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THE ROLE OF POTASSIUM IN IMPROVING DROUGHT TOLERANCE IN UPLAND COTTON (*GOSSYPIUM HIRSUTUM* **L.)**

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SUMMARY

Cotton (*Gossypium hirsutum* L.) is a vital cash crop in Pakistan, but climate change scenarios threatened its production by biotic and abiotic stress, especially drought. Nutrient management, specifically potassium (K) fertilization, typically alleviates the effects of drought. To this end, a greenhouse experiment evaluated the genotypes for drought stress tolerance and its management by K fertilization. The experiment consisted of 70 cotton genotypes factorially combined with two water levels (standard irrigation and drought stress) and two potassium levels (control and 102 mg/kg of potassium). Data collection occurred for shoot and root lengths, fresh shoot and root weights, dry shoot and root weights, root shoot ratio, total dry matter production, and K uptake after 45 days of germination. Results depicted that mean squares for genotypes, drought, potassium, and their interaction were significant for shoot and root lengths, fresh shoot and root weights, dry shoot and root weights, total dry weight, and potassium uptake, while some traits showed nonsignificant differences. Based on the principal component analysis, membership function value, and genotypic diversity, five genotypes emerged as tolerant: CIM-496, IR-3701, Cp-15/2, FH-113, and CIM-1100, and three, i.e., 4-F, MNH-129, and FH-1000, as susceptible. Tolerant and susceptible genotypes can further benefit breeding programs to develop cotton genotypes adaptable to drought stress and with better K uptake.

Keywords: Cotton (*G. hirsutum* L.), drought, potassium, PCA, cluster, the membership function value

Key findings: The five upland cotton (*Gossypium hirsutum* L.) genotypes, CIM-496, IR-3701, Cp-15/2, FH-113, and CIM-1100, performed better for potassium uptake under drought stress conditions. The three susceptible genotypes were the FH-1000, 4-F, and MNH-129.

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INTRODUCTION

Cotton (*Gossypium hirsutum* L.), also called white gold, is a fiber and edible oil source, making it a valuable cash crop (Salimath *et al.,* 2021). The top five cotton-producing countries with a share in global production are China (24%), India (22%), Brazil (13%), the USA (12%), and Pakistan (6%), and the global production of cotton was 113,458 million bales (480 lb.) (USDA, 2023). Its total value added to agriculture is 1.4%, and its share in Pakistan's GDP is 0.3%. Cotton production in 1991–1992 peaked at 12.822 million bales but declined due to the Cotton Leaf Curl Virus. Subsequent recovery observed an increase to 14.27 million bales in 2004–2005. However, cotton production decreased to 13.60 million bales in 2011–2012 and declined to 4.91 million bales in 2022–2023. For 2022–2023, the cotton sowing areas increased by about 10.7%, but the yield declined (Pakistan Economic Survey, 2022–2023).

The primary reasons behind the reduction of yield were abiotic and biotic stresses, improper fertilizer use, limited genetic diversity, rain patterns, and floods exacerbated by climate change. Climate change caused significant damage to Pakistan's cotton crop during the 2022–2023 season (the season began with a heatwave and ended with floods, drastically affecting cotton yield). An extreme heatwave resulted from a rise in temperature and a decrease in rainfall, affecting cotton germination, leaf withering, and seedling development (Khalid *et al.,* 2022). Cotton plants require a temperature range of 23 °C –32 °C, the best thermal kinetic window for successful metabolic activities (Sajid *et al.,* 2022), sufficient sunlight, and 60–120 cm of rain.

