

Growth, Water Relations And Chlorophyll Enhancement By Potassium Application In Intercropping System Of *Gossypium Hirsutum* L. And *Cajanus Cajan* (L.) Millsp. Under Different Irrigation Regimes

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Abstract

Introduction: Plant encounters various biotic and abiotic stress throughout their life cycle. Among abiotic stress, drought stress decreases growth and photosynthetic rate in crop plants which in turn decreases overall yield of food crops.

Methods: The present study investigated the impact of potassium on plant height, fresh and dry weight of plant, leaf area, leaf area index, relative water content, membrane stability index, electrolyte leakage and photosynthetic pigments in intercropping system of *Gossypium hirsutum* L. (RCH-773 variety) and *Cajanus cajan* (L.) Millsp. (Manak variety). The treatments were as given i.e. control (full irrigation), early water stress (no irrigation at vegetative stage), late water stress (no irrigation at flowering stage) and stress (no irrigation) along with three potassium concentration K₀, K₁, K₂ (0, 20, 60 kg/acre respectively). The sampling was done at vegetative stage (40 days after sowing).

Results: A long progressive water stress significantly reduced plant height, fresh and dry weight, leaf area, leaf area index, relative water content, membrane stability index and photosynthetic pigments. But potassium fertilizer enhanced all these parameters at a par value. The other parameter i.e. electrolyte leakage was increased in stress conditions. But after potassium application, electrolyte leakage showed a remarkable decrease in control as well as water stressed plants.

Conclusion: The findings of present study clearly shows that potassium alleviates the negative impact of drought stress. It means potassium is a strong osmo-regulator, maintains cation-anion balance and helps plant withstanding drought stress conditions.

KEYWORDS: Carotenoids, Electrolyte leakage, Leaf area, Water stress.

INTRODUCTION: Deficiency of water is the main hurdle in productivity of agriculture.^[1] Decreased capacity of photosynthesis and increased leaf senescence indicates a water deficit condition which in turn reduces crop yields.^[2] It may also leads to growth reduction and generates a situation of nutritional imbalance which may alleviated by applying some nutritional elements in soil profile.^[3]

Being a perennial crop, *Gossypium hirsutum* L. is grown as an annual crop in various countries. Mostly used parts of cotton plant are cotton buds. Cotton fibres are considered as the basic raw material for textiles product, paper industry, edible oil and medicinal compounds.^[4] After lubrication, remnants of hulls or seed meal can be utilised for livestock feed.^[5]

Cajanus cajan (L.) Millsp. is the second major legume crop after chickpea in tropical and sub-tropical countries. It can be eaten as a fresh vegetable in Karnataka, Gujarat and Maharashtra and commonly consumed as split dal. Apart from these, the pigeonpea is used as a vegetable in tribal areas.^[6]Pigeonpea can be grown as a sole crop or intermixed with other cereal crops.

As an ancient technique, intercropping is a practice of growing two or more distinct crops in rows simultaneously in a particular growing period.^[7,8] Intercropping has well documented biological and ecological benefits. Competitiveness of crops and resource usage is increased on a particular area in case of intercropping.^[9] Xu et al. ^[10] reported that intercropping is also called as the “new green revolution” and it can be used to attain sustainable agricultural intensification by increasing land use efficiency.

Various biochemical and physiological processes in plants are controlled by an essential element i.e. potassium which affects growth, reproduction and development of crop plants. Amelioration of biotic or abiotic stresses is mainly done by potassium application. Potassium is also involved in carbohydrates metabolism, protein synthesis, osmoregulation, energy transfer, movement of water and cation-anion balance.^[11] The involvement of potassium in water relations and stomatal regulations has confers its vital role in water stress resistance in plants.^[12]

MATERIALS AND METHODS:

Experimental site and plant material: Intercropping of RCH-773 and Manak, was done in a field trial experiment in kharif season of 2021 and 2022 from April to November in Kurukshetra University, Kurukshetra, India (29°95’N; 76°82’E). The randomised complete block design along with three replicates was applied on field having block size 2m x 2m. Row intercropping of ratio 1:1 was chosen having interspacing of 25 x 15 cm. The seeds of both the crops were collected from cotton and pigeon pea sections of CCSHAU (Chaudhary Charan Singh Hisar Agricultural University), Hisar, Haryana.

Treatments: Treatments were done with two factors i.e. irrigation regimes and potassium application “Table 1”.

