13.5e	43.6e	45.3e	29.6f
	K_{100}	38.6d	15.2d	48.5d	50.9d	36.3d
	K_{150}	35.4e	14.1de	45.5e	47.09e	32.8e
Drought stress	NA	45.08c	16.7c	50.6c	70.7c	49.3c
	WA	45.8c	17.0c	50.8c	70.9c	49.5c
	K_{50}	47.4bc	20.0b	54.5b	75.1b	53.3b
	K_{100}	51.6a	24.5a	59.7a	78.6a	57.9a
	K_{150}	49.5ab	23.6a	55.6b	76.9ab	54.6b
LSD		2.52	1.44	1.98	2.64	2.15

NA=No application, WA=Water spray, K_{50}, K_{100} and K_{150} represent potassium application at the rate of 50, 100 and 150 mg L^{-1}, respectively. Numerical values sharing typical letters within the same column do not differ significantly (*P* ≤0.05) according to the LSD test.

Potassium-induced antioxidant defense

The results of the analysis of variance revealed that exogenous application of K and DS had a significant ($P \leq 0.05$) effect on the antioxidant activities in black gram. The DS had a significant impact on the antioxidants, such as catalase, peroxidase and superoxide dismutase in the leaves of black gram compared to the control (Table 3). Under DS, exogenous K application increased the activities of antioxidants such as superoxide dismutase, catalase and peroxidase by 9.45, 8.82 and 7.49%, respectively, in comparison to the control (no potassium application) treatment. On the other hand, when compared to the control, the highest antioxidant activity was found at K_{100} followed by K_{150} and K_{50}. Compared to control plants without potassium supplementation, the K-supplemented plants increased the superoxide dismutase activity by 22.9%, 13.9% and 8.17% by the K_{100}, K_{150} and K_{50}, respectively (Table 3). The 100 mg/L treatment showed comparably better performance for boosting antioxidant activity. However, the antioxidant enzymes increased significantly in response to drought stress after K supplementation, which assisted plants to survive under DS.

Oxidative stress mitigation

Regarding the stress indicators in black gram, an analysis of variance revealed a significant ($P \leq 0.05$) effect of K exogenous application on membrane stability by accumulating more malondialdehyde and H_2O_2 and inflicting more damage to cell membranes in DS conditions than in control with no stress (Table 4). Results showed that MDA content increased up to 12 folds while H_2O_2 and cell membrane injury in leaf tissues increased by 42.2 and 44.6%, respectively, under DS as compared to control. When compared to control with no potassium applied, K application reduced the accumulation of malondialdehyde, H_2O_2 and cell membrane injury. Malondialdehyde, H_2O_2 and cell membrane damage under DS were all reduced by 12.7%, 8.70% and 17.6%, respectively, when K was applied than the control treatment. Maximum reduction of MDA content, H_2O_2 and cell membrane injury at K_{100} followed by K_{150} and K_{50}. Malondialdehyde content in black grams significantly increased because of the interaction between DS and K application.

In this study, K supplementation in varying concentrations ameliorated significantly ($P \leq 0.05$) the negative effects of DS in black gram. Shoot length, leaf area and shoot fresh and dry weight all drastically decreased in response to DS, while root length was pronouncedly increased. With K supplementation, black gram yield and yield components improved in a water-limited environment, likely because K served as a primary osmotic solute for plants under DS. Loss of turgidity is directly related to cell expansion and growth, so water stress has a major impact on plant growth. Likewise, the results showed that the leaf area and the photosynthetic pigments were negatively impacted by DS, leading to a decrease in photosynthetic efficiency. Black gram's chlorophyll content and leaf area were decreased due to water scarcity, which could have diminished the plants' light-harvesting efficiency and led to an excess of oxygen radicals produced by the photosynthetic apparatus (Ihsan *et al.,* 2013). The higher yield was primarily associated with greater leaf area, which provided more surface area for light interception and photosynthesis. Plants under DS were unable to uptake sufficient water, resulting in a reduction in pressure potential and, in turn, a decrease in turgor potential. This impeded plant growth by drying out the protoplasm. Under DS, plant water status and gas exchange attributes were negatively affected, as shown by a disruption in the source and sink ratio in this study, which indicated that leaf water content had a strong correlation with plant's drought tolerance (Khorsand *et al.,* 2021). Further, under DS, stomata begin to close, decreasing stomatal conductance and, consequently, reducing CO_2 availability in the chloroplast, which may result in diminished carbon assimilation. The K application increased stomatal opening and plant growth in response

Table 4: Effect of potassium on oxidative stress of black gram under drought stress conditions.

Treatment	Potassium application (K)	H_2O_2 (µmol g^{-1} FW)	MDA (µmol g^{-1} FW)	Cell membrane injury (%)
Control	NA	0.63d	0.09e	31.4c
	WA	0.63d	0.09e	29.5d
	K_{50}	0.59e	0.08e	25.2e
	K_{100}	0.55f	0.07e	16.1g
	K_{150}	0.57ef	0.08e	21.2f
Drought stress	NA	0.90a	11.8a	41.7a
	WA	0.89a	11.7a	40.2a
	K_{50}	0.82b	10.6b	36.4b
	K_{100}	0.79c	9.32d	28.1d
	K_{150}	0.81bc	9.99c	32.2c
LSD		0.023	0.46	1.30

H_2O_2 (hydrogen peroxide), MDA (malondialdehyde). NA=No application, WA=Water spray, K_{50}, K_{100} and K_{150} represent potassium application at the rate of 50, 100 and 150 mg L^{-1}, respectively. Numerical values sharing typical letters within the same column do not differ significantly ($P \leq 0.05$) according to the LSD test.

to DS by increasing osmotic water uptake and preserving cell turgidity. The DS disrupted the plant's metabolism and reduced crop productivity, whereas exogenously applied K downregulated the lipid peroxidation (malondialdehyde content), H_2O_2 concentration and cell membrane injury along with upregulating gas exchange attributes, plant water relations and antioxidant defense system (Kumar *et al.,* 2020).