Water stress affects crop development and reduces 30 to 90 percent of the yield depending on the crop (Hussain *et al.,* 2019). Drought substantially influenced plant germination to maturity, causing several physiological changes. Drought reduced cotton yield and growth by roughly 50% and dry matter accumulation by 70% due to osmotic stress, while some genotypes show tolerance (Dash *et al.,* 2022). The root length of some

plants increases under drought, resulting in a reduction of shoot length and more utilization of photosynthates (Khan and Manzoor, 2023). Drought stress also affects the plant's physiology, including changes in stomata conductance, leaf rolling, and root-to-shoot ratio. Water stress-tolerant genotypes exhibit different morpho-physiological changes as compared with susceptible genotypes, such as root and shoot fresh length, lateral root numbers, root and shoot lengths, photosynthesis, and stomatal conductance (Zahid *et al.,* 2021).

Nutrient management and genotype variability can control drought severity. The potassium (K) application can easily manage water stress severity. Potassium (K) is a primary nutrient for various physiological, biochemical, and metabolic functions. It promotes stress tolerance (by regulating the physio and biochemical process) and crop resilience to insects and diseases in a variety of host plants (Wang *et al.,* 2019). A K deficiency hampers cotton plants' development and growth, fresh and dry biomass, fruiting growth distribution, yield, and stress resistance (Fontana *et al.,* 2020).

Cotton is more vulnerable to K than other crops and shows deficiency symptoms due to its shallow root structure. Previous experiments showed that potassium application aids plants in decreasing the adverse effects of water stress on sunflowers (Dar *et al.,* 2021), maize (Ul-Allah *et al.,* 2020), and cotton (Akhtar *et al.,* 2022). Studies also revealed that the available germplasm is more susceptible to stress because of the selection pressure; these genotypes have narrow genetics. Complex genetic processes limit the development of drought-tolerant genotypes, and plants are vulnerable to severe environmental changes (Areej *et al.,* 2021). Developing drought-tolerant cotton genotypes requires screening and selecting suitable parents from the germplasm. Cotton genotype classification used various methods, including the principal component analysis.

The limited genotypes' evaluation studied the impact of potassium uptake under water stress (Zahid *et al.,* 2021; Akhtar *et al.,* 2022). Prior studies have not explored the role of potassium under water stress in seedling stages. Hence, this study evaluated the 70 cotton genotypes collected from all regions of Pakistan against the potassium uptake under drought stress. This information will be helpful in the selection of K-efficient cultivars under drought stress.

MATERIALS AND METHODS

Experiment location and soil

The experiment commenced in the Department of Plant Breeding and Genetics greenhouse, Faculty of Agricultural and Sciences Technology (FAST), Bahauddin Zakariya University, Multan (30°26′ E longitude and 71.50′ N latitude) in September 2020. The experimental material comprised 70 upland cotton (*G. hirsutum* L.) cultivars collected from various research

institutes in Punjab; a list of germplasm utilized in this experiment is available in Table 1.

After removing stones, leaves, and other unwanted elements, the soil sustained sieving and pulverizing (2 mm). The standard method for examining the physicochemical properties of soil (Estefan *et al.,* 2013) also used the soil texture hydrometer method. Electrical conductivity (EC) and pH measurements used the pH meters. For available phosphorus and nitrogen, the study employed Olsen (Olsen, 1954) and Kjeldahl techniques (Bremner, 1996). A Flame Photometer helped determine potassium. The Walkley-Black method with potassium dichromate determined organic matter. The soil has an EC of 30.00, pH of 7.50, 0.32% organic matter, 110.50 ppm potassium, 6.10 ppm phosphorus, 310 ppm nitrogen, a 31.00% saturation rate, and a loam texture.

Table 1. A list of cotton genotypes used in the study.