Sr No.	Treatment	Code	Details of Operations
1.	T ₁	C + K ₀	Control (four irrigation at all critical stages)+K ₀
2.	T ₂	C + K ₁	Control (four irrigation at all critical stages)+K ₁
3.	T ₃	C + K ₂	Control (four irrigation at all critical stages)+K ₂
4.	T ₄	EWS + K ₀	Early water stress (no irrigation at vegetative stage)+K ₀
5.	T ₅	EWS + K ₁	Early water stress (no irrigation at vegetative stage)+K ₁
6.	T ₆	EWS + K ₂	Early water stress (no irrigation at vegetative stage)+K ₂
7.	T ₇	LWS + K ₀	Late water stress (no irrigation at flowering stage) +K ₀
8.	T ₈	LWS + K ₁	Late water stress (no irrigation at flowering stage) +K ₁
9.	T ₉	LWS + K ₂	Late water stress (no irrigation at flowering stage) +K ₂
10.	T ₁₀	S + K ₀	Stress (no irrigation)+K ₀
11.	T ₁₁	S + K ₁	Stress (no irrigation)+K ₁
12.	T ₁₂	S + K ₂	Stress (no irrigation)+K ₂

“Table I: Here T₁.....T₁₂ are treatments, C(control), EWS(early water stress), LWS(late water stress), S(stress) and K₀, K₁, K₂ are 0, 20, 60 kg/acre respectively.

Soil testing report and Weather information: The field was properly ploughed before sowing of plants and soil testing was done in soil testing lab, Karnal, Haryana. Soil phytochemical analysis showed organic carbon 0.84(%), phosphorus>39.74(ppm), potassium=376(ppm), sulphur=15.54(ppm). The soil was having a pH of 7.5. The average day and night temperatures were $44^{\circ}\pm 5$ and $24^{\circ}\pm 6^{\circ}$ C respectively.

Plant characterisation and data analysis:

Growth parameters: Plant height, fresh and dry weight of plant was estimated by measuring scale and weighing balance respectively. Leaf area was measured by leaf area meter and leaf area index by using formula: $LAI = \text{Leaf area} / \text{Land area occupied by a plant}$.

Water relations: Relative water content (RWC) was calculated by Weatherly^[13], method. For this, uppermost leaf was weighed. The turgid weight was calculated after placing leaf in distilled water for 8 h. After that oven drying of leaf at 80°C for 72 h was done and then computation of dry weight (DW) was done. Then formula was applied: $RWC (\%) = \frac{FW - DW}{TW - DW} \times 100$. For estimating MSI, 100 mg leaf samples with 10 ml double distilled water were taken in two test tubes. One test tube placed in water bath at 40°C for 30 minute for EC_1 . The second test tube was boiled at 100°C for 10 minute and EC_2 was measured by electrical conductivity meter. Then, Membrane stability index (MSI) was determined by Deshmukh et al. ^[14] i.e. $MSI (\%) = [1 - (EC_1/EC_2)] \times 100$. Electrolyte leakage (EL) was calculated by Lutts et al. ^[15] method. 1 g of leaves was slashed into tiny pieces and discharged all electrolytes. Dip these pieces into 20ml of distilled water and autoclaving was done for 20 minutes at 120°C and measured EC_1 . Now the solution was cooled to around 25°C and EC_2 was calculated. $EL (\%) = EC_1 \times EC_2 / EC_2$.

Photosynthetic pigment analysis: Chlorophyll (Chl) content was measured by Arnon ^[16] method. 100 mg of leaf samples were grounded in 10 ml of 80% acetone along with a pinch of CaCO_3 . Then, centrifugation was done at 5000 rpm for 20 minutes, supernatant was collected and pallet was repeatedly extracted with 80% acetone. Now, absorbance was taken at 645nm for Chl a, 663nm for Chl b and 480nm for carotenoids.

$$\text{Chl a} = \frac{12.3 (A_{663}) - 0.86 (A_{645}) \times V}{\alpha \times 1000 \times w} \quad \text{Chl b} = \frac{19.3 (A_{645}) - 3.6 (A_{663}) \times V}{\alpha \times 1000 \times w}$$