Foliage application caused a K influx within the stomatal guard cells, which in turn caused a buildup of water, swelling of the guard cells and a stomatal opening that allowed CO_2 and transpired water vapors to freely move into, out of and throughout the plant tissues (Zhang *et al.,* 2023). The K efflux from the guard cells was stopped and the pores were securely closed to prevent any water loss that would have hampered plant development and cell turgidity. However, the optimized concentration of K boosted plants' ability to absorb and use water under DS. The results showed that K treatments enhanced leaf water relations by decreasing cumulative transpiration water loss. The K foliar feeding supported the critical osmotic pull that aided in drawing water from the roots, whereas its deficiency increased plant susceptibility to adverse effects of DS (Zahoor *et al.,* 2017).

In this study, it was recorded that the oxidative stress indicators such as the MDA concentration increased as a signal of deterioration in plant cells owing to the biosynthesis and excessive accumulation of reactive oxygen species (ROS) by 42.2%. Likewise, the membrane stability was also adversely affected due to the high amount of MDA, which indicated the extent of lipid peroxidation in black gram. Moreover, over-accumulation of reactive oxygen species (ROS) has also been reported as a vital indicator of oxidative damage and the cytotoxic products produced due to lipid peroxidation (Alkhsabah *et al.,* 2018). Overproduction of reactive oxygen species (ROS) caused serious injuries and caused biological membranes to become leaky, allowing electrolytes to move freely within the cell. To reduce the negative effects of drought, black gram plants increased accumulation of plant metabolites such as total phenolics, total soluble proteins and enzymatic antioxidants such as catalase, peroxidase and superoxide dismutase under DS. The findings were consistent with those of Helaly *et al.* (2017), who found that malondialdehyde and hydrogen peroxide were toxic under DS and were responded to by a significant increment in the concentration of enzymatic antioxidants with K supplementation.

These findings corroborated previous research whereby DS caused oxidative stress whereas K application mitigated its deleterious effects by triggering the biosynthesis of antioxidant enzymes and a wide range of plant metabolites (total phenolics, superoxide dismutase, total soluble sugars, peroxidase and catalase) (Jothimani and Arulbalachandran, 2020). By converting O_2 into H_2O_2, which peroxidase also helped to eliminate, superoxide dismutase assisted in the detoxification of O_2. Additionally, peroxidase enzymes are essential for the detoxification of hydrogen peroxide, the oxidation of phenolics and the regulation of cell elongation (Martineau *et al.,* 2017). It was also found that the antioxidant system regulated the delicate balance of oxygen radicals' production and detoxification, which inhibited lipid peroxidation and improved plant water status, gas exchange characteristics, crop growth and seed yield. Moreover, plants with an exogenously applied K had a higher capacity to scavenge ROS (Ul-Allah *et al.,* 2020).

The K supplementation exhibited pronounced potential to protect chlorophyll content and biological membranes under DS by upregulating water relations. The application of K has been shown to reduce ROS biosynthesis by blocking electron transport from photosynthesis to molecule O_2 (Kumar *et al.,* 2019). In an experiment with sunflower (*Helianthus annuus* L.), an adequate supply of K greatly reduced the MDA concentration, demonstrating the importance of K in reducing oxidative stress (Soleimanzadeh *et al.,* 2010). The outcomes have shown that several K-induced mechanisms, including (i) efficient photosynthetic rate and maintenance of turgor pressure, (ii) prevention of drought-induced accumulation of malondialdehyde content, reactive oxygen species (ROS) and cell membrane injury and (iii) upregulation of antioxidant defense system, contribute to the mitigation of DS in black gram.

CONCLUSION

The results were per the postulated hypothesis as black gram under drought stress responded significantly to the exogenous potassium application. The recorded findings of this study imply that the K supplementation holds the potential to alleviate the deleterious impacts through improvements in water relation, morpho-physiological and biochemical traits of black gram, which led to DS tolerance. This study further explored the optimum dose (100 mg/L) of K that could be useful for inducing antioxidant defense and plant fitness against DS. This study provides a mechanistic overview of K involving the enhancement of plant fitness, agronomic yield and DS tolerance in black gram. Exogenous supplementation of K induced a series of morpho-phycological and biochemical traits, which helped to improve plant robustness, agronomic yield and DS tolerance in black gram. These findings might be helpful to pulse breeders and farmers in improving plant fitness and drought adaptation. However, the limitation of this study necessitates conducting future studies to explore the underlying K-mediated mechanisms that impart tolerance against drought stress by neutralizing or minimizing the deleterious effects of reactive oxygen species.

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Disclaimers

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Informed consent

No animals or humans were subjected to experimental treatments in this trial.

Conflict of interest

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