Experiment treatment and design

Genotype seedlings sown in polythene bags received soil filling, with seeds soaked in water for 12 h before sowing. Each polythene bag (25 cm \times 15 cm) had four seeds of cultivars planted at 3 cm deep. One seedling remained per bag after eight days of germination. The recommended dose of Diammonium phosphate and nitrogen applied was 0.0625 and 0.125 g, respectively. The experiment comprised three treatment factors, namely, irrigation (two levels = standard, with soil moisture kept at field capacity and stress, with soil moisture kept at 50% of the field capacity), potassium (two levels= control and potassium at 102.5 mg/kg of soil), and genotypes (70). All the treatments had a completely randomized factorial design with three replications. Potassium sulfate (K_2SO_4) application began during sowing, and the drought stress application started after 25 days. When a sign of wilting appeared (five days after watering), the drought-stressed plants received irrigation using four drought cycles. All recommended agronomic and protection methods ensured a healthy crop stand. A weekly rotation of the bags ensured all experimental units had uniform conditions.

Data collection

Data recording proceeded from each genotype per replication for shoot length (SL), root length (RL), fresh and dry root weight (FRW, DRW), fresh and dry shoot weight (FSW, DSW), total dry matter production (TDW), root-shoot ratio, and K uptake. Cotton seedlings 45 days after planting proceeded to be extracted from the polythene bags, rinsed with water to remove soil debris, and dried using tissue paper. Samples brought to the laboratory had their shoots detached from the roots by cutting the shoot and root intersection with a cutter. The SL and RL calculations used a centimeter scale (cm) for each replication. Afterward, measuring FSW and FRW continued by placing the sample in a weighing balance in grams (g). Then, the plant sample sustained oven drying at 80 °C until reaching a constant weight. After drying, the samples placed in a

weighing balance for each replication had their DRW and DSW measured.

The content of K determination started by digesting finely powdered shoot and root samples (0.5 g) in a dual acid solution containing perchloric acid (HCLO4) and nitric acid (HNO3). The resulting extract helped determine K with a flame photometer (Hu *et al.,* 2015). The formula served as a sample to compute the K uptake (Zhang *et al.,* 2007), and the formula is:

K uptake = Dry weight \times K concentation

Statistical analysis

The data analysis for its variance (ANOVA) used a completely randomized factorial design (Steel *et al.,* 1997) to establish the significance level of differences in studied traits. The genotypes' grouping used the cluster analysis (CA) and principal component analysis (PCA) methods (Sneath and Sokal, 1973) and the membership function value (MFV) approach (Kumar *et al.,* 2020). Employing R studio obtained ANOVA, visualization, MFV, PCA, and CA on data from all characteristics.

RESULTS

The study on the effects of upland cotton (*G. hirsutum* L.) genotypes, potassium, and drought levels on morphological characteristics and potassium uptake (KU) used a three-factor ANOVA. The mean squares (MS) for genotypes, drought, potassium, and their interaction were significant for all studied traits, while some features showed nonsignificant differences, as shown in Table 2.

The mean performance of SL showed that the highest mean SL was notable under well-watered with K application (14.88 cm), and the lowest mean SL appeared under drought stress with no potassium applied (9.62). A similar pattern occurred for fresh and dry shoots and root weight. However, for RL, the highest mean value emerged under water stress with no K application, and the lowest mean resulted in well-watered, showing RL with no potassium applied (9.238). The results

Table 2. Mean squares (ANOVA) of morphological and physiological traits in response to genotype, application of drought, potassium treatments, and their interactions**.**

Significance level: *: 5%, **: 1%, SL: Shoot length, RL: Root length, KU: Potassium uptake, FSW: Fresh shoot weight, DSW: Dry shoot weight, FRW: Fresh root weight, DRW: Dry root weight, TDW: Total dry weight, and RS: Root shoot ratio.

Table 3. Descriptive statistics for morphological and physiological traits under well-watered and drought-stress conditions, with and without potassium applications.