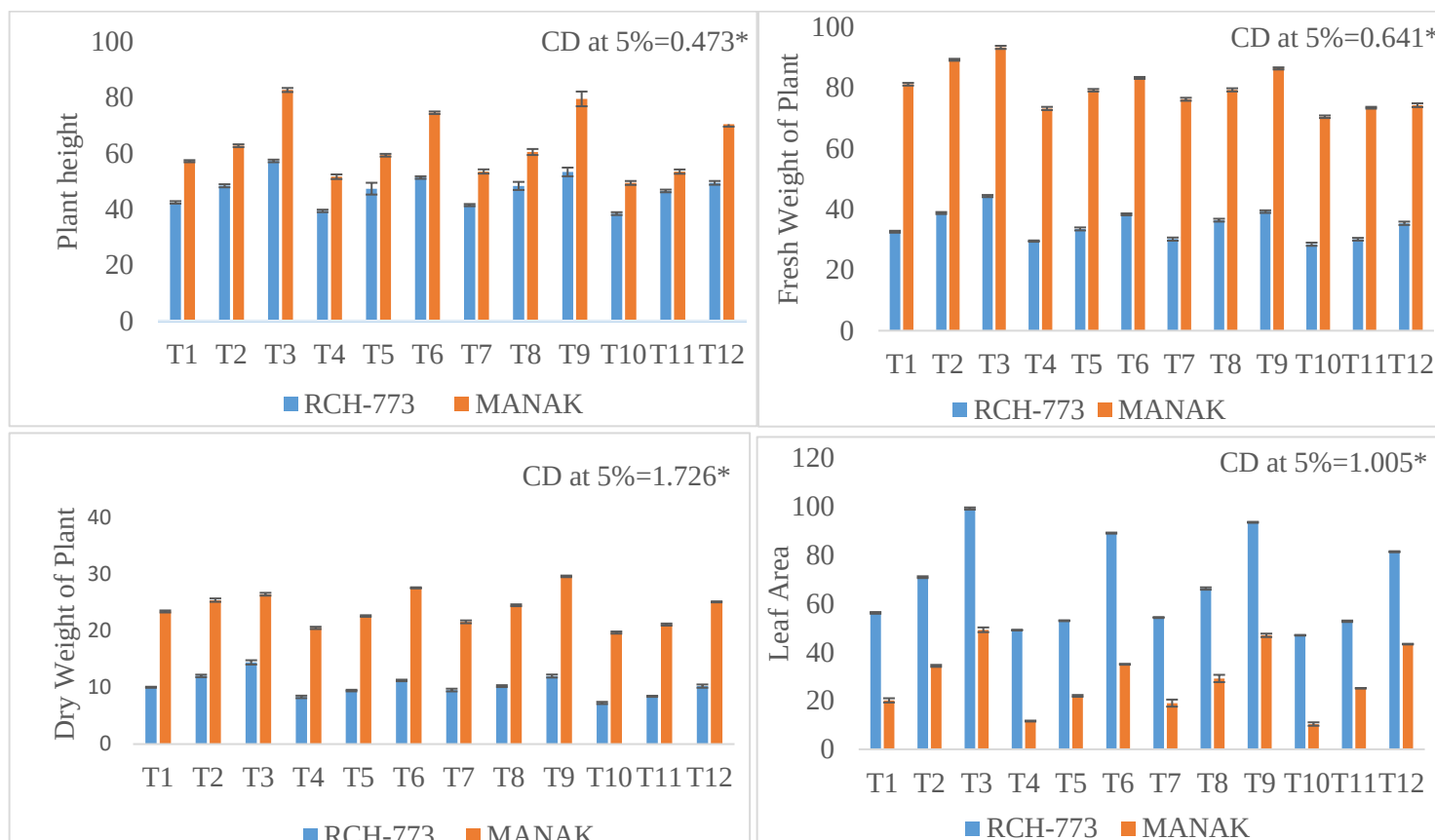
Total Chl = Chl a + Chl b

$$\text{Carotenoids} = \frac{7.6 (A_{480}) - 1.49 (A_{510}) \times (V/d \times 1000 \times W)}{\alpha \times 1000 \times w}$$

Statistical Analysis: The analysis of data was done by software OPSTAT 2.0 of Chaudhary Charan Singh Haryana Agricultural University (CCSHAU), Hisar. The critical difference was used to compare means and was calculated at the 5% level of significance.

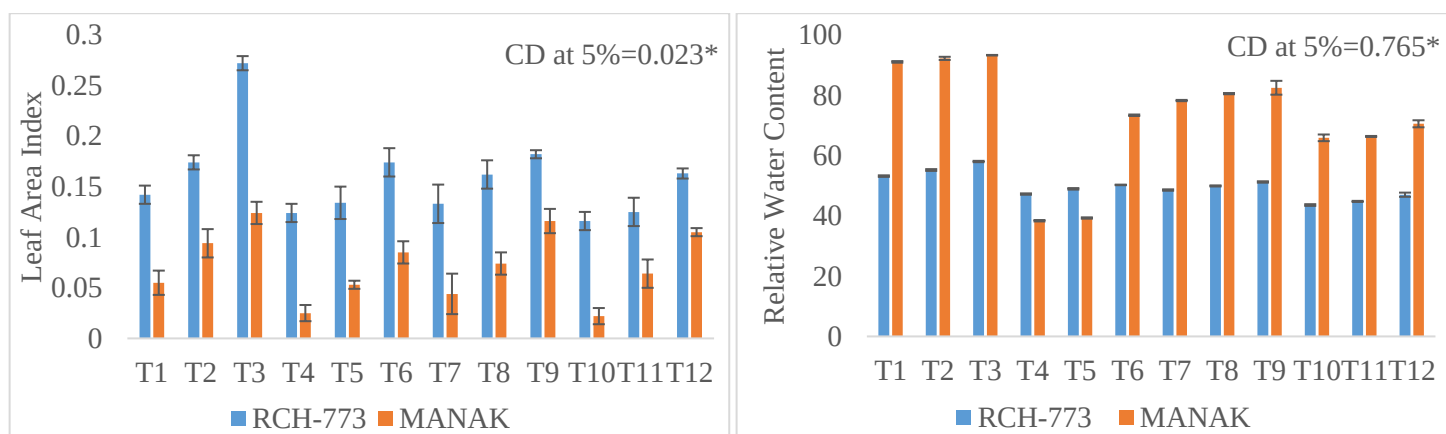
RESULTS:

A significant interaction was seen in potassium and irrigated water on vegetative growth characteristics. Water deficit conditions resulted in decreased plant height in both the cultivars “Figure 1”. The plant height was reduced from 42.473 ± 0.44 to 38.457 ± 0.49 cm in RCH-773 and 57.203 ± 0.371 to 49.423 ± 0.696 cm in Manak variety.



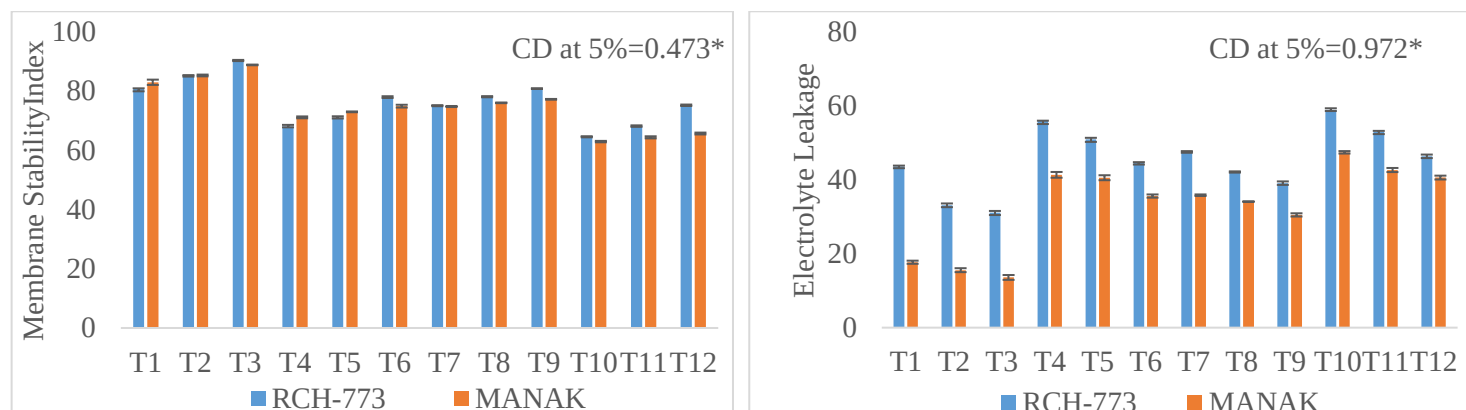
“Figure 1: Plant height (cm), FWP (gm), DWP (gm) and LA (cm²) of RCH-773 and Manak variety under different water stress treatments. Small bars shows standard error, critical difference (CD) was calculated at P<0.05. Symbol* indicates the interaction of genotype×water treatment×fertilizer treatment, T represent treatments.”