	Well-Watered				Drought Stress			
Traits	No Potassium applied		Potassium applied		No Potassium applied		Potassium applied	
	Mean \pm SD	Range	Mean \pm SD	Range	Mean \pm SD	Range	Mean \pm SD	Range
SL	13.46 ± 1.68	$9.45 - 17.94$	14.88 ± 1.71	$10.45 - 18.77$	9.62 ± 2.19	$4.78 - 14.36$	11.32 ± 2.22	$5.62 - 15.86$
RL	9.3 ± 2.99	$2.5 - 16.5$	9.28 ± 2.66	$2.83 - 16.47$	10.34 ± 3.22	$2.8 - 17$	9.65 ± 3.41	$3.8 - 16.5$
FSW	1.9 ± 0.56	$0.41 - 2.96$	1.99 ± 0.52	$0.91 - 3.07$	1.04 ± 0.54	$0.1 - 2.3$	1.3 ± 0.56	$0.32 - 2.99$
FRW	0.17 ± 0.07	$0.02 - 0.33$	0.2 ± 0.05	$0.08 - 0.32$	0.09 ± 0.06	$0.01 - 0.29$	0.13 ± 0.07	$0.01 - 0.33$
DSW	1.7 ± 0.56	$0.21 - 2.76$	1.78 ± 0.51	$0.71 - 2.87$	0.84 ± 0.54	$0.01 - 2.1$	1.09 ± 0.53	$0.1 - 2.57$
DRW	0.15 ± 0.06	$0.02 - 0.28$	0.18 ± 0.05	$0.06 - 0.29$	0.08 ± 0.06	$0 - 0.27$	0.11 ± 0.06	$0.01 - 0.29$
RS	0.1 ± 0.05	$0.01 - 0.25$	0.12 ± 0.04	$0.04 - 0.3$	0.1 ± 0.05	$0.01 - 0.3$	0.1 ± 0.05	$0 - 0.3$
TDW	1.19 ± 0.6	$0.14 - 2.78$	1.95 ± 0.52	$0.88 - 3.03$	0.92 ± 0.58	$0.02 - 2.27$	1.85 ± 0.57	$0.25 - 3$
KU	0.48 ± 0.26	$0.04 - 1.29$	0.78 ± 0.24	$0.28 - 1.35$	0.36 ± 0.31	$0.01 - 1.02$	0.74 ± 0.26	$0.13 - 1.32$

of TDW displayed the highest mean value under the application of K either under wellwatered (1.95) or under water stress conditions (1.85). The mean KU was highest under well-watered and K application at 0.78, while the lowest mean value was evident for drought stress and no K application (0.36), as shown in Table 3.

Principal component analysis

Evaluating the performance of 70 cultivars used the principal component analysis (PCA) under potassium and drought stress. The susceptibility index application performed the PCA. PCs with Eigenvalues greater than one can be reliable cultivars (Ara *et al.,* 2018). The Eigenvalues of 10 PCs with variation are available. The first three PCs were choices because their drought (DSI) and potassium susceptibility index (KSI) Eigenvalues are greater than one. The first three principal components explained about 78.72% of the overall variation for the DSI, accounting for a cumulative variance of 78.726%.

In comparison, the first four major components account for 90.38% of the total variation in KSI, meaning that they explain almost 90% of the overall variance (Table 4). In the first PCA, DSW, FSW, and KU show a high and positive contribution; for the second PC, the SL contribution to variability was high

in DSI. However, for KSI, the DSW, FSW, SL, and KU positively contributed to variability in the first PC; for the second PC, DRW, FRW, and KU provided high variability, as given in S2. The biplot analysis displayed that cultivars CIM-506, NIAB-78, MNH-552, and CP-15/2 were suitable for SL for DSI, cultivars MPS-11 and CIM-496 were fitting for SL, MNH-886, NIAB 78, BH-172, and BH-118 were more appropriate for FRW and DRW for KSI, as shown in Figure 1.