Same dramatic decline was also reported in FWP, DWP, LA, LAI, RWC and MSI under moisture stress. Lowest RWC and MSI were observed in T₁₀ treatment i.e., 43.577±0.281% for RCH-773 and 65.863±1.107% for Manak, 64.623±0.131% for RCH-773 and 62.947±0.238% for Manak, respectively “Figure III”. The reduction in MSI was due to membrane disorganisation. Moreover, control plants with full irrigation maintained a better MSI than water stressed plants.



“Figure II: LAI and RWC (%) of RCH-773 and Manak under different water treatments. Small bars in figure shows standard error, critical difference (CD) was calculated at P<0.05. Symbol* indicates the interaction of genotype×water treatment×fertilizer treatment, T represent treatments.”

However, potassium fertilizer increased all these parameter at a great extent. Maximum increase in height was demonstrated in T₃ treatment (57.298±0.466 cm in RCH-773 and 85.657±0.721 cm in Manak variety). Highest FWP, DWP, leaf area “Figure 1” LAI, RWC “Figure II” and MSI “Figure III” were also estimated in T₃ treatment i.e., 44.365±0.046 gm in RCH-773 and 93.346±0.015 gm in Manak, 14.458±0.378 gm in RCH-773 and 20.487±0.447 gm in Manak, 99.093±0.433 cm² in RCH-773 and 49.19±0.966 cm² in Manak and 0.272±0.017 in RCH-773 and 0.124±0.051 in Manak, 43.577±0.281% for RCH-773 and 65.863±1.107% for Manak, 90.25±0.28% for RCH-773 and 88.837±0.116% for Manak, respectively. But the effect of potassium on plant height of Manak was more pronounced than RCH-773 which is clearly visible from the results.



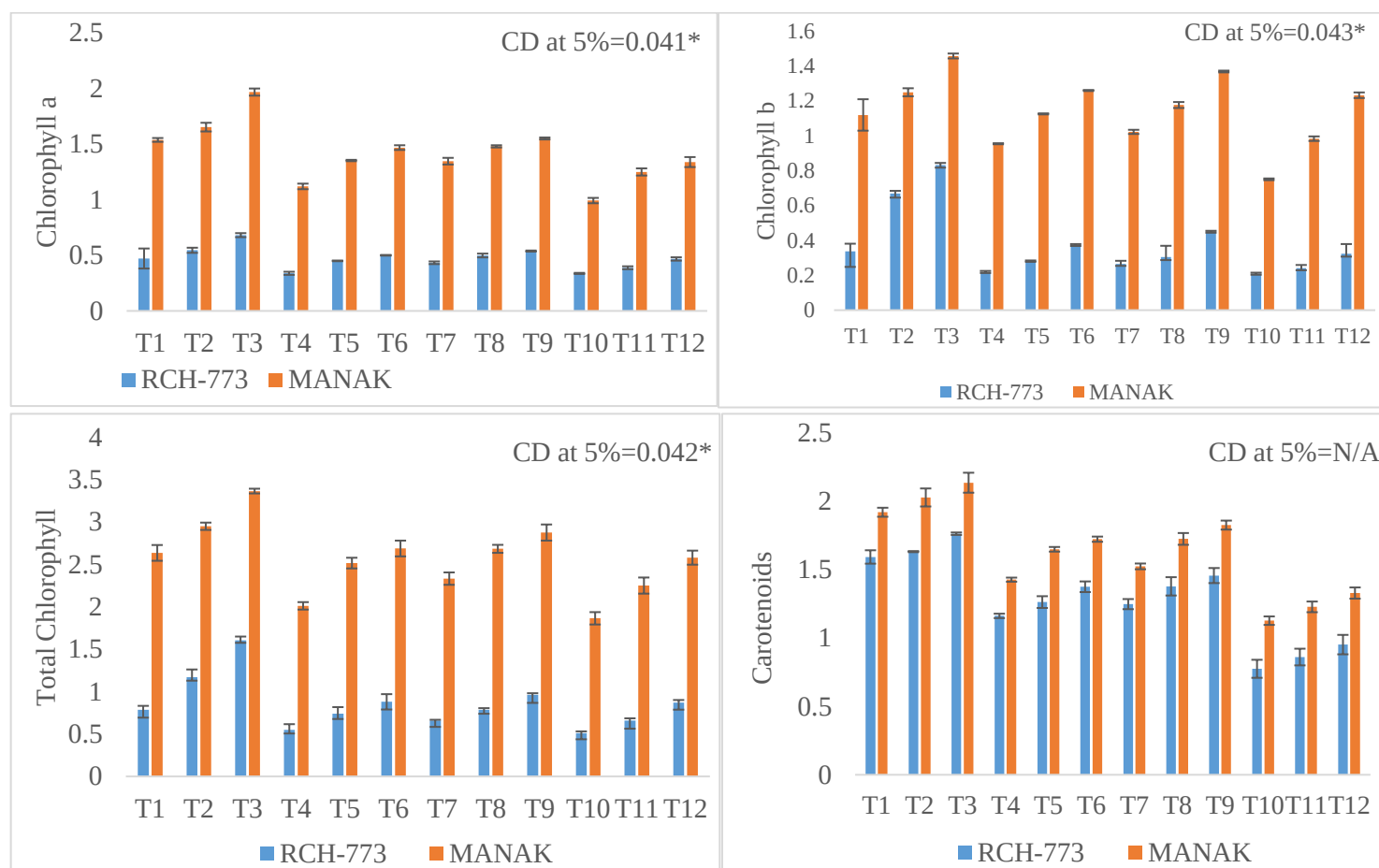
“Figure III: MSI (%) and EL (%) of RCH-773 and Manak under different water stress treatments. Small bars in the figure shows standard error, critical difference (CD) was calculated at $P < 0.05$. Symbol* indicates the interaction of genotype×water treatment×fertilizer treatment, T represent treatments.”

Apart from above parameters, electrolyte leakage “Figure III” was increased in water deficit conditions. Here, potassium application decreased electrolyte leakage significantly both in well-watered and stressed plants. Electrolyte leakage was highest in T₁₀ treatment (58.953±0.01% for RCH-773 and 53.198±0.033% for Manak) and lowest in T₃ treatment (31.13±0.028% for RCH-773 and 22.358±0.054% for Manak). It simply means that disorganisation of membrane is also associated with higher ions leakage from plant leaves.

Photosynthetic pigments:

Chlorophyll:

The present investigation showed a clear interaction of potassium fertilizer and different irrigation schedules in both varieties. Chlorophyll content was dramatically declined in severe water stress. Long progressive water stress decreased photosynthetic pigments such as Chl a, Chl b, total Chl and carotenoids as shown in “Figure 1”. The same level of decrement was observed in treatment combination T₄ and T₁₀ for both the cultivars. But a progressive increase in photosynthetic pigments was observed after supplementation of potassium. Highest increase was observed in T₃ treatment where 60kg/acre potassium was applied.



“Figure IV: Chl a (mgg^{-1}FW), Chl b (mgg^{-1}FW), Total Chl (mgg^{-1}FW) and Carotenoids (mgg^{-1}FW), of RCH-773 and Manak under different water stress levels along with potassium concentration of K_0 (0 kg/ acre), K_1 (20 kg/acre), and K_2 (60 kg/acre). Small bars in figure shows standard error, critical difference (CD) was calculated at $P < 0.05$. Symbol* indicates the interaction of genotype $\times$ water treatment $\times$ fertilizer treatment, T (Treatments), N/A(not applicable).”

Chl a was increased from 0.337 ± 0.005 (SK_0) to 0.467 ± 0.015 (SK_2) mgg^{-1}FW for RCH-773 and 0.992 ± 0.023 (SK_0) to 1.338 ± 0.045 (SK_2) mgg^{-1}FW for Manak, Chl b from 0.208 ± 0.007 (SK_0) to 0.323 ± 0.056 (SK_2) mgg^{-1}FW for RCH-773 and 0.992 ± 0.023 (SK_0) to 1.232 ± 0.016 (SK_2) mgg^{-1}FW for Manak, total Chl from 0.511 ± 0.02 (SK_0) to 0.870 ± 0.031 (SK_2) mgg^{-1}FW for RCH-773 and 1.864 ± 0.073 (SK_0) to 2.579 ± 0.083 (SK_2) mgg^{-1}FW for Manak and carotenoids from 0.775 ± 0.066 (SK_0) to 0.952 ± 0.071 (SK_2) mgg^{-1}FW for RCH-773 and 1.127 ± 0.03 (SK_0) to 1.329 ± 0.041 (SK_2) mgg^{-1}FW for Manak in stress conditions where no irrigation was given but highest amount of potassium fertilizer was added. Though treatment combinations T₁, T₇ and T₅, T₁₂ was having approximately same level of photosynthetic pigments.