Cluster analysis

The cluster analysis indicated that Cluster 2 contains the highest average value for SL (13.29), RL (12.62), FRW (0.29), FSW (1.83), DRW (0.27), DSW (1.63), and KU (0.83), based on DSI compared with the other three clusters (Table 5). Cluster 2 contains 18 genotypes for DSI and 28 for KSI. However, based on the K susceptibility index, Cluster 2 consists of the maximum average value for SL (12.97), RL (11.89), FRW (0.24), FSW (1.75), DRW (0.22), DSW (1.55), and KU (0.76). The genotypes' selection relied on Cluster 2 because these genotypes' performance was high compared with other clusters (Table 5). The genotypes for Cluster 2 under DSI and KSI were CIM-707, CIM-1100, FH-113, MPS-11, and CP-15/2 (Figures 1c and d).

Table 4. Eigen values and Eigen vectors for four PCs for cotton genotypes under the drought and potassium susceptible index.

Figure 1. Biplot analysis and Dendrogram illustrating hierarchical clustering of morphological parameters under the drought susceptibility index (A, C) and potassium susceptibility index (B, D). Arrows indicate individual traits, and points represent genotypes. Abbreviations: SL (shoot length), RL (root length), FSW (fresh shoot weight), FRW (fresh root weight), DSW (dry shoot weight), DRW (dry root weight), TDW (total dry weight), RS (root-shoot ratio), KU (potassium uptake).

Table 5. Mean performance of traits within clusters based on the drought susceptibility index (DSI) and potassium susceptibility index (KSI).

SL: Shoot length, RL: Root length, FSW: Fresh shoot weight, FRW: Fresh root weight, DSW: Dry shoot weight, DRW: Dry root weight, TDW: Total dry weight, RS: Root shoot ratio, KU: Potassium uptake.

Membership function value (MFV)

The estimated MFV values of the 70 cultivars under two water levels (well-watered and control conditions) and K application (no K applied and K applied) achieved classification into four groups. The results of the MFV showed that the drought tolerance (DT) was 17, the moderate drought tolerance (MDT) was 37, and the drought susceptible (DS) was 16. Only 15 genotypes attained potassium efficient (KE) classification, 45 as moderate K efficient (MKE), and 10 as K inefficient (KI). The top five performing genotypes are CIM-496, IR-3701, Cp-15/2, FH-113, and CIM-1100; these genotypes fall in the tolerance group, and susceptible genotypes are MNH-129, 4-F, and FH-901 (Figure 2).

DISCUSSION

Drought stress has drastically affected cotton growth and production. Climate change makes it worse. Water stress can be manageable through nutrition management or by

developing new cotton cultivars with more genetic potential to withstand drought stress. Prior studies showed that applying potassium (K) helps plants survive drought stress (Akhtar *et al.,* 2022). Cotton plants are more vulnerable to K deficiency than other crops (Denton *et al.,* 2023). It is essential to plant development and growth; plant dry matter ranges from 2% to 10% (Tian *et al.,* 2023).

The results showed that significant variation was evident for all studied characteristics, and their interaction proved substantial. Enough variation resulted in genotypes under water and their interactions with the K application. The average performance of all cultivars revealed that SL decreased during drought stress compared with normal conditions. SL increased in water stress and well-watered conditions when applied with K. By effectively utilizing available water and controlling stomata movement, SL helps cotton plants endure droughts by improving their ability to minimize water stress and transpiration losses (Shavkiev *et al*., 2022).

Figure 2. Comparison of average membership function values (MFVs) across drought (tolerant, moderately tolerant, and susceptible) and potassium use efficiency (efficient, moderately efficient, and inefficient) groups and individual genotypes.

A similar pattern also emerged for FRW, FSW, DSW, and DRW (Zahid *et al.,* 2021). The K application aids plants in lowering the effects of stress (Hussain *et al.,* 2019). Under drought conditions, K treatment has manifested linkages to enhanced FSW, DSW, RL, and DRW. It could refer to the K's role in cellular activity and water uptake, which aids plants in dealing with the negative impacts of water stress (Studer *et al.,* 2017).