DISCUSSION:

The reduction of plant height may be due to protoplasm degradation, decreased relative turgidity, cell division and cell expansion. [17] The present results were also in concurrence with *Gossypium barbedence* L. in which all growth parameters of plant (dry weight, plant height, relative water content and leaf area) were significantly increased in salt stress by potassium citrate application. It means that potassium is amplifying plant growth by CO_2 assimilation, increasing leaf efficiency, regulating cellular pH, activating enzymatic system, increasing N_2 uptake and activating enzymatic system. [18]

The same findings were also reported in Faba beans in which highest values of branching number, plant height, dry matter and leaf area were calculated in control condition with 150kg/ha of potassium fertilizer (K_2O) for the two seasons. [19] Same findings were seen in *Brassica juncea* (L.) Czern cultivars in which all morphological parameters i.e. the plant height, leaf area, FW and DW were increased in blocks having highest concentration of potassium sulphate. [20] The results simply points out that block with potassium level of 60kg/acre resulted in increased growth parameters of both the varieties. The biochemical characteristics (proline and soluble sugars) and physiological attributes

(WUE, RWC, MSI and chlorophyll content) also showed a positive reaction in response to introducing water stress along with potassium application. ^[21]

A significant interaction was also seen between potassium treatment and chlorophyll content under control and water stress conditions. There was drastic decline in chlorophyll content during long progressive stress which was due to lower capacity of light harvesting. Hsu and Kao, ^[22] also found a dramatic decline in total chlorophyll amount in moisture stress induced by PEG in rice leaves. This reduction in chlorophyll may be attributed to generation of reactive oxygen species (O₂ and H₂O₂) which causes peroxidation of lipids and destruction of chlorophyll changing leaf green colour to yellow. Hassanzadeh et al. ^[23] reported that Chl a is less sensitive than Chl b. But potassium application increases Chl a, Chl b, total Chl and carotenoids in well-watered and water stressed plants. It means potassium increases the chlorophyll efficiency, thus increases photosynthesis. Potassium also inhibits the transporters and porins in membrane of thylakoid in chloroplasts which in turn increases carotenoids content ^[24,25] That's why the total chlorophyll and carotenoids was increased in both the cultivars of our study after potassium application.

CONCLUSION:

The present investigation found a profound effect of water stress on growth, photosynthetic and water related parameters. The study evaluated a great enhance effect of all parameters under potassium application. All these results show that potassium plays an important role in alleviating the negative impact of water deficit conditions in both the cultivars of intercropping as it is involved in various biochemical and physiological activities like photosynthesis, synthesis of protein, metabolic enzymes activation, regulation of stomata opening and closing and photo-assimilates translocation into sink organs.

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REFERENCES

1. Nyawade S, Gitari HI, Karanja NN, Gachene CKK, Schulte-Geldermann E, Parke M. Yield and evapotranspiration characteristics of potato-legume intercropping simulated using a dual coefficient approach in a tropical highland. *Field Crop Res.* 2021 274: 108327
2. Raza MA, Gul H, Wang J, Yasin HS, Qin R, Khalid MHB, et al. Land productivity and water use efficiency of maize-soybean strip intercropping systems in semi-arid areas: A case study in Punjab Province, Pakistan. *J. Clean Prod.* 2021 308: 127282.
3. Nyawade S, Gitari HI, Karanja NN, Gachene CK, Schulte-Geldermann E, et al. Enhancing climate resilience of rain-fed potato through legume intercropping and silicon application. *Front Sustain. Food Sys.* 2020 4:566345
4. Loka DA, Oosterhuis DM, Baxevanos D, Noulas C, Hu. Single and combined effects of heat and water stress and recovery on cotton (*Gossypium hirsutum* L.) leaf physiology and sucrose metabolism. *Plant Physiol. Biochem.* 2020 148: 166–1793.
5. Gao X, Guo H, Zhang Q, Guo H, Zhang L, Zhang C. Arbuscular mycorrhizal fungi (AMF) enhanced the growth, yield, fiber quality and phosphorus regulation in upland cotton (*Gossypium hirsutum* L.). *Sci. Rep.* 2020 10: 1–1230.
6. Saxena KB, Kumar RV, Gowda CLL. Vegetable pigeonpea – a review. *Journal of Food Legumes.* 2010 23: 91-98
7. Lithourgidis A, Dordas C, Damalas CA, Vlachostergios D. Annual intercrops: an alternative pathway for sustainable agriculture. *Australian Journal of Crop Science* 2011 5:396-410
8. Federer WT. Statistical design and analysis for intercropping experiments. 2012 Vol. 1
9. Chang X, Yan L, Naeem M, Khaskheli MI, Zhang H, Gong G. Maize/soybean relay strip intercropping reduces the occurrence of *Fusarium* root rot and changes the diversity of the pathogenic *Fusarium* species. *Pathogens* 2020 3: 211.
10. Xu Z, Li C, Zhang C, Yu Y, Van der Werf W, Zhang F. Intercropping maize and soybean increases efficiency of land and fertilizer nitrogen use. A meta-analysis. *Field Crop Research.* 2020 246:107661.
11. Wang M, Zheng Q, Shen Q, Guo S. The critical role of potassium in plant stress response. *Int. J. Mol. Sci.* 2013;14:7370- 7390
12. Bahrami-Rad S, Hajiboland R. Effect of potassium application in drought-stressed tobacco (*Nicotiana rustica* L.) plants: Comparison of root with foliar application. *Annals of Agricultural Sciences.* 2017. 62:121–130.
13. Weatherley P. Studies in the water relations of the cotton plant. The field measurement of water deficits in leaves. *New Phytol.* 1950 49: 81-87.
14. Deshmukh PS, Sairam RK, Shukla DS. Measurement of ion leakage as a screening technique for drought resistance in wheat genotypes. *Ind. J. Plant Physiol.* 1991 34: 89-91.
15. Lutts S, Kinet JM, Bouharmont J. NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. *Ann Bot.* 1996 78: 389-398.
16. Arnon DI. Copper enzymes in isolated chloroplasts, polyphenoxidase in *Beta vulgaris*. *Plant physiol.* 1949 24: 1-15.

17. Hussain M, Malik MA, Farooq M, Ashraf MY, Cheema MA. Improving drought tolerance by exogenous application of glycinebetaine and salicylic acid in sunflower. *J. Agron. Crop Sci.* 2008 194: 193-199
18. Sadak MS, Orabi SA. Improving thermos-tolerance of wheat plant by foliar application of citric acid or oxalic acid. *International Journal of Chem. Tech. Research* 2015 8(1), 333-345
19. Fawzy S, Samie A, Ali AH, Rady MOA, Abd El –Mageed SA. Integrative effect of potassium level and deficit irrigation on physiological response, yield and water use efficiency of Faba bean under salt affected soil. *Fayoum Journal of Agricultural Research and Development.* 2021 35:348-362
20. Rani P, Saini I, Singh N, Kaushik P, Wijayal L, Al-Barty A, et al. Effect of potassium fertilizer on the growth, physiological parameters, and water status of Brassica juncea cultivars under different irrigation regimes. *Plos One* 2021 <https://doi.org/10.1371/journal.pone.0257023.t001>
21. Islam MR, Kamal MM, Alam MA, Hossain J, Soufan W, Skalicky M. Physiochemical Changes of Mung Bean [*Vigna radiata* (L.) R. Wilczek] in Responses to Varying Irrigation Regimes *Horticulturae*. 2021 7:565
22. Hsu SY, Kao CH. Differential effect of sorbitol and polyethylene glycol on antioxidant enzymes in rice leaves. *Plant Growth Regul.* 2003 39:83-90.
23. Hassanzadeh MA, Ebadi M, Panahyan-e-Kivi AG, Eshghi S, Jamaati-e-somarin M, Saeidi R. Evaluation of drought stress on Relative water content and chlorophyll content of Sesame (*Sesamum indicum* L.) genotypes at early flowering stage. *Res. J. Environ. Sci.* 2009 3 (3):345-360.
24. Ahmad P, Jaleel CA, Salem MA, Nabi G, Sharma S. Roles of enzymatic and nonenzymatic antioxidants in plants during abiotic stress. *Crit Rev Biotechnol.* 2010 30(3):161–75. <https://doi.org/10.3109/07388550903524243> PMID: 20214435
25. Kohli SK, Khanna K, Bhardwaj R, Abd_Allah EF, Ahmad P, Corpas FJ. Assessment of Subcellular ROS and NO Metabolism in Higher Plants: Multifunctional Signaling Molecules. *Antioxidants.* 2019 8 (12):641. <https://doi.org/10.3390/antiox8120641> PMID: 3184238