A high mean RL value was prominent under water stress compared with wellwatered conditions, while applying K reduced the RL (Khan and Manzoor, 2023). The plant response to stress caused the increase in RL, helping plants absorb water from deeper soil layers. It will help the plant resist drought by improving its ability to absorb water and nutrients from the soil. Furthermore, genetic factors may influence RL's growth, as some cultivars seemed to have longer roots under water stress (Shahzad *et al.,* 2021). The studies revealed that K application might increase the electrical conductivity of soil, adversely affecting root growth (Zhao *et al.,* 2020).

The K application improved the KU under well-watered conditions, followed by drought stress. The MFV helped select droughttolerant and potassium-efficient cotton genotypes. In prior studies, they reported the successful use of MFV to select droughttolerant genotypes in sugar cane hybrids (Jin *et al.,* 2020). Similarly, the MFV applied in soybeans aided in identifying water stresstolerant genotypes during the vegetative and reproductive growth phases (Yan *et al.,* 2020). Developing K-efficient genotypes under drought stress tolerance was not an easy process. Principal component analysis (PCA) served to select genotypes with better K uptake under stress because it helps identify genetic diversity against drought tolerance. PCA is handy when choosing from a large germplasm with multiple traits simultaneously (Mari *et al.,* 2022). The RL and SL showed significant decreases by water stress, but genotype differences were apparent. Most cotton varieties perform significantly worse under drought stress, while CIM-707, CIM-1100, FH-113, MPS-11, and Cp-15/2

performed best under normal and drought stress.

Root and shoot characteristics perform/respond less efficiently due to insufficient water availability. Prior studies reported the association of root characteristics with enduring drought stress (Iqbal *et al.,* 2010). When subjected to drought stress, the tolerant cultivars displayed longer roots, which explains their capacity to survive stress; a similar trend occurred in the presented study. The ability of plants to tolerate stress indicated the reliability of roots, even though they are more sensitive than shoots. Drought stress reduces stem elongation, the root-shoot ratio of cotton plants, and leaf size (Pettigrew, 2004). The results of our experiment supported those of other research (Reddy *et al.,* 2017), which found that water stress reduces shoot growth. Applying K increased SL and K uptake, and a discovery showed that K content in the shoot has a K rate and uptake regulating it (Fageria *et al.,* 2011). Another study reported that cotton's nitrogen and K content lowered under water stress (McWilliams, 2003).

Additionally, it was noteworthy that tolerant cultivars had deeper roots than susceptible ones. Drought reduces SL, dry root, and shoot weight (Ashraf and Foolad, 2007). As a result, cultivars with improved RL, SL, and KU are choices for future breeding to reduce the negative impact of water stress on cotton by applying K. Based on PCA, cluster, and membership function value, eight genotypes were selections. The five tolerant genotypes were CIM-496, IR-3701, Cp-15/2, FH-113, and CIM-1100, and three susceptible genotypes were 4-F, FH-1000, and MNH-129. Tolerant and susceptible genotypes can benefit further breeding programs to develop cotton genotypes adaptable to climate change scenarios.

CONCLUSIONS

Drought stress is an emerging threat to cotton, and climate change worsens it. Potassium (K) fertilization has proven to alleviate the effects of drought. The results suggested significant differences for all traits, and the highest mean shoot length occurred under well-watered conditions with potassium application. The lowest was under drought stress without potassium in upland cotton (*G. hirsutum* L.) genotypes. The principal component analysis of 70 cultivars under potassium and drought stress revealed that the first three principal components explained about 78.72% of the overall variation for the drought susceptibility index, and for potassium susceptibility index was 80.48%. Cluster analysis indicated that Cluster 2, containing 18 genotypes for DSI and 28 for KSI, had the highest average values for most traits. Membership Function Value (MFV) classified the cultivars into four groups based on drought and potassium efficiency. The top performers were CIM-496, IR-3701, Cp-15/2, FH-113, and CIM-1100.

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